

Review

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Review

Rules of Life at the Interface of Calcium Signaling and Mechanobiology

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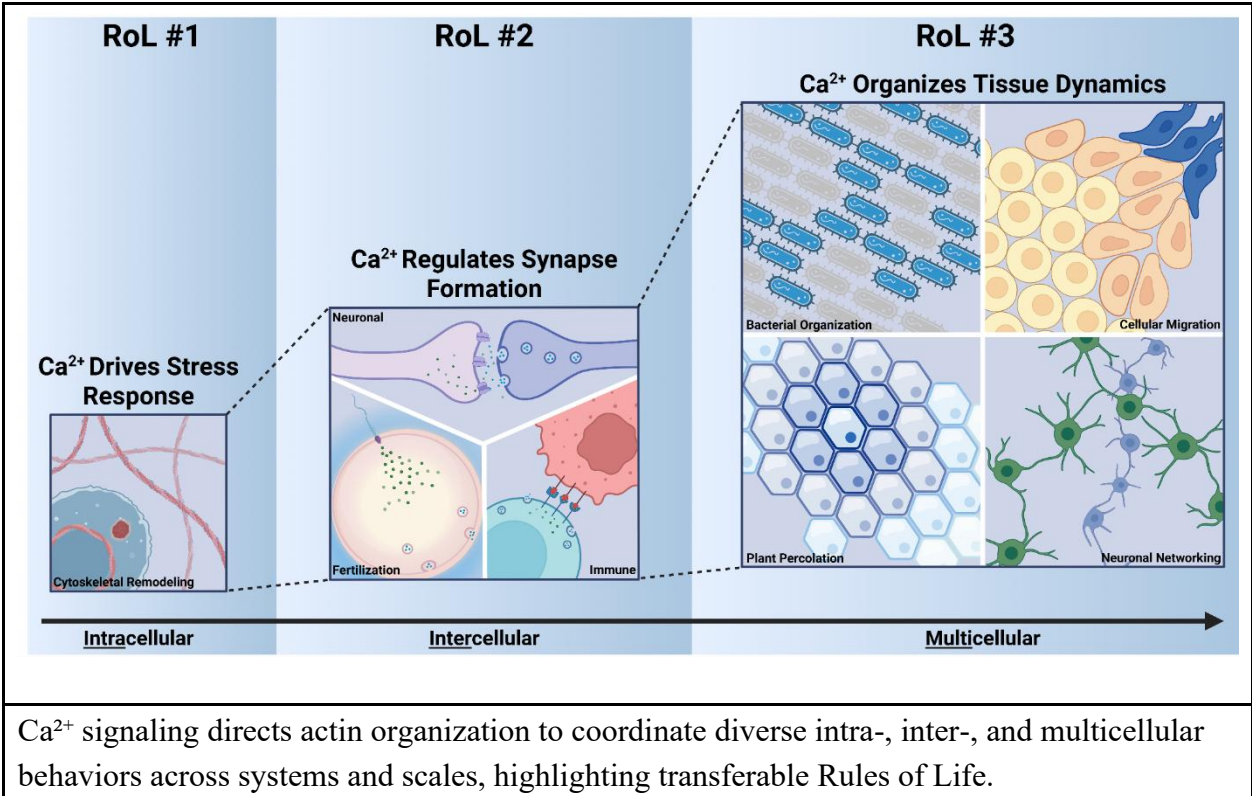
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Abstract: Living systems process a broad range of internal and external stimuli, respond to environmental constraints, and adapt to various conditions. The functional interactions between components and mechanisms operating at each scale - from molecules to organisms - define the collective “Rules of Life” (RoLs) that govern the emergent properties of biological systems. This review highlights calcium ions (Ca^{2+}), key signaling mediators, which serve as core integrators by facilitating the organization and emergence of structure and function through information encoded in its dynamics. Despite its diverse roles, calcium signaling follows consistent and conserved principles that establish regulatory RoLs that operate across various biological contexts. Here, we examine the key components and functional interactions at the interface of calcium signaling and cellular responses, focusing on calcium-cytoskeleton coupling—how calcium signaling regulates and is regulated by cytoskeletal dynamics. Throughout, we emphasize the importance of computational modeling for the integration of large scale biological data and propose defined RoLs as a modular framework to efficiently capture emergent, multiscalar dynamics of cellular and tissue mechanics across diverse systems. We introduce three RoLs: i) Ca^{2+} directs cytoskeletal reorganization following stress and damage, ii) Ca^{2+} modulates actin dynamics to control synapse-like processes across neuronal, immune, and fertilization contexts, and iii) Ca^{2+} exhibits diverse spatial patterns that both influence and reflect cellular function, contributing to role-specific behaviors. Finally, we address current limitations in integrating cell responses and calcium signaling and outline future directions to develop predictive computational models that unify chemical signaling with cellular organization, including translational applications.

Graphical Abstract



Highlights

The emergent features of biological robustness, signal integration, and organization are encoded by the dynamics of second messengers such as calcium ions (Ca²⁺) to regulate cellular processes such as cytoskeletal organization from the cellular to organismal level.

Introduction

Living systems are remarkably adaptive, capable of reorganizing after injury, communicating complex information, and self-organizing for robust performance. Underlying these emergent properties are the modular interactions between mechanical cues and chemical signals, which can be described as “Rules of Life” (RoLs)—principles that define how biological systems behave across contexts and scales. Many such RoLs center on the integration of physical cues with biochemical pathways, and one particular focus is through calcium-mediated regulation of the cytoskeleton [1–6]. As ubiquitous second messengers, calcium ions (Ca²⁺) link environmental inputs to biological processes and are central for coordinating cellular responses, tissue organization, and organism behavior [7–9].

A systems-level understanding of cell and tissue mechanics depends on deciphering the coupling between Ca²⁺ signaling and cytoskeletal dynamics. This Ca²⁺-cytoskeletal coupling not only reveals how cells process mechanical and chemical inputs, but also further informs the development of computational models for accelerated progress in bioengineered tissues, self-organizing biomaterials, and predictive digital twins [10–14]. Such models range from mechanochemical reaction-diffusion systems for signal regulation [15] to mechanistic models simulating force generation and mechanical behavior [16]. These frameworks are often built on ordinary and partial differential equations (ODEs/PDEs) and are solved using agent-based finite element simulations [17,18]. By integrating with experimental work, these simulations reveal how calcium signaling and cytoskeletal remodeling generate force and direct cellular function across multiple biological systems and spatial scales [5,19].

To organize this complexity, we apply the RoLs framework, traditionally used to explain organismal traits (development, function, and environmental response), and distill them into transferable computationally defined rules that map input-output relationships across biological scales [20–22]. A major challenge lies in understanding how these RoLs operate across diverse spatial and temporal contexts. Developing predictive, quantitative RoL frameworks allows researchers to simulate how biological systems integrate multiple cues, enabling hypothesis testing, perturbation analysis, and iterative refinement through the integration of modeling and experimentation. Furthermore, multiple RoLs can be combined into a collection of modular computational models that incorporate specific initial and boundary conditions, enabling the generation of hypotheses that are testable through quantitative experimental methods [22].

In this review, we examine the functional roles of how Ca^{2+} -cytoskeleton coupling shapes biological functions across multiple contexts and propose three initial Ca^{2+} -cytoskeleton Rules of Life:

- **RoL #1:** Ca^{2+} regulates cytoskeletal reorganization after stress and damage. A rapid Ca^{2+} influx initiates cytoskeletal remodeling, which is essential for cell responses such as migration, wound healing, and regeneration.
- **RoL #2:** Ca^{2+} regulates actin dynamics to control synapse-like processes including neuronal, immune, and fertilization synapses, supporting both synapse formation and exocytosis.
- **RoL #3:** Ca^{2+} exhibits diverse spatial patterns that both influence and are influenced by cellular dynamics, resulting in a division of cellular roles.

By defining these RoLs and illustrating their cross-context applications, this review describes a conceptual foundation for computational modeling, experimental hypothesis generation, and the engineering of living systems that are robust, adaptive, and responsive to their environments.

Background: The Calcium-Cytoskeleton Toolkit

Calcium ions (Ca^{2+}) function as critical intracellular messengers, enabling cells to respond to various stimuli [23,24]. For instance, ligand binding to plasma membrane receptors such as G protein-coupled receptors (GPCRs) activates signaling pathways that often regulate cytoplasmic Ca^{2+} levels with specific spatiotemporal dynamics, termed Ca^{2+} signatures [25]. Upon GPCR stimulation by ligands (neurotransmitters, hormones, growth factors, etc.), phospholipase C (PLC) is recruited to cleave phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 then binds its receptor (IP_3R) on the endoplasmic reticulum (ER), triggering the release of Ca^{2+} to the cytosol from the ER lumen [24,25]. These increases in cytosolic Ca^{2+} concentration are essential for a wide range of cellular responses to stimuli, including dynamic actin reorganization, which in turn supports the formation and function of critical cellular and tissue structures [5,7,8,26–28].

Proteins that respond to Ca^{2+} activities—either by direct binding or via Ca^{2+} -binding partners like calmodulin—undergo conformational changes that decode Ca^{2+} signals to regulate processes such as exocytosis, contraction, metabolism, egg activation during fertilization, and morphogenesis [9,29–33]. This interplay between calcium signaling dynamics and the cytoskeleton spans across kingdoms. For example, between plants and animals, Ca^{2+} plays a crucial role in cell growth, development, and response to environmental stimuli [8,27,33–35]. In the following subsections, we present examples from a range of biological systems to illustrate how organisms transduce stimuli into Ca^{2+} signals and interpret those signals to regulate cytoskeletal organization.

Calcium Regulation of Actin Dynamics

As a core cytoskeletal component, actin exists in two primary forms: monomeric globular actin (G-actin) and polymeric filamentous actin (F-actin). F-actin undergoes continuous cycles of polymerization and depolymerization, enabling significant remodeling essential for cellular processes such as migration, division, and maintaining cell shape [36,37]. Structurally, monomeric G-actin polymerizes into right-handed helical filaments (F-actin) in an ATP-dependent manner, with

growth occurring at the barbed (+) end and disassembly at the pointed (-) end. This process known as actin “treadmilling” facilitates turnover [36]. Calcium signaling plays a pivotal role in regulating actin filament dynamics, both directly and indirectly, by interacting with actin-binding proteins (ABPs) [4,6,38–40]. External stimuli create changes in intracellular Ca^{2+} levels, and through ABPs, are essential for maintaining cellular morphology and allow for structural reorganization by influencing the severing, capping, and crosslinking of actin [6,41,42].

For example, Ca^{2+} binding activates gelsolin by inducing a conformational change that enables it to sever F-actin and cap newly formed barbed ends, halting further elongation [39,43]. Similarly, elevated Ca^{2+} levels reduce the actin crosslinking ability of α -actinin, altering filament bundling and overall cytoskeletal organization [40,44]. Other ABPs, such as plastin/fimbrin and EFHD2/Swiprosin-1, also respond to Ca^{2+} via EF-hand domains [38,45,46]. In plastin/fimbrin, Ca^{2+} binding induces a conformational change that weakens or misaligns their actin-binding domains, reducing their ability to bundle filaments [45]. This intricate regulation by Ca^{2+} -responsive proteins provides cells with a versatile toolkit for rapid assembly and disassembly of the actin cytoskeleton, enabling multiple processes not limited to synaptic plasticity, and tissue morphogenesis [6,47–49].

Beyond direct regulation, Ca^{2+} indirectly controls actin architecture through signaling cascades involving Ca^{2+} -binding proteins such as calmodulin (CaM), which activates downstream effectors such as Calcium/Calmodulin-dependent protein kinase II (CaMKII), Protein Kinase C (PKC), and phosphatases like calcineurin [24,50]. These enzymes regulate, in turn, the activity of small Rho GTPases (RhoA, Rac1, Cdc42) by modulating the phosphorylation of guanine nucleotide exchange factors and GTPase-activating proteins (GAPs), altering actin polymerization, branching, or crosslinking [51,52]. For example, back-propagating action potentials in neurons elevate intracellular Ca^{2+} concentration levels, activating CaM. In turn, CaM binds proteins like Cordon-Bleu to drive actin branching at or near the submembrane region of neuronal dendrites, facilitating the remodeling and extension of dendritic processes and thereby demonstrating how Ca^{2+} governs cytoskeletal flexibility [53,54].

In plants, a striking example of Ca^{2+} -actin crosstalk occurs in organizing actin dynamics during key growth processes, such as the elongation of highly polarized structures like pollen tubes and root hairs. At the growing tip, localized Ca^{2+} oscillations drive rapid cell expansion. ABPs such as villin, gelsolin, and fragmin (homologs to animal villin/gelsolin family members) respond to these Ca^{2+} fluctuations by severing and capping actin filaments, promoting both filament bundling and depolymerization [55–57]. In pollen tubes, a “tip-focused calcium gradient” forms as Ca^{2+} levels rise at the growing tip, triggering actin depolymerization through Ca^{2+} -bound calmodulin, which activates villin. This enhances actin turnover and facilitates continued tip growth [58]. Ca^{2+} also integrates with RhoGTPase signaling pathways, such as the RHO of plant (ROP) family, to regulate actin polymerization and coordinate pollen tube growth [56]. Additionally, Ca^{2+} works with auxin signaling in root hairs to regulate ABPs and support root outgrowth [26,59].

While the functional outcomes and the specific intermediary Ca^{2+} -actin interactions vary across species and cell types, the overarching role of Ca^{2+} in actin regulation is conserved. Animal cells exploit rapid Ca^{2+} transients to drive dynamic actin reorganization during cell migration, adhesion, and synaptic plasticity processes. While plant cells also exhibit transient Ca^{2+} signals, they also use stable or oscillatory Ca^{2+} gradients to support sustained, directional growth and nutrient transport across large, vacuolated cells. Despite these differences, both systems utilize conserved mechanisms involving CaM and RhoGTPases, highlighting the shared importance and signaling network architecture of Ca^{2+} regulation in actin dynamics and cytoskeletal control across species.

Ca^{2+} Regulation of Actin-Interacting Motors

Myosins are a family of contractile proteins critical for cytoskeletal organization. Myosin proteins support diverse cellular functions, including apical constriction, cell mobility, cytokinesis, cargo and organelle transport, and muscle contraction. Myosin activity relies on actin binding and is regulated by chemical and mechanical cues, with calcium signaling playing a central role [60–66].

Myosin proteins share common structural domains, including motor, neck and tail domains. The neck domains contain IQ motifs defined as a series of isoleucine (I) and glutamine (Q) residues, which bind essential and regulatory light chains (ELCs, RLCs) and Ca^{2+} -dependent calmodulin (CaM). Both the tail and neck domains vary across families [60,67]. Among the 30+ myosin families, myosin II (also known as non-muscle myosin II) is the most studied and relies on Ca^{2+} -bound CaM to activate myosin light-chain kinase (MLCK) to phosphorylate its RLCs, enabling motor activation and filament assembly [65,67]. RhoA-associated Rho-kinase (RhoA/ROK) inhibits myosin light-chain phosphatase (MLCP), which dephosphorylates the RLC and deactivates myosin II. RhoA/ROK sustains RLC phosphorylation, enhancing myosin II sensitivity to MLCK and Ca^{2+} levels [68]. This regulation is crucial for processes like muscle contraction, cytokinesis, and tension regulation across species, including mammals [30,65,69], zebrafish [70], *Drosophila melanogaster* [71], *Caenorhabditis elegans* [72], *Dictyostelium discoideum* [73], and yeasts [74], among others [65].

Unconventional myosins (myosins other than myosin II), such as myosin V and VI, exhibit distinct Ca^{2+} -dependent regulatory mechanisms specialized for cargo transport [75–81]. Myosin V, a processive motor, transports organelles and vesicles along actin filaments before dissociating [77,82–84]. Its activity is regulated by Ca^{2+} -CaM binding at IQ motifs, which controls cargo attachment and actin interaction [75,85]. Interestingly, reduced Ca^{2+} levels during active transport enhance myosin V motility speed [75]. Myosin V functionality is conserved across various species, including mammals [77,86], yeasts [82,87], and *Drosophila* [83,88], among others. Myosin VI, unique for its reversed polarity and movement toward actin's pointed (-) end [66,89–91], similarly relies on Ca^{2+} -CaM to release its tail for cargo binding. However, motility is only initiated once Ca^{2+} levels return to a reduced level [76]. Myosin VI functions in vesicle transport, endocytosis, lysosome maturation, autophagy, cell migration, and cell division [76,78–81], with functional roles conserved across species [92–94]. Other myosins, such as myosin I [65,95], VII [65,96], and plant-specific myosin VIII and XI [97,98], also display varying degrees of Ca^{2+} sensitivity, underscoring the intricate regulation of myosin activity by calcium signaling and associated factors like MLCK, MLCP, RhoA/ROK, CaM, and IQ motifs. This fine-tuned control of myosin activity via Ca^{2+} signatures enables precise cytoskeletal dynamics and intracellular transport.

Computational Models as Tools to Condense and Transfer Essential Biological Mechanisms

As discussed above, the intricate interplay of calcium signaling and cytoskeletal dynamics is pivotal in a wide range of cellular processes. This highlights the versatility of Ca^{2+} as a second messenger. The ability of Ca^{2+} signaling to regulate cytoskeletal organization underscores its foundational role in both biological relationships and broader Rules of Life.

Quantitative data-driven representations of hypothesized RoLs, expressed through computational models, can serve as powerful tools for integrating the complexity of biological signaling systems. Such models aim to recapitulate components of a system with functional-interdependent variables. In biological applications, computational modeling enables simulation of system dynamics and the generation of predictive outcomes, often with greater specificity and capability, providing insight that can be difficult or impossible to acquire from wet lab experimentation [99], such as at the inference of physical parameter values [100]. These models can test biological hypotheses, perform cross-system optimizations and translations, and transform our understanding of how Ca^{2+} governs cytoskeletal dynamics and their underlying mechanisms. To do this, the simulation frameworks should capture spatiotemporal dynamics of calcium signaling, actin reorganization, and motor protein activity. This, in turn, demands a multiscale approach that incorporates both localized Ca^{2+} spikes and global cytoskeletal reorganization.

Computational models also enable a comparative analysis across systems, revealing both shared principles and comparing context-specific variations in calcium signaling. Ultimately, they lay the foundation for deeper insight into mechanisms of health and disease linked to Ca^{2+} dysregulation.

Rules of Life (RoLs), which describe functional relationships between biological components, link systems across hierarchical levels to predict responses to perturbations. By unifying reductionistic mechanisms into a single modeling framework [21,22], RoLs condense vast datasets into generalized heuristics, facilitating knowledge transfer across scientific fields. For example, **Figure 1** shows a representative pathway connecting experimental data with mathematical models. Here, actin polymerization is modeled as a reversible reaction using rate constants representing polymerization (k_+) and depolymerization (k_-), respectively (Eqn. 1). To account for Ca^{2+} feedback, these rate constants can be further parametrized as functions of Ca^{2+} concentrations (Eqn. 2a, 2b). This often uses linear or Hill equations. These coupling functions constitute relationships, and their parameterization requires data from biological systems. Analyzing these system-specific parameters reveals underlying rules and dynamics associated with Ca^{2+} regulation of actin polymerization.

$$\frac{d[F.\text{Actin}]}{dt} = k_+[G.\text{Actin}][F.\text{Actin}] - k_-[F.\text{Actin}] \quad (1)$$

$$k_+ = f(\text{Ca}^{2+}) = \alpha \frac{[\text{Ca}^{2+}]^n}{\beta^n + [\text{Ca}^{2+}]^n} \quad (2a)$$

$$k_- = g(\text{Ca}^{2+}) = \gamma \frac{[\text{Ca}^{2+}]^m}{\delta^m + [\text{Ca}^{2+}]^m} \quad (2b)$$

Directly measuring the parameters involved in biological processes remains a key bottleneck for validating computational models of biological systems. Techniques such as Fluorescence Recovery After Photobleaching (FRAP) and Raster Image Correlation Spectroscopy (RICS) provide powerful experimental assays for parameter estimation to extract kinetic parameters like diffusion coefficients and binding rates, respectively [101–104]. When integrated with partial differential equation (PDE)-based models of reaction-diffusion dynamics, both FRAP and RICS can help infer the underlying biochemical parameters that define ligand-receptor and protein-protein interactions [105,106]. Nonetheless, parameter values can be estimated with quantifiable levels of uncertainty through model calibration to experimentally obtain metrics. Parameter calibration uses sensitivity analysis, prediction testing, and comparison of model results over a variety of scenarios [107]. Commonly, parameter screening explores defined biologically feasible parameter spaces to find the best-fit parameter value (or parameter distribution) for a given set of experimental data; however, this approach is often computationally expensive depending on the model complexity and size of the parameter space [108,109].

Recent advances in Artificial Intelligence (AI) and Machine Learning (ML), including Neural Networks (NNs), Physics-Informed Neural Networks (PINNs), and Bayesian integrative models, have improved the efficiency of parameter optimization in biological-computational modeling, particularly in their application of surrogate models and active parameter range searching [108,110–112]. Multisystem optimization has improved our understanding of how shared biological molecules or mechanisms function across different systems, offering insights into conserved processes and their variability across species [113–116]. One study using a core Smad pathway model combined with Pareto front analysis showed that by tuning non-conserved parameters, the BMP-Smad pathway can be optimized for multiple performance objectives, such as response speed, noise amplification, and output sensitivity, but not all objectives can be idealized simultaneously. This tradeoff can instead be addressed through Pareto-optimal balance, which varies across species [117]. Advances in artificial intelligence and computational power further open new opportunities to bridge experimental and computational approaches, helping transform RoLs into quantifiable and practical frameworks for biological research [118,119].

As computational power increases, the formulation of biochemical-biomechanical models enables the quantitative application of RoLs across diverse cellular and tissue processes. By harnessing computational techniques to reverse-engineer the emergent biological mechanisms, we can uncover how the combinatorial coupling of RoLs varies across different biological contexts and

systems, providing insights into their impact on robustness and organization in those complex systems.

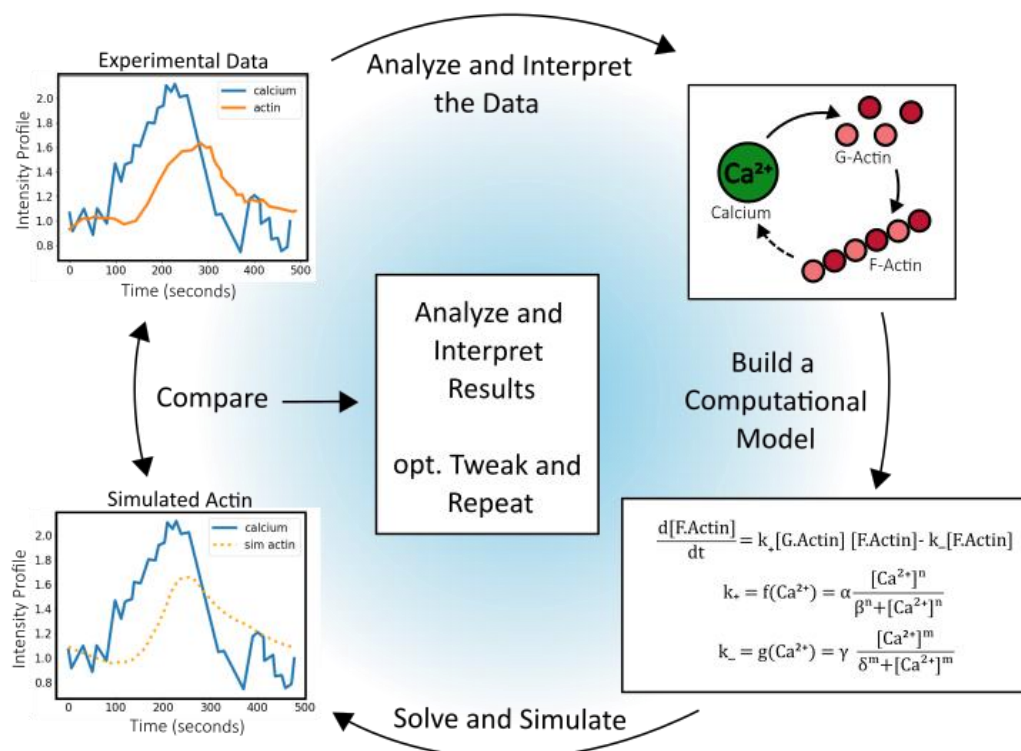


Figure 1. Design-test-iterate, coupling computational and experimental workflows. Experimental Ca²⁺ and actin observations are shown with a simple model to predict actin response from input Ca²⁺ data. Beginning with the experimental data, relationships among network components can be mapped, such as the impact of Ca²⁺ on G-actin and F-actin. A hypothesized interplay of F-actin on Ca²⁺ is included as a dashed line. Next, a system of equations is defined and integrated computationally. Comparison and interpretation of the experimental and computational results can be used for calibration, sensitivity analysis, and model comparison to test predictions across systems.

Candidate Set of Rules of Life for Calcium-Cytoskeleton Coupling

Building on the biological and computational discussions above, we propose candidate Rules of Life pertaining to calcium-cytoskeleton coupling. These proposed rules have wide zones of influence and we discuss specific application contexts for each proposed rule. We also lay a conceptual framework for systematically testing RoL candidates through iteration between quantitative experiments and simulations.

I. RoL 1: Ca²⁺ Triggers Cytoskeletal Reorganization After Stress and Damage

Ca²⁺ mediates cellular responses to various stressors, including physical damage, toxins, environmental extremes, pathogens, and antigens. A disruption in cellular homeostasis triggers an immediate calcium signal, elevating cytosolic Ca²⁺ levels and prompting a restructuring of the cell's actin-myosin cytoskeleton structure. This mechanism is shared across numerous cell types and species [120,121]. For instance, in mammals, stressors like ATP or damage-associated molecules lead to Ca²⁺ influx in endothelial cells [122–124]. As additional examples, fungi, and yeast show distinct Ca²⁺ signaling patterns in response to osmotic shock and oxidative stress [125,126]. Similarly, plants rely on Ca²⁺ to respond to both abiotic stress, such as drought, salinity, and extreme temperatures, and biotic stresses caused by pathogen invasion or damage from insects and parasites [127–130],

where Ca^{2+} influx initiates systemic defense signaling. Other perturbations such as stimulation with G-protein-coupled receptor ligands [131], thapsigargin-mediated ER Ca^{2+} release [132–134], and mechanical disruptions like atomic force microscopy or laser-induced ablation, induce rapid Ca^{2+} waves across cells in diverse organisms [5]. Thus, calcium signaling regulates stress and injury responses across kingdoms.

Calcium-Mediated Actin Reorganization

Calcium-mediated actin reorganization occurs due to a sudden increase in intracellular Ca^{2+} concentration [5], as observed in multiple mammalian cell types, including epithelial, mesenchymal, endothelial, and immune cells, and is triggered by diverse physiological stimuli [5,135]. In calcium-mediated Actin Reset (CaAR), a sudden Ca^{2+} influx induces the disassembly and reassembly of cortical actin around the nucleus (**Figure 2**). This dynamic rearrangement alters cell shape and mechanical properties, facilitating essential cellular functions such as the transient immobilization of organelles, cortex repair, cell spreading, wound healing, and regulation of gene expression crucial for cellular repair [5]. In immune responses, neutrophil activation requires Ca^{2+} -driven actin reorganization to promote the phagocytosis-promoting complement receptor, in response to a pathogen encounter. This allows the cells to engulf invaders at sites of infection [136].

In plants and fungi, rapid actin remodeling occurs in response to stress or cell damage. For example, fungal or oomycete pathogen penetration induces dense actin patches and focal arrays [137], while mechanical stimuli, such as touching the plant surface with a microneedle, trigger Ca^{2+} waves and local actin aggregation within 1–2 minutes [138,139]. Furthermore, in response to microbe-associated molecular patterns, actin remodeling and an increase in global filament abundance are induced in the *Arabidopsis* epidermal cell cortex [137]. Although direct evidence linking Ca^{2+} influx to actin remodeling is lacking in these studies, it likely influences actin dynamics indirectly by modulating reactive oxygen species (ROS), lipid production, or calcium-dependent protein kinases (CPKs). For instance, during plant pattern-triggered immunity, Ca^{2+} activates ROS bursts, which along with phosphatidic acid (PA), stimulate actin assembly and regrowth by inhibiting the Capping Protein [140]. Additionally, CPK3 targets the Actin Depolymerizing Factor 4 during the immune response against bacterial pathogens [141]. In the pollen self-incompatibility response, which prevents self-fertilization, high Ca^{2+} influx triggers actin severing and depolymerization leading to pollen cell death [142], with a gelsolin-like protein identified as the Ca^{2+} target [143]. Fungi also demonstrate instances of Ca^{2+} -mediated actin reorganization, specifically in *Aspergillus nidulans*, a filamentous fungi in the phylum Ascomycota. Here, pulsed Ca^{2+} influxes regulate temporally controlled actin assembly and exocytosis, resulting in stepwise cell extension [29]. These examples demonstrate how Ca^{2+} acts as a conserved and versatile signal that transduces environmental stress into actin remodeling, enabling diverse cell types across organisms to adapt rapidly to stimulus.

Ca^{2+} Signal Initiation of Wound Healing and Regeneration

Ca^{2+} channels also play a crucial role in wound repair by mediating the influx of Ca^{2+} into cells) [144–146]. In *Caenorhabditis elegans*, skin wounding triggers a Ca^{2+} -dependent signaling cascade with two key functions: facilitating wound closure and activating the innate immune response. After injury, a rapid and sustained elevation of Ca^{2+} levels are observed in the epidermis, which is essential for organism survival. This influx relies on the transient receptor potential channel of the melastatin family (TRPM), known as GTL-2, which senses mechanical or chemical stress and mediates Ca^{2+} influx from the extracellular space into the cytosol, and is amplified through IP_3 -mediated Ca^{2+} release from internal stores (**Figure 2C**). Xu et al. identified an epidermal signal transduction pathway involving $\text{G}\alpha_q$ - Ca^{2+} and its effector $\text{PLC}\beta$ (EGL-30 and EGL-8 in *C. elegans*, respectively), which drives actin-dependent wound closure, independently of known innate immune responses; loss of this pathway impairs survival post-injury [147].

After injury, mammalian cells, such as fibroblasts, immune cells, and epithelial cells exhibit a rapid increase in intracellular Ca^{2+} that can propagate through tissue via signaling receptors or gap junctions [144]. Similar calcium signaling dynamics occur during tissue-level responses in human wound healing. For example, fibroblasts depend on Ca^{2+} influx in response to deep dermal wounds for proper response, which can influence both healing outcomes and scar formation. Blocking this signaling with the Ca^{2+} chelator EGTA, Jiang et al. observed reduced scarring by fascia fibroblasts [148], suggesting Ca^{2+} modulation can influence scarring via the gelsolin family of actin-severing proteins [149]. Additionally, traditional wound treatments, like honey, induce extracellular Ca^{2+} influx in keratinocytes through TRPM2 and Orai1 channels, an effect that varies with honey's botanical source due to differential H_2O_2 production [150]. Moreover, both calcium and ROS species transcriptionally regulate tissue regeneration. The conserved Ca^{2+} -calcineurin signaling pathway activates transcription factors such as the nuclear factor of activated T-cells (NFAT) and myocyte enhancer factor-2 (MEF2), initiating immune responses [31,151–154]. H_2O_2 can regulate actin cytoskeleton dynamics by modulating actin-binding protein (ABPs) expression such as Profilin, ARP-3, MRLC, and 14-3-3 ζ , therefore facilitating wound closure [155,156]. Elevated Ca^{2+} and H_2O_2 levels activate inflammatory transcription factors, such as activator protein 1 (AP-1) and nuclear factor kappa B (NF- κ B), to promote wound healing [157–160]. Ultimately, calcium signaling acts as a central regulator of both immediate wound responses and long-term regeneration outcomes, coordinating cytoskeletal remodeling, immune activation, and transcriptional reprogramming across species. All these processes ultimately require coordination between cells and organs, and multicellular calcium signaling enables the systemic-level inter-organ communication through modulation of systemic and hormonal signals [161,162].

Feedback Between Actin and Ca^{2+} in Stress Response

At the molecular scale, Ca^{2+} and actin participate in a feedback regulation loop that enhances cellular responses to stress. For instance, actin dynamics significantly influence Ca^{2+} levels, playing a pivotal role in a plant's response to salt stress. The Arp2/3 complex, which initiates actin filament nucleation, is central to modulating this adaptive mechanism. Disrupting actin organization in stressed plants results in elevated Ca^{2+} levels [120,163]. This actin- Ca^{2+} connection is highlighted by *Mimosa pudica*'s well-known rapid leaf-folding response to touch, which serves as a defense against herbivores. The rapid bending results from changes in forces within the plant cell that pushes the plasma membrane against the cell wall (turgor pressure), driven by ion flux and partially regulated by Ca^{2+} -mediated actin dynamics. Electrical stimulation experiments in this species confirm that changes in actin dynamics directly affect Ca^{2+} levels, highlighting the interconnectedness of these components in the plant's response to stimuli [164]. Furthermore, Annexin proteins like AtANN5 and AnxGb6, further mediate Ca^{2+} -actin interactions by serving as scaffolding proteins for binding F-actin. These proteins associate with membranes displaying negatively charged phospholipids in a Ca^{2+} -dependent manner, influencing Ca^{2+} -permeable channel function during stress in cotton plants [165].

In mammals, actin dynamics contribute to Ca^{2+} homeostasis by interacting with ion transport channels on the plasma membrane [166]. One key calcium pump in this process is the plasma membrane calcium ATPase (PMCA), crucial for maintaining low Ca^{2+} concentrations in eukaryotic cells [166,167]. In red blood cells and blood platelets, G-actin directly binds to PMCA to release Ca^{2+} from the cell [166,167]. Maintaining low intracellular Ca^{2+} levels in platelets is essential for preventing premature activation, a process facilitated by PMCA pumps [166,168]. Upon platelet activation by agonists such as thrombin in response to vascular damage, PMCA is phosphorylated, leading to its inhibition and a subsequent rise in intracellular Ca^{2+} . This elevation in Ca^{2+} triggers conformational changes that promote clot formation [169]. In contrast, when F-actin binds to PMCA, it deactivates the channel, preventing Ca^{2+} removal, highlighting a complex and dynamic interplay between actin and Ca^{2+} [167]. Together, these findings illustrate a tightly regulated calcium-cytoskeleton coupled

mechanism in which actin and actin binding proteins and Ca^{2+} -related proteins dynamically modulate one another, enabling rapid and precise cellular responses to environmental needs.

Computational Modeling of Actin Dynamics Following Stress

Ca^{2+} acts as a central mediator of cellular responses to diverse stressors, triggering actin cytoskeleton remodeling to support regeneration, immune defense, and structural adaptation [5,120,121]. This interplay between calcium signaling and actin dynamics highlights a conserved mechanism for coordinating repair and stress responses across kingdoms, enabling cells to adapt efficiently to environmental and physiological challenges. Computational models have been applied to capture the role of Ca^{2+} signals in wound healing, for instance, [Stevens et al.](#) presents a multi-scale calcium signaling model for epithelial wound healing in a *Drosophila* pupal tissue. It combines a tissue injury model with detailed cellular calcium dynamics [170]. Meanwhile, mechanical models have been developed to explore how these actin-driven processes contribute to wound closure efficiency. [Bai and Zeng](#) introduced a finite-element (FE) based mechanical model of wound healing, treating an epithelial cell sheet as a continuum but with cellular-level resolution [171]. However, existing modeling approaches often struggle to fully integrate the signaling network with mechanical models [172]. As noted earlier, one promising strategy is explicitly linking calcium dynamics to actin polymerization rates. For example, as shown in **Figure 2**, a single-cell model can be used to test and predict the change in actin concentration following a rapid rise in Ca^{2+} levels [173]. This approach, ultimately, enables mechanistic testing of the factors that regulate cellular behaviors such as cell shape changes, cell migration, and wound repair.

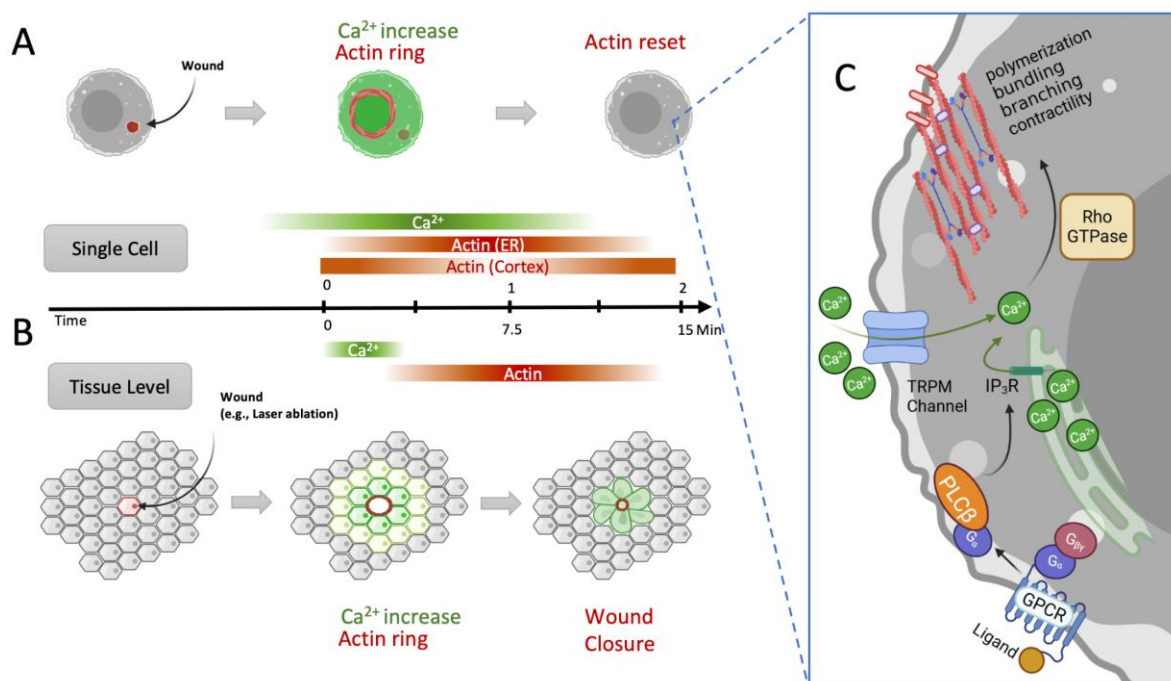


Figure 2. Calcium signaling and actin dynamics during wound response. A) Ca^{2+} and actin dynamics at the single-cell level in response to wounding. Localized wounding induces a rapid increase in Ca^{2+} concentration, which triggers the formation of an actin ring around the nucleus, followed by an actin reset to restore normal cytoskeletal organization once healed. B) Ca^{2+} and actin dynamics at the multi-cell level in response to wounding. Tissue wounding triggers a Ca^{2+} increase in surrounding cells, propagating a Ca^{2+} wave across the tissue. This leads to the coordinated formation of an actin ring at the wound site, promoting collective wound closure. C) General intracellular Ca^{2+} dynamics upon wounding across species. Intracellular Ca^{2+} levels rise through calcium channel influx across the plasma membrane or release from the endoplasmic reticulum (ER). Meanwhile, G protein-coupled receptors (GPCRs) can detect cellular damage by recognizing Damage-Associated Molecular Patterns (DAMPs) and chemokines, activating G protein pathways. Activated G_α subunits subsequently recruit $\text{PLC}\beta$ to the plasma membrane, hydrolyzing PIP_2 into IP_3 . The generated IP_3 then binds to IP_3R on the ER membrane, triggering Ca^{2+} release into the cytosol. The elevated intracellular Ca^{2+} levels remodel actin dynamics through multiple pathways, including Rho GTPase and ROS signaling. Activated GTPases such as

Rac1, RhoA, and Cdc42 regulate actin polymerization and actomyosin contractility. Cytoskeletal remodeling, in turn, can trigger Ca^{2+} responses, creating a positive feedback loop between calcium signaling and cytoskeletal dynamics. Created in BioRender.

II. RoL 2: Ca^{2+} Oscillations Drive Actin Reorganization Facilitating Synapse Formation

Synapses connect different biological compartments, facilitating vital interactions between cells [174,175]. A prominent example is the neuronal synapse, which transmits and integrates signals between neural cells [176]. Synapse-like structures are not limited to the nervous system; they also appear in other biological contexts, such as the immunological synapse between T-cells and APCs or target cells, and the fertilization synapse between sperm and egg [174,175,177,178]. Despite differences in structure and function, these synapse-like structures surprisingly share key organization and molecular features, including adhesion molecules, signaling cascades, secretion, and precise localization of molecular arrangements [174,175]. In this section, we review a specific example of common signaling cascades in synapse-like structures, focusing on how calcium signaling dynamics, triggered by synapse formation, induce actin cytoskeleton reorganization in three distinct specialized synapse-like structures: i) neuronal synapse, ii) immune synapse, and iii) fertilization synapse (**Figure 3**).

Calcium-mediated Actin Regulation in Neuronal Synapses

In neuronal synapses, communication requires actin reorganization, which allows the synapse to modify cytoskeletal structures in response to stimuli [179–181]. While signaling pathways differ between presynaptic and postsynaptic terminals [182–184], intracellular Ca^{2+} serves as a key modulator of these changes by influencing F-actin dynamics through actin-binding proteins (ABPs) like cofilin [185], $\text{CaMKII}\beta$ [186], and drebrin (DBN) [187]. These interactions are further shaped by signaling cascades involving kinases like LIM Kinase 1, which regulates cofilin activity [188].

In the presynaptic terminal, Ca^{2+} -dependent exocytosis of glutamate (Glu) from synaptic vesicles (SV) plays a critical role in signal propagation across the synaptic cleft (**Figure 3**). This process includes spontaneous "mini" release, asynchronous release, and action potential-driven neurotransmitter release, regulated by synaptotagmin (Syt) and complexin [189]. Cytoskeletal proteins such as F-actin and associated motor proteins are essential for SV trafficking and positioning within distinct pools, particularly the reserve and readily releasable pool. The movement of SVs from the reserve pool to the readily releasable pool is a critical step in exocytosis [190–193]. Ca^{2+} -mediated actin filament dynamics, such as actin depolymerization, facilitate SV movement into the active zone (AZ) of the readily releasable pool, where exocytosis occurs [194]. Further, Rab-3 and SNARE (soluble N-ethyl-maleimide-sensitive fusion protein (NSF) attachment protein receptor) proteins orchestrate vesicle trafficking, with Rab-3 aligning and docking vesicles at the membrane and the SNARE complex (syntaxin, SNAP-25, and VAMP) mediating their fusion. Ca^{2+} binding to synaptotagmin rearranges this complex, allowing vesicle and plasma membrane to merge and neurotransmitter release to occur. Following exocytosis, NSF and α -SNAP disassemble the SNARE complex, ensuring vesicle recycling [195–197].

At the postsynaptic terminal, Ca^{2+} influx from the synapse through NMDA (N-methyl-D-aspartate) receptors (NMDARs) initiates actin remodeling that is critical for dendritic spine morphology. NMDAR activation via glutamate binding dislodges Mg^{2+} ions in the receptor and initiates Ca^{2+} influx from the extracellular space. This increase in Ca^{2+} triggers signaling cascades involving $\text{Ca}^{2+}/\text{CaM}$, RhoGTPase proteins, and actin depolymerizing factors (ADFs) [198–201]. Additionally, this Ca^{2+} influx recruits NMDARs and AMPA receptors (AMPA receptors) to the postsynaptic terminal via exocytosis, accelerating future dendritic spine depolarization [202,203]. Proper localization of scaffolding proteins like PSD95 [204], GKAP [205], Shank [206,207], and Homer [208,209] at the postsynaptic terminal, is essential for actin cytoskeletal dynamics and cellular morphology, as they support effective synaptic signaling. For example, disruptions in Homer1b through gene silencing show reduced NMDAR- and AMPAR-mediated excitatory postsynaptic

currents (EPSCs) and spine density [207,209]. Further evidence with knockout studies of ArpC3, a subunit of the actin-related protein 2/3 (Arp2/3) complex involved in F-actin branching, highlights the role of actin in structural plasticity, as these knockouts exhibit abnormal dendritic spine loss following stimulation [210]. Additionally, splice variants of SNAP-25 in neurons result in fewer primed vesicles compared to their native counterpart [211]. However, most experiments draw conclusions through either individual protein silencing, knockout, or protein homologs. A key open question relates to the systematic characterization of cytoskeletal dynamics. Consequently, computational models that incorporate experimental data and finetune parameters are needed to test systems-level behavior. For example, recent computational models demonstrate that the geometry of the synaptic cleft itself can influence neurotransmitter diffusion and NMDAR opening timing, highlighting the coordination between Ca^{2+} and cytoskeletal proteins for synapse formation [212]. In summary, precise regulation of Ca^{2+} signaling, together with cytoskeletal protein function, is essential for neuronal synapse formation.

The Interplay Between Calcium and Actin in Immune Synapses

The immune synapse forms at the interface between T cells and either antigen-presenting cells (APCs) or target cells such as cancerous or virally infected cells (**Figure 3**). The remodeling of the actin cytoskeleton within T cells plays a central role in establishing and maintaining the T-cell receptor (TCR) microclusters, which are critical for initiating downstream immune responses [213–218]. TCRs recognize peptides presented on the surface of infected cells or APCs by major histocompatibility complex (MHC) molecules, forming peptide-MHC complexes that trigger a signaling cascade leading to the recruitment of phospholipase C gamma 1 (PLC γ 1) and subsequent Ca^{2+} release from the ER through the IP $_3$ pathway [213,219–221]. When ER Ca^{2+} stores are sufficiently depleted, the Ca^{2+} sensor STIM1 translocates to ER–plasma membrane junctions, where it activates calcium release-activated calcium (CRAC) channels composed of Orai1 subunits [222,223]. Elevated cytosolic Ca^{2+} then activates calmodulin, CaMKII gamma (CaMKII γ), calcineurin, and Rho-family GTPases including Rac1 and Cdc42, which are essential for actin remodeling and dynamics [222,224–226]. These Ca^{2+} -dependent signals promote the activation of actin nucleators including Arp2/3 and formins, which drive the formation of distinct actin networks such as distal (dSMAC), peripheral (pSMAC), and central (cSMAC) supramolecular activation clusters (SMAC), as well as small actin foci distributed within the dSMAC and pSMAC [213,227,228].

TCR microclusters are initially assembled within the branched actin network in the dSMAC and then move further into the pSMAC. An inward retrograde flow of F-actin in the dSMAC drives the centripetal retrograde movement of TCR microclusters toward the center of the cell, where their signaling is terminated and Ca^{2+} levels are decreased. This process plays a critical role in modulating the strength and duration of TCR signaling [213,227,229]. Interestingly, while Ca^{2+} is essential for actin dynamics and retrograde flow, retrograde flow and the formation of TCR microclusters are, in turn, necessary to sustain Ca^{2+} signaling. Studies have shown that freezing retrograde flow preserves preformed TCR microclusters, but prevents the formation of new ones, leading to the loss of PLC γ 1 activation and intracellular Ca^{2+} [221,230].

One of the major T cell effector functions driven by Ca^{2+} -actomyosin dynamics in cytotoxic T cells (CTLs) is the polarized secretion of lytic granules (LGs)—specialized lysosomes containing cytotoxic molecules like perforin and granzymes—at the immunological synapse [231–233]. LG secretion occurs at the cSMAC, but requires a coordinated sequence of cytoskeletal events. First, integrins cluster and concentric actomyosin arcs form within the pSMAC, creating a tight adhesive seal between the T cell and its target. Second, actin-binding scaffold proteins are cleared from the cSMAC, enabling centrosome polarization and docking at the plasma membrane [213,234]. Lytic granules are then transported along microtubules toward the polarized centrosome, transfer onto actin filaments near the synapse under guidance of myosin IIA [234], and finally dock at the plasma membrane with the assistance of tethering proteins [213,234]. In the mature cSMAC, a dense actin meshwork initially acts as a barrier to the granule release. Upon T cell activation, actin remodeling

increases the porosity of the network, enabling the final approach and fusion with the membrane, facilitating the targeted secretion of LGs at the immunological synapse [213]. Once released, cytotoxic molecules enter the target cell either via endocytosis (internalization model) or directly through perforin-formed pores in the plasma membrane (pore formation model), ultimately leading to apoptosis of the target cell [234]. Natural killer (NK) cells use a similar mechanism for granule release, relying on the same cytoskeletal dynamics in the transition from microtubule-based transport to actin-guided movement and Ca^{2+} -driven actin remodeling and fusion, despite differing in how they recognize their targets [235]. As in neuronal synapses, LG exocytosis at the immune synapse involves conserved machinery—such as synaptotagmin, Rab proteins, and SNAREs—which respond to Ca^{2+} dynamics to trigger granule release [236].

The Role of Calcium on Actin in the Fertilization Synapse

The fertilization synapse is formed by sperm-egg membrane interaction, a process that leads to increased cytosolic Ca^{2+} concentrations in the egg (**Figure 3**) [175]. The spatiotemporal cytosolic Ca^{2+} signaling dynamics during fertilization vary by species and experimental conditions, particularly in pattern (overall temporal profile), frequency (occurrence of waves or oscillations over time), and amplitude ($[\text{Ca}^{2+}]$ change) [33,237–239]. In many non-mammalian species, fertilization typically triggers one or a few Ca^{2+} waves lasting minutes (e.g., *Caenorhabditis elegans*, *Drosophila melanogaster*) [237], while in certain marine invertebrates oscillations can persist 30–60 minutes (e.g., the mollusk *Septifer virgatus*), and in mammals, fertilization initiates a large initial transient followed by shorter oscillations lasting several hours with intervals ranging from a few to tens of minutes [237]. In most invertebrates studied to date, this Ca^{2+} rise at fertilization is mediated by an influx through voltage-gated long-lasting (L-type) Ca^{2+} channels [237]. However, in mammals, this fertilization-induced rise of intracellular Ca^{2+} originates instead from the ER, triggered by a signal transduction cascade initiated by the sperm. The sperm introduces a unique form of phospholipase C (PLC), PLC zeta ($\text{PLC}\zeta$), which stimulates Ca^{2+} release from the ER through the IP_3 pathway [240].

This initial release is essential for propagating Ca^{2+} waves across the egg; however, sustained Ca^{2+} oscillations depend on continued Ca^{2+} influx from the extracellular space, which is mediated by different channels depending on the species. During mammalian fertilization, several Ca^{2+} entry pathways have been implicated. One of the best characterized pathways is Store-Operated Calcium Entry (SOCE), mediated by STIM1/2 and Orai1 upon ER Ca^{2+} depletion [237,241]. However, its role in fertilization remains debated and appears species-specific [237]. In mice, channels such as Cav3.2, TRPM7, and possibly TRPV3 also contribute to Ca^{2+} influx [175,242–244]. The resulting rise in cytosolic Ca^{2+} acts as a key trigger for both signaling and actin remodeling, driving essential events such as cortical granules exocytosis, meiotic resumption, and cytoplasmic reorganization via effectors like calmodulin, $\text{CaMKII}\gamma$, gelsolin, PKC, and myosin II [245,246].

Prior to fertilization, Golgi-derived cortical granules (CGs) migrate along actin filaments, nucleated by formin-2 and Spire 1/2, toward the oocyte cortex in preparation for exocytosis [247,248]. In metaphase II eggs, which are competent for fertilization, CGs are positioned just beneath the plasma membrane and are anchored to the cortical actin cytoskeleton with the assistance of the subcortical maternal complex (SCMC), which creates passages for CG docking to enable rapid exocytosis at fertilization [248]. Non-muscle myosin IIA, associated with CGs, generates contractile forces to help clear actin and facilitate CGs docking and exocytosis [246,249]. Ca^{2+} -dependent actin depolymerizing proteins like gelsolin also facilitate actin clearance [246]. Following sperm-egg fusion, the CGs exocytose the metalloendopeptidase ovastacin, which cleaves zona pellucida (ZP) glycoprotein 2 (ZP2), modifying the ZP to prevent further sperm penetration and thus blocking polyspermy [250,251].

Similar to synaptic vesicle (SV) release in neurons, exocytosis of CGs involves proteins such as synaptotagmin, synapsin I, Rab3, Rabphilin-3A, and SNAREs. Synaptotagmin and SNAREs, in particular, regulate vesicle fusion in response to Ca^{2+} . While these proteins are expressed in oocytes and localized to the egg cortex, their specific roles in CG exocytosis remain to be fully confirmed.

Nonetheless, their presence suggests they may be key conserved players in the Ca^{2+} -dependent exocytotic process during fertilization [246].

Computational Modeling of the Conserved Characteristics Between Synapses

Although neuronal, immune, and fertilization synapses operate in distinct biological contexts, they share many core features highlighting their conserved organization and function. One example is the use of Ca^{2+} -triggered exocytosis, which is tightly regulated by actin cytoskeletal dynamics across all three synapse types (**Figure 3**). In each case, Ca^{2+} -signaling drives actin remodeling, enabling the transport, positioning, and fusion of secretory vesicles with the plasma membrane—whether releasing glutamate in neurons, lytic granules in cytotoxic T cells, or ovastacin in oocytes. Limitations in a cell’s ability to remodel its structure lead to dysfunction and disease. Understanding the signaling cascades governing Ca^{2+} -driven cytoskeletal changes enable the development of a generalized computational model based on RoL principles. Recent advancements in super-resolution microscopy techniques, such as single-particle tracking photoactivated localization microscopy (sptPALM), enable researchers to measure a variety of actin-binding protein diffusion rates within dendritic spines [204]. These quantitative measurements can calibrate cell-to-cell communication models to test how Ca^{2+} signaling dynamics impact the cytoskeletal and communication junctions between cells. For example, agent-based models simulate CaMKII phosphorylation dynamics in response to calcium signals, while stochastic models analyze how spine geometry modulates Ca^{2+} influx and its effects on structural plasticity [252,253]. Additionally, molecular simulations combined with experimental data have reconstructed CaMKII/F-actin bundle architectures to explain Ca^{2+} -triggered cytoskeletal remodeling [254].

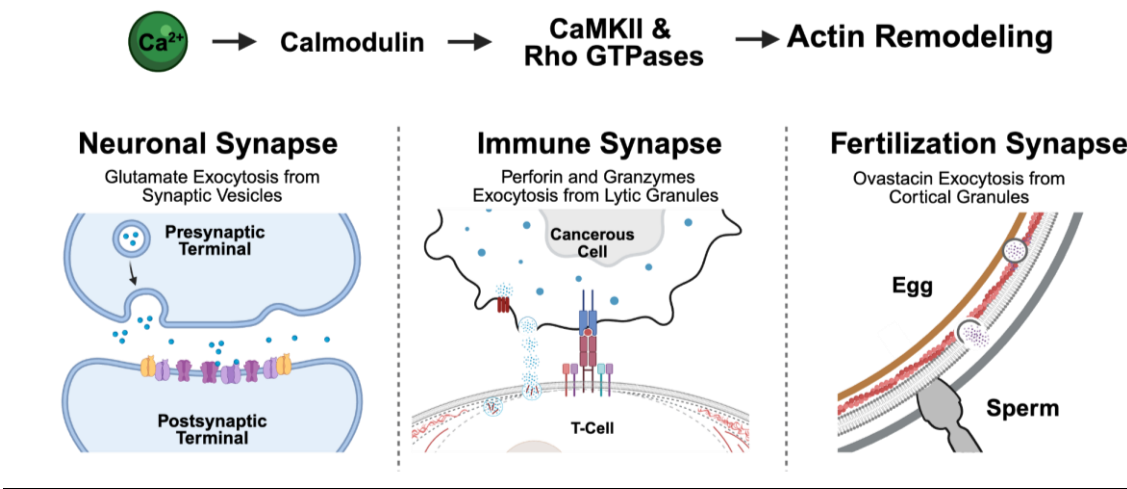


Figure 3. A unifying model of Ca^{2+} -regulated actin remodeling that leads to vesicle exocytosis at the fertilization, immune, and neuronal synapse. Upon synapse formation, a rise in intracellular Ca^{2+} activates calmodulin, CaMKII, and Rho GTPases, leading to reorganization of the actin cytoskeleton and exocytosis of specialized vesicles: synaptic vesicles in neurons, lytic granules in T cells, and cortical granules in oocytes. This conserved pathway suggests a common Ca^{2+} dependent mechanistic rule of life for organizing synapses between cells. Created in BioRender.

III. RoL 3: The Functional Subdivision of Cell Populations Enables Improved Cellular Performance

From the pack mentality in wolves to the cellular communication within diverse bacterial communities in biofilms, collective behavior and intelligence [255] is a fundamental phenomena observed throughout the natural world [256]. Quantifying collective activities is complex and context-dependent, requiring diverse methodologies. For example, collective intelligence is assessed in human groups by evaluating individual and group task performance across various settings [257]. Similarly, collective behavior is quantified in biological systems by measuring individual parameters such as cell velocity in cell migration [258]. These approaches link individual performance to

emergent collective properties using meta-analytic models, providing insights into intelligence across different contexts [257,258]. Understanding the mechanisms driving collective behavior can lead to innovative approaches for manipulating them, with potential applications in tissue development, wound healing, cancer treatment, and other health-related fields [259].

In multicellular organisms, ion-based electrochemical signaling delineates cellular roles and facilitates tissue-wide signaling [28,260–262]. Less complex communities, like bacterial biofilms, use potassium ions to facilitate percolation—signal propagation through heterogeneous mediums. This process segregates cellular roles into active and passive cells, enabling coordinated organization and optimized survival [260]. This phenomenon is preserved in larger complex organisms like metazoans where calcium signaling plays crucial roles in similar biological pathways. This has resulted in a division between cell populations into firing/non-firing [260,261], initiator/standby [28], and leader/follower (**Figure 4**) [262,263]. Ca^{2+} exhibits diverse patterns that shape and are shaped by cellular dynamics, creating functional separations that facilitate signaling within and between cells and between tissues and organs. Because of this, calcium signaling is pivotal in regulating cellular behaviors and coordinating multicellular processes.

Firing and Non-firing

Tissue-wide, organ-to-organ, whole-body signaling is accomplished in metazoans through the use of complex cell types, structures, and agonists like neurons, axons, and chemokines. These provide the framework that facilitates long-range signaling throughout an organism [28,146,260,263]. However, similar signal coordination and self-organization can occur in simpler yet still complex biological systems, such as diverse cell populations found in bacterial biofilms. This phenomenon of percolation, signal propagation through heterogeneous mediums, arises from a separation of cellular roles between active (firing) and passive (non-firing) cells (**Figure 4A**). By self-separating, bacterial communities can achieve the benefits of tissue-wide organization, decreasing the average individual cost of survival. They do this by channeling electrochemical cell-to-cell signaling from the growth center to the biofilm periphery creating a connected cluster of cells and a conduit for signal diffusion of potassium ions. Ultimately, this results in reduced nutrient consumption at the edges, while allowing increased nutrient availability and consumption at the interior [260]. This is not the only example of role segregation in biology. Ca^{2+} , a universal messenger with a diverse set of signaling patterns, has been found to facilitate percolation in more complex systems.

Percolation also occurs in plants. Here, plants use Ca^{2+} to facilitate cell-to-cell communication with diverse Ca^{2+} kinetics and patterns [261]. This can be modeled using stochastic two-cell and multi-cell scenarios, incorporating the origin of the signal and the number of initiating cells (**Figure 4B**) [261]. Such models properly simulate experimentally observed calcium dynamics of fire-diffuse-fire, where localized Ca^{2+} release triggers further release from neighboring cells as the signal diffuses. This can result in a single cell Ca^{2+} spark (a local stochastic event compared to more prolonged spiking), propagating through a tissue [264]. These sparks in Ca^{2+} don't always propagate, however, but demonstrate nonequilibrium phase transitions between propagating and non-propagating, suggesting that the same set of scaling laws defines the fire-diffuse-fire phenomenon as those describing percolation [265].

In animals, a related signaling behavior is observed in neurons through “sparse coding,” which efficiently translates complex environmental stimuli, such as light and sound, by reducing dimensionality [266]. Sparse coding achieves this by relying on a small subset of active neurons within a population to represent information (**Figure 4C**). These active neurons exhibit changes in membrane potential, producing temporally coordinated spikes of various ions like Ca^{2+} [267,268]. The percolation patterns of signal propagation governed by sparse coding have been demonstrated in systems like *Drosophila* [269]. This strategy offers advantages similar to bacterial biofilm communication, including energy efficiency, enhanced storage capacity (reduced cross-talk enables greater memory), and improved data processing capabilities. In neurons, processing involves sequentially breaking information into components processed by distinct sets of neurons [270].

Together, these studies highlight the utility of percolation theory in describing multicellular communication across both prokaryotic and eukaryotic systems.

Initiator and Standby

Cell populations often result in a subdivision into multiple cell classes based on cell signaling or morphological distinctions. For example, in epithelia, quantifying signaling dynamics have revealed the distinction between two types of cells, initiator and standby cells, which are defined by their specific roles in calcium signaling. Calcium's diverse tissue patterns involve rapid spiking and long transient signaling within single cells and extended wave responses as well as sharp fluttering across tissues [8,10,28,31,271]. These dynamic patterns create separations in cellular behavior. For example, Soundarajan et al. 2021 demonstrated the existence of initiator cells—responsible for initiating calcium signals—and standby cells—waiting to propagate these signals—in *ex vivo* experiments on developing wing imaginal discs in *Drosophila melanogaster*, which is similar to what is observed in plants (**Figure 4B**) [28,272]. Their findings also suggest a “Goldilocks zone” for calcium signaling, where either too low or too high of a Ca^{2+} level, disrupts tissue growth and development. This phenomenon was modeled by mimicking natural observations, with initiator cells identified by increased phospholipase activity, leading to rapid Ca^{2+} signaling [28].

Distinct calcium signaling events in plants have also been reported, each carrying different physiological relevance [27]. For example, tip-focused Ca^{2+} oscillations during pollen tube and root hair elongation provide different signaling cues that facilitate rapid cell growth essential for elongation [26,56]. Additionally, in response to infection, transient cytoplasmic increases of Ca^{2+} within local cells activate plant defense responses in an initiator vs. standby pattern [272,273]. Salt stress signals also rely on high-speed Ca^{2+} waves capable of triggering molecular responses in distant parts of the plant and are capable of traveling through root cortical and endodermal tissue layers (**Figure 4B**) [274]. Such phenomena exemplify the role of Ca^{2+} signaling in facilitating long-range communication and percolation across cellular networks.

Structures such as gap junctions in animals, insects, and plasmodesmata in plants, facilitate direct intercellular communication and regulate Ca^{2+} signaling, representing additional examples of RoL 2 communication beyond classical synapses [275–277]. Gap junctions form continuous pathways between adjacent cells by connecting two hemichannels, enabling the exchange of ions and small molecules essential for physiological processes. In *Drosophila*, the gap junction protein Inx2 regulates Ca^{2+} flux, influencing the cell fate of border cells during oogenesis [278]. Similarly, plasmodesmata mediate molecular and ionic exchanges between plant cells through cell walls [279,280]. Notably, plasmodesmata permeability is regulated by Ca^{2+} ; changes in cytosolic Ca^{2+} concentrations lead to plasmodesmata closure, controlling Ca^{2+} flux [281,282]. This regulation is crucial in plant defense, as studies in *Arabidopsis thaliana* show that Ca^{2+} influx and plasmodesmata closure are integral to pathogen perception and response [283]. Ultimately, calcium enables complex signaling within and between cells across tissues and organs by assigning cellular roles.

Leaders and Followers

Following this trend, cellular migration relies on calcium signaling, facilitating cellular organization in many critical areas like wound healing and immunity [146]. Like the division of firing/nonfiring and initiator/standby roles, multicellular migration divides cells into distinct subpopulations as either leaders or followers. Leader cells are, by definition, located at the leading edge of the migrating ensemble and have the characteristics of directing and attracting other cells while inhibiting any leader activities in the cells that succeed them. In contrast, follower cells are defined as following the leaders, however, they do have the potential of becoming leaders themselves if the leading edge expands or a follower outcompetes the leader(s) (**Figure 4D**) [1,262,263,284]. This type of cellular organization is observed in many different systems from insects, mammals, and zebrafish [285–288].

Multicellular migration depends on the separation of roles between leader and follower cells, driven by polarized signaling and physical asymmetry [262,263,284]. Leaders exhibit asymmetric contact with their environment, forming a protrusive leading edge, while followers maintain symmetric contact with surrounding cells (**Figure 4D**). Higher cadherin recycling rates along leaders' lateral sides enhance migration speed and thick myosin-actin bundles prevent followers from overtaking them. Leaders additionally have reduced Notch1 levels due to lower internal tension than higher-tension follower cells [263].

This polarization of signaling in leader-follower dynamics is mirrored in internal Ca^{2+} levels, where a gradient of Ca^{2+} across the cell drives persistent forward migration [1]. Cells maintain reduced Ca^{2+} at the front with elevated plasma membrane Ca^{2+} -ATPase (PMCA) activity compared to the rear. This polarization preserves myosin light chain kinase (MLCK) sensitivity to localized Ca^{2+} signals via receptor tyrosine kinase and phospholipase C pathways. Repetitive signaling from these pathways depletes Ca^{2+} from the ER, stimulating STIM1 (stromal interaction molecule 1), which is then transported to the cell front by the growing ends of microtubules, facilitating Ca^{2+} entry via Orai1. Ca^{2+} is then restored to the ER by SERCA (sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase), sustaining local Ca^{2+} signaling [1,289]. This dynamic signaling supports actin polymerization and recycling at the front and actin turnover at the rear, enabling persistent forward migration.

The leader-follower dynamic seen in multicellular systems can also be observed within single-cell migration. A single cell also exhibits distinct spatial Ca^{2+} dynamics to regulate its migration differently from surrounding cells. Similar to multicellular migration, single cells create local Ca^{2+} pulses near the leading edge and maintain a back-to-front Ca^{2+} gradient [289]. These calcium signaling events drive the processes of polarization, protrusion, retraction, and adhesion. However, underlying mechanisms driving the leader-follower structure, in which the activity of one cell influences Ca^{2+} signal propagation across tissues, remain unclear. For example, during wound healing, ATP (adenosine triphosphate) serves as an early damage signal. A recent computational study revealed that distinct Ca^{2+} responses to ATP could be attributed to variations in how Ca^{2+} and IP_3 (inositol trisphosphate) are sensed by the IP_3 receptor (IP_3R) [290]. These variations may arise due to differences in IP_3R expression within the endoplasmic reticulum. In epithelial tissues, functional diversity may arise during wound healing, as cells with unique physiological roles exhibit distinct calcium signaling activity to meet specific functional demands [291]. These findings suggest that differential expression of Ca^{2+} receptors like IP_3R may play a key role in determining which cells initiate and propagate signals during processes like wound healing.

Computational Modeling for Organized Tissue Responses

Multicellular computational models simulate and capture collective behaviors across diverse biological contexts, offering insights into how calcium signaling scales from internal cellular dynamics (RoL 1) to cell-cell interactions (RoL 2) and tissue-wide coordination (RoL 3). While RoL 1 and RoL 2 describe localized, cell-to-cell calcium dynamics that influence direct interactions, expanding to RoL 3 reveals how these signals propagate across larger cellular networks. Computational models developed for *Drosophila* wing discs show the importance of variability in PLC production and gap junction permeability in regulating spatiotemporal patterns of Ca^{2+} [28]. Similarly, a multi-scale approach combining agent-based methods and partial differential equation (PDE) models in plant systems illustrate how localized Ca^{2+} release propagates through tissues using Ca^{2+} -activated Ca^{2+} channels, generating emergent patterns [272]. Computational modeling plays a critical role in elucidating the signaling mechanisms in these systems, and the core details of these models can be applied to other biological settings, creating computational building blocks that can be used across species and kingdoms with little to no adaptation [292,293]. These models also help identify thresholds and feedback mechanisms determining whether cells function as initiators or standby cells. Since calcium signaling shapes and is shaped by cellular processes including actomyosin remodeling, integrating experimental and computational approaches will be essential for deepening our understanding of calcium's role in regulating cellular behaviors and coordinating

multicellular processes. This will simultaneously guide Ca^{2+} -targeted therapeutic applications in regenerative medicine and disease treatment.

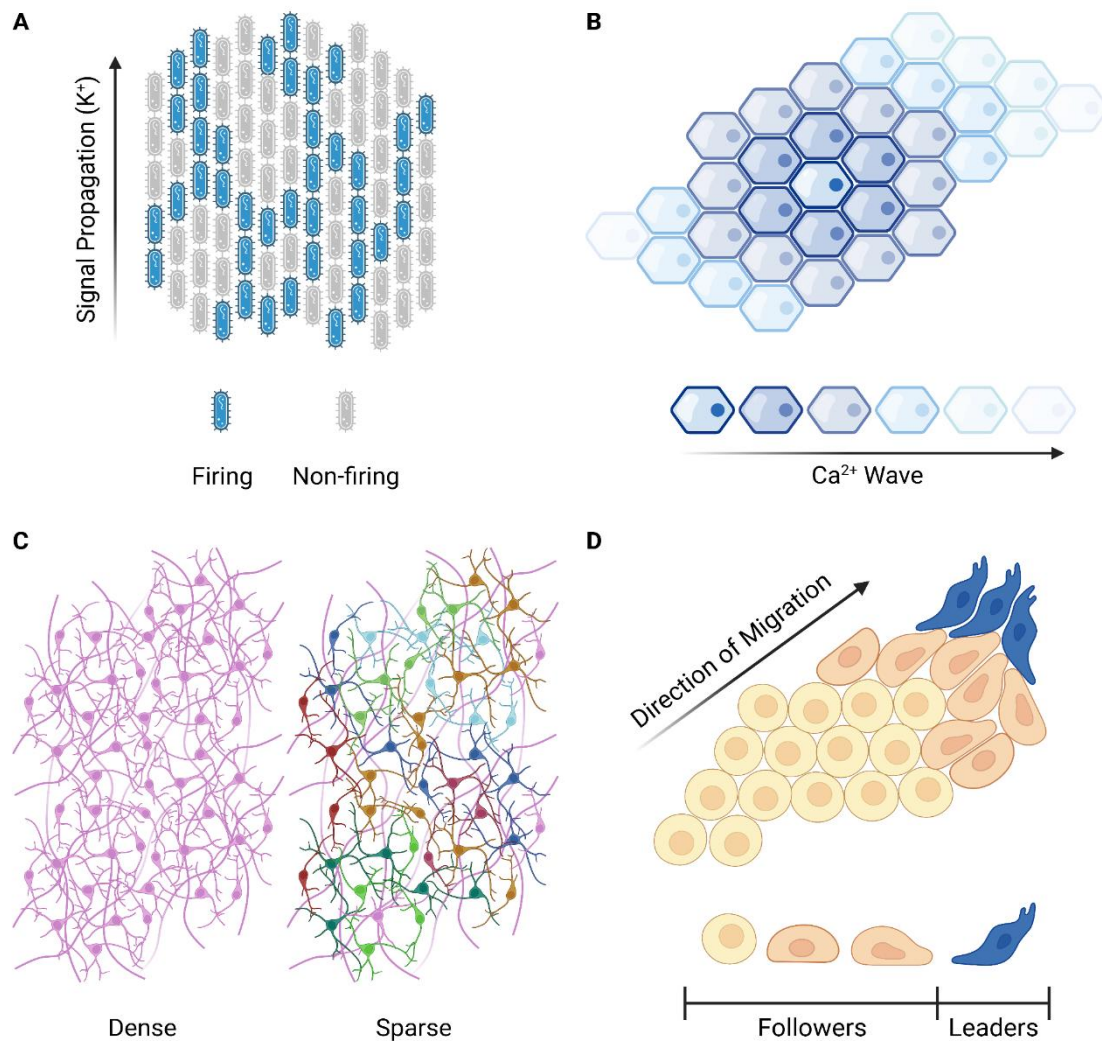


Figure 4. Subdivision of Cell Populations. **A) Firing and nonfiring in bacterial organization.** Bacteria demonstrate signal percolation with distinct active (firing - blue) and passive (non-firing - gray) cells, through potassium ion use, organizing their communication. **B) Initiator and standby dynamics in plant percolation.** In plants, Ca^{2+} ions are similarly used to propagate signals across tissue layers, generating a Ca^{2+} wave that travels across the tissue, originating from the initiating cell (darker blue) and diffusing through the standby cells (fading blue). **C) Neuronal dimensional reduction.** In neurons, complex information is dimensionally reduced through sparse coding, organizing neurons from a single (dense) group into (sparse) sub-populations and networks (denoted by color), each representing unique stimuli or information, reducing cross-communication. **D) Leader and follower segregation in multicellular migration.** Leading cells (blue) direct cell migration forming a protrusive leading edge, while followers (tan) maintain more symmetric contact with surrounding cells while following the leaders. Created in BioRender.

Conclusions

Defining the “Rules of Life” (RoLs) as mechanistic, data-driven relationships provides a foundational framework for understanding how calcium signaling and cytoskeletal dynamics coordinate biological responses across systems and scales. This forward-looking review identifies widespread roles for Ca^{2+} in regulating cytoskeletal remodeling in stress response, cellular communication, and tissue organization—demonstrating how RoLs unify predictive models for cellular responses while remaining adaptable to molecular and environmental contexts.

By developing modular computational functions grounded in experimental data, researchers can build adaptable computational models that simulate system-specific behaviors—capturing both shared principles and specialized mechanisms. In this way, the framework addresses a major challenge in systems biology—translating conserved signaling components into predictive, transferable models despite species specific differences in channel kinetics, effector sensitivity, feedback regulation, and spatial organization, even when homologous or orthologous proteins are involved [294–298]. These models also support closed-loop discovery by guiding experimental planning through model-based design of experiments (MBDoE) techniques [299,300].

This computational framework aligns with the RoLs presented here, where modular logic is derived from conserved signaling patterns and adapted to context.

- RoL 1 demonstrates how Ca^{2+} signaling mediates stress responses by triggering cytoskeletal remodeling across organisms. However, the regulatory feedback between Ca^{2+} , actin dynamics, and pathways like reactive oxygen species and G-protein signaling, remain poorly understood. Computational models, integrated with advanced live-imaging, are critically needed to further capture this modularity across stress-response systems.
- RoL 2 illustrates how Ca^{2+} -triggered actin remodeling spans neuronal, immune, and fertilization synapses—communication systems that share conserved features, like exocytosis, but also exhibit distinct molecular mechanisms. This combination of specialized signaling dynamics with shared core processes shows how biology implements modular components to accomplish context-specific functions. Replicating this principle in modular computational functions and integrating them with expanding *in vivo* studies and bioengineering approaches, will enhance our understanding of cellular communication and biological junctions.
- RoL 3 underscores the versatility of Ca^{2+} signaling as a second messenger. Systematic expansion of *in vivo* studies across a broader range of model organisms, combined with centralized bioinformatic resources, featuring live-imaging data, will accelerate the identification of additional organizational RoLs, supporting flexible and extensible modeling frameworks.

Finally, RoL-based computational modules can inform digital twins for Regulator Science Tools in drug evaluation [301–304], or help connect translational medicine between model organisms and humans through predictive systems modeling [292,305,306]. In synthetic biology and tissue engineering, modular RoLs may accelerate the design of engineered tissues and biomaterials with context-aware signaling capabilities that respond to their local environmental conditions [307–309]. Finally, as advances and applications of artificial intelligence and advanced machine learning with bioengineering applications raise ethical, legal, and social implications (ELSI) [310–312], a RoL framework offers a formal, modular basis for articulating design intent, tracing system behaviors, and guiding responsible innovation in emergent biological technologies [313–315]. Overcoming each of these challenges ultimately requires interdisciplinary collaborations [316]. By unifying experimental insight with modular computational models, this RoL-centered approach lays the groundwork for cross-scale, cross-species understanding of Ca^{2+} -actin dynamics—advancing both fundamental biology and its engineering applications.

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