Microorganisms possess diverse mechanisms to regulate investment into individual cellular processes according to their environment. How these regulatory strategies reflect the inherent trade-off between the benefit and cost of resource investment remains largely unknown, particularly for many cellular functions that are not immediately related to growth. Here, we investigate regulation of motility and chemotaxis, one of the most complex and costly bacterial behaviors, as a function of bacterial growth rate. We show with experiment and theory that in poor nutritional conditions, *Escherichia coli* increases its investment in motility in proportion to the reproductive fitness advantage provided by the ability to follow nutrient gradients. Since this growth-rate dependent regulation of motility genes occurs even when nutrient gradients are absent, we hypothesize that it reflects an anticipatory preallocation of cellular resources. Notably, relative fitness benefit of chemotaxis could be hypothesized that it reflects an anticipatory preallocation of cellular resources. Notably, relative fitness benefit of chemotaxis could be observed not only in the presence of imposed gradients of secondary nutrients but also in initially homogeneous bacterial cultures, suggesting that bacteria can generate local gradients of carbon sources and excreted metabolites, and subsequently use chemotaxis to enhance the utilization of these compounds. This interplay between metabolite excretion and their chemotaxis-dependent reallocation is likely to play an important general role in microbial communities.

Bacterial responses to environmental changes range from adjustment of metabolism to induction of different stress protection mechanisms and tactic behavior. In most cases, there is an apparent qualitative benefit of investment into a cellular function under particular environmental conditions, e.g., uptake of a specific carbon source when it is available. However, demonstrating that a regulatory strategy exactly follows the physiological benefit of the respective cellular function remains challenging, although several recent studies suggested that allocation of metabolic resources may indeed optimize growth (1–6). Most prokaryotes can swim by rotating flagellar filaments, powered by the proton motive force. Swimming bacteria can typically follow various chemical gradients in their environment, by modulating the frequency of cell reorientations (tumbles) dependent on their swimming direction relative to the gradient (7, 8). The core of the chemotaxis signaling pathway includes stimulus-specific chemoreceptors (Tar, Tsr, Trg, Tap, and Aer in *Escherichia coli*), which regulate the cytoplasmic histidine kinase CheA and thereby phosphorylation of the response regulator CheY. Phosphorylated CheY controls rotation of flagellar motor; in the case of *E. coli*, promoting its directional switching that induces tumbles. *E. coli* chemotaxis pathway further includes CheZ, the phosphatase of CheY, and the adaptation enzymes CheR and CheB.

Flagellar motility consumes several percent of total cellular protein and energy budget (9, 10), which is primarily spent for the biogenesis of flagella and powering of their rotation, respectively, and to a lesser extent for the chemotactic signaling. Consistent with this high cost of motility, expression of flagellar genes can significantly reduce growth (11). Nevertheless, *E. coli* motility is up-regulated in poor carbon sources, as a part of the region controlled by cyclic adenosine monophosphate (cAMP) and its receptor protein (CRP) (1, 12, 13). Although this investment strategy could potentially enhance chemotaxis-mediated nutrient uptake in heterogeneous environments (14), thus enabling risk-prone foraging behavior (12), under which conditions it might be advantageous remained unclear, particularly considering that selection pressure on motility and chemotaxis is environment specific (15).

In this study, we investigated the relation between costs and benefits of motility in *E. coli* cells grown in a number of carbon sources of different quality (Fig. 1A). Our results suggest that bacteria invest in swimming behavior in proportion to the fitness benefit provided by chemotaxis, both of which depend on the growth rate. This demand-dependent investment could be recapitulated by mathematical modeling that considers growth-dependent proteome partitioning, chemotaxis, and competition for nutrients. Tactic enhancement of nutrient utilization was observed in externally introduced or in self-generated gradients of nutrients, suggesting that it is important across a wide range of environments.

Significance

*To which extent gene regulatory programs are optimized by evolution is one of the fundamental biological questions.* Swimming motility is one of the costliest bacterial behaviors, but expression of motility genes nevertheless increases when bacteria grow slower on poor carbon sources. Here, we show that competitive fitness benefit provided by the ability of motile bacteria to follow gradients of secondary nutrients shows similar negative dependence on the growth rate as the investment in motility. Thus, bacteria appear to pre-invest into the motile behavior in proportion to the anticipated benefit that can be provided by chemotaxis when nutrient gradients become available in their environment.

Author contributions: B.N., R.C., H.L., R.G.E., and V.S. designed research; B.N., R.C., H.L., and R.G.E. performed research; B.N. and R.C. analyzed data; and B.N., R.C., H.L., R.G.E., and V.S. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1910849117/-/DCSupplemental.

First published December 23, 2019.
both strains were initially inoculated at a 1:1 ratio. 

**Results**

**Growth-Rate Dependent Investment in Motility.** We first assessed regulation of motility dependent on the growth rate, by culturing *E. coli* MG1655 Δflh (deleted for the major surface adhesin to minimize cell aggregation) with shaking in different carbon sources. We observed that, similar to other cAMP-CRP regulated genes (5), activity of the chemotaxis (Ptar) or the flagellin (PflhC) gene promoters showed a negative relationship with the growth rate (Fig. 1B and SI Appendix, Fig. S1A). The growth rate on a particular sole carbon source under these conditions of well-mixed culture was defined as the nutritional value of this carbon source (N-value), and it was used as a carbon source identifier throughout the manuscript. The up-regulation of motility genes at low N-value was reflected at the phenotypic level as a proportional increase of the cell swimming speed (SI Appendix, Fig. S1B) and of the chemotactic efficiency of a bacterial population (SI Appendix, Fig. S1C), which could be explained by the increased number (SI Appendix, Fig. S1D) and length (SI Appendix, Fig. S1E) of flagella.

Next we used the mathematical model that is based on the previous theory of the growth-dependent protein resource allocation (5, 16) to describe how motility gene expression varies with the growth rate. This model assumes that a fixed finite size of the protein budget can either be dedicated to nutrient uptake or growth, i.e., protein synthesis, but trade-offs determine optimal growth rate (Fig. 1A, see SI Appendix, Supplementary Text for details). Indeed, the model could well reproduce the observed growth-rate dependence of motility gene expression (Fig. 1B, solid line).

**Relative Fitness Cost of Motility Depends on Carbon Source.** In order to directly evaluate the costs and benefits of motility as a function of the N-value, we used pairwise growth competition between strains to assess their relative reproductive fitness under particular conditions (11, 17). To monitor the ratio of 2 strains in co-cultures using flow cytometry, we respectively labeled them with either cyan or yellow fluorescent proteins, CFP or YFP (SI Appendix, Supplementary Text). Cartoon in D and E illustrates emergence of gradients of casein hydrolysate from an introduced bead. In all competition experiments, both strains were initially inoculated at a 1:1 ratio.

**Fig. 1.** Nutrient-dependent costs and benefits of motility in the presence of introduced gradients. (A) Schematics illustrating growth-rate dependent partitioning of the cellular protein budget into different functional sectors, as well as catabolization of 2 different nutrients (triangles and squares) regulated by cAMP (small circles). Also indicated is chemotaxis-mediated enhancement of nutrient uptake. (B) Relation between growth rate, denoted as nutritional (N-) value of the carbon source and motility gene expression. Cells were cultured with shaking (180 r.p.m.) in minimal medium containing different carbon sources as indicated (Rib: 20 mM ribose, KG: 12 mM 2-ketoglutarate, Suc: 15 mM succinate, Gro: 0.4% glycerol, GlcA: 20 mM gluconate, Fru: 20 mM fructose, Mal: 0.2% maltose, Ara: 20 mM arabinose, Glc: 0.4% glucose). Expression of Ptar-GFP reporter and growth rate were measured at log phase of growth using plate reader (see Methods and SI Appendix, Supplementary Text). Solid line shows resource allocation model with 2 fitting parameters as detailed in SI Appendix, Supplementary Text; zero promoter activity is predicted at the N-value of 1.03. (C and D) Growth competition in the coculture of motile WT cells (expressing CFP) and nonmotile ΔflhC cells (expressing YFP), with flhDC expressed either under native promoter (solid diamonds) or under Pmam (open diamonds) promoter induced with 10 μM IPTG. Shown is fraction (in %) of the WT cells in a mixed population grown for 72 h without shaking in the absence (C) or in the presence (D) of a bead containing 12% casein hydrolysate. N-values were taken from B. (E) Same as D, but for competition between WT and ΔcheY cells. Solid lines show fit to the data using simulations of 2-compartment chemotaxis model (black) and prediction of the full 2D model (green) as detailed in SI Appendix, Supplementary Text. Cartoon in D and E illustrates emergence of gradients of casein hydrolysate from an introduced bead. In all competition experiments, both strains were initially inoculated at a 1:1 ratio.
To test whether the increased relative fitness cost of motility at low N-values is due to the elevated expression of flagellar genes, we placed the fisDC operon encoding transcriptional activator of the entire flagellar regulon under control of the synthetic P_{ac} promoter that is inducible by β-thio-galactoside (IPTG), but independent of cAMP-CRP regulation (18). Although in this background the expression of motility genes could be elevated above their native levels and largely decoupled from the growth rate (SI Appendix, Fig. S2G), the fitness cost of motility remained proportional to gene expression (SI Appendix, Fig. S2H).

**Competitive Advantage of Chemotaxis in Presence of Introduced Nutrient Gradients.** To explore the potential fitness benefits of motility and chemotaxis under conditions of competitive growth, nutrient gradients were introduced into the unstirred culture by using small (40 μL) agarose beads that contain 12% casein hydrolysate, a mixture of amino acids (SI Appendix, Fig. S3A). In the presence of these gradients and in poor carbon sources, the fraction of WT cells in the coculture with ΔfisC strain was >50% (Fig. 1D and SI Appendix, Fig. S3B), suggesting that the ability to locate nutrient gradients exceeded the high fitness cost of motility. In contrast, in carbon sources with high N-value, motility remained overall disadvantageous, despite its comparatively low fitness cost.

Confirming that this advantage of motility in the presence of nutrient gradients was due to chemotaxis, the WT outcompeted the motile but nonchemotactic ΔcheY strain in the same growth-rate dependent manner (Fig. 1E). Since these 2 strains have similar investment in motility, the competitive reproductive fitness advantage of the WT relative to ΔcheY provides a direct measure for the relative fitness benefit provided by chemotaxis under our experimental conditions. Swimming itself provided no advantage but rather carried significant cost, as ΔcheY strain was outcompeted by the flagellated but nonmotile ΔmotAB strain (SI Appendix, Fig. S3C). Importantly, a similar growth-rate dependent advantage of motility and chemotaxis was observed in P_{ac-fisDC} background (Fig. 1D and E), despite a very different dependence of gene expression on the carbon source (SI Appendix, Figs. S2G and S3D). This demonstrates that the increased benefit of chemotaxis in poor carbon sources is due to the increased importance of following nutrient gradients rather than to the higher investment in motility.

We next investigated how relative fitness benefit provided by chemotaxis depends on the concentration of the secondary nutrient. For *E. coli* grown on ribose, it exceeded the fitness cost of motility for the levels of casein hydrolysate in beads above 5% (SI Appendix, Fig. S3B). This corresponds to ~20 mM aspartate and 10 mM serine, the 2 strongest attractants and at the same time preferentially consumed amino acids for *E. coli* (19). This fitness benefit of chemotaxis was nearly identical in experiments with 5% and 12% casein hydrolysate in beads over the entire range of the N-values (SI Appendix, Fig. S5E). In poor carbon sources, the trade-off between this benefit and the cost of motility was also similar for both these levels of casein hydrolysate (SI Appendix, Fig. S5G). However, at higher N-values where chemotaxis generally confers less benefit, the cost of motility apparently exceeds the benefit of chemotaxis toward lower levels of casein hydrolysate (SI Appendix, Fig. S5G). Consistently, this balance between the relative fitness cost of motility and benefit of chemotaxis was affected by up-regulation of flagellar genes, using P_{ac-fisDC} construct: Although the benefit of chemotaxis remained the same or even increased slightly in the P_{ac-fisDC} background (Fig. 1E and SI Appendix, Fig. S3F), it was apparently outweighed by the higher cost of motility for most carbon sources, particularly at the lower level of casein hydrolysate (Fig. 1D and SI Appendix, Fig. S3H).

Computational modeling was then used to rationalize how the advantage provided by chemotaxis to the secondary nutrient depends on the N-value of the primary carbon source (Fig. L4 and SI Appendix, Fig. S4 A and B and Supplementary Text). Growth was described using the previously proposed model (20), which predicts the growth rate on 2 coutilized nutrients based on the growth rates on individual substrates. The primary carbon source was initially spatially homogeneous, while the secondary nutrient formed a gradient (SI Appendix, Fig. S4A). The N-value of the secondary nutrient, 1.0 h−1, was in the range of the growth rates observed at varying concentrations of casein hydrolysate (SI Appendix, Fig. S4C). Chemotactic cells were assumed to both spread due to random swimming and follow nutrient gradients, while nonchemotactic ones only spread randomly. Both cell types grew at the same rate on given nutrients. Here we simulated 2 different models: First, we described the process of gradient formation and chemotaxis using full spatial 2-dimensional (2D) simulations based on partial differential equations (PDEs; see SI Appendix, Supplementary Text), where the second nutrient leaked at constant rate from one side of the simulation box, mimicking diffusion out of the bead (SI Appendix, Fig. S4 B and G). Since the simulated gradient of secondary nutrient was confined to the immediate proximity of its source (SI Appendix, Fig. S4G), we also used a simplified model based on ordinary differential equations (ODEs) to fit experimental data. In this latter model, space is divided into 2 compartments, with the second nutrient being confined to one compartment while cells moved between the 2 compartments. Simulations of both models could qualitatively (prediction of the PDE model) and even quantitatively (data fitting by the ODE model) reproduce the observed dependence of the benefit of chemotaxis on the N-value (Fig. 1E). In our simulations, the reproductive fitness benefit of being able to localize the regions with higher concentration of the secondary nutrient is larger at low N-values, because in poor carbon sources the secondary nutrient is comparatively more nutritionally advantageous (SI Appendix, Fig. S4D). Consistent with experimental results (Fig. 1E), this dependence on the N-value was observed even without taking into consideration dependence of motility on the carbon source.

**Competitive Advantage of Chemotaxis in Absence of Introduced Gradients.** Surprisingly, we observed that chemotaxis could benefit bacteria even without imposed nutrient gradients, as revealed when coculturing the WT with ΔcheY or with similarly nonchemotactic ΔcheB strain without shaking (Fig. 2A and SI Appendix, Fig. S5A). The competitive advantage of the WT largely decreased at moderate mixing and was entirely abolished at strong mixing (Fig. 2B), indicating that it is caused by—apparently self-generated—gradients of nutrients. Equal reproductive fitness of the WT and ΔcheY cells in the stirred culture further confirms that the cost of the chemotactic signaling, incurred by the CheY phosphorylation cycle, is negligible compared to the overall cellular energy budget. In the unstirred culture, the enrichment of the nutrient formed a gradient (SI Appendix, Fig. S4C), while nonchemotactic ones only spread randomly. Both cell types grew at the same rate on given nutrients. Here we simulated 2 different models: First, we described the process of gradient formation and chemotaxis using full spatial 2-dimensional (2D) simulations based on partial differential equations (PDEs; see SI Appendix, Supplementary Text), where the second nutrient leaked at constant rate from one side of the simulation box, mimicking diffusion out of the bead (SI Appendix, Fig. S4 B and G). Since the simulated gradient of secondary nutrient was confined to the immediate proximity of its source (SI Appendix, Fig. S4G), we also used a simplified model based on ordinary differential equations (ODEs) to fit experimental data. In this latter model, space is divided into 2 compartments, with the second nutrient being confined to one compartment while cells moved between the 2 compartments. Simulations of both models could qualitatively (prediction of the PDE model) and even quantitatively (data fitting by the ODE model) reproduce the observed dependence of the benefit of chemotaxis on the N-value (Fig. 1E). In our simulations, the reproductive fitness benefit of being able to localize the regions with higher concentration of the secondary nutrient is larger at low N-values, because in poor carbon sources the secondary nutrient is comparatively more nutritionally advantageous (SI Appendix, Fig. S4D). Consistent with experimental results (Fig. 1E), this dependence on the N-value was observed even without taking into consideration dependence of motility on the carbon source.
in motile behavior to be proportional to the potential benefit provided by chemotaxis (see Discussion).

To further understand whether the relative fitness benefit of chemotaxis in the absence of introduced gradients is limited to a specific growth stage, we used flow cytometry to measure the composition of the coculture during growth on ribose (Fig. 2D), α-ketoglutarate (SI Appendix, Fig. S6A), gluconate (SI Appendix, Fig. S6B), or glucose (SI Appendix, Fig. S6C). The WT cell fraction increased steadily throughout the early (SI Appendix, Fig. S6A, Inset) or entire (Fig. 2D, Inset and SI Appendix, Fig. S6B, Inset) growth phase, with WT cells growing almost twice as fast as ΔcheY cells on ribose (Fig. 2E). No significant difference between the WT and ΔcheY was observed during growth on glucose (SI Appendix, Fig. S6C), confirming that at high N-value chemotaxis
Gradients of Carbon Sources and Excreted Metabolites Could Explain Advantage of Chemotaxis. We hypothesized that chemotaxis enables WT bacteria to follow beneficial (most likely nutrient) gradients that transiently emerge in unstirred bacterial cultures. Oxygen could be excluded as a possible gradient source, since sealing the cocultures with heavy mineral oil to prevent aeration, with WT cells competed against strains individually deleted for the deletion of Tar-dependent competitive advantage during growth on succinate, α-ketoglutarate, and glycerol (Fig. 3C), since Tar mediates attractant responses to all of these compounds (SI Appendix, Fig. S8 E–G). In contrast, Tsr apparently mediates repellent response to succinate and α-ketoglutarate (SI Appendix, Fig. S8 H–J), which might explain why during growth on these 2 carbon sources the deletion of tsr even increases the advantage of chemotaxis (SI Appendix, Fig. S7C).

We speculated that the ensuing higher efficiency of resource exploitation provides competitive advantage to the chemotactic bacteria in the coculture, enabling them to actively withdraw nutrient resources from the nonchemotactic cells, and that this fitness benefit provided by chemotaxis increases with the extent of nutrient limitation. In the absence of competition, however, nonchemotactic cells can eventually consume these nutrients and thus achieve similar growth. To test whether the lack of chemotaxis might indeed result in a less efficient consumption of the carbon source, we used a previously described assay of the pathway activity based on the Förster (fluorescence) resonance energy transfer (FRET) (21) to compare chemotactic response of the WT cells to supernatant derived from either WT or ΔcheY cultures. The WT cells, but not Δtg cells, responded much more sensitively to the ΔcheY supernatant than to the WT supernatant (Fig. 3E and F and SI Appendix, Fig. S8 A–D), indicating that the former indeed contains significantly higher levels of a Trg ligand—most likely less efficiently consumed ribose. Chemotaxis toward the carbon source gradients could also at least partly account for the Tar-dependent competitive advantage during growth on succinate, α-ketoglutarate, and glycerol (Fig. 3C), since Tar mediates attractant responses to all of these compounds (SI Appendix, Fig. S8 E–G). In contrast, Tsr apparently mediates repellent response to succinate and α-ketoglutarate (SI Appendix, Fig. S8 H–J), which might explain why during growth on these 2 carbon sources the deletion of tsr even increases the advantage of chemotaxis (SI Appendix, Fig. S7C).
**Succinate Is a Major Excreted Chemoattractant.** Following gradients of extracellular metabolites excreted by bacteria themselves appears to be comparably important for the observed fitness benefit. Such chemotaxis toward extracellular metabolites—mediated by Tar—could explain why ΔcheY cells compete equally with the WT during growth on ribose (Fig. 3B), despite clearly lacking response to this sugar (SI Appendix, Fig. S8 K–M). It is also likely to explain the advantage of chemotaxis during growth on carbon sources that do not themselves elicit a chemotactic response, such as gluconate (SI Appendix, Fig. S8 M and N).

Consistent with this hypothesis, we detected comparatively high (10–100 μM) levels of several potential chemoattractants and nutrients in the culture supernatant of WT cells growing on ribose, α-ketoglutarate, gluconate, or glucose (Fig. 4A and SI Appendix, Fig. S9A and Datasets S1 and S2), and culture supernatant could both elicit a chemotactic response (Fig. 3E) and enhance growth of an *E. coli* culture (SI Appendix, Fig. S9B) at dilutions of up to $10^{-3}$ to $10^{-4}$. In particular, high levels of succinate, which is sensed by *E. coli* as a chemoattractant at concentrations above 1 μM (SI Appendix, Fig. S8F), were excreted by ribose-, gluconate- or glucose-fed cells (Fig. 4A and SI Appendix, Fig. S9A). Secretion of succinate indicates that, under our conditions, these carbon sources are at least partly catabolized via mixed acid fermentation (22), presumably due to low levels of oxygen in the unstirred culture. Addition of 5 mM succinate was observed to promote culture growth, dependent of the N-value of the primary carbon source (SI Appendix, Fig. S9C). It further seems that succinate and several other extracellular metabolites are secreted into the culture medium and not released from lysing cells, since levels of these compounds were similar in the supernatant and in the sample from the whole culture broth (SI Appendix, Fig. S9D). Levels of external succinate were lower in the α-ketoglutarate-fed cultures, but high levels of another potent Tar-specific chemoattractant, t-glutamate, were detected instead (SI Appendix, Fig. S9A).

**Emergence of Nutrient and Cell Gradients in the Culture.** How gradients of carbon sources and extracellular metabolites emerge in the culture? One possible origin of transient gradients, predicted by a previous theoretical study (14), could be local fluctuations in the nutrient consumption by bacteria. Another likely origin is gradual sedimentation of bacteria, which is common in aquatic habitats and could establish a nonuniform profile of cell density and therefore of nutrient consumption in the unstirred culture. Consistent with this second scenario, an increasing fraction of bacteria could be found in the sediment at the later stages of culture growth (Fig. 4B). This fraction was significantly smaller in the WT than in the ΔcheY culture, indicating that chemotaxis in emergent gradients reduces sedimentation of the WT cells. This difference was even more pronounced in the coculture, where the fraction of the WT was markedly higher in the upper layers of the culture than near the sediment (Fig. 4C).

To better understand this interplay between emergent gradients of secreted and consumed metabolites and chemotaxis, we modified our numerical models to include excretion of the secondary nutrient as a byproduct of the carbon source consumption (SI Appendix, Fig. S10 and Supplementary Text). This secondary nutrient is then channeled along the primary nutrient for growth. In the full 2D simulations, cell sedimentation due to gravity was also explicitly considered. We observed that chemotaxis toward the secondary nutrient was alone sufficient to gain an N-value dependent competitive growth advantage (Fig. 4D). The 2-compartment ODE model that considered only chemotaxis toward the secondary nutrient and assumed constant chemotactic efficiency could then be used to quantitively reproduce experimental data (Fig. 2A). Notably, nearly identical advantage of chemotaxis was observed when the efficiency of motility and chemotaxis was assumed to depend on the N-value (Fig. 4D), consistent with experiments (Fig. 2A).

The 2D simulations provided further insights into spatial dynamics that might be at play during our experiments (Fig. 4E and SI Appendix, Fig. S10G). We observed that sedimentation favored the initial nucleation of dense patches of chemotactic cells, formed at the bottom of the simulation box via chemotaxis toward self-generated gradients of excreted metabolites. These patches later merged and eventually traveled as a band from the bottom to the top of the box, leaving behind a partially depleted medium. Although chemotaxis toward the primary carbon source had itself little importance in our simulations because its gradients were relatively weak (Fig. 4D and SI Appendix, Fig. S10G), chemotaxis toward the secondary nutrient nevertheless increased availability of the primary carbon source to the WT cells since at the later time points both gradients were coincident. Chemotactic response to the primary carbon source became more important when cell sedimentation was assumed to moderately increase in our simulations, which resulted in much steeper carbon source gradients (SI Appendix, Supplementary Text). Thus, the combination of feeding on higher levels of secondary nutrients and reaching higher levels of the primary carbon source in the upper layer of culture gives the WT cells a growth advantage over the nonchemotactic cells, which were denser at the bottom (SI Appendix, Fig. S10G).

**Discussion**

Although microorganisms evolved various regulatory strategies to reallocate their resources dependent on the environment (23), quantitative comparison of costs and benefits of a particular investment strategy for microbial fitness is rarely available. It thus typically remains unclear whether the amount of resources allocated to a particular function follows a specific evolutionary logic of optimization (6). One notable exception here is the regulation of bacterial metabolism, where relative investment into catabolism versus biosynthesis, coordinated by cAMP-CRP signaling, appears to be balanced to maximize growth (1–3, 5). Similarly, at least in some cases, the uptake of a carbon source has been apparently optimized for maximal growth (5). However, bacterial growth on many carbon sources is clearly suboptimal (24), indicating that in those cases the regulation of resource allocation did not evolve to maximize growth but rather toward other evolutionary objectives (6). These might include pre-allocation of resources into biosynthetic or metabolic machinery that may become beneficial when bacteria become suddenly exposed to nutrients, or investment into other functions that are not apparently related to growth (25, 26). Particularly for these latter types of investment, conditions under which they provide fitness benefit and the quantitative relation between the extent and cost of investment and its benefit are unknown.

Here, we investigated costs and benefits of resource allocation into motile behavior, the major cAMP-CRP-dependent cellular function that is not immediately related to *E. coli* growth. Similarly to other cAMP-regulated genes, the expression of motility genes increases linearly with the decrease of growth rate on poorer carbon sources (1, 5). This results in a proportional increase of the length and number of flagellar filaments, and consequently of cell swimming velocity and chemotaxis. The relative fitness cost of motility, i.e., growth disadvantage of motile cells under conditions where motility provides little or no benefit, was also proportionally higher at lower growth rates. This increasing cost was primarily a consequence of higher investment into the biosynthesis of flagella, whereas the cost of swimming was also measurable but lower and only weakly dependent on the growth rate, and the cost the chemotactic signaling was negligible. Thus, linearly increased investment in motility at lower growth rates results in a proportionally linear increase of both the functionality and the cost of motility.
The same negative dependence on the $N$-value of the carbon source was observed for the relative fitness benefit that is provided by chemotaxis, when chemotactic and nonchemotactic cells are cocultured in the presence of nutrient gradients. This dependence could also be seen when expression of chemotaxis and flagellar genes was largely decoupled from the growth rate, implying that increased benefit of chemotaxis at low $N$-values is not the consequence of increased investment but rather due to greater importance of locating additional nutrients when the primary carbon source is poor. The growth-rate dependent regulation of motility genes thus apparently ensures that investment in motility, including chemotaxis, is proportional to the fitness benefit that chemotaxis could provide when nutrient gradients are encountered in a particular carbon source. However, since motility gene regulation was also observed in a shaking culture where gradients cannot form, we conclude that the correlation between potential benefit and investment in motility and chemotaxis is predictive (anticipatory) rather than causal (23). We hypothesize that in the case of chemotaxis such predictive investment might have emerged because chemotaxis operates on a much shorter timescale than gene expression (7) and microscale gradients of nutrients in many natural environments are highly variable and short-lived (27), making direct regulatory coupling of gene expression to the benefit provided by chemotaxis unreliable. Precallocation of increasing amounts of resources in motility and chemotaxis at low growth rates—when the payoffs are likely to be larger—might be a superior strategy, which could explain why motility genes are regulated by cAMP-CRP. The trade-off between the relative fitness benefit and the cost of motility is ultimately dependent on the abundance and quality of encountered nutrient gradients.

The growth-rate dependent fitness benefit of chemotaxis could be observed not only when gradients of nutrients were introduced into bacterial culture, but also in self-generated gradients of the excreted secondary metabolites and of the primary carbon source.

Bacterial sedimentation was the likely initial source of nonuniform distribution of bacteria in a culture under our experimental conditions, followed by chemotaxis-dependent redistribution of bacteria toward higher levels of both primary and secondary nutrients. Similar interplay between sedimentation and motility may play an important role in natural (e.g., aquatic) habitats, along with numerous other sources of microto mesoscale heterogeneity (28). Excretion of metabolites, including amino acids, sugars, and organic acids into the environment is also a common phenomenon in bacteria and other microorganisms (29). It might serve to optimize protein allocation during growth (2), but excreted metabolites have also been shown to mediate (mutualistic) metabolic interactions (30) or signaling (31) in various microbial communities, as well as chemotactic self-concentration of bacteria (32–34). Our work thus suggests that chemotaxis may provide a general ecological advantage in competition for reutilization of excreted metabolites, even in communities consisting of single species as long as spatial heterogeneity is allowed to emerge. Such competition for local sources of metabolites is likely to be even more important in structured natural communities (27, 28), and we hypothesize that the regulation of *E. coli* motility has evolved under this selection.

**Methods**

Detailed description of experimental methods and mathematical modeling can be found in SI Appendix.

**Data Availability.** Metabolomics data and codes used for ODE and PDE simulations are available as Datasets S1–S4.

**ACKNOWLEDGMENTS.** We thank Thomas Heimerl, Seigo Shima, and Gangfeng Huang for their help with experiments. This research was supported by the Max Planck Society, European Research Council grant 294761-MicRobE to V.S. and Biotechnology and Biological Sciences Research Council grant BB/N00065X/1 to R.G.E.

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