Title: Toward bacterial bioelectric signal transduction

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Abstract
Bacteria are electrically powered organisms; cells maintain an electrical potential across their plasma membrane as a source of free energy to drive essential processes. In recent years, however, bacterial membrane potential has been increasingly recognized as dynamic. Those dynamics have been implicated in diverse physiological functions and behaviors, including cell division and cell-to-cell signaling. In eukaryotic cells, such dynamics play major roles in coupling bioelectrical stimuli to changes in internal cell states. Neuroscientists and physiologists have established detailed molecular pathways that transduce eukaryotic membrane potential dynamics to physiological and gene expression responses. We are only just beginning to explore these intracellular responses to bioelectrical activity in bacteria. In this review, we summarize progress in this area, including evidence of gene expression responses to stimuli from electrodes and mechanically induced membrane potential spikes. We argue that the combination of provocative results, missing molecular detail, and emerging tools make the investigation of bioelectrically induced long-term intracellular responses an important and rewarding effort in the future of microbiology.

Introduction

Bacteria maintain electrical potentials across their membranes. They use the energy stored in these voltage gradients to drive essential processes, including ATP synthesis, flagellar rotation, and active transport. These essential homeostatic functions of the membrane potential necessarily underly all of bacterial physiology. In recent years, however, bacterial membrane potential has been increasingly recognized as dynamic even under steady conditions, not just after stressful stimuli such as antibiotic treatment[1,2]. Those dynamics have been implicated in diverse physiological functions and cell behaviors, including cell division[3], cell-cell signaling[4], coordination of metabolism[5], and environmental sensing[6].

Bacteria are experts at sensing and responding to their environments, possessing diverse mechanisms to transduce signals from their internal and external environment into changes in gene expression. Coupling of electrical stimuli (changes in membrane potential) to changes in gene expression is well-known in neurons[7] and to a lesser extent in non-excitable eukaryotic cells[8]. The extent to which bacteria regulate gene expression (and how) in response to changes in their membrane electrical potential remains largely unknown.

Here, we draw attention to mechanisms by which electrical stimuli could be transduced to gene regulation in bacteria. We focus on two areas in which progress has been made to identify specific response pathways: redox-coupled electrical sensing and gene expression responses to transmembrane ion fluxes. Much is still unknown, but powerful tools are emerging to investigate bacterial bioelectrical signal transduction; we highlight outstanding questions and promising areas of future investigation.

Redox-coupled electrical sensing

Membrane potential dynamics and metabolism are necessarily intimately coupled in bacteria, which lack internal membrane-bound organelles[9]. Eukaryotic cells, in contrast, use the

mitochondrial membrane potential for energy generation and the plasma membrane for electrical signaling. In bacteria, therefore, we suspect that respiration and the internal redox state are sensitive to membrane potential dynamics, as well as likely involved in generating them. Bacteria employ many mechanisms to sense and respond to changes in redox state and respiratory activity[10,11]. Bacteria are also known to regulate diverse behaviors according to redox state, including biofilm formation, sporulation, and motility[12], raising the intriguing possibility that electrical signaling could regulate cell fates and social behaviors. We highlight here evidence from the study of electrochemically active bacteria for electrical control of gene expression via redox sensors.

Electrochemically active bacteria – those that can interact with external electrodes as electron acceptors or donors – provide an opportunity to ask how changes in external electrical potential influence physiology and gene expression (Fig. 1A). A recent study by Hirose *et al*. demonstrated that *Shewanella oneidensis* cells respiring an external electrode sense changes in electrode potential and alter expression of metabolic genes through the Arc (anoxic redox control) system[13]. The Arc system is a two-component signal transduction system originally characterized in *Escherichia coli*; it is composed of the sensor ArcB (functionally split into ArcS and HptA in *Shewanella*) and response regulator ArcA, a transcription factor activated by phosphorylation[14]. In *E. coli*, ArcB senses the quinone pools of the electron transport chain and either phosphorylates (anaerobic conditions) or dephosphorylates (aerobic conditions) ArcA[15,16]. In *S. oneidensis*, the Arc system responds to the cell's interaction with biased electrodes (Fig. 1B), however, non-electrode-specific stimulation of ArcA due to oxygen limitation needs to be ruled out. Pirbadian *et al*. demonstrated that the membrane potential of *S. oneidensis* does in fact change in response to external electrode potential, suggesting that the biological effects of electrode potential are associated with changes in membrane potential[17]. Additionally, the utility of redox sensors to couple electrical signals to regulation of gene expression has been exploited to engineer bacteria to express particular genes in response to electrode-driven stimuli. For example, Tschirhart, *et al*. demonstrated the control of *E. coli* motility genes with an engineered, electrode-coupled redox system[18].

We hypothesize that co-opting of redox sensors (or rather, electrical alteration of redox state) is likely a general mechanism that couples electrical signals to gene regulation. An interesting outcome is that electrical signaling—or simply membrane potential dynamics in the absence of true cell-to-cell signaling—could stimulate metabolic responses typical of an energy-limited state (like that induced by oxygen limitation), possibly contributing to metabolic heterogeneity within populations of cells.

Ion flux-dependent gene regulation

Bacteria possess a variety of ion channels, including ligand-gated and voltage-gated channels[19]. Structural and mechanistic characterizations of bacterial ion channels have contributed greatly to our understanding of electrophysiology in neurons and other electrically excitable cells. However, the physiological roles of ion channels in bacteria remain largely unknown. Due to the small capacitance of the plasma membrane in bacteria, the flux of even a small number of ions across the membrane is sufficient to significantly change the membrane potential[9,19]. Membrane potential dynamics could also influence ion flux by regulating voltage-gated ion channels as well as altering electrochemical gradients across the membrane. Bacteria

possess diverse strategies to sense and respond to changes in the concentrations of ions both inside and outside the cell. In this section, we focus on two ions, K^+ and Ca^{2+}, highlighting their potential roles in electrical signaling and the capacity of bacteria to respond at the level of gene expression to changes in their concentrations.

Potassium (K^+) is the major intracellular cation in bacteria and eukaryotes[20]. Propagating waves of K^+ efflux and membrane depolarization in biofilms of *Bacillus subtilis* constitute the first form of cell-to-cell electrical communication discovered in bacteria. A metabolically-gated potassium channel, YugO, allows bacteria to communicate their metabolic state and link metabolic processes with distant cells[4,5]. It remains to be seen if and how the dynamics of potassium concentrations and membrane potential under these conditions leads to changes in gene expression. In *B. subtilis*, potassium is one signal sensed by the multi-component "phosphorelay" pathway that regulates multicellular behaviors including biofilm formation[21] and sporulation[22]. López *et al*. found that self-production of surfactin causes K^+ to leak through the membrane, triggering biofilm formation in a KinC-dependent manner[23]. Lundberg *et al*. found that the YugO potassium channel is required for robust biofilm formation, as it promotes K^+ efflux at high cell density[24]. In the YugO-mediated electrical signaling system, not all cells in the population participate in propaging the signal[25]. This raises the interesting possibility that electrical signaling contributes to phenotypic heterogeneity in biofilms.

Calcium-mediated conveyance of electrical signals to regulation of gene expression and epigenetic modification is well-characterized in neurons[26]. Bacteria have many major ingredients to execute similar intracellular bioelectric signaling strategies: they tightly regulate calcium concentration in the sub-100 nM range[27], possess calcium channels[28], and numerous calcium-sensitive proteins[29]. The Kralj lab has pioneered the investigation of calcium fluxes in bacteria using genetically encoded calcium sensors[6]. Bruni, et al. found that *E. coli* exhibit transient, seconds-scale calcium spikes. By coupling their calcium sensor to a genetically encoded voltage reporter[30], they discovered that membrane depolarization induced calcium dynamics (Fig. 1C). They further found that these membrane potential dynamics manifested in response to mechanical perturbation. By monitoring cellular protein concentrations with a reporter library, they found that *E. coli* change the levels of several proteins in response to mechanically-induced bioelectrical dynamics. Bruni et al's results suggest that bacteria use bioelectric signal transduction mechanisms to effect gene expression changes in response to mechanical stimuli, opening up an entirely new avenue to investigate mechanobiology in microbes. To further develop our understanding of how bacteria transduce calcium signals, we will need to systematically investigate the roles of calcium-sensitive proteins[31] in response to membrane potential dynamics and uncover new gene regulatory mechanisms.

Conclusions

Though the study of bacterial electrophysiology is in its nascency, it is clear that bacteria sense and respond to membrane potential dynamics and ion fluxes. The mechanisms described above highlight that electrical activity in bacteria is likely to have global effects on physiology; there are likely many more direct and indirect mechanisms by which bacteria interpret electrical signals at the level of gene expression. It will likely be challenging to determine cause-and-effect relationships in electrical signaling, since membrane potential is highly integrated in all bacterial physiology. However, the study of membrane potential and ion dynamics in bacteria will resolve

critical questions in microbiology, including: What are the physiological roles of ion channels in bacteria? How sensitive are cells to membrane potential dynamics at the level of gene expression and how are the signals transduced? How widespread is cell-to-cell electrical communication in bacteria and what aspects of cell physiology are regulated as a function of that communication?

We still critically lack specific molecular pathways for the intracellular transduction of bioelectric signals in bacteria. This stands in stark contrast to eukaryotic response systems, where molecular players have been fleshed out in great detail[7,26]. Identification of the genes and molecules that make up bacterial bioelectric transduction networks will have major impacts on both basic microbiology and synthetic biology: we will understand entirely new ways that bacteria sense and respond to their environments, and those mechanisms will provide new tools for engineering. These tools will be especially exciting because they will directly couple engineered microbes to powerful electronics[32].

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Authorship Confirmation Statement
J.M.J. and J.W.L. conceived of and wrote the manuscript. All co-authors have reviewed and approved of the manuscript. The manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

Figures

Figure 1

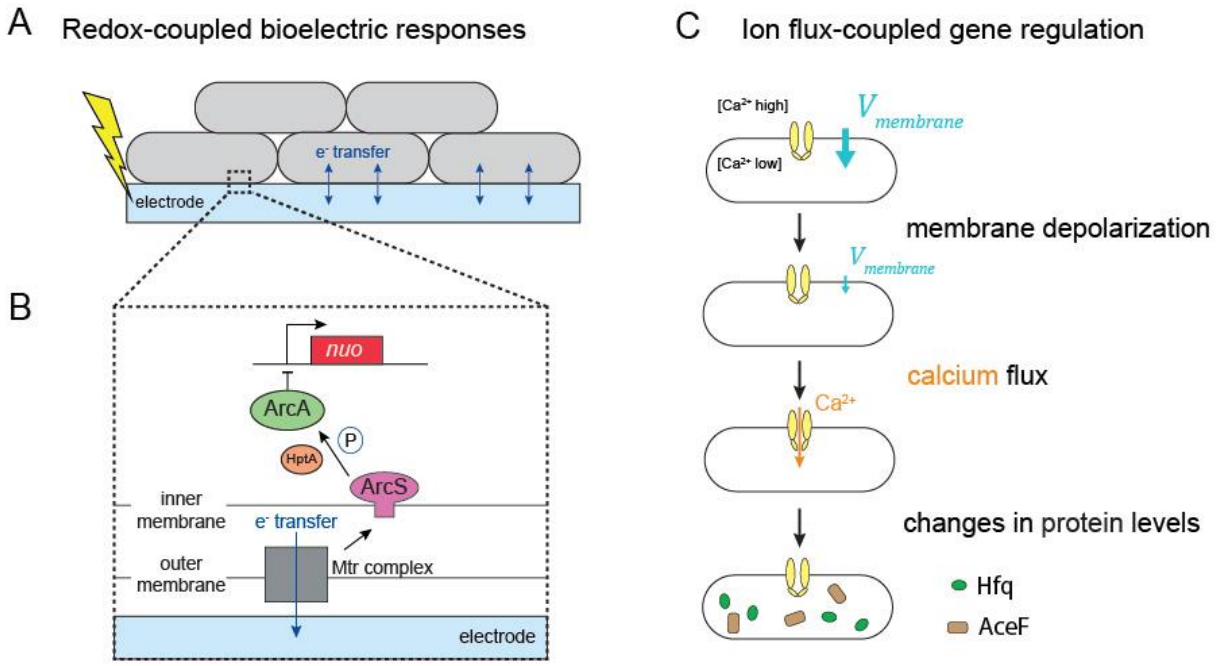

Figure Legends

Figure 1. Schematic of known bioelectrical gene expression responses in bacteria. **A**. Redox-coupled bioelectric responses in bacteria. Electrochemically active *S. oneidensis* can transfer electrons to extracellular electrodes. **B**. In response to electrochemical interaction with biased extracellular electrodes, *S. oneidensis* cells effect metabolic gene expression responses through the two-component Arc system. The sensor kinase ArcS transfers a phosphate (P circle) to the transcription factor ArcA through the phosphotransfer protein HptA. ArcS then effects gene expression changes, for example repressing the ubiquinone oxidoreductase *nuo* genes. **C**. Ion flux-coupled gene regulation. *E. coli* maintains a much lower calcium concentration inside the cell compared to outside. Membrane depolarization (represented by shrining cyan membrane voltage arrow) leads to transient calcium influx and eventual increase in the level of multiple proteins, including the RNA-binding protein Hfq and the pyruvate dehydrogenase aceF (Bruni, et al. 2017).

Author Disclosure Statements
No competing financial interests exist.