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Current Opinion in Microbiology

Engineered bacteria to report gut function: technologies and implementation

Tanmay Tanna[1,2,5], Raghavendra Ramachanderan[1,5] and Randall J Platt[1,3,4]

Advances in synthetic biology and microbiology have enabled the creation of engineered bacteria which can sense and report on intracellular and extracellular signals. When deployed *in vivo* these whole-cell bacterial biosensors can act as sentinels to monitor biomolecules of interest in human health and disease settings. This is particularly interesting in the context of the gut microbiota, which interacts extensively with the human host throughout time and transit of the gut and can be accessed from feces without requiring invasive collection. Leveraging rational engineering approaches for genetic circuits as well as an expanding catalog of disease-associated biomarkers, bacterial biosensors can act as non-invasive and easy-to-monitor reporters of the gut. Here, we summarize recent engineering approaches applied *in vivo* in animal models and then highlight promising technologies for designing the next generation of bacterial biosensors.

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Introduction

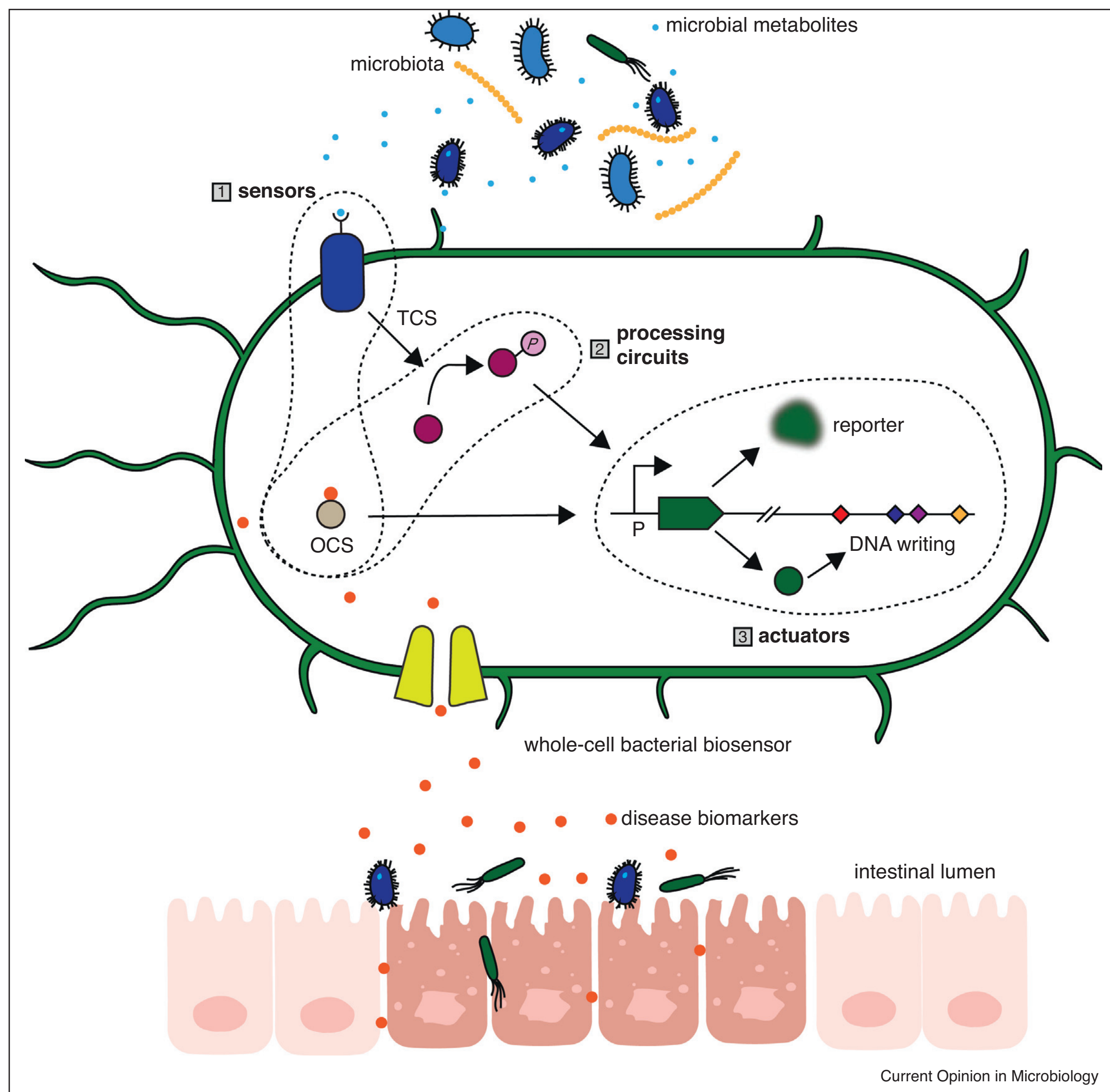
Bacteria have evolved complex mechanisms to sense and respond to diverse external signals which can be harnessed to repurpose them to monitor and modulate their environments. While a plethora of whole-cell bacterial biosensors have been described for food and environmental monitoring [1,2], their development as *in vivo* diagnostics and therapeutics has lagged behind owing to the complexity of the environment and regulatory hurdles. Recent advances in synthetic biology combined with an increased understanding of host–microbe and microbe–microbe interactions, particularly within the gut, enable exciting applications for engineered bacteria as living medicines, which have been extensively reviewed recently [3•,4,5•], and as reporters of *in vivo* environments, which is the focus of this review.

The human gut provides a rich source of biomarkers of health and disease which are readily accessible to microbes within the intestinal lumen [6]. Alterations in the composition of gut microbiota and the dynamics of gut metabolites have been associated with several pathological states, including metabolic disorders, immune diseases, cancer, neurological diseases, and behavioral disorders [7]. Although certain biomarkers such as lactoferrin, C-reactive protein, and inflammatory molecules like cytokines and metabolites found in stool samples are used as indicators of disease, they are non-specific, transiently present, and inadequate to reliably predict disease progression [8,9]. Omics-based measurements of fecal material have uncovered several associations between disease states and gut microbial composition, but the gut is spatially heterogenous and houses several distinct niches whose composition and dynamics are influenced by diet and lifestyle but not captured in stool samples [10–12]. Indeed, analyses of fecal microbiota are largely uninformative about the proximal intestine, and are even remarkably different to the contents of the lower digestive tract which it should most closely represent [13]. While surgical, endoscopic- and microdevice-based methods for collecting intraluminal samples are also utilized, these approaches can be highly invasive and are also easily contaminated, cannot detect transient signals, and generally require disruptive methods that confound interpretations [14,15•].

Bacterial biosensors can be deployed to eavesdrop on the chatter between the host and its microbiota for monitoring gut health, since they widely interact with the host as well as each other, can readily explore gut niches, and can be retrieved without disruptive or invasive approaches. The recently expanded toolkit for genetic manipulation, including CRISPR-Cas technologies, as well as systems-level insights into prokaryotic biology have yielded new synthetic genetic circuits and bacterial cell-based sensors that can detect disease biomarkers with specificity and are functional in the gut [16–19,20•].

Non-invasive monitoring of the gut through fecal testing requires engineered microbes equipped with three key elements (Figure 1): 1) biosensors that detect transient or localized signals; 2) processing circuits for converting the stimulus into a predefined downstream response; and 3) actuators or DNA-based storage mediums that report on the presence of the original stimulus. Sensing elements in bacteria are typically one-component or two-component systems. One-component systems (OCSs) are allosteric transcription factors (TF) that activate downstream gene expression upon ligand binding. Unlike OCSs, two-component systems (TCSs) typically sense extracellular signals through transmembrane histidine kinases which phosphorylate cytoplasmic response regulator proteins that control gene expression. Synthetic processing circuits come in many flavors depending on the target application and are generally built to assess whether a stimulus is present (binary detection), how much of a stimulus is present (analog detection), or how much of a stimulus was present throughout time (DNA writing-based detection). While actuator elements utilize colorimetric, luminescent or fluorescent reporter proteins to enable rapid field-deployable digital readouts, DNA-based storage medium elements utilize DNA-modifying enzymes to enable the conversion and storage of stimulus information into heritable alterations in nucleic acid sequences, which upon

Figure 1



Components of whole-cell bacterial biosensors in the gut: Engineered bacteria that can report on environmental signals in the intestinal lumen consist of sensor components such as one-component systems (OCSs) or two-component systems (TCSs), processing circuits for transducing inputs into outputs, and actuator elements such as reporter proteins or DNA-writers for generating outputs that are detectable from fecal samples.

sequencing can illuminate a record of the stimulus over time [21•]. Below we summarize a few selected approaches that have been successful in developing robust *in vivo* bacterial biosensors, and highlight promising technologies for developing future bacterial reporters in the gut.

Demonstrated applications of engineered microbes in the gut

Successful bacterial biosensors need to access target environmental niches and survive transit through the gut, and thus require sensing modules whose sensitivity is robust to unpredictable environmental perturbations as well as genetic circuits that stably propagate over time without imposing fitness burdens on the bacterial chassis. Synthetic memory circuits that retain information of past events are critical for capturing transient signals in this regard. Early circuits leveraged OCSs to sense environmental signals and regulate reporter proteins, but suffered from leaky expression and a low dynamic range as well as decreased strain fitness and the inability to retain information past a few days [17,22]. The latter is particularly critical to develop stable biosensors that can monitor disease biomarkers long-term.

Kotula *et al.* built a memory circuit that overcame these limitations by using the *cI/cro* toggle switch from bacteriophage λ (Figure 2a) [23]. This circuit expresses cI in its default state but flips to a Cro-dependent state upon exposure to anhydrotetracycline (aTc), triggering expression of β-galactosidase (*lacZ*) providing a colorimetric readout. An *Escherichia coli* (*E. coli*) strain NGF-1 harboring this memory circuit reported aTc-induced Cro-switching within the murine gut, detectable up to a week after aTc was removed. In a follow-up study, the authors coupled the circuit with the TtrR/TtrS TCS from *Salmonella typhimurium* to trigger Cro expression upon sensing tetrathionate, a marker for gut inflammation (Figure 2a) [24]. Upon colonizing the murine gut – in mice pretreated with the broad-spectrum antibiotic streptomycin to circumvent colonization resistance – their engineered bacteria could report on inflammation for up to 200 days. While this circuit represents a promising advance, the tetrathionate sensing module is susceptible to confounders — particularly nitrate and oxygen (Figure 2a) [25], and high Cro expression can lead to self-repression. In a subsequent study, the authors overcame the latter limitation by using a dominant negative mutant of cI instead of Cro, and used this modified circuit to screen a library of known *E. coli* promoters for novel stimuli in the gut [26].

In an effort to find more reliable sensing modules for gut inflammation, Daeffler *et al.* identified a TCS for sensing the inflammation biomarker thiosulfate from the marine bacterium *Shewanella halifaxensis*, a γ-proteobacterium like *E. coli*. This TCS was optimized for performance, did not undergo cross-regulation by nitrate, and was only weakly regulated by oxygen. Remarkably, an *E. coli* Nissle 1917 biosensor strain harboring this TCS was able to report on inflammation present within the gut without colonization or antibiotic pre-treatment, leaving the native microbiota mostly intact [27]. These studies illustrate that by judiciously selecting and screening for parts, the performance characteristics of circuits can be tuned for functionality in the gut.

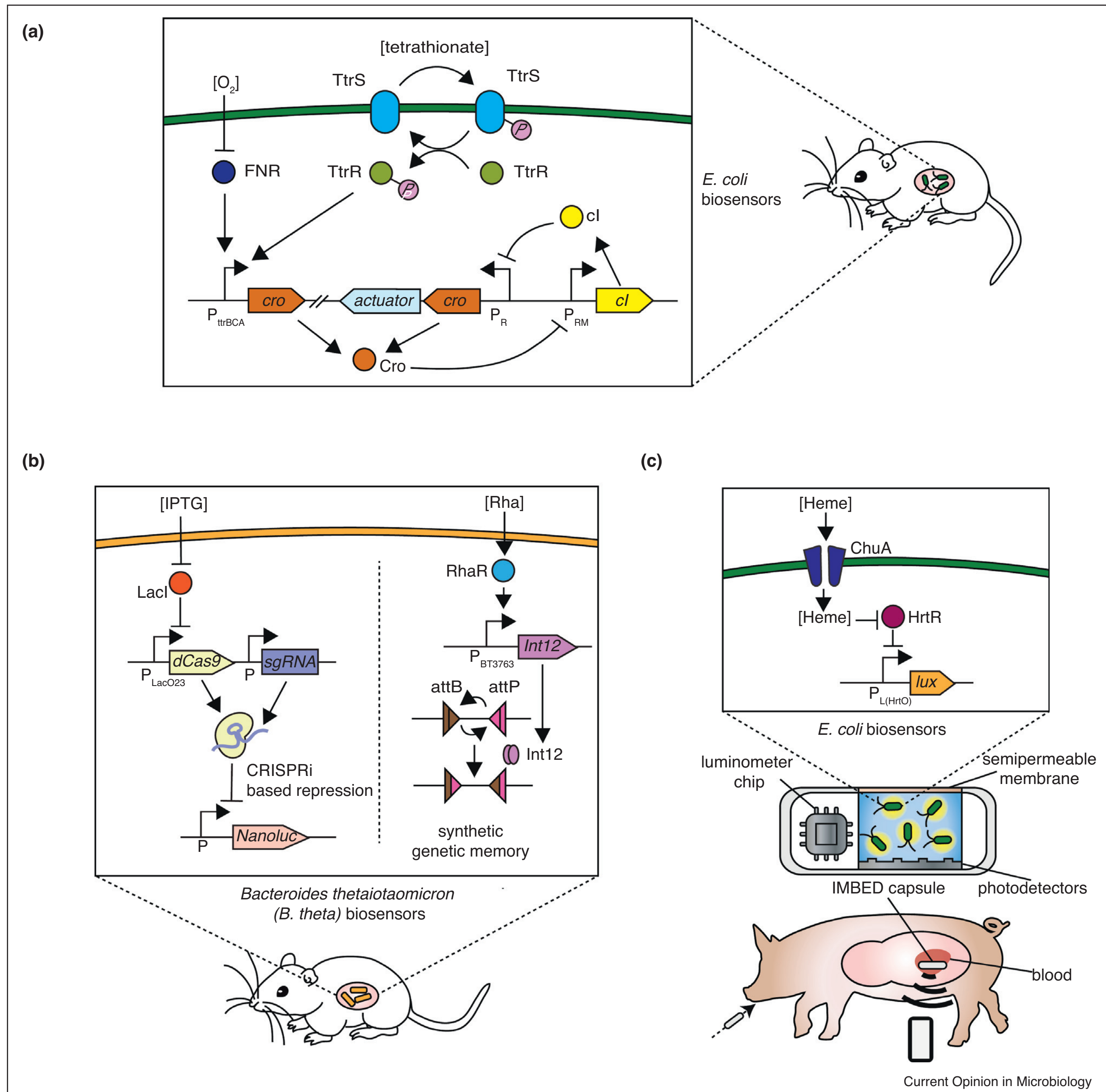
Although tools to engineer *E. coli* are the most established, *E. coli* are not major constituents of the gut microbiota across adult human populations and only poorly colonize the gut — indicating that the use of other commensal bacterial chassis could provide a complementary toolbox for interrogating gut function. In a study by Mimee *et al.*, the abundant human commensal *Bacteroides thetaiotaomicron* (*B. theta*) was engineered for *in vivo* applications by constructing novel genetic circuit elements, and shown to sense stimuli in the murine gut using both OCSs and TCSs (Figure 2b) [28]. Notably, this study demonstrated the first application of CRISPR-based components and DNA-writing elements for detecting signals in the gut (Figure 2b). While certain technical limitations still persist, including high levels of leaky expression, this study highlights the potential of non-model organisms as chassis for whole-cell biosensors.

Signal processing and reporting modules need not be restricted to bacterial circuitry. For instance, ingestible miniature electronic sensors are being increasingly used to monitor the gastrointestinal tract, but their function is restricted to imaging and sensing broad signals such as pH, temperature, and pressure [15•,29]. In one exciting study, the sensitivity of bacterial sensing was coupled to an electronic sensor, exploiting advances in building ultra-low-power microelectronic devices to create an ingestible micro-bio-electronic device (IMBED) platform that can detect signals *in vivo* and report them wirelessly in real-time from the mammalian gut [30••]. As a proof-of-concept, the authors incorporated heme-sensing *E. coli* cells into an IMBED capsule which was used to report on the presence of blood in the porcine gastrointestinal tract (Figure 2c). This approach could allow novel biomarkers to be rapidly reported *in vivo* and also be integrated with other modules to, for example, notify the patient or implement a closed-loop therapeutic system. While these devices are still too large for use in humans, they nonetheless offer a unique and promising approach to monitor the gut using bacterial sensing.

New approaches and technologies

Despite significant advances in bacterial biosensor development, the critical bottleneck remains the difficulty in identifying biomarkers as well as mining and optimizing sensor elements that detect them. Recent work describes multiple approaches to address this hurdle through both

Figure 2



Demonstrated examples of *in vivo* bacterial biosensors.

(a) Development of a tetrathionate-sensing memory circuit functional within the gut: The positive feedback loop between Cro and cI from the λ lytic lysogenic switch was exploited to build a low-burden memory element. This memory element was coupled with the TtrS/TtrR TCS which triggers transcription of Cro upon exposure to tetrathionate, flipping the switch from a cI-state to a Cro-state. The system is however confounded by other molecules such as oxygen via the transcriptional regulator FNR.

(b) Development of *Bacteroides thetaiotaomicron* (*B. theta*) as a chassis for gut biosensing: Multiple genetic components, including OCS-based and TCS-based sensors, transcriptional regulators, actuators and memory elements were described and engineered for use in *B. theta*. Particularly interesting was the IPTG-inducible CRISPRi system used to repress reporter luciferase activity, representing the first use of CRISPR-based components in gut biosensors, and the use of a Rhamnose-sensing OCS to express the site-specific recombinase Int12, which catalyzed the inversion of its binding site (*attB*/*attP*), leading to the formation of a genetic memory of Rhamnose exposure.

(c) Demonstration of an ingestible micro-bio-electronic device (IMBED): *E. coli* Nissle 1917 biosensor cells were enclosed by a semipermeable membrane in a cavity within the IMBED capsule. These cells were equipped with the ChuA outer-membrane transporter to facilitate the transit of extracellular heme through the cell envelope and the heme-responsive transcriptional repressor HrtR for regulating the expression of the luciferase operon (*lux*). Luciferase activity was detected by phototransistors below the cavity, processed by a luminometer chip and could be wirelessly transmitted to external devices in real-time.

OCS-based and TCS-based sensing [31,32] as well as through fundamentally new approaches.

Biosensing through one and two component systems

OCSs have been widely implemented as sensing modules because of their simplicity and ubiquity. While novel OCSs can be identified bioinformatically, their input stimuli have been challenging to predict. Hanko *et al.* leveraged the knowledge that LysR-type TFs and their regulated genes are transcribed in divergent orientations for discovering novel OCSs along with their ligands and target promoters from published metagenomic data (Figure 3a) [33,34••]. Using this approach, the authors identified and characterized numerous novel OCSs from the metabolically versatile *Cupriavidus necator*, including TFs for gut-relevant ligands such as sugars and amino acids, and demonstrated their functionality when transplanted into other bacteria such as *E. coli*.

Recent work has also demonstrated that the performance characteristics of OCSs can be optimized. Meyer *et al.* used a dual selection scheme to optimize the performance of 12 small-molecule sensing TFs, and genomically integrated these into *E. coli* 'marionette' strains, allowing independent and orthogonal induction of 12 sense-and-respond circuit elements [35]. Using an engineering approach, Mannan *et al.* formulated general phenomenological models for biosensors, showing that sensor response thresholds and dynamic ranges are interdependent, and tuning biosensor characteristics by altering ligand-TF affinity or the affinity of the TF to the promoter [36].

Although numerous TCSs for sensing various stimuli – such as peptides, carboxylic acids, metal ions, or quorum sensing signals – have been described [37], TCS-based sensing is limited by insufficient functional characterization: the input stimuli or target promoters for most systems are unknown. A recent study by Schmidl *et al.* discovered that the DNA-binding domains (DBDs) and phosphorylation domains of TCS response regulator proteins are separable, facilitating modular function and portability (Figure 3b) [38••]. The authors swapped the DBD of the *E. coli* nitrate sensing NarX/NarL TCS with one native to *Bacillus subtilis* and showed it to be functional in *B. subtilis*. They further screened TCSs from *Shewanella oneidensis* in *E. coli* with their DBDs swapped to a known *E. coli* DBD and identified a pH-sensing system. However, such modularity is not complete — not all DBDs can be swapped this way, and the optimal position to separate the domains for a given response regulator might have to be individually tested. Additionally, the input dynamic range varies depending on the precise amino acids at which the two proteins are fused.

Addressing TCS response tunability, a different study by Landry *et al.* showed that the input detection thresholds and dynamic ranges of TCSs are tunable by mutating a single conserved amino acid in the histidine kinase that affects phosphatase activity [39]. Another study generated orthogonal kinase-regulator pairs with minimal cross-talk by mutating a subset of less than 11 amino acids lining the interaction surface between them. The authors exploited this feature to design multiple orthogonal TCS-based sensing pathways, demonstrating potential for multiplexed biosensing [40]. Together, these advances show that both OCSs and TCSs are highly tractable.

Novel biosensors based on antibodies

An orthogonal approach for potentially developing biosensors for any ligand of interest uses fusions of DBDs to single-domain antibodies which dimerize upon ligand binding to regulate gene expression (Figure 3c) [41,42••,43]. Single-domain antibodies function as either transmembrane or cytosolic receptors and can bind varied ligands, making this approach potentially modular and scalable. However, this approach still suffers from a low signal-to-noise ratio and the only ligands currently known to induce antibody dimerization are caffeine and its metabolites, but structural studies indicate that this mode of binding may not be uncommon [44]. Parenthetically, when integrated within TCSs, induced dimerization of response regulators in the absence of phosphorylation from the cognate histidine kinase is sufficient to trigger DNA binding [45], and split-protein reporter systems such as those based on T7 have already been developed [46]. This approach demonstrates that biosensor designs need not be limited to existing mechanisms in bacteria.

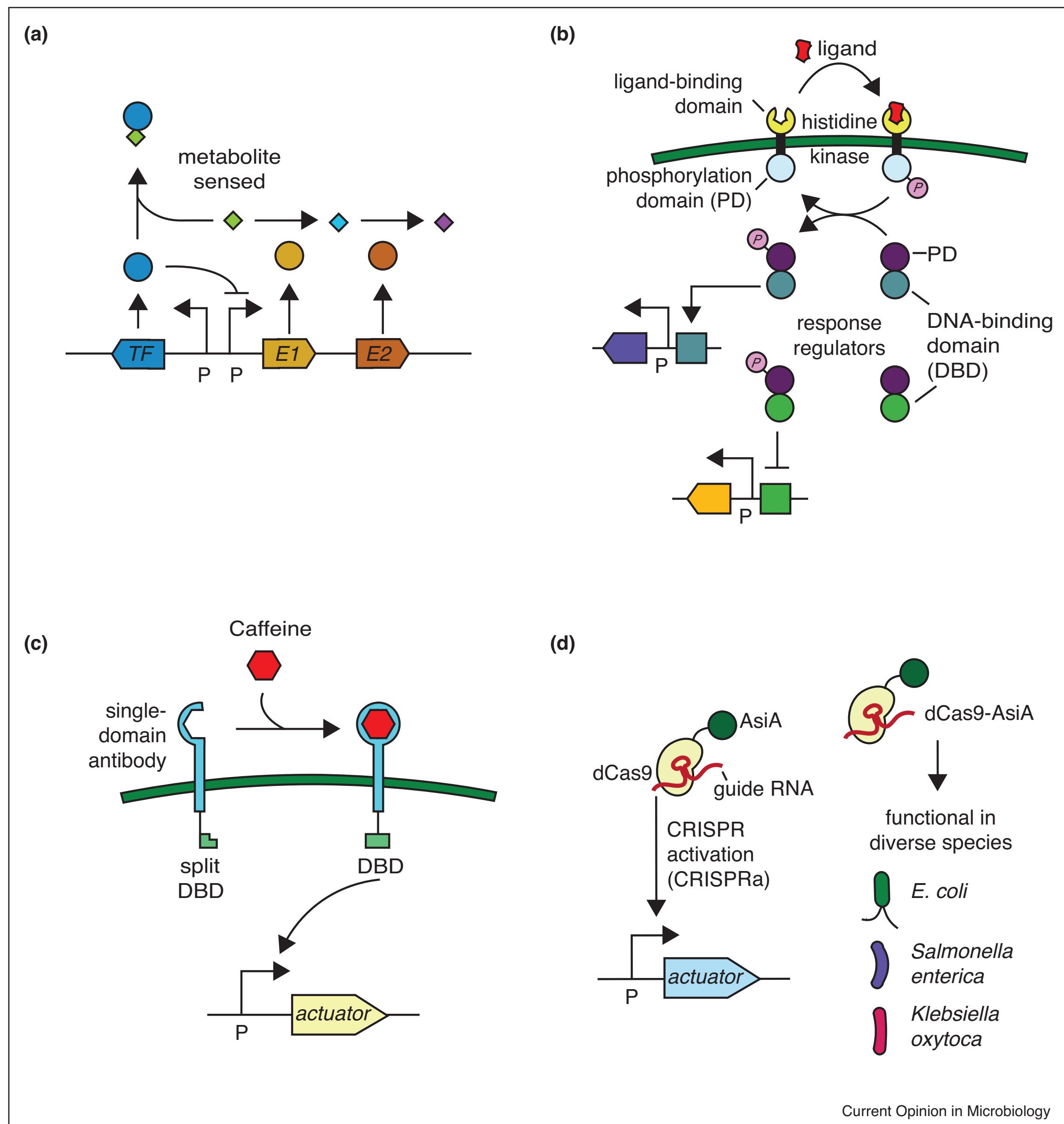
Leveraging bacterial population behaviors for biosensing

Effective diagnostic biosensors will necessitate processing several inputs and orchestrating downstream computation, but complex designs decrease strain fitness [22]. While several strategies for engineering circuits have been recently reviewed [47,48], one exciting approach explores circuits beyond single cells and uses quorum sensing to express the actuator only at high cell densities of the sensing bacterium [49]. This allows for the tuning of sensor response characteristics while distributing the cell burden of signal processing across the population. In future systems, this could potentially be combined with 'pseudotaxis' – engineering bacteria to respond and move toward specific signals – endowing biosensing bacteria with population-level behaviors [50].

New synthetic biology parts based on CRISPR-Cas9 system components

CRISPR-Cas technologies offer innovative and flexible strategies for engineering bacteria, particularly in terms of gene regulation through transcriptional inhibition (CRISPRi) or activation (CRISPRa). CRISPRi functions by blocking transcription through the binding of catalytically inactive Cas (dCas) proteins at target sites and has been

Figure 3



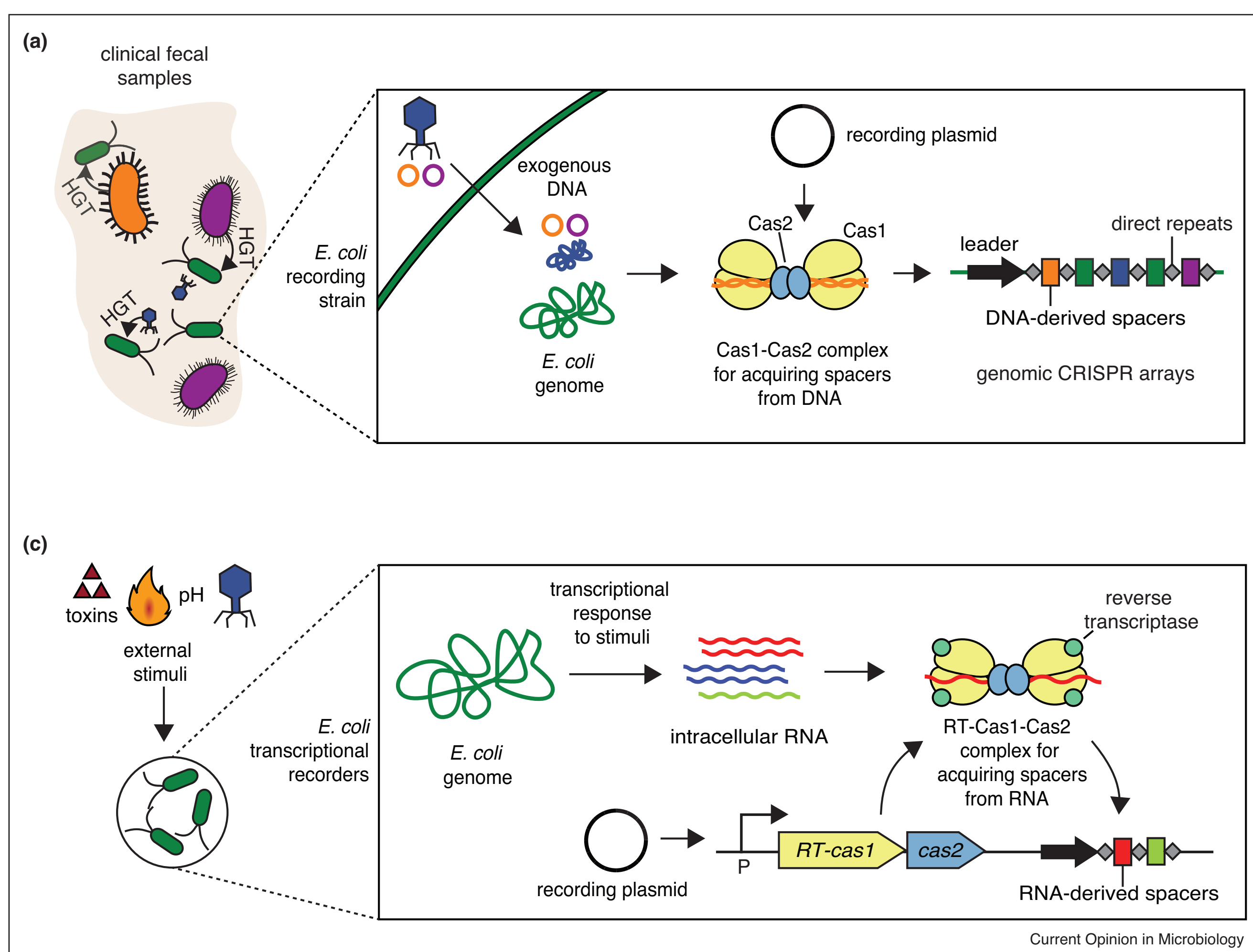
Promising technologies for future implementations of *in vivo* bacterial biosensors.
(a) Strategies to mine OCSs and their cognate ligands: OCSs were mined by a targeted search for operons of metabolic enzymes (E) which were adjacent to genes encoding transcription factors (TFs) transcribed in a divergent fashion. By identifying the likely metabolites processed by the enzymes, the regulator of the TF could be accurately identified.
(b) Modularizing TCSs: The response regulator in TCSs can be decomposed into phosphorylation domains (PDs) and DNA-binding domains (DBDs) that function modularly. Swapping of the DBDs allows TCSs to be rewired to regulate diverse promoters positively or negatively across species.
(c) Rethinking bacterial signaling: Ligand-induced dimerization of the single-domain anti-caffeine camelid antibody was used to regulate transcription of downstream actuators by fusing the antibody to split (monomeric) DBDs, which dimerized upon antibody dimerization and regulated downstream elements.
(d) Transcriptional activation using dCas9-AsiA: A fusion of dCas9 to the phage protein AsiA (dCas9-AsiA) was shown to induce gene activation in *E. coli* by up to 200-fold using guide RNAs targeting ~200 bp upstream of the transcription start site. dCas9-AsiA could activate thousands of promoters from a metagenomic library and was functional in diverse bacterial species.

used for gene repression in microbial gut biosensors (Figure 2b) [28,51]. CRISPRa functions by recruiting transcriptional machinery to target promoters through linking dCas proteins to TFs and has also been achieved in bacteria [52,53]. A recent paper by Ho *et al.* describes a generalizable strategy for developing CRISPRa effectors through screening dCas9-TF fusion libraries for transcriptional activation of fluorescent reporters and antibiotic resistance markers, followed by engineering identified proteins using directed evolution [54•]. Using this strategy, the authors identified the phage TF AsiA (Figure 3d), which they then showed was functional in several bacterial species and could activate hundreds of endogenous promoters, highlighting the multiplexability and portability of their approach. CRISPR-based regulatory components allow for precise control of gene expression and will play a central role in designing synthetic circuits for future bacterial biosensors.

DNA-writing with CRISPR-Cas9-based effectors

As an alternative to conventional binary readouts, DNA-writing offers unprecedented scalability and multiplexing capabilities for memory and actuator components [21•]. DNA-writing using recombinases has been demonstrated in gut biosensors (Figure 2c) [28], but recombinases can only generate a limited number of DNA modifications at specific sites, restricting the reporter to digital readouts. This can be particularly limiting in the context of the gut where, for instance, drug compounds are actively metabolized by gut microbiota, leading to variations in their

Figure 4



CRISPR spacer acquisition-based molecular recording in the gut microbiota.

(a) CRISPR spacer acquisition-based DNA recorders detect extensive horizontal gene transfer (HGT) in clinical fecal samples: *E. coli* recording cells overexpressing a Cas1–Cas2 DNA spacer acquisition complex were shown to capture endogenous and exogenous DNA-derived spacers and used to create genetic records of HGT events in the fecal microbiota of clinical samples.

(b) Record-seq uses the *Fs*RT–Cas1–Cas2 RNA spacer acquisition complex to record intracellular RNA-derived spacers in CRISPR arrays on a recording plasmid. This was leveraged for creating long-term records of the transcriptional response of sentinel cells to transient external stimuli such as pH, oxidative stress or herbicide exposure as well as phage-derived transcripts, and can help broadly illuminate environmental states.

effective concentrations in the guts of different individuals. Measuring such variations necessitates analog reporters [55]. CRISPR-based DNA writers offer a solution to these limitations given their flexibility and programmability. For example, DNA-writing effectors based on base editors – dCas9-cytidine deaminase fusions which generate mutations at specific sites – can be programmed to encode digital and analog information into DNA with temporal resolution [56,57], and hold promise for future gut reporters.

DNA-writing with CRISPR spacer acquisition

In addition to Cas9-based DNA-writing, CRISPR spacer acquisition complexes have been engineered to create versatile DNA writers capable of iterative multiplexed molecular recording of intracellular DNA or RNA [21•] — offering a provocative entry point to time-resolved massively multiplexed biosensing. DNA recording complexes comprise Cas1 and Cas2 proteins (Cas1–Cas2) that integrate short DNA fragments as spacers within CRISPR arrays [58], and can be leveraged to record analog information, including arbitrary DNA sequences encoded with user-defined information such as a short movie [59], metabolite concentrations [21•,59,60], or horizontal gene transfer in fecal microbiota (Figure 4a) [61••].

RNA recording complexes comprise a reverse transcriptase (RT)-fused Cas1–Cas2 (RT–Cas1–Cas2) that converts short RNA fragments into DNA spacers within CRISPR arrays [62]. Leveraging the RNA recording complex from the human commensal bacterium *Fusicatenibacter saccharivorans* (*Fs*RT–Cas1–Cas2), we developed 'transcriptional recording' [63••,64], a technology that enables bacteria to record their own transcriptomes, which through Record-seq can reveal the gene expression history of a population of cells. We demonstrated that Record-seq can reveal transcriptional states of *E. coli* in response to environmental stimuli like oxidative or acid stress as well as transient exposure to the herbicide paraquat (Figure 4b). This showcases the potential of Record-seq for developing fundamentally new types of massively multiplexed bacterial biosensors that are highly scalable and do not necessarily require specific components for, or even knowledge of, each stimulus of interest — overcoming a major bottleneck in bacterial biosensors and providing a promising tool to interrogate gut function. A key current technical limitation is the low efficiency of spacer acquisition – 1 new spacer for several thousands of cells – which necessitates bulk analyses and limits the temporal resolution of recordings. Approaches such as protein engineering, as well as mechanistic insights into RT–Cas1–Cas2 spacer acquisition and the role of host factors, may help improve acquisition efficiencies and facilitate transcriptional recordings with small populations of cells or even at the single-cell level.

Outlook

Engineered bacteria show great promise as non-invasive *in vivo* diagnostics but must meet several conditions before clinical translation [5•,65,66•]. An immediate concern is potential environmental release and proliferation of engineered microbes — to prevent this, biocontainment strategies must be incorporated into bacterial biosensor designs. Further, as a regulatory requirement, engineered microbes must be devoid of antibiotic resistance and mobile genetic elements such as plasmids for clinical applications to prevent the horizontal transfer of synthetic genes into the native human microbiota.

A major design consideration for engineering bacterial biosensors is whether to use a colonizing or non-colonizing strain. While colonizing reporter strains can enable stable long-term monitoring, they also represent an alteration to the host microbiota and could result in unintended side effects, making non-colonizing strains generally preferable for diagnostic applications if they are capable of sensing relevant biomarkers during their transitory flow of the intestine. As a corollary, another important factor is the choice of bacterial chassis — while *E. coli* strains are commonly used due to availability of genetic circuit components, more gut-abundant genera such as *Bacteroides* or *Lactobacillus* might be better suited for applications that benefit from the colonization of specific environmental niches in the gut. If different bacterial chassis are to be deployed in different contexts, the modularity and portability of sensor and effector components become essential considerations for future genetic circuit design. Synthetic genetic components also impose metabolic burden on the bacterial chassis, and the fitness and genetic stability of the system must be pre-tested for clinical efficacy. A key challenge for *in vivo* bacterial reporters, particularly non-colonizing strains, will be to provide spatially localized information about the gut environment and to link it to detected stimuli.

Rapid progress in creating synthetic biology tools with platform technologies such as CRISPR will enable the development of future *in vivo* bacterial biosensors with the capability to monitor and respond to multiple stimuli in the gut. In the long run, we envision that this will facilitate the creation of *in vivo* bacterial reporters that offer personalized diagnostics and help uncover novel biology in the gut.

Conflict of interest statement

R.J.P. is an inventor on patent application WO2020053299A1 filed by ETH Zurich relating to work described in this article. All other authors have no competing interests.

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