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Review

The Microbiologist's Guide to Membrane Potential Dynamics

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All cellular membranes have the functionality of generating and maintaining the gradients of electrical and electrochemical potentials. Such potentials were generally thought to be an essential but homeostatic contributor to complex bacterial behaviors. Recent studies have revised this view, and we now know that bacterial membrane potential is dynamic and plays signaling roles in cell–cell interaction, adaptation to antibiotics, and sensation of cellular conditions and environments. These discoveries argue that bacterial membrane potential dynamics deserve more attention. Here, we review the recent studies revealing the signaling roles of bacterial membrane potential dynamics. We also introduce basic biophysical theories of the membrane potential to the microbiology community and discuss the needs to revise these theories for applications in bacterial electrophysiology.

Highlights

Bacterial membrane potential is dynamic, with the ability to hyperpolarize and depolarize.

The dynamics of bacterial membrane potential mediate signaling at the single-cell and biofilm levels.

Bacterial electrophysiology is different from neural electrophysiology because of the size of bacteria and their membrane structure.

Techniques have been developed and utilized to measure bacterial membrane potential quantitatively and temporally.

Membrane Potential Is Important for Bacterial Functions

Across the cellular membrane there is an **electrical potential** (see Glossary) difference, akin to a conventional battery. This electrical potential across the membrane, membrane potential (a.k.a. transmembrane voltage), is a source of **free energy** which enables cells to do chemical and mechanical work. Due to its well-known importance in fundamental cellular functions such as ATP synthesis [1,2], this potential was generally assumed to be homeostatic. However, recent studies revealed that the bacterial membrane potential is dynamic – it can act as a tool for information signaling and processing. It is now evident that membrane potential regulates a wide range of bacterial physiology and behaviors, for example, pH homeostasis [3,4], membrane transport [5], motility [6,7], antibiotic resistance [8], cell division [9], electrical communication [10,11], and environmental sensing [12–14]. Here, we review the physiological roles of bacterial membrane potential as a source of free energy and as a means of information signaling and processing (Figure 1). The roles of membrane potential in bioenergetics are well documented in textbooks (e.g., [15]). Thus, our main focus is on recent studies reporting the dynamic signaling.

While we introduce the basic biophysical theories of membrane potential that are critical for microbiological investigations, in-depth biophysical analyses and concepts of membrane potential, including dipolar potential and electrodiffusion, are beyond the scope of this article. This is due to our focus here on microbiological context. For these topics, we recommend the reviews [16–19]. This review focuses on studies at the cellular level. Readers interested in studies on the molecular dynamics of prokaryotic **ion channels** are directed to the reviews [20–22]. They are only superficially mentioned because of our focus on the cell-level phenomena. Membrane potential dynamics is not the only electrical process in cells. The other important electrical and electrochemical cellular processes, such as redox metabolism, external electron transfer (EET), and direct interspecies electron transfer (DIET), are out of the scope of this review. For these topics, readers are directed to the electromicrobiology reviews on the mechanisms [23–25] and their applications for biotechnology and synthetic biology [26,27].

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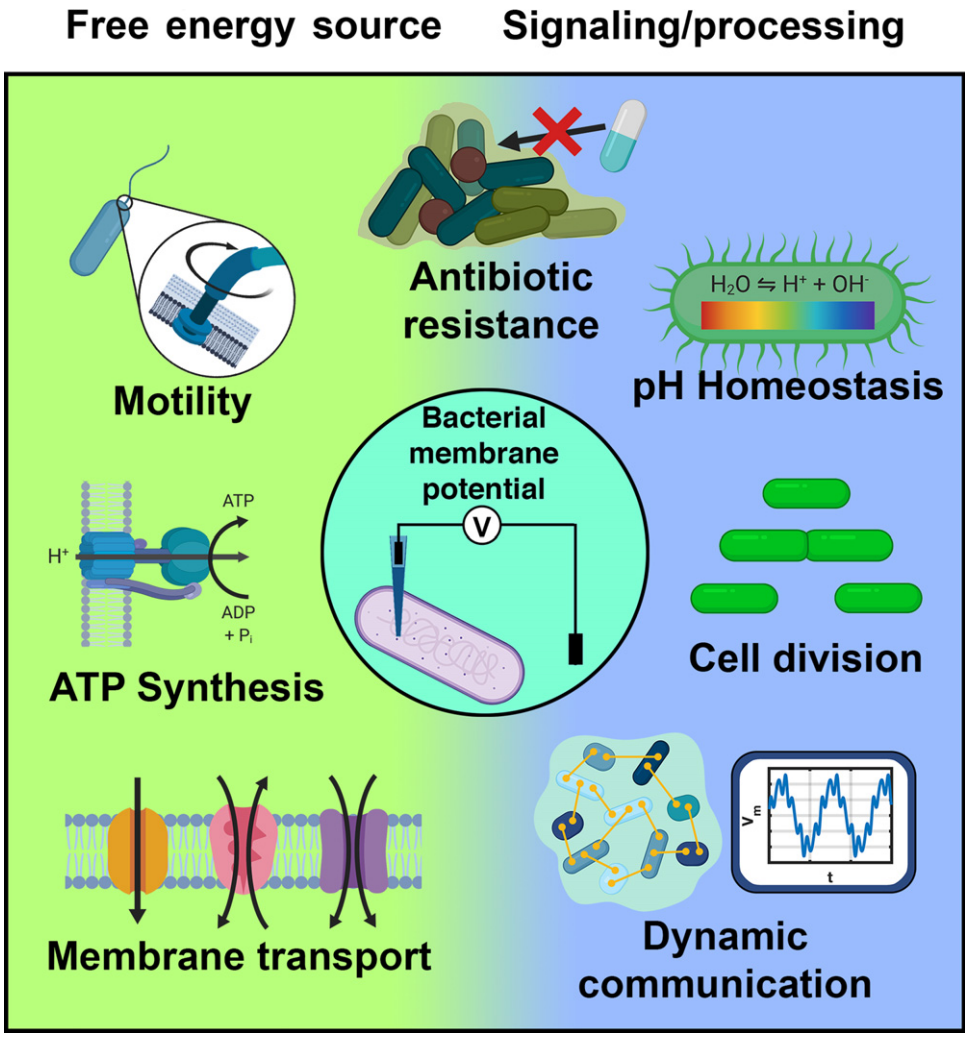

Figure 1. Graphic of the Various Bacterial Processes Known to Be Associated with the Membrane Potential. Membrane transport, ATP synthesis, motility, and antibiotic resistance are all processes driven by ion motive forces (equation 1), the energy gained by translocating ions across the membrane. Bacteria also utilize their membrane potential as a means of signaling and processing information. By depolarizing the membrane potential, bacteria can enter a 'persister' state wherein they are more resistant to antibiotics. Homeostasis of pH is controlled by proton pumps, which maintain a PMF, and is thus dependent on the membrane potential. Cell division requires a membrane potential for the proper localization of the division site and can be inhibited by native protonophores. Lastly, bacterial membrane potential can respond dynamically to molecular and electrochemical signals, thus allowing for intercellular communication. Figure was created with BioRender.

Free Energy for Bacteria from Ion Translocation

An important and well-known role of the membrane potential is that it makes up a part of the electrochemical gradient of ions across the membrane, known as ion motive force (IMF) [15]. For any ion with charge z, its IMF is:

$$IMF = V_m - \frac{RT}{zF} \ln \frac{C_{out}}{C_{in}} \quad [1]$$

where V_m is membrane potential, R is the gas constant, T is absolute temperature, F is the Faraday constant, and C_{in} and C_{out} are the concentrations of the ion inside and outside, respectively.

Glossary

Capacitance: the capability to store electrical charge. It is measured in Farads.
Debye length: the length scale at which a charge becomes screened in an electrolytic solution, from Debye–Hückel and Guoy–Chapman theory.
Debye–Hückel constant: the inverse of Debye length.
Depolarization: when the membrane potential becomes less negative.
Electrical potential: the energy required to move a Coulomb of charge in an electric field. It is measured in Volts.
Free energy: the energy in a system that can be used to do work. It is measured in Joules.
Ion channels: membrane molecules that allow the free diffusion of ions, with the potential to be ion selective.
Ion pumps: active membrane transporters with ion and direction specificity.
Ionophore: a chemical which increases the permeability of one or more ions.
Symporter: a membrane protein which couples the energy-favorable transport of a chemical with the energy-unfavorable transport of another, in the same direction.

This electrochemical potential is given in units of volts and is proportional to the energy released by translocating an ion across the membrane. The sign of the IMF indicates which direction the ions flow according to the potential difference. Explicitly, if the sign is negative then the flux is inwards, and outwards if the sign is positive.

The ion flux down this electrochemical gradient provides a source of free energy to drive biologically important but thermodynamically unfavorable reactions. For example, ATP synthesis can be driven by proton motive force (PMF) or sodium motive force (SMF) [28,29]. ATP synthesis driven by PMF is an important and well-studied role of membrane potential in all orders of life [30]. Exposure to indole, which can act as a proton **ionophore**, inhibits cell division in *Escherichia coli*, indicating the importance of PMF to cell physiology [31]. Although the necessity of PMF for cell division and growth appears to be condition dependent, when glucose is present at pH 7.5, bacteria can still grow and divide without PMF [32,33]. PMF and SMF can also fuel flagellar motor rotations [7].

IMF is also crucial for membrane transport against the concentration gradient, such as glucose uptake, antibiotic uptake, or antibiotic efflux [34]. For example, lactose permease (LacY) couples the IMF of hydrogen ions with the inward translocation of β-galactoside in a **symporter** fashion [35]. The uptake or accumulation of positively charged aminoglycoside antibiotics (e.g., gentamicin and streptomycin) is also driven in part by the IMF. In *Staphylococcus aureus* and *Bacillus subtilis*, membrane potential linearly correlates with the intracellular antibiotic levels upon a threshold membrane potential [36,37]. The drug efficacy of aminoglycosides depends on membrane potential: **depolarization** by a +70 mV change increases the resistance up to a 100-fold [8]. Pretreating cells with CCCP and salicylate, which depolarizes cells, increases the emergence of persister phenotypes by 1000-fold [38,39]. The persister formation mediated by P-loop GTPase Obg leads to a collapsed membrane potential which gives a greater tolerance against ofloxacin and tobramycin [40]. Although these studies suggest that lower IMF could result in greater resistance against antibiotics, it is likely more complicated because many multidrug efflux pumps are driven by SMF or PMF in an antiporter fashion [41].

While it has been widely acknowledged that bacterial membrane potential is important for the physiology of cells, the general perspective has been that membrane potential is a homeostatic background of complex cellular behaviors. However, recent findings have challenged such a view.

Dynamic Electrical Signaling from One to Many Bacteria

Most prokaryotes have carried genes encoding ion channels since the early stages of evolution [21,42–44]. For example, prokaryotic potassium channels may have evolved as early as 4000 million years ago [43]. Structural biology studies of these prokaryotic ion channels have shaped our basic understanding of neural electrical signaling – a classic example being the first structural study of a bacterial potassium channel, for which Rod MacKinnon was granted a Nobel prize in 2003 [45]. As early as the 1970s, Franklin Harold discussed 'the ubiquitous distribution of ion currents and their presumptive evolutionary antiquity encourages one to wonder about their involvement in other cellular activities…' [46]. However, the endogenous functional roles of prokaryotic ion channels and ion currents in signaling were left unexplored for several decades. In 2002, Iyer *et al.* wrote 'prokaryotes use ion channels in roles more adaptive than providing high-quality protein to structural biologists' [47]. In this study, Iyer *et al.* revealed that bacterial chloride channels have a role in acid stress response [47]. More recently, Lundberg *et al.* showed that the deletion of the gene encoding a potassium ion channel prevents *B. subtilis* biofilm formation [48]. While these studies showed that bacterial ion channels

have functional roles in signaling, the question of whether membrane potential could be dynamic and mediate signaling – like neurons – remained unclear.

In 2015, Prindle *et al.* showed that the potassium ion channel YugO mediates electrical signaling within a *B. subtilis* biofilm [11]. By combining time-lapse microscopy, mathematical modeling, and the use of genetic mutants, the authors revealed that metabolic stress induces an opening of the potassium channels, which triggers a relayed event of depolarization of neighboring cells. The mechanism is reminiscent of the neural action potential. This electrical cell–cell interaction enables long-range coordination of glutamate metabolism within a biofilm, which gives rise to a fitness advantage and avoids extinction of the biofilm community when exposed to hydrogen peroxide [49]. Several mathematical modeling frameworks have recapitulated the oscillation dynamics of biofilm growth and electrical signaling [50–52]. A follow-up study further revealed that only a subpopulation of cells within a biofilm participates in the electrical signaling [53]. Intriguingly, the cells that show excitable hyperpolarization pulses are spatially organized into a percolated network, which compromises the cost–benefit trade-off associated with the excitable pulse [53]. In addition to these optical measurements, the measurements by multielectrode array (MEA) have shown electrical spikes from biofilms [54], although, whether these MEA measurements are related to bacterial membrane potential remains unknown.

The electrical interactions are not necessarily confined within a biofilm. Beyond the cell–cell interactions within a biofilm, the electrical signaling mediates the colony–colony interactions which allow time-sharing of available resources between adjunct biofilms [55]. Intriguingly, the potassium waves, as a result of biofilm electrical signaling, also allow cross-species interactions between *B. subtilis* biofilms and swimming *Pseudomonas aeruginosa* cells [56]. Several microbial and animal cells exhibit electrotaxis (also known as galvanotaxis) [57,58], which may suggest that this form of cross-species and cross-kingdom interaction may be common in nature.

The electrical signaling also plays a role at the single-cell level by mediating the cellular sensing of the environment. Kralj *et al.* published a pioneering work in 2012, reporting the electrical spiking of transient depolarization in *E. coli* [59]. This unexpected discovery generated excitement and established a foundation for bacterial cell electrophysiology [60,61]. However, attempts to understand physiological and functional roles of the electrical spiking were inconclusive at the time. A 2017 study by Bruni *et al.* tackled this question and revealed that the electrical spiking is involved in mechanosensation [13]. A mechanical stimulus induces Ca^{2+} influx, which then triggers spiking membrane potential dynamics via opening of ion channels. Furthermore, in spore-forming *B. subtilis* cells, electrical polarization is coupled with the environmental and developmental sensing, which determines the fate of developing spores [12]. The electrical polarization enables the integrative and adaptive quality-control of developing spores. Such an electrical polarization may underpin the phenotypic plasticity and memory of spores [62,63]. In both cases, the genes encoding the ion channels that mediate cellular sensation of external and internal stimuli are yet to be identified.

The dynamic response of membrane potential to stimuli is inherently linked to cellular capacity to proliferate because of its function as a free energy source. Using a bespoke experimental tool, Stratford *et al.* showed that actively proliferating cells and growth-inhibited cells respond to an electrical stimulation in apparent opposite directions [64]. A phenomenological mathematical model, based on the FitzHugh–Nagumo neuron model [65], provided a mechanistic understanding of the observed response dynamics. This finding also offered technology for rapid bacteria detection. Krasnopeeva *et al.* quantitatively investigated the membrane potential changes in response to chemical and optical stimuli [66]. By combining single-cell measurements and a

mathematical framework, the authors found that butanol acts as an ionophore. The authors proposed that the mode of action of perturbations can be determined by analyzing membrane potential dynamics. Furthermore, a recent study suggested that cells may use the change in membrane potential as a means of protection: when exposed to an antibiotic stimulus, *B. subtilis* cells either hyperpolarize and die or maintain their membrane potential to survive [67]. Combining experimental data and computational simulations, Lee *et al.* determined that bacteria use magnesium influx to cope with ribosomal stress and tolerate ribosome-targeting antibiotics [67].

The discoveries of ion-channel-mediated bacterial electrical signaling at the single-cell and the community levels provoke many questions, such as the following. What is the origin of electrical signaling? How common is this form of electrical interaction? Libby and Dworkin suggested that many environmental bacteria and archaea have YugO homologs, and thus electrical cell–cell communication may be conserved [68]. Glutamate is a well known neurotransmitter [69], and the finding that glutamate is the gating molecule for the YugO channel is interesting in view of the fact that glutamate receptors mediate electrical signaling in plants [70]. Glutamate is also

Box 1. Membrane Potential Arises from the Separation of Charges

Membrane potential can be modeled as a separation of charges across a membrane, and thus can be expressed as:

$$V_m = \frac{q}{C} \quad [I]$$

where V_m is membrane potential, q is charge amount in Coulombs and C is the membrane capacitance in Farad. Various sources of electrical potential integratively give rise to resting membrane potential (Figure I).

The most well-known component of membrane potential is the separation of charges through heterogeneous permeabilities in ion channels and active **ion pumps** (Figure IA). In cells, [K^+] is kept higher inside a cell, while [Na^+] is higher outside. Maintenance of this electrical potential requires maintaining the ion concentration gradient, particularly the one with high permeability. This means that constant investments of energy are needed to maintain this potential. The potential that arises from this effect can be modeled with the Goldman–Hodgkin–Katz equation:

$$V_G = \frac{RT}{F} \ln \frac{\sum_i p_i \left[ion_i^+\right]_{out} + \sum_j p_j \left[ion_j^-\right]_{in}}{\sum_i p_i \left[ion_i^+\right]_{in} + \sum_j p_j \left[ion_j^-\right]_{out}} \quad [II]$$

This equation depends on the permeabilities, $p_{i,j}$, and internal and external concentrations, $[ion]_{in,\,out}$, of the dominant ions across membranes. R is the gas constant, T is the absolute temperature, and F is the Faraday constant.

Additionally, there is the Donnan potential (V_D), which arises from charges impermeable to the membrane (Figure IB). These fixed charges include DNA, RNA, ribosomes, and proteins; all contribute a negative electrical potential. The Donnan potential does not need active transport to be maintained. It also contributes ~30 mV of the outer membrane potential in Gram-negative bacteria [98]. This potential may affect the accumulation of cell-wall-targeting antibiotics, and thus influences the efficacy of antibiotics.

The lipid heads of the membrane bilayer are also negatively charged. Asymmetry in the strength of the charge on either side of the membrane introduces a membrane potential (V_s) (Figure IC). A Guoy–Chapman model with all the charge directly on the surface gives a potential difference of:

$$V_s = \frac{4\pi}{\epsilon\kappa}(\sigma_{in} - \sigma_{out}) \quad [III]$$

where ϵ is the dielectric constant, κ is the **Debye–Hückel constant**, and σ is the charge density on the membrane surfaces [81]. Alternatively, the charge can be modeled using Donnan theory where the charge is distributed over a distance larger than the Debye length, which is usually around a nanometer with the physiological ionic strength [99]. This asymmetry can be caused by modifications to the membrane, which are common in bacteria, most notably lipopolysaccharides and peptidoglycans, and thus influence voltage-sensitive machinery across the membrane.

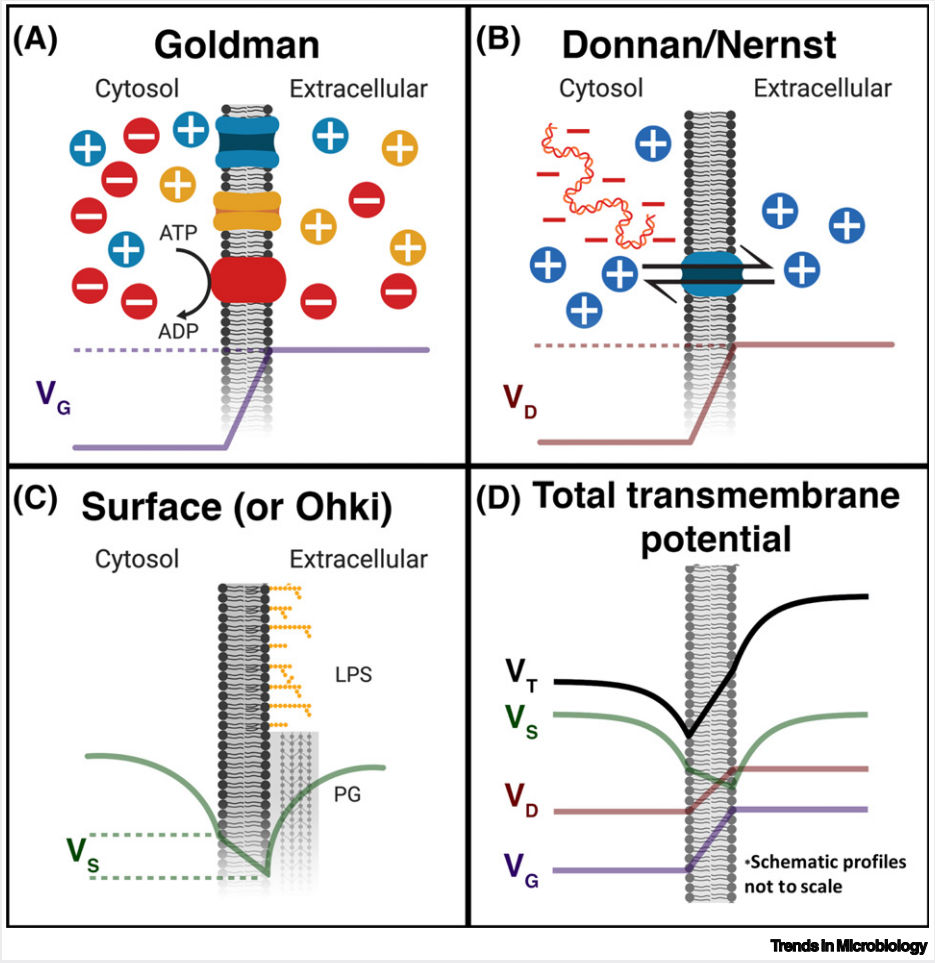

Figure I. The Main Sources of Bacterial Membrane Potential. (A) The Goldman–Hodgkin–Katz model of charge separation is driven by the heterogeneous permeabilities of ions, as well as active transportation of ions across the membrane through pumps. (B) The Donnan potential arises when charges are impermeable to the membrane (for example DNA), while Nernst potential is the consequence of the equilibrium state of a permeable ion. (C) The Ohki solution to asymmetric surface potential on either side of the membrane, which can arise from different phospholipid heads as well as chemical surface modifications. (D) Summing the potentials together gives the total transmembrane potential difference V_T. Note that the curves on these graphs are illustrative and not plotted to scale. The amplitudes of $V_{S,D,G}$ do not correspond to the cellular condition. Figure was created with BioRender.

important for animal bioelectricity during tissue development and regenerations [71]. Systematic phylogenetic analysis of different ion channels, including YugO, would be an important step towards elucidating the evolutionary origin of bioelectrical signaling.

While appreciating the similarities between bacterial and animal electrical signaling is important, recognizing the differences is equally so. The fundamental biophysical frameworks and models of cellular electrophysiology could highlight some of the unique features of bacterial electrophysiology.

Size Matters! Prokaryotic and Eukaryotic Electrophysiology Are Different

Bacterial cells are much smaller than neurons. This size difference has many interesting consequences for electrophysiological dynamics, as also discussed by Cohen and Venkatachalam

[18]. A typical bacterium has the volume of a femtoliter (10^{-15} l) and the surface area of 6 μm^2 [72,73]. Comparing that with typical eukaryotic dimensions – a picoliter (10^{-12} l) and 1600 μm^2 respectively [74,75] – there are three orders of magnitude difference. This means that the membrane **capacitance**, which depends on its surface area, is much less in bacteria than in neurons. Since the membrane potential arises from the separation of charges (Box 1), a smaller difference in capacitance gives rise to higher membrane potential changes. Approximating the membrane capacitance to be 1 $\mu F/cm^2$, indicates that it only takes the transfer of ~100 monovalent ions to change a bacterial membrane potential by a millivolt, which means that 1000-fold fewer ions cross the membrane than for an equivalent potential change in mammalian cells [76].

Another consequence of the cell size is that the actual number of ions is smaller in prokaryotes than in eukaryotes, and thus intracellular ion concentrations are subject to stochasticity and biological noise [77]. When intracellular pH is 7–8, there are only approximately 10 to 100 free protons per cell. Cytoplasmic Ca^{2+} concentration of 100 nM is equivalent to only ~100 ions per bacterium. The maintenance of ion concentrations is sustained by pumps and the varying permeability of the ions, which affect the membrane potential (see Equation II in Box 1). Permeability is controlled by the opening and closing of ion channels, and when opened, they conduct ions at a rate of ~10^6 ions per second per channel [78]. This means, in an extreme instance, that the opening of a single channel with a pico-amp current can deplete the entire stock of less abundant ions within seconds. Hence, minor fluctuations in the number of ion channels expressed and opened could give rise to significant changes in membrane potential and heterogeneities in the permeability of ions in a bacterial population.

In neuronal action potential, changes in membrane potential arise from ion concentrations in the vicinity of membranes, while the ion concentrations within the cell at large remain stable [79]. Because of this, the electrical and chemical potential (i.e., each term in Equation 1) can be treated as independent to each other. This is in contrast to bacteria, where membrane potential changes could lead to chemical potential changes, and vice versa, due to a small number of total ions per cell as described above. This suggests that there may be more complex coupling between the changes in electrical and chemical potential across the whole bacterial cell, which may be an important point when considering IMF and the IMF-associated physiological processes.

Additionally, prokaryotes lack organelles that give rise to multiple membrane intracellular structures within eukaryotes, leading to more phases of cytosol in these cells. One such organelle is the mitochondrion, which shares an evolutionary past with prokaryotes. Despite some similarities due to their heritage, mitochondrial inner membranes have a much larger surface area than the outer membranes. This allows for many more ATPases, thus maximizing utilization of free energy in the mitochondrial membrane potential for ATP production [80]. Furthermore, ATP synthesis and electrical signaling occur in different membranes in eukaryotes, whereas prokaryotes use the same membrane for both functions. This suggests that electrical signaling and metabolism are tightly coupled in bacteria. Consistent with this observation, our group showed in a recent study that electrical stimulation induces opposite responses in proliferative and inhibited cells [64]. When scaled up to the level of a biofilm, electrical signaling is indeed coupled with metabolic activity [49]. This integrated nature of bacterial membrane potential deserves additional attention. Physicochemical and biological frameworks, specifically for bacterial electrophysiology, that differ from eukaryotic paradigms have yet to be developed.

Another key difference between eukaryotic and prokaryotic cells is the structure of the membrane and its surroundings. For example, Gram-negative bacteria have negatively charged lipopolysaccharide on their surface and peptidoglycan in the intermembrane space. Gram-positive bacteria

have peptidoglycan, lipoteichoic acids, teichoic acids, and other surface proteins, all negatively charged. These molecules integratively give rise to electrical potential across the cell membrane (see Equation III in Box 1) [81]. Physicochemical models must be developed for gaining quantitative insights into bacterial electrophysiology. Surface potentials are generally assumed to act only locally within the **Debye length** (~1 nm with a cellular condition), but the complex nature of the chemical modifications and ion fluxes at the surface suggests that this model is insufficient for describing cellular surface potentials. Several experimental reports suggest that the Debye length in a eukaryotic cell is indeed much larger than typically assumed [82,83]. Our study on *B. subtilis* sporulation also suggested that the Debye length may be extended due to ion flux across the membrane [12]. The considerations of Debye length are particularly important for the investigations into the accumulations of antibiotics at the intermembrane space in Gram-negative bacteria [84].

Electrophysiological studies have focused on mammalian systems, which has led to a fundamental framework and models of cellular electrophysiology (Box 1). As discussed here, the prokaryote world is different for several reasons and, hence, the basic assumptions made by existing electrophysiological models may need careful reconsiderations when applied to bacteria. Acquiring experimental measurements of bacterial membrane potential dynamics is essential to advance our understanding of how to apply these models to bacteria.

How Can We Measure Bacterial Membrane Potential?

We hope that readers will consider the possible roles of membrane potential in conjunction with their topic of microbiological research and interest. To facilitate such, this section overviews three experimental techniques for measuring membrane potential, namely, (i) optical probes, (ii) flagellar rotation, and (iii) patch clamps. Note that this is by no means a comprehensive list of available techniques. For example, impedance spectroscopy has been applied to determine bacterial membrane potential [85]. Scanning electrochemical microscopy (SECM) is another promising technique for measuring membrane potential, as well as other electrical and electrochemical potentials [86,87].

Fluorescent Probes

The optical probes for membrane potential measurements can be categorized into three main types: (i) Nernstian dye, (ii) membrane-bound dye, and (iii) genetically encoded voltage indicators (GEVIs) [19,88]. The most widely used is Nernstian dye [89]. When a cationic molecule is permeable to the membrane its distribution across a membrane follows the Nernst equation:

$$V_m = \frac{RT}{zF} \ln\left(\frac{C_{out}}{C_{in}}\right) \quad [2]$$

Accordingly, membrane potential can be determined by measuring the ratio between the fluorescence intensities inside and outside the cell. Lipophilic fluorescent cationic dyes, such as tetramethylrhodamine, methyl ester (TMRM), have been used to probe bacterial membrane potential [11,12,59,64,90,91]. Nernstian dyes present a particularly powerful and convenient tool for microbiological investigations, although their use for quantitative biophysical investigations requires more careful calibrations [91,92]. A potential drawback of Nernstian dyes is that their permeabilities tend to be low with Gram-negative bacteria, which requires pretreatments of cells with EDTA to chelate divalent cations [90]. Low permeability would also mean that they are not suitable for capturing fast dynamics. Another point to consider is that, when used at high concentrations, Nernstian dyes can become invasive to cellular membrane potential [12,91]. Membrane-bound dyes for measuring membrane potential, such as aminonaphthylethenylpyridinium (ANEP)

dyes, are a preferable choice for capturing fast dynamics, although they suffer from a weaker signal-to-noise ratio. di-4-ANEPPS is one of such indicators and has been used with bacteria [93]. ANEP dyes shift their excitation spectra accordingly to the membrane potential. Lastly, while many GEVIs are available for the study of eukaryotic cells, only one GEVI, proteorhodopsin optical protein sensor (PROPS), has been specifically applied to research into bacterial membrane potential dynamics [13,59]. An advantage of GEVIs is that they can be conjugated with other fluorescence reporters for dual monitoring. Bruni *et al.* developed CaPR by fusing PROPS with a calcium indicator protein GCaMP6f [13,94]. Fusion and expression control can also allow monitoring membrane potential of specific cell types and/or specific sites of the membrane. A potential drawback of GEVIs is the requirement for transformation, which may be a limiting factor with some bacterial species or strains.

Flagellar Rotation

Because the speed of flagellar rotation relates linearly to the PMF within a certain range, membrane potential can be determined by measuring the speed of flagellar rotation when the pH outside the cell is kept the same as that in the cytoplasm. This approach has been applied to assess the mode of action of chemical and optical perturbations [66]. It allows quantitative determination of membrane potential at single-cell levels. However, this approach requires low-throughput and labor-intensive assays with a high technical barrier that requires specialized equipment. Another potential drawback is that this technique cannot be used with cells that are not expressing flagella motors.

Patch Clamp

Patch clamping is widely used in neuroscience as the most direct way of measuring membrane potential [79,95]. While this technique allows direct measurements of electrical potential, it is highly invasive and cannot be used on live bacterial cells due to their small sizes and their cell wall. Patch-clamp technique has been applied only with isolated bacterial membranes or giant spheroplasts and protoplasts [22,96]. Therefore, while patch clamp is a powerful technique for studying prokaryotic ion channels, it is not suitable for functional studies of membrane potential dynamics under physiological conditions. It is yet to be seen whether advanced nanotechnology may be able to overcome this technical challenge.

Concluding Remarks and Future Perspectives

For several decades, electrophysiology was remote from the concerns of most microbiologists [46]. The recent realization of signaling roles of bacterial membrane potential dynamics has begun to draw the attention of researchers to the roles of membrane potential dynamics in the microbiological phenomena of interest, many of which remain unexplored. For example, it is now conceivable that electrical signaling may mediate host–microbiota interactions. We also foresee a future where bacterial cellular behaviors and functions can be controlled using electricity – in a similar manner by which neurons and muscles are controlled. Such technologies may offer an electrical approach to treat antimicrobially resistant pathogens. It could also allow precise spatio-temporal control of industrial bioreactors for improved productivity. Development of electrical interfaces to bacteria and electrobacterial hybrid systems would facilitate the convergence of bioelectronics and synthetic biology [97]. Yet, electrophysiology is still a largely uncharted territory in microbiology (see Outstanding Questions). For this reason, we argue that bacterial electrophysiology approaches hold unrealized promise of making exciting new scientific discoveries and societally valuable technology developments.

Outstanding Questions

How universal are the electrical cell–cell interactions among different microbial species?

Does membrane potential mediate cross-kingdom interactions? If so, how common is it?

How do bacteria generate and maintain their membrane potential? Can this be modeled and demonstrated experimentally?

How much information can be encoded by bacterial membrane potential dynamics?

What is the origin of excitability and electrical signaling?

Can we control the membrane potential artificially?

By perturbing the membrane potential, can we control gene expression and cell phenotype?

Are there more bacterial functions dependent on membrane potential?

What other methods can be used to measure the membrane potential?

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