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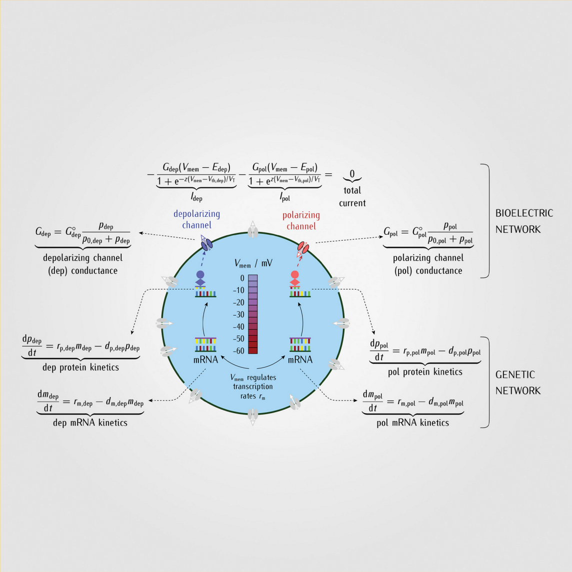

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# Synchronization of Bioelectric Oscillations in Networks of Nonexcitable Cells: From Single-Cell to Multicellular States

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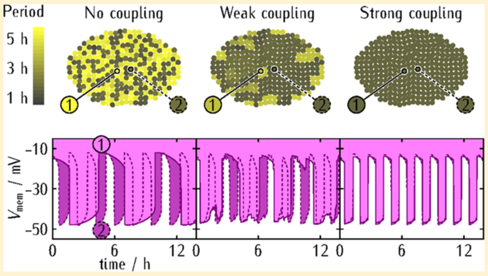
**ABSTRACT:** Biological networks use collective oscillations for information processing tasks. In particular, oscillatory membrane potentials have been observed in nonexcitable cells and bacterial communities where specific ion channel proteins contribute to the bioelectric coordination of large populations. We aim at describing theoretically the oscillatory spatiotemporal patterns that emerge at the multicellular level from the single-cell bioelectric dynamics. To this end, we focus on two key questions: (i) What single-cell properties are relevant to multicellular behavior? (ii) What properties defined at the multicellular level can allow an external control of the bioelectric dynamics? In particular, we explore the interplay between transcriptional and translational dynamics and membrane potential dynamics in a model multicellular ensemble, describe the spatiotemporal patterns that arise when the average electric potential allows groups of cells to act as a coordinated multicellular patch, and characterize the resulting synchronization phenomena. The simulations concern bioelectric networks and collective communication across different scales based on oscillatory and synchronization phenomena, thus shedding light on the physiological dynamics of a wide range of endogenous contexts across embryogenesis and regeneration.



## ■ INTRODUCTION

Pattern regulation, in embryogenesis and regeneration, requires complex spatiotemporal coordination of cell behavior to achieve a long-range anatomical order.[1,2] Robustness of patterning and physiology is the result of complex signaling pathways mediated by not only biochemical but also by bioelectrical signaling: note the use of ion channel- and pump-mediated ion flows and voltage gradients by all cells (not only neurons and muscle) to communicate on the tissue and organ levels.[3,4] Mutation of electrogenic proteins often leads to characteristic birth defects,[5] and targeted modulation of the endogenous bioelectric patterns result in downstream changes of gene expression, cell differentiation, and morphogenesis. Thus, recent progress in this field has highlighted the importance of this control system for regenerative medicine,[6] cancer,[7] immunology,[8] and synthetic biology.[9,10] Despite this progress in the molecular details of how voltage patterns control downstream events,[3,4] crucial knowledge gaps remain about the bioelectric code itself: the global spatiotemporal dynamics of bioelectrical signaling within tissues. This is a prerequisite for understanding the evolutionary implications of developmental bioelectrics and for the design of rational strategies for biomedical intervention.[11,12]

There exists a significant interplay between the bioelectrical and biochemical signals of nonexcitable cells both at the intra- and intercellular levels, though the mechanisms involved are challenging to study because of the complex feedback between these different signals. While emphasis is usually made on positional information and spatial bioelectric prepatterns,[12] oscillatory phenomena and synchronization are basic components in computational procedures[13] and can also be exploited in collective information processing. Oscillations are characteristic of cell populations and developmental patterning,[14,15] as well as in primitive cognition in slime molds.[16,17] In particular, oscillatory cell membrane potentials have been observed in glioma cells,[18] bacterial communities,[19] and pancreatic islets[20] where specific ion channel proteins contribute to the coordination of large cell populations. Recently, bioelectric oscillations and dynamic gap junction networks have been experimentally characterized in the development of chicken embryos.[21] Describing collective oscillations is difficult, however, because of the complex spatiotemporal patterns that emerge at the multicellular level from single-cell dynamics.[22] At these different levels of description, two closely related questions immediately arise: (i) What single-cell properties are relevant to multicellular behavior? (ii) What biophysical properties defined at the multicellular level can allow an external control of the system dynamics?[3,23−25]

Conceptual approaches for describing bioelectric oscillatory phenomena in networks of nonexcitable (non-neural) cells can be based on the ion channel proteins inserted in the cell membrane.[24] These approaches have a solid experimental basis because ion channels form the aqueous pores at the cell membrane that are responsible for the exchange of biological

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information between the cell inside and the outer environment, including the neighboring cells.[26,27] In particular, these channels regulate the transmembrane potential $V_{\text{mem}}$ defined as the electrical potential difference through the cell membrane, which plays a crucial role in proliferation and differentiation.[5,7,23] While persistent deviations of $V_{\text{mem}}$ from the steady physiological values are not likely to occur because of the feedback mechanisms that regulate $V_{\text{mem}}$,[7,27] transcriptional and translational changes affecting key ion channel proteins could compromise this control, though cells can also control these channels at the post-translational level. Also, since bioelectrical signals are shared between neighboring cells, a key question is to find out if individual transcriptional and translational processes could be influenced by changes of ensemble-averaged cell membrane potentials. Multicellular electric potentials can exert an endogenous control on the molecular random kinetics of cell biochemistry because they constitute ensemble-averaged macroscopic magnitudes defined at a higher level than single-cell characteristics.[24,25,28,29] The causal structure of physical systems can be studied at different spatial and temporal scales and it is an open question as to whether this structure could be fully captured from the most detailed microscale descriptions.[30] This is an important issue because efforts to control bioelectric dynamics in contexts such as tumor normalization[31] or repair of birth defects[32] need to be informed by models that facilitate the design of interventions at the most effective and causal level.

Although it has long been known that bioelectric events can determine cell behavior and gene expression, the large-scale code mapping between spatiotemporal bioelectric patterns and specific anatomical outcomes is still unknown.[3,23] It is clear however that transduction mechanisms enable bioelectrical signals to be integrated with other, often downstream, means of intercellular communication such as biomechanical and biochemical signals.[7,21,23,33] For instance, a perturbation of $V_{\text{mem}}$ can be transduced in modifications of the distribution of negatively charged phospholipids over the membrane.[33] In turn, these conformational changes may influence the clustering of signaling proteins with positive residues around the negative lipids. Finally, these intracellular rearrangements can indirectly impact on downstream transcriptional pathways regulating cell proliferation.[33] Other end-effects on transcription can also result from the spatiotemporal distribution of signaling ions and molecules such as calcium and serotonin, which depend markedly on $V_{\text{mem}}$.[3,23,29,34] Moreover, changes in $V_{\text{mem}}$ have often a post-translational effect because of the opening/closing of specific voltage-gated channels.[26] These experimental facts suggest that bioelectrical signals and cell states are not a mere consequence of biochemical processes but may also have an instructive role in the observed feedback between biological mechanisms occurring at different levels.[3,35,36] In this way, while $V_{\text{mem}}$ may not be a transcription factor itself, it can indirectly modulate transcription via other biochemical and biomechanical effects.[5,7,23,33,34]

In a different context, local potentials and currents allow information processing in electronic devices composed of many interconnected individual components, which suggests that bioelectrical signals should also be especially suited to provide information exchange in multicellular ensembles,[3,24] as is the case of neural networks. Following this analogy, it is conceivable that the spatiotemporal map of local $V_{\text{mem}}$ values may allow some control of multicellular ensembles.[3,24,28,29] In particular, the intercellular gap junctions can provide a short-range connectivity for neighboring cells complementary to the spatiotemporal maps of signaling molecules involved in positional information and multicellular patterning processes. Note that molecular diffusion-reaction processes alone are relatively slow and messy for the precise regulation of these maps over long distances.[37,38] Moreover, signaling ions and molecules such as calcium and serotonin carry electrical charges and thus their distribution is markedly influenced by the map of $V_{\text{mem}}$ values. It is then of interest to explore theoretically other long-range biomechanical[39] and bioelectrical[24] mechanisms that may coordinate spatially separated cells.

Recently, we have studied the collective properties of gap junction-coupled multicellular ensembles. When a high enough number of cells in the ensemble share a common bioelectric state, they could impose this particular state to the rest of cells following a threshold mechanism[28,40,41] due to system-level bioelectric responses to local transcriptional changes. The multicellular oscillations are possible because the single-cell state is modulated not only by the individual membrane potential but also by the potential difference relative to the neighboring cells. These theoretical predictions have an experimental basis,[3,24,36,42] suggesting that collective regulatory mechanisms of single-cell bioelectric states could be possible,[20,40] as in the cases of biomechanical oscillations in monolayers of coupled cells[39] and biochemical networks relevant to cell differentiation and tumorigenesis.[22] We aim here at exploring the coupling mechanisms that may allow and sustain bioelectric oscillations in multicellular networks based on the interplay between biochemical prepatterns and oscillatory cell potentials. In particular, we consider the spatiotemporal oscillatory patterns and synchronization phenomena that arise when the average electric potential makes cell groups to act as a coordinated multicellular patch.[24,28,29] It is tempting to speculate that these modes of bioelectric communication across different scales may act as distributed biological memories in nonexcitable cells.[42] Instead of focusing on a particular biological problem, we describe general results relevant to intercellular bioelectric communication that can be applicable to different contexts, emphasizing functional rather than structural aspects.

New biophysical approaches are timely in view of the recently available experimental techniques. For instance, spatially distributed gene expression can be monitored using microfluidic devices where confined compartments allow establishing the gene expression spatiotemporal patterns.[43] Also, patterns of cell potentials can be dynamically studied using electrically gated field-effect transistor biosensors,[44] nanoparticle binding to cell membranes,[45,46] and protein fluorescence based on electrically dependent optical activity.[9] Note, in addition, that the cell membrane potential can currently be associated with the molecular characteristics of key ion channel proteins.[3,32] In this way, optogenetic and pharmacological techniques such as local injection of particular mRNAs that encode ion channel and gap junction proteins can modulate the cell polarization states.[8,23,24,31] Also, the electrical activity of specific channels can be suppressed by specific pharmacological inhibitors.[18,47]

## ■ THEORETICAL METHODS

Genetic and bioelectric networks are interrelated in an instructive way, as shown in a multitude of experimental contexts. For instance, the local concentrations of signaling

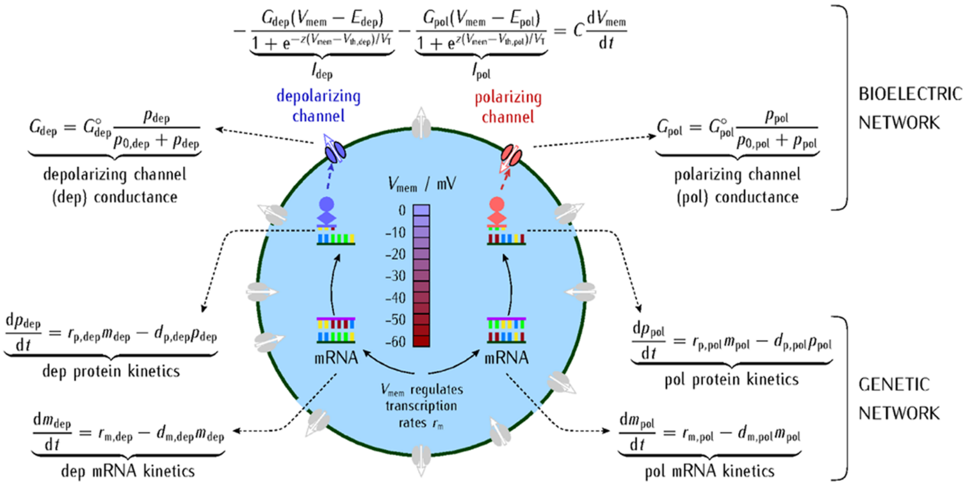

**Figure 1.** Single-cell description of the genetic and bioelectrical feedback is shown for two channel proteins that regulate opposite cell polarizations, an extension of the model previously described in ref 24. The kinetic equations describe the protein transcription and translation. Two specific mRNAs of concentrations $m_{dep}$ and $m_{pol}$ regulate the respective protein concentrations $p_{dep}$ and $p_{pol}$; these magnitudes are relative values that depend on the biochemical problem considered. As a first approximation, molecular diffusion within the cell is ignored because it is fast compared with genetic processes. The rate constants for mRNA transcription ($r_{m,k}$), protein translation ($r_{p,k}$), and degradations ($d_{m,k}$ and $d_{p,k}$) are effective values that may correspond to multiple kinetic steps. In the text, the protein transcription rates are written as $r_{m,k}$ ($V_{mem}$) (k = pol, dep) to emphasize that they depend on $V_{mem}$ because this cell potential can influence the concentrations $S_{pol}$ and $S_{dep}$ of the respective signaling ions or molecules. Note that this dependence provides the feedback between the genetic and the bioelectric layers of description. In the figure, $C$ is the cell capacitance and we consider the Hill kinetics $G_k = G^o_k p_k/(p_{0,k} + p_k)$ for the dependence of the k channel conductance on the protein concentration $p_k$, where $G^o_k$ is the maximum conductance and $p_0 = p_{0,k} = 60$ corresponds to $G^o_k/2$. Note that the cell transmembrane potential $V_{mem}$ is modulated by the dep and pol conductances $G_{dep}$ and $G_{pol}$ that act to establish the respective depolarized and polarized potentials, $E_{dep} = 0$ mV and $E_{pol} = -60$ mV. Note that the cell is assumed to be in contact with an external microenvironment that acts as a bioelectric buffer in the sense that the extracellular ionic concentrations are approximately constant and thus these potentials do not change with time.

ions and charged molecules such as calcium and serotonin that influence transcriptional, translational, and post-translational processes depend on the spatiotemporal map of cell electric potentials.[3,25,26,29,36] Also, the relationship between the membrane potential and cell proliferation and differentiation processes suggests that multicellular bioelectric states are significant to gene expression patterns in embryogenesis, regeneration, and tumorigenesis.[3,48−51] In these experimental contexts, a phenomenological description of the feedback between the biochemical and bioelectric networks can complement bottom-up molecular approaches with alternative top-down mechanisms.[3,24,48,50,51]

There exists an electrical potential difference between the cell cytoplasm and the extracellular medium (Figure 1) that is regulated by the electrical conductance of specific ion channels in the membrane together with the intracellular and extracellular ionic concentrations.[7,52−55] For the particular case of zero current $I_{pol} + I_{dep} = 0$ between the external microenvironment and the cell cytoplasm, the transmembrane potential as shown in Figure 1 would reduce to the steady-state resting potential,[26,28] which constitutes a bioelectric read-out of the cell state. For instance, differentiated cells tend to show relatively high values of $|V_{mem}|$ while proliferating cells are characterized by abnormally low values of $|V_{mem}|$,[7,49,56] although the role of $V_{mem}$ in tumorigenesis is still a matter of controversy. Interestingly, a minimal model based on two effective channels promoting low (depolarized, dep) and high (polarized, pol) values of $|V_{mem}|$ allows a useful qualitative description of $|V_{mem}|$.[24,28,53−55]

Because of the feedback between the genetic and bioelectric descriptions,[3,24,25,29,36,57,58] the kinetic equations for the concentrations of mRNAs ($m_{dep}$ and $m_{pol}$) and ion channel proteins ($p_{dep}$ and $p_{pol}$) are coupled with the cell potential $V_{mem}$ (Figure 1). Therefore, the upregulation/downregulation of a specific ion channel depends on the other functional channels via the cell bioelectric state. Experimentally, this feedback between channels can lead to different compensatory and control mechanisms.[34,53,59] In addition to acting on the ion channel expression, $V_{mem}$ can also manifest post-translationally by closing voltage-gated channels or by driving the channel blocking with specific molecules.[26,28,47,60]

Figure 1 is an extension of a biophysical model described in detail previously.[24] In this case, however, both the dep and pol channel protein concentrations are influenced by $V_{mem}$ because of the potential-dependent local concentrations $S_{dep}$ and $S_{pol}$ of the respective signaling ions and molecules that regulate the transcription rate constants $r_{m,dep}$ ($V_{mem}$) and $r_{m,pol}$ ($V_{mem}$) of the respective ion channel proteins, as shown in Figure 2. The typical input values assumed for the model parameters have already been justified.[25,40] Figures 1 and 2 constitute a minimal

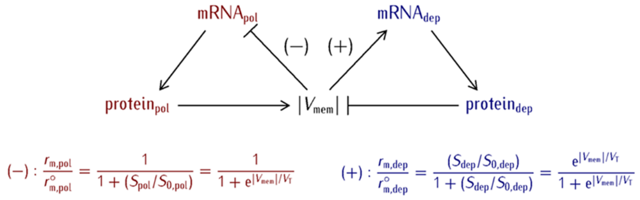

**Figure 2.** Feedback between the mRNA transcription rate and the absolute value of the transmembrane potential regulates the protein concentrations, with $r_{m,k}$ ($V_{mem} = 0$) $= r^o_{m,k}/2$ (k = pol, dep). The potential regulation is negative (−) for the pol channel protein and positive (+) for the dep channel protein. These bioelectric regulations thus allow the spontaneous emergence of the single-cell oscillations without the need of an outside pacemaker.

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model for the complex feedback between the genetic and bioelectric layers of description. This feedback scheme involves only a small number of basic concepts and can be extended further to more realistic cases.[24,29,61] Interestingly, the biophysical description of Figure 1 can provide a bistable memory based on depolarized and polarized single-cell states.[25,54,55] The bistable dynamics are associated with the cell membrane potential, as shown in Figure 2 of ref 25 and Figures 2−4 and 6 of ref 54.

The phenomenological equations for the currents $I_{\mathrm{dep}}$ and $I_{\mathrm{pol}}$ as shown in Figure 1 allow a qualitative description of typical current-voltage curves in terms of a reduced number of experimental parameters: the effective charge $z = 2$ for channel gating and the channel threshold potentials $V_{\mathrm{th,pol}} = V_{\mathrm{th,dep}} = -V_{\mathrm{T}}$, where $V_{\mathrm{T}} = RT/F = 27$ mV is the thermal voltage, where $T$ is the temperature, $R$ is the gas constant, and $F$ is the Faraday constant.[26,28] The different contributions of the dep and pol channels to the membrane conductance regulate the cell potential $V_{\mathrm{mem}}$. For instance, when the conductance ratio $G_{\mathrm{dep}}/G_{\mathrm{pol}}$ is high, $V_{\mathrm{mem}}$ decouples from the normal polarized potential $E_{\mathrm{pol}}$ and takes abnormally low potentials close to the depolarized potential $E_{\mathrm{dep}}$.[28,54]

The concentration $S_{\mathrm{k}}$ of a signaling ion influencing the protein transcription rates can be regulated by $V_{\mathrm{mem}}$.[29,34,62,63] The model of Figure 2 assumes that the protein transcription rates can follow positive or negative Hill kinetics. For positive regulation (*dep* channel), the potential-dependent transcription rate is $r_{\mathrm{m,dep}}\,(V_{\mathrm{mem}}) = r^{\mathrm{o}}_{\mathrm{m,dep}}(S_{\mathrm{dep}}/S_{0,\mathrm{dep}})/[1 + (S_{\mathrm{dep}}/S_{0,\mathrm{dep}})] = r^{\mathrm{o}}_{\mathrm{m,dep}}\,e^{|V_{\mathrm{mem}}|/V_{\mathrm{T}}}/(1 + e^{|V_{\mathrm{mem}}|/V_{\mathrm{T}}})$ and for negative regulation (pol channel) $r_{\mathrm{m,pol}}\,(V_{\mathrm{mem}}) = r^{\mathrm{o}}_{\mathrm{m,pol}}/[1 + (S_{\mathrm{pol}}/S_{0,\mathrm{pol}})] = r^{\mathrm{o}}_{\mathrm{m}}/(1 + e^{|V_{\mathrm{mem}}|/V_{\mathrm{T}}})$, where $S_{0,\mathrm{k}}$ (k = pol, dep) is a reference concentration.[25] In this way, the biochemical and bioelectric processes become coupled: the cell potential $V_{\mathrm{mem}}$ regulates the channel protein concentrations $p_{\mathrm{k}}$ of conductances $G_{\mathrm{k}}$ and, in turn, the conductances $G_{\mathrm{k}}$ regulate the potential $V_{\mathrm{mem}}$. Figure 2 shows that the potential $V_{\mathrm{mem}}$ has a central role in the interplay between the mRNAs, proteins, and cell potential because it regulates the feedback between the genetic and bioelectric layers of description.

Figure 3 shows that the single-cell states as shown in Figure 1 can be extended to the multicellular case by means of voltage-gated gap junctions[25,64,65] of conductance $G_{ij}$ that couple two neighboring cells $i$ and $j$ through intercellular currents $I_{ij} = G_{ij}(V_i - V_j)$, where we omit the subscript (mem) in $V_i$ for clarity. Experimentally, these junction conductances depend on the potentials of the neighboring cells and are involved in development, regeneration, and cancer growth and progression.[65−67] The multicellular ensemble is a planar monolayer of $N = 304$ cells assumed to be initially at the same cell potential $V_i$ $(t = 0)$, $i = 1,\ldots, N$. The initial mRNA and protein concentrations correspond to the depolarized potential case of the respective equations in Figure 1.[25] At time $t > 0$, the multicellular state changes according to the $N$ equations for the cell potentials $V_i(t)$. Electrical responses are characterized by a time $C_i/G_{\mathrm{ref}}$ of the order of 1 s for a cell capacitance $C_i = 100$ pF and a reference channel conductance $G_{\mathrm{ref}} = 0.1$ nS.[25] In contrast, the genetic processes are much slower because transcription and translation rate constants in the range 0.1−1 min$^{-1}$ give characteristic time responses between 0.02 and 0.2 h, whereas degradation rate constants in the range 0.003−0.1 min$^{-1}$ give times between 0.1 and 5 h.[25,58,61]

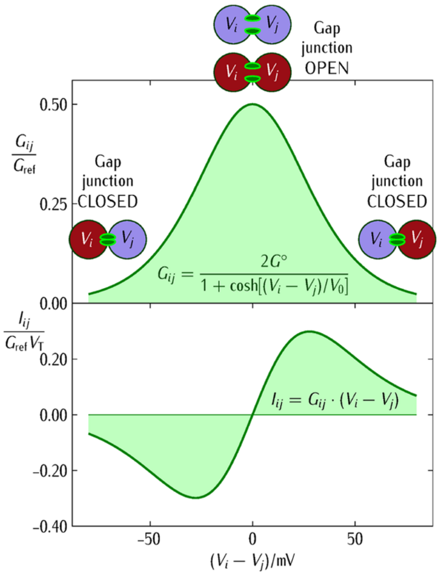

**Figure 3.** Feedback between the genetic and bioelectric descriptions of the cell can be extended to the multicellular level by introducing intercellular gap junctions of conductance $G_{ij}$ that couple two neighboring cells $i$ and $j$ (up). Experimentally, $G_{ij}$ shows a bell-shaped dependence with the intercellular potential difference $V_i - V_j$, as in the figure. The maximum junction conductance $G^{\mathrm{o}} = 0.5G_{\mathrm{ref}}$ and the reference potential $V_0 = 18$ mV characterize the experimental distribution of conductances. In this model, the cell potential $V_i$ changes with time $t$ according to the intercellular current $I_{ij}$ regulated by $G_{ij}$ and $V_i - V_j$ (bottom) and the single-cell currents $I_{\mathrm{pol},i}$ and $I_{\mathrm{dep},i}$ of Figure 1. We incorporate only the nearest neighbors around the central cell $i$ in the sum over the surrounding cells $j$.[24] To characterize the relative contributions of the intercellular $G^{\mathrm{o}}$ and single-cell $G^{\mathrm{o}}_{\mathrm{pol}} = G^{\mathrm{o}}_{\mathrm{dep}}$ conductances to the cell potential, all these conductances are scaled to the common reference value $G_{\mathrm{ref}}$.

## RESULTS AND DISCUSSION

**Single-Cell.** Figure 4 shows the single-cell oscillations of the transmembrane potential, the pol and dep channel protein

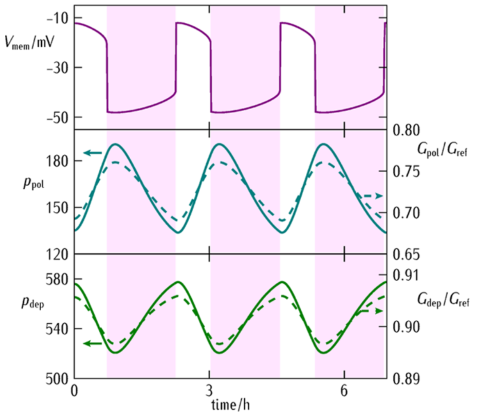

**Figure 4.** Single-cell bioelectric oscillations corresponding to the transmembrane potential (up) and the channel protein concentrations (left axis) and conductances (right axis) for the system of Figure 1. The pol and dep protein transcription and degradation rate constants are $r^{\mathrm{o}}_{\mathrm{m,k}} = r_{\mathrm{p,k}} = 0.8$ min$^{-1}$ and $d_{\mathrm{m,k}} = d_{\mathrm{p,k}} = 0.03$ min$^{-1}$ (k = pol, dep). Note the time delay between the oscillations. Note that the electric potential regulations of Figure 2 explain the emergence of the single-cell oscillations directly from the physiological model circuit without any external pacemaker.

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concentrations, and the respective channel conductances. Note the close correspondence between protein concentrations and channel conductances as well as the effects of these conductances on the potential. The results show clearly the feedback between the genetic and bioelectrical layers of description at the single-cell level (Figures 1 and 2).[25,57,58] Experimentally, the nonlinear coupling of ion channel proteins with local cell potentials plays a role in morphogenetic phenomena.[3,23]

While electrical responses are relatively fast compared with diffusional and transcriptional processes, Figure 4 shows that the coupling between all these processes can give times on the order of hours.[24,25,41] Indeed, the ratio $C/G_{ref}$ corresponds to single-cell electrical times in the second time scale for conductances $G_{ref} = 0.1{-}1$ nS and capacitances $C = 10{-}100$ pF. In contrast, diffusional processes across multicellular systems and protein transcription, translation, and degradation processes take times on the order of hours. Therefore, the multicellular system response can be dictated by the relatively slow spatial redistribution of signaling ions and charged molecules (e.g., $Ca^{2+}$, serotonin, and butyrate) in the hour time scale. Eventually, the influence of the signaling molecules local concentrations on transcriptional processes occurring in long time scales should produce the final biological outcomes.

Figure 5 establishes the space phase region of genetic rate constants where the above oscillatory phenomena can be obtained for the particular biophysical model of Figures 1 and 2. The numbers in the curves correspond to the oscillation periods obtained. For a better understanding of the essential trends of the model, all transcription and translation rate constants take the same values, as is the case of the degradation constants. The ranges of rate constants where coupled bioelectrical and biochemical oscillations exist are given in the central region of Figure 5. Phase diagrams of increasing complexity can be constructed for the particular experimental parameters characteristic of each biological problem.[41]

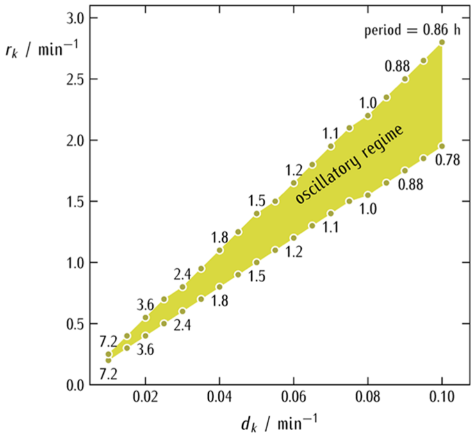

**Figure 5.** Phase diagram showing the pol and dep protein transcription and degradation rate constants where single-cell coupled bioelectrical and biochemical oscillations are obtained (central region). The numbers in the curves correspond to the oscillation periods (in hours, h). The system parameters are those given in the caption of Figure 1. Note that we assume $r^{o}_{m,k} = r_{p,k} = r_k$ and $d_{m,k} = d_{p,k} = d_k$ (k = pol, dep) with $r_{pol} = r_{dep}$ and $d_{pol} = d_{dep}$ for simplicity.

The phase diagram of Figure 5 establishes the region of oscillatory cell states as a function of genetic rate constants. In particular, Figure 5 associates individual oscillation frequencies with groups of rate constants. Because of cell heterogeneity, a wide range of individual time responses should then be expected for an uncoupled multicellular ensemble where no intercellular communication exists. It could be anticipated that intercellular connectivity will assure reliable average responses despite the individual cell variability. In other words, the intercellular coupling could act as an error-minimizing mechanism because the different frequency states resulting from the synchronization of the genetic and bioelectric layers at the single-cell level can be coupled together at the multicellular level. We consider this important question in the next sections.

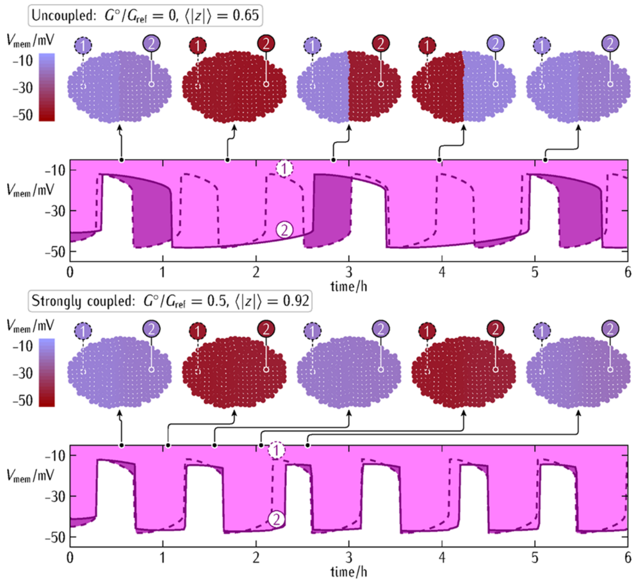

**Figure 6.** Snapshots showing the oscillations of the transmembrane potentials in the multicellular ensemble for the gap junction conductance $G^{o}/G_{ref} = 0$ (zero intercellular coupling, up panel). Note that the periods of the left and right regions of the ensemble differ by a factor 2 approximately. The ensemble oscillates as a whole for the gap junction conductance $G^{o}/G_{ref} = 0.5$ (strong intercellular coupling, down panel). As in Figure 5, the single-cell rate constants for the pol and dep proteins are equal, $r^{o}_{m,k} = r_{p,k} = 0.8$ min$^{-1}$ and $d_{m,k} = d_{p,k} = 0.03$ min$^{-1}$ (left region of the ensemble) and $r^{o}_{m,k} = r_{p,k} = 1.7$ min$^{-1}$ and $d_{m,k} = d_{p,k} = 0.07$ min$^{-1}$ (right region of the ensemble), k = pol, dep, with $G^{o}_{pol}/G_{ref} = G^{o}_{dep}/G_{ref} = 1$. Initially ($t = 0$), the membrane potentials take the polarized value. Biochemical oscillations of the mRNA and protein concentrations are coupled to these bioelectric oscillations (see Figure 4). The time average $\langle|z|\rangle$ of the order parameter $z$ is also included in the insets.

**Homogeneous Multicellular Ensemble.** Figure 6 shows the transmembrane potential oscillations in a multicellular ensemble characterized by zero (up) and non-zero (bottom) gap junction conductances simulating weak and strong intercellular coupling, respectively. In both cases, the rate constants are in the oscillatory region of Figure 5. However, these rate constants take different values in the left and right regions of the multicellular ensemble to allow for different oscillatory periods. As a consequence, the oscillations in the left and right regions are independent in the absence of intercellular coupling. In contrast, the ensemble can oscillate as a whole for strong coupling despite the fact that the ion channel protein rate constants take different values in the left and right regions. These limiting cases suggest that a range of intermediate oscillatory patterns characterized by different

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spatiotemporal regionalization of the rate constants should be possible; particular examples showing different flexible bioelectric topologies are given in ref 41.

Figure 6 includes an order parameter $z$ as a measure of the system synchronization. This parameter is defined using an individual period obtained as the time between two consecutive protein concentration maxima (see Figure 4), $T_n = t_{n+1} - t_n$. The single-cell effective period is not, in general, constant and allows defining an individual phase $2\pi\,(t - t_n)/T_n$ at an intermediate time $t$. From the $j$ single-cell phase $\theta_j$, the complex order parameter of the ensemble can be written as $z(t) = \sum_{j=1}^{N} \exp(i\theta_j)$. Order parameters are useful to characterize synchronization as a function of the coupling strength.[68,69]

Experimentally, single-cell cycle regulation[49,62,70] and multicellular functions[20,39,42,52,71,72] may involve the coordination of oscillatory bioelectrical and biochemical signals. In this context, the above results provide clear physical insights at the single-cell (note the feedback between the genetic and bioelectric layers of description in Figures 1, 2, and 4) and multicellular (note the intercellular coupling supporting the bioelectric oscillations of Figure 6) levels. For instance, Figure 6 suggests that the single-cell oscillation of Figure 4 consisting of two alternating polarized/depolarized cell states[40] can be extended at the multicellular level by: (i) changes in the spatial distribution of the signaling molecules that regulate the different protein rate constants assumed in the left and right regions of the ensemble and (ii) modifications of the intercellular coupling between cells.[41]

Endogenous bioelectric gradients are instructive factors in morphogenetic processes[3,36] that can be regulated by a dynamic intercellular connectivity during embryonic development.[42,73] For instance, the transduction of distant bioelectric signals through active gap junctions can influence the developing brain because local and distant bioelectric signals may have counter-acting actions.[32,73] Also, the transmembrane potential of somatic cells appears to influence oncogene-mediated tumorigenesis at long-range.[71] Figure 6 suggests that a distant oscillatory control should also be possible by showing two limiting cases for the bioelectric coupling of single-cell membrane potentials over a two-region ensemble. While long-distance developmental mechanisms in real biological cases are regulated by the spatiotemporal distribution of signaling ions and molecules, the local concentrations of electrically charged messengers such as calcium, butyrate, and serotonin are influenced by the spatial maps of cell potentials.[24]

**Heterogeneous Multicellular Ensemble.** Self-organization of biochemical oscillators into spatiotemporal patterns have been previously observed during embryo development.[74,75] However, the possible role of biochemical and bioelectric coupling on the multicellular oscillations is not usually considered, despite the fact that ensemble-averaged electric potentials may exert an endogenous control on the single-cell biochemical kinetics,[24,25,29] as shown in the case of pancreatic islets.[76] Individual variability and network stochasticity are inherent to multicellular aggregates. Therefore, it is important to show that synchronized bioelectric oscillations at the multicellular level are also possible in a heterogeneous ensemble.

We study the oscillation and synchronization of the cell potentials as a function of the intercellular coupling for a bimodal distribution of single-cell frequencies, which are randomly distributed in the multicellular ensemble (Figure 7). For increasing values of the gap junction conductance $G^o/G_{ref}$, the initially assumed bimodal distribution for the individual cell frequencies eventually collapses into one multicellular effective frequency at a long time. The ensemble shifts to lower periods (higher frequencies) because the average electric potential makes groups of cells to act as a coordinated multicellular patch. Note here that: (i) single-cell electrical responses are much faster than transcriptional and translational processes[25,28] and (ii) these biochemical processes are regulated by the cell transmembrane potentials in the model of Figure 1. Consequently, those cells with higher intrinsic frequencies eventually dominate the oscillatory behavior of the whole ensemble.

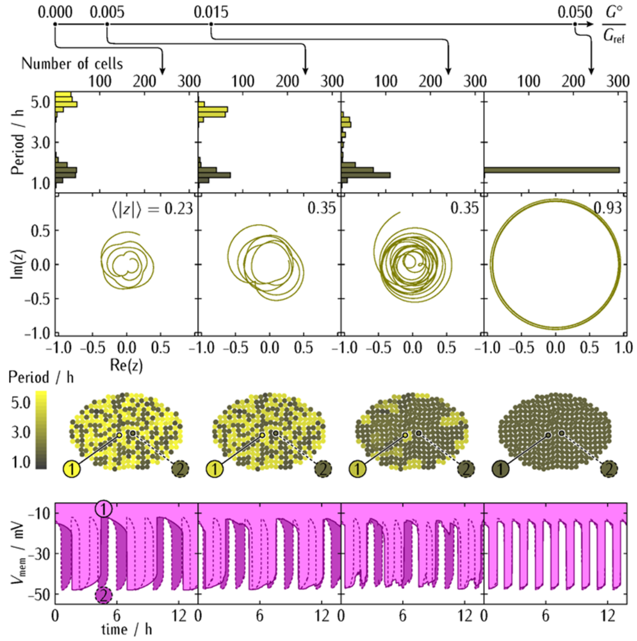

**Figure 7.** Synchronization of cell transmembrane potentials is shown as a function of the dimensionless gap junction conductance $G^o/G_{ref}$ (up) for the case of a spatially random bimodal distribution of single-cell frequencies at zero coupling (left) that collapses into one effective ensemble frequency for the case of high intercellular coupling (right). The trajectories of the multicellular ensemble order $z$ parameter in the complex plane are shown together with the corresponding frequency maps. The maps show self-organized clusters of different frequencies that merge into a synchronized multicellular ensemble at high intercellular coupling. The transmembrane potential oscillations of two particular cells in the ensemble are also shown for different values of $G^o/G_{ref}$ (bottom).

To reveal this collective synchronization, Figure 7 also shows the trajectories of the order parameter $z$ in the complex plane Re($z$)−Im($z$) for different values of the intercellular coupling strength. In the incoherent regime of zero coupling, the individual phases are uncorrelated. By increasing the intercellular conductance multicellular synchronization is gradually achieved, as shown by the limit circle[77] corresponding to the highest conductance. The frequency maps obtained at long times and the transmembrane potentials of two particular cells in the ensemble are also shown in Figure 7 for different values of $G^o/G_{ref}$.

Figure 8 considers the case of a spatially structured L−R distribution of single-cell characteristics instead of the spatially random distribution of Figure 7. This case is of biological relevance: the rate constants describing ion channel protein transcription can be spatially regionalized in early devel-

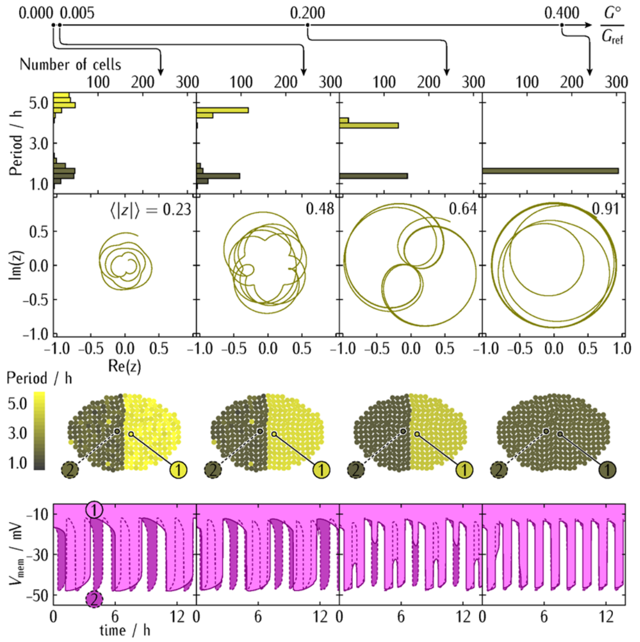

**Figure 8.** Synchronization of cell transmembrane potentials is shown as a function of the gap junction conductance for the case of a spatially structured L−R distribution of single-cell frequencies. The trajectories of the multicellular ensemble order $z$ parameter in the complex plane are shown together with the corresponding frequency maps. The transmembrane potential oscillations of two particular cells in the ensemble are also shown for different values of $G^\circ/G_{\text{ref}}$.

opmental processes and the establishment of axial body asymmetry (L−R or head-tail).[23,42] Remarkably, the cells can coordinate their individual states not only with regard to the spatial coordinates but also with regard to timing. As in the case of Figure 7, the ensemble synchronization to a high effective frequency (low effective period) is achieved by increasing the gap junction conductance. However, relatively high intercellular conductances are now needed for frequency locking: compare the $G^\circ/G_{\text{ref}}$ scale of Figure 8 with that of Figure 7. This result occurs because the high period right (R) ensemble of Figure 8 can resist better the driving effect of the low period left (L) ensemble than in the case of the high period small microdomains of Figure 7. This difference is also clearly shown in the two-circles (Figure 8) and one-circle (Figure 7) trajectories of the order parameter $z$. In both cases, however, desynchronization should be possible by changing the dynamic intercellular connectivity that modulates the spatial patterns; particular examples of this reset function are given in Figures 3 and 4 of ref 41.

From an experimental viewpoint, enhancing the intercellular coupling is analogous to increasing the density in quorum sensing bacterial ensembles[19,78] and cell populations,[15] which leads to collective behaviors at different levels of complexity. In this context, the results of Figure 8 can also be extended to the case of spatially structured oscillatory cell populations[15,74,75] by introducing prepatterns of genetic rate constants and gap junction conductances.[24,41] These patterns can define spatially isolated domains in the multicellular ensemble where the cells coupled within a particular domain share the same oscillatory state which is different from those in the neighboring domains (see ref 41 for specific examples).

Note that the protein concentrations $p_k$ (see Figures 1 and 2) should also synchronize with the cell potential oscillations of Figures 7 and 8, as shown in Figure 4. Increasing experimental data show a significant interplay between the transmembrane potential and the progression through the cell cycle.[51,62,70,79] Therefore, the ensemble synchronization of electric potentials should also have feedback effects concerning the collective regulation of individual cell cycles. In a different context, the genes encoding the ion channels and synaptic proteins that regulate the local electric potentials in the brain are involved in the modulation of oscillatory patterns during memory encoding in particular areas, suggesting experimental correlations between gene expression and oscillating brain states.[80] Synchronization and oscillatory coherence can contribute to memory consolidation by acting on the expression of plasticity-related proteins.[81,82]

In summary, the feedback between the bioelectric and genetic descriptions (Figure 1) allows oscillatory and synchronization phenomena at the single-cell (Figure 4) and multicellular (Figures 6−8) levels. Remarkably, these collective modes of information processing emerge in a natural way without assuming any input periodic function for the biological magnitudes involved at the single-cell and multicellular levels. The voltage-dependent intercellular conductances of Figure 3 allow coherent multicellular states because the average transmembrane potential acts as an ensemble controller that couple together individually different single-cell frequencies to produce a double biochemical/bioelectrical coherence (Figures 4 and 7) for high enough junction conductances.

Biorhythms and synchronization phenomena are ubiquitous to systems biology.[83] Specific examples concern coupled enzyme systems and glycolytic models,[84,85] developmental[74] and synthetic[86] genetic oscillators, and cellular and circadian rythms.[87,88] More complex synchronized behaviors such as 2:1 synchronization patterns are also typical of genetic oscillators, brain, and brain and body oscillations.[85,89,90] In our case, however, we have focused on bioelectrical phenomena, where oscillatory collective communication across different scales arise in aggregates of glioma cells,[18] bacterial communities,[19] pancreatic islets,[20] and the possible coupling between the expression of plasticity-related proteins and oscillating brain states.[80,81] In all these cases, specific ion channel proteins contribute to the coordination of large cell populations by regulating endogenous electric pulses. The potential maps of Figures 6−8 can be seen as bioelectrical templates for the spatiotemporal distributions of signaling ions and molecules that influence downstream biochemical pathways. It is then conceivable that ensemble-averaged bioelectric magnitudes may assist in the control of single-cell characteristics by improving the system reliability at the multicellular level,[24,91] which suggests that efforts to control the system-level activity of cellular ensembles can target specific electric potentials and currents.[3,23,24,32,45,92]

In the above context, it is tempting to speculate whether the interplay between genetic prepatterns and cell potentials, regulated by the intercellular coupling, could provide a sort of bioelectric software implementing distributed biological memories via slow oscillatory patterns.[12,29] In the synapses of neural networks, the functional feedbacks between biochemical and electrical signals allow for information processing and memory.[93] Following this analogy,[3,36,94] we note that the intercellular junctions that couple two neighboring cells in the model of Figure 3 are regulated by voltage-dependent conductances that change dynamically with the bioelectric states of the connected cells. From a biological hardware viewpoint, these conductances provide the junctional plasticity[24] that is needed for a distributed memory where the network nodes are locally adapted to the dynamic state of the different neighboring regions.[41]

Most models of networks are based on stable attractors. However, the regulation of large-scale synchrony by a dynamic intercellular connectivity[41] may also allow multiple attractors as transient spatial patterns reminiscent of brain oscillatory states.[81,82] In our case, the collective states are regulated by bioelectrical and genetic layers of multicellular control showing both intralayer and interlayer synchronizations because of the controller role of ensemble-averaged transmembrane potentials.

## CONCLUSIONS

Oscillations, synchronization, and pattern formation occur in biology across a range of spatial and temporal scales.[95,96] We have explored theoretically the bioelectric coupling between the biochemical patterns of ion channel protein expression and the cell potential maps as a plausible mechanism to control multicellular networks of non-neural cells. Because of its simplicity and independence from many structural details, the model suggests a generic ion channel and gap junction dynamics that can be relevant to a range of functional interactions at the transcriptional and post-translational levels through the spatiotemporal maps of cell potentials.[23,31,32,36,48,51,60] Interestingly, the model predicts that coherent oscillations can emerge in a heterogeneous ensemble of non-neural cells without any centralized coordination.

The control of transmembrane potentials can be a powerful experimental strategy because patterns of electric potentials and currents are central to information processing not only in neural cells[81,82,97] but also in bacterial communities and non-neural cells.[18−20,33,42,52,92,98] In addition, the role of intercellular coupling on the regulation of multicellular dynamical states is crucial in cell differentiation and tumorigenesis.[22,42,99] The final goal here would be to devise new methods of acting on multicellular ensembles in terms of a small number of average bioelectric magnitudes as a collective approach complementary to manipulating individual cell characteristics.[3,24,36,100]

We have suggested that a memory based on bioelectric patterns could permit a top-down control complementary to the dominant bottom-up approach based on biochemical pathways and individual cell genetics because multicellular ensemble-averaged properties such as the electric potential can be used to gain network control, as shown in experiments on model animals.[3,23,32,36,60] In our case, the simulations show that long lasted (several hours) electrical rhythms can synchronize feedback processes between model genetic and bioelectrical networks. Future work can extend this theoretical approach to include specific molecular mechanisms of feedback at both the single-cell and multicellular levels.[24,40]

The model results emphasize that experimentally testable actions concerning slow oscillatory phenomena in non-neural cells should consider the feedback between bioelectric and genetic networks. It is well-known that potassium channels can influence cell proliferation using different mechanisms.[62] In particular, slow electrical oscillations in the cell polarization are associated with phase-specific changes in the cell cycle (see Figure 2 of ref 62). Remarkably, a reduced number of key ion channels contribute to these transient hyperpolarization and depolarization processes characteristic of the cell cycle (see Figure 1 of ref 70). In a different context, neuron models of gene expression incorporate biochemical schemes with an activity-dependent ion channel expression.[63] The feedback between bioelectric and biochemical pathways is included here by channel mRNAs that are produced at a rate that depends on a calcium concentration-activated factor (see Figures 1 and 2 of ref 63 for the case of a $Ca^{2+}$ integral controller). This approach shows that biophysical models incorporating a minimal number of biologically consistent assumptions can be useful.[34]

In summary, the effects that local ion concentrations and electric fields can exert on signaling cascades and gene expression constitute a question of current biophysical interest.[23−25,101] For practical interventions seeking to control oscillatory phenomena, however, systems with a limited number of ion channels and gap junctions whose functional role is known[9,10,52,97,102] should be used to avoid exceedingly complex nonlinear interactions. In real biological problems, a detailed identification of the particular channels entering the genetic and bioelectric networks, together with a global understanding of the resulting feedback circuits, will be essential ingredients to developing control strategies for bioelectric signaling in applications.[21,32,48,103]

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### Notes

The authors declare no competing financial interest.

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