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**Review**

# Integrating Bioelectrical Currents and $Ca^{2+}$ Signaling with Biochemical Signaling in Development and Pathogenesis

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**Abstract**

Roles of bioelectrical signals are increasingly recognized in excitable and nonexcitable non-neural tissues. Diverse ion-selective channels, pumps, and gap junctions participate in bioelectrical signaling, including those transporting calcium ions ($Ca^{2+}$). $Ca^{2+}$ is the most versatile transported ion, because it serves as an electrical charge carrier and a biochemical regulator for multiple molecular binding, enzyme, and transcription activities. We aspire to learn how bioelectrical signals crosstalk to biochemical/biomechanical signals. In this study, we review four recent studies showing how bioelectrical currents and $Ca^{2+}$ signaling affect collective dermal cell migration during feather bud elongation, affect chondrogenic differentiation in limb development, couple with mechanical tension in aligning gut smooth muscle, and affect mitochondrial function and skeletal muscle atrophy. We observe bioelectrical signals involved in several developmental and pathological conditions in chickens and mice at multiple spatial scales: cellular, cellular collective, and subcellular. These examples inspire novel concept and approaches for future basic and translational studies.

**Keywords:** collective cell migration, chondrogenic differentiation, smooth muscle alignment, neuromuscular degenerative disease

## Introduction

Eight decades after the first measurements of action potentials in squid giant axon were published,[1] bioelectrical signals in the form of flowing ions, including $Ca^{2+}$, $H^+$, $Na^+$, $K^+$, $Cl^-$ and charged large molecules (such as serotonin and butyrate) are recognized to play roles in a myriad of biological processes that have long-lasting impact on tissue/organ structure and function, which are beyond the scope of classical electrophysiology. For example, functional perturbation of a series of ion channels and gap junctions resulted in permanent changes of vein patterns developed in Drosophila wings and in the anterior/posterior body axis in planaria upon regeneration.[2–4]

Gap junctions are intercellular channels formed by two end-to-end hexameric hemichannels (composed of connexin protein in chordates) on closely apposed plasma membrane.[5] The channels allow passages of small molecules up to 1 kDa, such as $Ca^{2+}$, $IP_3$, glutamate, glutathione, ADP, ATP,[6] and serotonin.[7–10] Electrical coupling between cells by gap junctions have been demonstrated to be crucial for large-scale patterning processes, such as left/right symmetry breakage, brain region specification during Xenopus embryogenesis,[7–11] and bone growth in the fins of zebrafish.[12]

The cell behaviors modulated by bioelectrical signals, beside fate specification and proliferation in the processes mentioned above, also include migration/shape change. For example, biofilms formed by *Bacillus subtilis* were recently found to emanate self-sustained electrical field oscillations due to $K^+$ currents, which attract other species of bacteria over long distances.[13] In eukaryotic cells, not only could applied electrical fields reorient the growth cones, axons, and even the direction of migration of cultured neurons/neural progenitor cells *in vitro*,[14–17] naturally occurring electrical fields *in vivo* under physiological and pathological conditions also could serve as axon guidance cues.[16,18] Additionally, chemically manipulating membrane potential ($V_{mem}$) in cellular collectives, which results in altered tissue electrical field, could alter axon growth, as in ivermectin-induced depolarization, which enhances axon growth toward eye primordia transplanted to ectopic locations in tadpoles.[19]

The electrotaxis phenomenon is not restricted to excitable cells—keratinocytes, corneal epithelial cells, fibroblasts, adipose-derived stromal cells, osteoblasts and

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osteoclasts, lymphocytes, macrophages, and endothelial cells have all been reported to exhibit directed migration in applied electrical fields *in vitro*.[20] Naturally occurring electrical signals, such as contact-dependent depolarization in zebrafish pigment cells, are involved in the repulsion of the xanthophores (yellow pigmented) from melanophores (black pigmented).[21] On the other hand, wound-induced electrical fields in cornea attract corneal epithelial cells to the wounded area.[22] More intriguingly, the strength of the wound-induced current is correlated with the rate of wound healing in both animal models and patients.[22,23] A recent study reported bacterial (*Salmonella*) infection-induced reversal of gut electrical fields in follicle-associated epithelium, providing electrotactic or electrorepulsive cues for macrophages based on their surface electrical properties.[24]

Thus, although less well explored compared with neurons and muscles, the importance of bioelectrical signaling in nonexcitable tissue cannot be ignored. This new frontier is gaining increasing attention due to both its basic research and its translational values. In this review, we will use several recent studies to demonstrate how bioelectrical signals engage $Ca^{2+}$ transients in developmental processes of organogenesis in both excitable and nonexcitable tissue and the pathological process of muscle atrophy during neuromuscular degenerative disease.

## $Ca^{2+}$-Mediated Crosstalk Between Bioelectrical and Biochemical Signaling

Bioelectrical signals are commonly observed to involve $Ca^{2+}$ ions, either as the carrier of the electrical signal or as a downstream second messenger. $Ca^{2+}$ ions are charged, rapidly diffusing, capable of passing through gap junctions between cells,[25–27] and can be translocated among the cytosol, organelles, and extracellular milieu using different channels and transporters (Fig. 1).

More specifically, $Ca^{2+}$ influx into the cells can occur through at least five routes: (1) Ligand-gated cation channels permeable to $Ca^{2+}$, such as purinergic (ATP) receptor channels, cyclic nucleotide-gated channels[28]; acetylcholine receptor channels, N-methyl-D-aspartate receptor channels, AMPA receptor channels, and kainate receptor channels[29–33]; (2) Mechanosensitive Piezo1 and Piezo2 channels[34–36]; (3) Voltage-gated $Ca^{2+}$ channels (VGCCs)[37]; (4) transient receptor potential (TRP) channels (gated by either ligands, mechanical force, or temperature variations)[38]; (5) $Ca^{2+}$ release-activated channels (CRACs) composed of $Ca^{2+}$ release-activated $Ca^{2+}$ channel protein (ORAI) on the plasma membrane and stromal interaction molecule (STIM) on the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR). They open upon ER/SR $Ca^{2+}$ store depletion.[39,40]

$Ca^{2+}$ efflux from cells can be carried out by plasma membrane $Ca^{2+}$ ATPases (PMCA) or $Na^+/Ca^{2+}$ exchangers.[41] Additionally, intracellular $Ca^{2+}$ level is heavily influenced by $Ca^{2+}$ release from ER/SR through the activation of ryanodine receptors (RYR) or inositol 1,4,5-trisphosphate receptors ($IP_3R$).[42] $Ca^{2+}$ influx from the extracellular milieu can activate both RYRs and $IP_3Rs$, leading to $Ca^{2+}$-induced $Ca^{2+}$ release .[42]

Under pathological conditions, cardiomyocyte SR can spontaneously release $Ca^{2+}$ due to $Ca^{2+}$ overload, leading to a phenomenon known as store-overload-induced $Ca^{2+}$ release.[43–49] $IP_3Rs$ can also be activated by $IP_3$, whose production is catalyzed by phospholipase C upon the activation by G-protein-coupled receptors on the plasma membrane.[50,51] This is one mechanism underlying purinergic receptor-mediated $Ca^{2+}$ wave propagation.[52–54] Reuptake of cytosolic $Ca^{2+}$ into ER/SR is primarily carried out by the sarcoendoplasmic reticulum $Ca^{2+}$ pump (SERCA).[55]

Cytosolic $Ca^{2+}$ signaling may be further modulated by mitochondria.[56,57] After influx into the mitochondrial matrix through mitochondrial $Ca^{2+}$ uniporters (MCU),[58] $Ca^{2+}$ may be reversibly sequestered by phosphate.[59] This $Ca^{2+}$ buffering effect may be more prominent in cells with larger mitochondrial volume, such as differentiated cardiomyocyte (30–40% of the cell volume).[60] Export of mitochondrial $Ca^{2+}$ may be through $Na^+/Ca^{2+}/Li^+$ exchangers or the mysterious permeability transition pore (PTP).[50,61] The molecular nature of PTP has been under debate for many years and recent studies indicate that it forms from dimers of ATP synthase.[62]

Due to the presence of such complex regulatory machinery, it is not surprising that $Ca^{2+}$ transients exhibit both an extremely diverse spatial range (from multicellular to subcellular) and temporal dynamics (from hours to less than a millisecond).[63–67] Meanwhile there exist numerous $Ca^{2+}$ effector proteins that activate different biochemical pathways, such as $\alpha$-actinin for actin filament crosslinking,[68] troponin for actin/tropomyosin filament contraction,[69] protein kinase C (PKC)-NF-$\kappa$B, calmodulin/calcineurin/NFAT and $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) for gene expression regulation.[67,70] Based on $Ca^{2+}$ association/dissociation kinetics, $Ca^{2+}$-binding sites, and subcellular localization of these effector proteins, they can specifically respond to $Ca^{2+}$ transients at a certain amplitude/frequency.[67,71,72] Therefore, it is not surprising that $Ca^{2+}$ signaling is involved in numerous biological processes, such as migration,[71] contraction,[73] endocytosis and exocytosis,[74] mitochondrial respiratory function regulation,[56] cell death,[56] proliferation,[75,76] and differentiation[77]).

It is worth noticing that fast (less than a second), ''superficial'' (usually close-to-plasma membrane) $Ca^{2+}$ transients more likely induce temporary phenomena such as exocytosis and contraction, while more profound changes like the regulation of gene expression or cell proliferation usually requires relatively slow (minutes to hours) and global (whole-cell) $Ca^{2+}$ level changes.[67] For bioelectrical currents to generate long-lasting impact on tissue/organ structure, engaging the slow, global $Ca^{2+}$ transients seem to be ideal. However, repetitive activation of the superficial $Ca^{2+}$ transients of sufficient duration, may trigger other signaling mechanism (such as cyclical contraction induced nonautonomous cell rearrangement, which will be discussed later) or creating a pathological condition, which may also produce permanent structural/functional changes.

We believe the study of developmental, physiological, and pathological events involving both bioelectrical currents and $Ca^{2+}$ signaling could yield critical information about how bioelectrical signals are linked to other signaling mechanisms, either biochemical, biomechanical, or transcriptional. We expect the elucidation of these crosstalk pathways will uncover a whole dimension of signaling in development and pathological conditions. The following four recent studies represent only the tips of the icebergs.

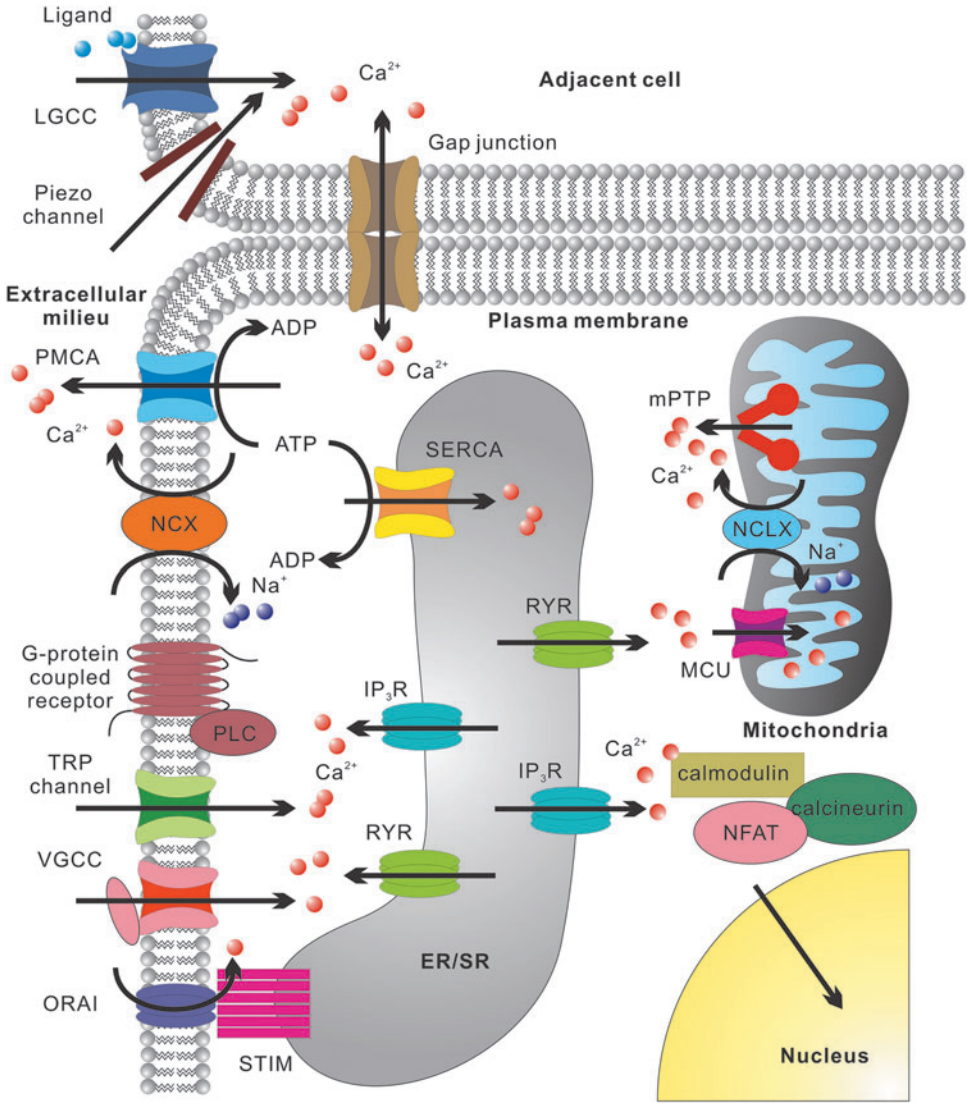


**FIG. 1.** Schematic representation of key regulators of cytosolic $Ca^{2+}$. $Ca^{2+}$ enters cytosol from the extracellular milieu through channels situated on the plasma membrane, such as LGCCs that are permeable to $Ca^{2+}$ (such as purinergic receptors, CNG channels, AChRs, AMPARs, NMDARs, KARs), mechanosensitive Piezo1 and Piezo2 channels, VGCCs, TRP channels, and ORAI (the plasma membrane part of CRAC). ORAI is activated by STIM upon ER/SR $Ca^{2+}$ depletion. $Ca^{2+}$ efflux can occur through PMCA or NCX. Intercellular $Ca^{2+}$ exchange can occur through gap junction channels. ER/SR $Ca^{2+}$ release through RYR can be activated by cytosolic $Ca^{2+}$ elevation or VGCC conformation change. $IP_3R$ $Ca^{2+}$ release can be initiated by cytosolic $Ca^{2+}$ elevation or $IP_3$ produced by PLC upon the activation of G-protein-coupled receptors. $Ca^{2+}$ influx into ER/SR can be mediated by SERCA. Mitochondrial $Ca^{2+}$ intake mainly occur through MCU, while the efflux can occur through NCLX or mPTP formed by dimers of ATP synthase. For nuclear $Ca^{2+}$ signaling, a representative example is the dephosphorylation of NFAT by calcineurin and its nuclear translocation. Calcineurin is activated by calmodulin upon $Ca^{2+}$ binding. AChR, acetylcholine receptor; AMPAR, AMPA receptor; CNG, cyclic nucleotide-gated; CRAC, $Ca^{2+}$ release-activated channel; ER/SR, endoplasmic reticulum/sarcoplasmic reticulum; KAR, kainate receptor; LGCCs, ligand-gated cation channel; MCU, mitochondrial $Ca^{2+}$ uniporter; mPTP, mitochondrial permeability transition pore; NCLX, $Na^+/Ca^{2+}/Li^+$ exchanger; NCX, $Na^+/Ca^{2+}$ exchanger; NMDAR, N-methyl-D-aspartate receptor; ORAI, $Ca^{2+}$ release-activated $Ca^{2+}$ channel protein; PLC, phospholipase C; PMCA, plasma membrane $Ca^{2+}$ ATPase; RYR, ryanodine receptor; SERCA, sarcoendoplasmic reticulum $Ca^{2+}$ pump; STIM, stromal interaction molecule; TRP, transient receptor potential; VGCC, voltage-gated $Ca^{2+}$ channel.

### Transient Bioelectrical Currents and $Ca^{2+}$ Signaling in Collective Mesenchymal Cell Migration During Feather Bud Elongation

The involvement of $Ca^{2+}$ in the motility of individual cells is well established. $Ca^{2+}$-binding proteins regulate actin filament assembly/disassembly, myosin activity and focal adhesion turnover.[71] The leading edges of migrating fibroblasts exhibit transient, intracellular $Ca^{2+}$ elevations in localized submembrane microdomains due to the activation of mechanosensitive TRPM7 channels, and these ''$Ca^{2+}$ flickers'' are involved in steering the migration direction.[64]

Yet, in many developmental and pathological processes, such as gastrulation, organogenesis, wound healing, chronic

inflammation, and cancer metastasis, cells move as a collective population.[78,79] Long-distance migration as a large cohort is difficult to explain by chemotaxis alone. Migration up a concentration gradient toward the source of released chemokine, implies that the gradient is steep and that a large cohort of separate cells will ''see'' different concentrations depending on whether cells are located close to or far from the source, so chemokine-stimulated movement would not be expected to be uniform. What mechanisms would ensure a more uniform signal across a large cell population?

A recent study of chicken feather elongation implicates a tissue-wide gap junction network[80] as a means to enable directional mesenchymal cell population migration in posterior/distal elongation of feather primordia.[81] Gap junctional coupling enables rapid and long-distance electrical signaling. Voltage-gated $Ca^{2+}$ and $Na^{+}$ channels regenerate signal strength as the signal travels from cell to cell. Higher-density channel expression assures nondecrementing (e.g., action potential) propagation over distances in the cm range, while far lower densities may be sufficient to enable shorter distance (100s of micrometers) propagation. As noted above, $Ca^{2+}$ serves double duty as both an electrical charge carrier and as a second messenger for biochemical pathways. Furthermore, other ions and molecules may traverse gap junctions, also serving as second messengers.

So, how does the signaling initiate in a gap junction-coupled syncytium? In heart muscle, the initial signal arises at a pacemaker that initiates an action potential, which then spreads outward like a wave. How about in the mesenchyme of feather primordia? How does current flow in mesenchyme initiate and spread? Before feather primordia elongation, a vibrating probe detects only inward electrical currents across the feather region.[82] Following the onset of elongation, the bioelectrical currents at the anterior part of the feather reverse direction. A local standing current then envelopes each primordium, with currents entering the feather bud tip and exiting at the anterior.[80] Inward currents are typically mediated by $Na^{+}$ and/or $Ca^{2+}$ channel currents, and outward currents are most often potassium channel currents.

Through transcriptomic profiling, candidate gene expression analysis and functional perturbation experiments, we have shown that a connexin-43-based gap junction network, VGCCs (likely Cav1.2), and Icrac (ORAI/STIM) likely contribute to this electrical circuit within feather mesenchyme. The migration process is blocked upon inhibition of VGCCs (by nifedipine) or the gap junctions (by carbenoxolone, PMA or shRNA against *connexin-43*), whereas ectopic $Ca^{2+}$ oscillations induced by photoactivatable CRACs enhanced this process.[80] The expression of *connexin-43* is regulated by the synergistic actions of Sonic Hedgehog and Wingless-INT signaling.[80] Thus, the establishment of the feather bioelectrical circuit and $Ca^{2+}$ oscillations rely on inputs from biochemical pathways (Fig. 2A).

### Bioelectrical Currents and $Ca^{2+}$ Signaling During Limb Chondrogenic Differentiation

Chondrogenesis is well known to be regulated by $Ca^{2+}$ signaling, as the calmodulin/calcineurin/NFAT pathway that can activate the expression of Sox9, which is crucial for mesenchymal cell condensation and the expression of several cartilage-specific extracellular matrix molecules.[83,84] Meanwhile $Ca^{2+}$-calmodulin is also known to promote Sox9 nuclear localization.[85] Rapid $Ca^{2+}$ transients ($\sim$1 min or less) have been observed in different chondrogenic models, while CRACs, TRP channels, as well as VGCCs (both L-type and T-type) have been demonstrated to contribute to these transients.[83,84,86,87] The involvement of VGCCs implies $V_{mem}$ changes, in other words bioelectrical signals, as an upstream event of chondrogenesis.

Indeed, a recent study of limb bud chondrogenesis used the potential sensitive fluorescent probe $DiBAC_4$ to demonstrate a depolarization of $V_{mem}$ in the chondrogenic region of the limb when chondrogenic differentiation starts.[87] The spatiotemporal correlation of depolarization and chondrogenic differentiation was observed in both limb slice culture and micromass culture, in which Col2, a cartilage-specific extracellular matrix molecule, was used as the chondrogenic differentiation marker.[87]

The importance of depolarization to chondrogenesis is further supported by the phenomenon that pharmacological inhibition of epithelial $Na^{+}$ channel, which is involved in depolarization, also blocks chondrogenic differentiation.[87–89] Additionally, an artificially induced depolarization achieved by exposing cells to an external solution containing high $K^{+}$ increased the frequency of $Ca^{2+}$ transients in the micromass culture, while nifedipine treatment has the opposite effect. Yet the nifedipine-mediated inhibition of $Ca^{2+}$ transients is limited to the first day of micromass culture, implying that VGCCs are important for the early but not late chondrogenic differentiation.[87] Nifedipine treatment of the micromass culture significantly reduced Alcian Blue-positive cells and Sox9 expression, while the $Ca^{2+}$ ionophore A23187 (Calcimycin) has the opposite effect.[87]

Finally, using a knockout mouse model, the authors pinpoint $Ca_V1.2$ as a VGCC critical for limb chondrogenesis and calmodulin/calcineurin/NFAT signaling is confirmed to be one of its downstream biochemical pathways that promote chondrogenic differentiation[87] (Fig. 2B).

### Bioelectrical Currents and $Ca^{2+}$ Signaling Couple Mechanical Tension and Smooth Muscle Alignment in the Intestinal Wall

For gastrointestinal (GI) smooth muscle cells, excitation/contraction (E-C) initiates from a depolarization that can be triggered by multiple input sources, including enteric neurons and intestinal cells of Cajal.[90,91] Depolarization-induced activation of VGCCs provides the majority of $Ca^{2+}$ ions required to initiate the spontaneous contraction, which is supplemented by storage-operated $Ca^{2+}$ release through $IP_3$ receptors.[90] $Ca^{2+}$ activates kinases like myosin light chain kinase (MLCK), which phosphorylates the myosin light chain for contraction. Meanwhile $Ca^{2+}$ has also been reported to inhibit myosin light chain phosphatase in a Rho kinase- and PKC-dependent manner, which also promotes contraction.[90] Blocking VGCC by nicardipine reduced the contraction force in both the circular and longitudinal layer of the GI smooth muscle.[90]

Gap junctions were implicated in electrical coupling of the GI smooth muscle cells, as carbenoxolone treatment reduced the amplitude of contraction.[91] However, the relationship between the spontaneous contraction and the alignment orientation of the smooth muscle has not been explored until

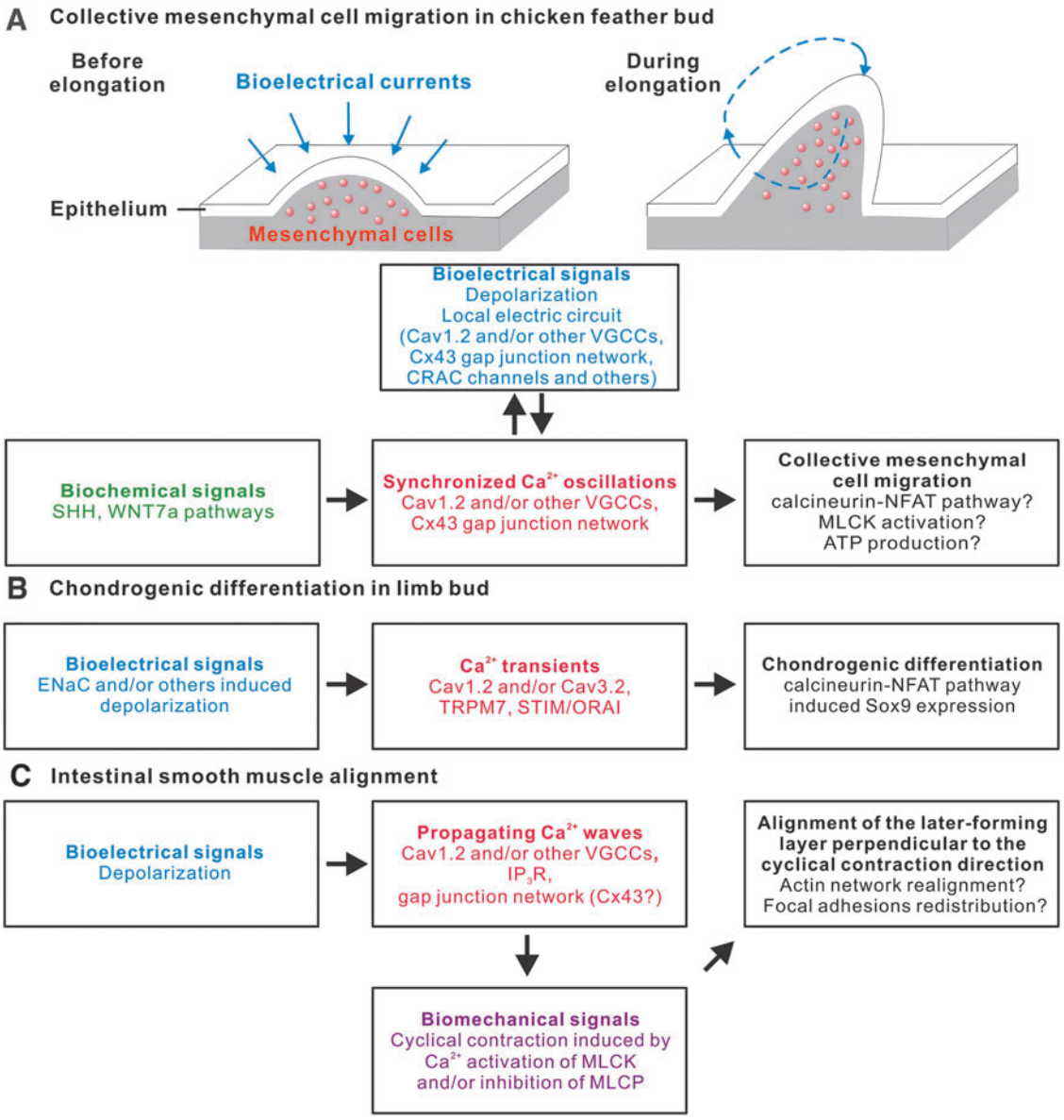


**FIG. 2.** Linkage of bioelectrical signals and $Ca^{2+}$ transients to other signaling mechanisms in different biological processes. **(A)** Biochemical WNT and SHH pathways promote the establishment of Cx43 gap junction network, facilitating the completion of a bioelectric circuit around individual feather buds. The depolarization-induced VGCC activation, the gap junction network, and CRACs contribute to the synchronized $Ca^{2+}$ oscillations in feather mesenchyme. These oscillations may promote cell migration either through transcriptional regulation (NFAT pathway), or MLCK activation to contract the actin/myosin network, or ATP production enhancement in mitochondria. **(B)** Early chondrogenic differentiation in limb involves ENaC-dependent depolarization, which initiates Cav1.2-dependent $Ca^{2+}$ transients. Meanwhile, some other $Ca^{2+}$ channels have also been reported to initiate $Ca^{2+}$ transients during chondrogenic differentiation. These transients activate NFAT pathway and elevate Sox9 expression. **(C)** Spontaneous contractions of intestinal smooth muscle cells are driven by propagating waves of $Ca^{2+}$. The formation of these waves requires depolarization-induced VGCC activation and gap junction network-based $Ca^{2+}$ exchange between cells. The cyclic contraction serves as a biomechanical signal aligning newly forming smooth muscle layer along the direction perpendicular to it, likely through modulating actin network and focal adhesions. Cx43, connexin-43; ENaC, epithelial $Na^{+}$ channel; MLCK, myosin light chain kinase; SHH, Sonic Hedgehog; WNT, wingless-INT.

recently, which reveals an intriguing coupling relationship between bioelectrical signals and mechanical force in tissue morphogenesis.[92]

The role of mechanical force in tissue morphogenesis has been recognized in the recent decade, as changes in the shape and arrangement of cells modify the mechanical forces, which may initiate signaling leading to changes in proliferation, differentiation, and gene expression.[93] Previous *in vitro* experiments demonstrate that mesenchymal cells, such as fibroblasts and smooth muscle cells, could reorient themselves roughly perpendicular to the direction of cyclically applied mechanical stretches.[94–98] During the development of intestinal smooth muscle, the circular layer forms earlier than the longitudinal layer and undergoes spontaneous contraction along the

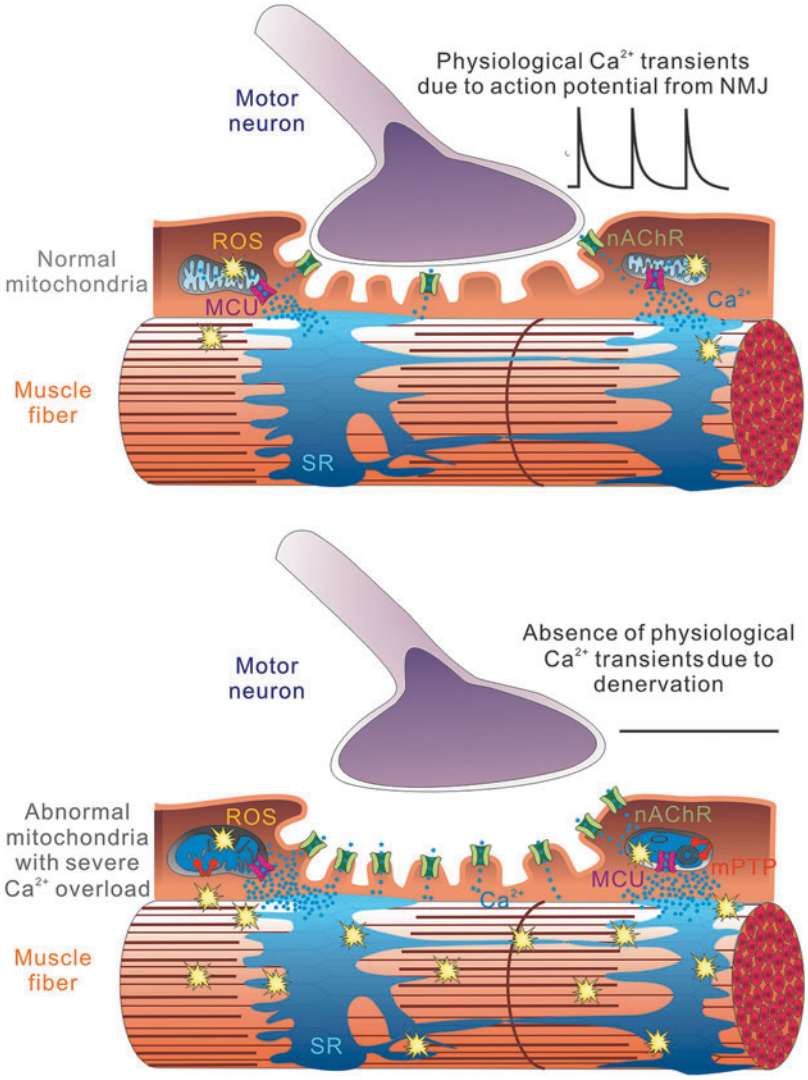


**FIG. 3.** Physiological $Ca^{2+}$ transients induced by motor neuron impulses help prevent mitochondrial $Ca^{2+}$ overload and ROS overproduction. After denervation, elevated nAChR expression likely contributed to the increase of cytosolic $Ca^{2+}$ in the skeletal muscle, which overloads mitochondrial matrix $Ca^{2+}$ content. This promotes the production of ROS, increases structural damage to the mitochondria, and facilitates mPTP opening. However, the elevated ROS production could be reversed by the application of electric field stimulation that simulates physiological $Ca^{2+}$ transients induced by neuronal impulses. nAChR, nicotinic acetylcholine receptor; ROS, reactive oxygen species.

circumferential axis,[99–103] bringing up the possibility that cyclical mechanical tension from the circular layer causes the longitudinal smooth muscle cells to differentiate later.

Consistent with this, the application of nifedipine, carbenoxolone, or ML-7 (an inhibitor of MLCK) to chicken intestinal explant culture before the formation of the longitudinal layer could all inhibit the longitudinal alignment. While treatment with the VGCC agonist (*S*)-(-)-BayK8644 had the opposite effect.[92] Furthermore, the application of cyclical longitudinal stretch to the explant culture (while inhibiting the endogenous contraction with nifedipine) led to the circumferential alignment of the later-forming smooth muscle layer, implying that cyclical mechanical stimuli are not only necessary but also sufficient to align the smooth muscle cells.[92] More interestingly, the authors also confirmed that alignment of the later-forming smooth muscle layer requires the spontaneous contraction of the earlier forming smooth muscle layer around the esophagus and ureter, which implies a broad applicability of this principle.[92]

In sum, the $V_{mem}$ fluctuations and a gap junction network allow $Ca^{2+}$-mediated contraction waves to occur in smooth muscle cells, producing cyclical mechanical stimuli as a tissue alignment signal (Fig. 2C).

### Bioelectrical Currents and $Ca^{2+}$ Signaling Affect Mitochondrial Function and Atrophy in the Skeletal Muscle

During development, mammalian skeletal muscles exhibit spontaneous $Ca^{2+}$ sparks, which are also observed in smooth and cardiac muscles.[104,105] However, in postnatal mammals, the sparks become rare (unless the muscle fibers are permeabilized), which may be explained by the loss of RYR3 and the maturation of t-tubule structure.[105–108] In adult mammalian skeletal muscle, motor neuron impulses at neuromuscular junctions are generally considered the sole input signal to initiate muscular action potential and induce contraction, a process termed E-C coupling.[109,110] Besides initiating contraction, bioelectrical signals (in the form of muscle action potential) also seem to be important for maintaining the skeletal muscle cytosolic $Ca^{2+}$ levels, mitochondrial integrity, and muscle mass. This is exemplified by the linkage between neurodegenerative disease (such as spinal muscular atrophy and amyotrophic lateral sclerosis [ALS]) and muscle atrophy.[111,112]

In the human $SOD1^{G93A}$-overexpressing mice (an animal model for familial ALS), the skeletal muscle fibers exhibited

collapsed mitochondrial inner membrane potential, reduced mitochondrial $Ca^{2+}$ uptake, and ectopic $Ca^{2+}$ waves under osmotic stress.[113,114] Furthermore, surgical denervation of the hindlimb skeletal muscle in rodent models triggered dramatic elevation of reactive oxygen species (ROS) production in the muscle mitochondria and ROS-related mitoflash events in just a few days, which posed a significant risk of oxidative damage to lipid, protein, and DNA.[115–117] In 2 weeks, the denervated muscle lost more than 30% of its original mass.[115,117] In contrast, electrical stimulation, as a physical therapy approach, has been shown to help preserve muscle mass and reverse the atrophy process in patients.[118–121]

Although the denervation-induced elevation of ROS production phenomenon has been known for more than a decade, the underlying molecular mechanism was poorly understood. Mitochondria are the major ATP provider for skeletal muscle, especially the slow oxidative muscle fibers, which heavily rely on oxidative phosphorylation for energy.[122] Yet the oxidative phosphorylation process is also a major producer of ROS, which is exacerbated under conditions of mitochondrial $Ca^{2+}$ overload.[56] Long-term denervation or disuse of skeletal muscle was reported to elevate the resting cytosolic $Ca^{2+}$ level.[123–125] This could result from decreased PMCA/SERCA function due to handicapped mitochondrial ATP production,[126] or $Ca^{2+}$ leak-in from nicotinic acetylcholine receptors (nAChRs),[30,127] as in denervated rodent models, nAChRs exhibit elevated expression and ectopic localization.[128,129]

The elevated cytosolic $Ca^{2+}$ likely increased resting mitochondrial $Ca^{2+}$ load, which promoted mitochondrial ROS production, structural damage, and mitochondrial PTP opening[116,130] (Fig. 3). In a recent study, when denervated muscles were exposed to electrical field stimulation, ROS-related mitoflash events were dramatically reduced. However, when mitochondrial $Ca^{2+}$ transients induced by the electrical stimulation were blocked by the MCU antagonist Ru360, this reduction effect was abolished.[116] More than a coincidence, virus-mediated overexpression of *MCU* was shown to have protective effect against denervation-induced skeletal muscle atrophy.[131,132] These discoveries unveil a seemingly counterintuitive, yet possible scenario that while mitochondrial $Ca^{2+}$ overload leads to excessive ROS production, the physiological mitochondrial $Ca^{2+}$ transients/uptake induced by the motor neuron impulse are required to keep the ROS production in check.[127]

## Conclusions

The four examples highlighted above, although seemingly unrelated, share a core mechanism in common: the changes in membrane potential activate VGCCs, which trigger the downstream $Ca^{2+}$-related physiological/pathological molecular processes. In each case, there are circumstantial evidences suggesting that VGCCs are crucial both for ensuring an electrical signal that coordinates events across populations of cells, and for generating intracellular changes in $Ca^{2+}$ that elicit the developmental or pathological change. Both the electrical signal and the intracellular $Ca^{2+}$ dynamics are thought to be necessary for proper development, differentiation, or in the last case, maintenance of tissue in a healthy state.

For the developmental process of organogenesis, understanding the cell/cell communication mechanisms coordinating collective behaviors of a population of cells is a major research direction and bears translational value. As shown in the feather mesenchyme and the intestinal smooth muscle studies, one possible solution for the large-scale intercellular communication is the establishment of the gap junction network.

In support of the idea that gap junctions are important for multicellular communications, studies of color stripe formation in zebrafish and Japanese quail have implicated gap junction channels in tuning the spatial organization of the pigmented cells.[133–135] For example, the blockage of gap junction communication by carbenoxolone specifically reduced the width of yellow stripes formed by pheomelanin-producing melanocytes in Japanese quail embryonic skin explants, whereas overexpressing *connexin-40* in melanocytes expanded the yellow stripes.[133] Interestingly, besides playing a role in determining color patterning, the gap junctions may also contribute to feather branching morphogenesis as membrane-localized connexin-43 was detected in the hooklet, but not pennulum region of barbules in chicken plumes.[136] Thus, the potential role of bioelectrical signals in the diversification of feather branching patterns for the adaptation to different ecospaces is worth future exploration among scientists interested in evo devo.

On the translational front, although electrical stimulation has become a well-established therapeutic approach,[118–121] the correlation between muscle action potential, mitochondrial $Ca^{2+}$ homeostasis, and ROS production is relatively underexplored. Further studies on the control of resting mitochondrial $Ca^{2+}$ level and ROS production could provide inspiration for developing new therapeutic approaches against aging and neuromuscular diseases.

On the other hand, it is also worth noticing that ROS production is not completely bad. It is an important signal from wounded tissue used to attract immune cells and stimulate regeneration in multiple animal species.[137–141] Recent studies also imply that ROS (more specifically $H_2O_2$) facilitates activation of voltage-gated $Na^+$ channels, promoting the reversal of the electrical field around the wound tissue, which induces regeneration.[137,138] We hope that insights from these studies will trigger more discoveries in this relatively unexplored new frontier.

## Author Contributions

C-.M.C. and A.L. designed the outline of this article. A.L., J.Z., R.B.W., R.H.C., and C-.M.C. contributed to the writing and editing of this article. All coauthors have reviewed and approved of the article before submission.

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