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Timing in Cellular Ca^{2+} Signaling

Review

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Calcium (Ca^{2+}) signals are generated across a broad time range. Kinetic considerations impact how information is processed to encode and decode Ca^{2+} signals, the choreography of responses that ensure specific and efficient signaling and the overall temporal amplification such that ephemeral Ca^{2+} signals have lasting physiological value. The reciprocal importance of timing for Ca^{2+} signaling, and Ca^{2+} signaling for timing is exemplified by the altered kinetic profiles of Ca^{2+} signals in certain diseases and the likely role of basal Ca^{2+} fluctuations in the perception of time itself.

Introduction

Crudely, the business of Ca^{2+} signaling is one of information delivery. How do biological systems interpret environmental cues to choreograph the generation of Ca^{2+} signals and thereby execute appropriate physiological responses? Understanding how inputs are processed and relayed via changes in cytoplasmic Ca^{2+} concentration ('encoding') to impact the activity of only a desired subset of Ca^{2+}-sensitive targets ('decoding') has proved to be a durable and mechanistically intriguing field of research, as well as one of increasing pathological pertinence.

Ca^{2+} signals display great spatiotemporal malleability. They are generated across wide spatial and temporal ranges — from nanometer to centimeter, microsecond to hour. This broad scope disguises additional flexibility in the size, source, spread, persistence and rhythm of cytoplasmic Ca^{2+} changes coordinated through the specific organization and properties of Ca^{2+} channels, pumps, buffers and exchangers in any given cell type. Therefore, as spatial and temporal controls are inseparable orchestrators of Ca^{2+} signals, our focus here on issues of 'timing' is somewhat contrived. Consequently, we direct readers to broader reviews [1–4] and discuss here solely principles of timing in Ca^{2+} signaling and examples that showcase their application.

Timing pervades all aspects of Ca^{2+} signaling, affecting how environmental information is compiled, encrypted and deciphered (an overview is provided in Figure 1). Temporal considerations govern: how incoming information is both processed at the cell surface and resolved by effectors ('interpreting inputs'); how reactions are set into motion with appropriate sequentiality and interdependence, cueing processes in an order determined by feedback from and dialog with other signaling pathways ('choreographing responses'); and how Ca^{2+}-dependent effectors are differentially activated ('targeting effectors') by temporal aspects of cytoplasmic Ca^{2+} signals, notably their frequency or duration ('temporal decoding'). These topics are discussed below.

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Interpreting Inputs

Cells are exposed to a multiplicity of environmental signals that may regulate their behavior depending on when and how these inputs are presented. Temporal considerations, including the rate, context and order of signal presentation are important in specifying discrete signaling outcomes and these principles are discussed below.

Single Inputs: Context

A first example of time-dependent sensitivity in Ca^{2+} signaling is context, i.e. scenarios in which responsiveness to a signal is state-dependent (Figure 2A). At one end of the spectrum, this represents gain-of-function scenarios where a signal is ineffective at evoking a response at one point in time, but not at another. More subtly, different outcomes may be associated with the same input when presented at different times (e.g. different antigen-evoked Ca^{2+} signals in naïve and primed lymphocytes [4,5]). If time is "that great gift of nature which keeps everything from happening at once", then context is the timekeeper that paces change via external or autonomous cues. In short, such contextual cues distill specificity from pervasive signals.

Clear-cut examples of state-dependent responsiveness are found during the natural temporal progression of development and cellular differentiation, and during acute refractory states in excitable tissue. Sperm-evoked Ca^{2+} signaling is ineffective at fertilizing oocytes until maturation (which can take between minutes and days) renders a competent egg. Depolarizing stimuli are ineffective at stimulating Ca^{2+} entry until the developmental timepoint when voltage-gated Ca^{2+} channels are expressed [6,7]. Defined periods of ligand sensitivity are seen with several Ca^{2+}-modulating agonists [7,8]. Windows of 'competency' to inductive signaling during embryogenesis exist transiently and execute irreversibly on receiving appropriate inputs. Since the activity of many inducing factors modulates and/or is modulated by local Ca^{2+} concentration [9–12], it is not surprising that embryonic Ca^{2+} transients or gradients have been implicated in patterning and specification [13–16]. Potential mechanisms that delimit these periods include temporal regulation of receptor transcription/translation and restricted spatial expression of Ca^{2+} channels [17,18]. Alternatively, targets may be present but inhibited, and time must pass before this attenuatory regulation is relieved [19,20]. Finally, it is important to point out that the functional readout of Ca^{2+} signaling networks may change over developmental time [13]: non-canonical Wnt (Wnt–Ca^{2+}) signaling early in development is associated with specification events (such as ventral patterning [15,16]), but later in development with morphogenesis (e.g. convergent extension [21]); also, Ca^{2+} oscillations can either initiate development or terminate cellular viability, depending on the age of the egg when fertilized [22].

Ca^{2+} signals observed during cellular differentiation [6,23,24] reinforce differentiative events and possibly impact their intrinsic timing. One example is the ordered production of first neurons, then glia from multipotent neuronal stem cells in the developing vertebrate cortex. This timing mechanism is contextual: environmental cues bias the outcomes, even though the intrinsic timing mechanism is hard-wired into isolated clones [25]. Spontaneous Ca^{2+} entry signals

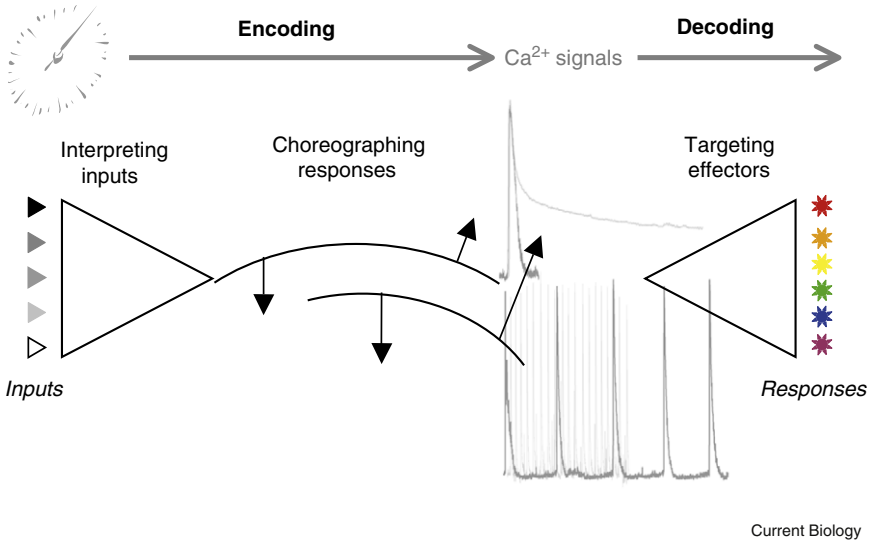

Figure 1. Kinetic orchestration of Ca^{2+} signals.

Schematic overview of the impact of timing in cellular Ca^{2+} signaling. Aspects of timing impact how extracellular signals are interpreted and choreographed into specific profiles of cytoplasmic Ca^{2+} signals ('encoding'). Different Ca^{2+} signals result depending on how cells interpret dynamic environmental cues ('integrating inputs', depicted as shaded triangles on the left) to contextualize and temporally order specific signaling processes ('choreographing responses'). Temporal aspects of the resultant cytoplasmic Ca^{2+} signal — notably, variability in duration (upper trace) and periodicity (lower trace) — are sensed by subsets of Ca^{2+}-sensitive effectors via Ca^{2+}-binding sites of varied affinity, kinetics and interdependence, resulting in their selective activation ('decoding: targeting effectors') to yield discrete responses (depicted as different colored stars on the right).

observed during neuronal progenitor differentiation [6,23,24] may impact switching between neurogenic and gliogenic states through global epigenetic control. Methyl-CpG-binding protein 2 (MeCP2), a transcriptional repressor with affinity for glial-specific promoters, is subject to a Ca^{2+}-influx-dependent phosphorylation that relieves transcriptional repression [12,25,26].

Multiple Inputs: Summation, Sequentiality, Coincidence and Memory

Timing provides further flexibility in information processing where multiple inputs are involved (Figure 2). Kinetic considerations ensure that two inputs do not invariantly produce only two outputs. Changes in the duration, sequentiality and temporal convergence of incoming signals ensure a multiplicity of biological outcomes from a pair of stimuli. Here, we have broadly corralled examples under the headings of summation (the effects of two Ca^{2+} signals compound to ensure a discrete outcome, Figure 2B), sequentiality (the order of presentation of two signals dictates different outcomes, Figure 2C), coincidence detection (the arrival of two different signals within a set timeframe ensures a discrete outcome, Figure 2D) and memory (certain combinations or sequences of repetitive signals associate with specific responses, Figure 2E). Although this categorization is fluid — for example, many coincidence detectors are also sensitive to the order in which inputs are presented — it provides a simple framework for discussion.

Summation is frequently the basis of amplitude modulation [4] in Ca^{2+} signaling (Figure 2B). When different inputs converge on the same signaling currency (Ca^{2+}) and their effects overlap, the resulting cytosolic Ca^{2+} increases compound to yield unique responses. Summation of Ca^{2+} signals is not only important for activating targets of progressively lower Ca^{2+} affinity, but also for triggering cellular Ca^{2+} signals. In the majority of cell types, the initiation of a Ca^{2+} wave is regulated by local summation of short-lasting Ca^{2+} fluxes that drive the ambient cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) toward a threshold at which Ca^{2+}-induced ('regenerative') Ca^{2+} release occurs [2,27,28]. As Ca^{2+} channels integrate diverse regulatory inputs, there can be considerable variability in the occurrence and duration of unitary Ca^{2+} release events that impact their summation. The local $[Ca^{2+}]_{cyt}$ threshold that is needed to evoke regenerative Ca^{2+} release is also variable, as it is related to the global behavior of the cytoplasm as an excitable medium, acting as an integrative gauge of second-messenger levels and the repleteness of cellular Ca^{2+} stores at any point in time. Therefore, temporal summation shapes both the generation of Ca^{2+} signals and the ensuing responses.

In paradigms of 'sequentiality' (Figure 2C), the order of presentation of inputs is important: certain input combinations render one response, whereas other combinations generate another response or no response at all. A classic paradigm involving sequentiality (and coincidence) is spike-timing-dependent plasticity (STDP), where the same pre-synaptic and post-synaptic action potentials can strengthen or weaken synaptic efficiency depending on their order [29,30]. At the molecular level, sequentiality is encoded by mechanisms that endow interdependence to binding or regulatory sites, such as overlap [31], conformational masking [32–35], *de novo* generation of binding sites or functionality by intermolecular assembly [36,37] or spatial translocation [33,38]. An elegant example of a scenario where one signal is ineffective unless another has been presented first is the sequential activation of conventional protein kinase C (PKC) isoforms. Diacylglycerol (DAG) analogs do not promote the translocation of full-length PKC to the membrane unless Ca^{2+} is elevated [33]. This is because the DAG-binding sites found within the $C1_2$ domain of the kinase are rendered inaccessible by a pseudosubstrate clamp until Ca^{2+} binds to the C2 domain of PKC to effect translocation to the plasma membrane and a series of stabilizing interactions (including DAG binding) that must be maintained to relieve kinase inhibition. Therefore overlapping Ca^{2+} signals (first step, translocation) and DAG signals (second step, activity), but neither signal alone, produces maximal kinase activity.

Many processes involved in Ca^{2+} signaling integrate different signals by detecting 'coincident' inputs (Figure 2D). In some cases, simple overlap of different signals is sufficient to evoke a supralinear response. In more stringent examples, the arrival of an input may define a set time window in which the coincident input must be received, or the ordering of coincident inputs is important. Most notably in STDP, the type and extent of synaptic modification is dependent on both the respective timing (millisecond discrimination [29]) and

ordering of pre- and post-synaptic action potentials [29,30,39]. Examples of molecular coincidence detectors include Ca^{2+} channels [30,34,39–41]), Ca^{2+}-regulated enzymes [42,43] and transcriptional regulators [44], all of which sense pairings of extracellular signals (such as agonist-mediated stimulation or depolarization), G proteins or second messengers (such as inositol (1,4,5) trisphosphate (IP_3), Ca^{2+}, cyclic AMP (cAMP) and DAG).

Finally, the involvement of Ca^{2+} signaling in 'memory' (Figure 2E) spans work on speculative mechanisms of memory deposition in individual Ca^{2+} sensors [38], through to intensely researched changes in local synaptic plasticity or connectivity that likely underpin local ('neuronal clique') and network firing patterns involved in the deposition and consolidation of memories [30,45,46]. Ca^{2+}-entry and Ca^{2+}-release signals regulate short-term activity-dependent changes (over periods of less than hours) in synaptic efficiency of pre-existing synaptic components, as well as longer term changes (over periods of more than days) in synaptic function and architecture [30,47–49]. Short term working memory (seconds), spatial memory (hours) and the reactivation of consolidated memories embedded via transcription/translational events harbor a common dependency on Ca^{2+} fluxes [48–50]. The increasing technical ability to monitor and manipulate the firing patterns of many neurons during mnemonic episodes [46,51] demonstrates how temporal precision, as determined by neuronal discharge frequency and latencies, is paramount for coupling clique dynamics and for preserving the fidelity of signals traversing neuronal networks.

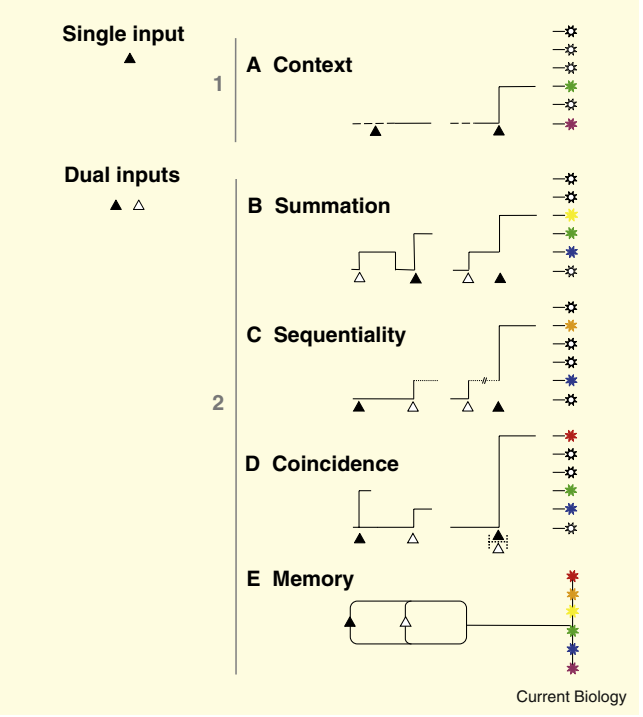

Figure 2. Temporal integration empowers diverse outcomes from limited inputs.

Principles of timing impact signal interpretation for single and multiple (in this case, dual) inputs, represented as ▲ and △. (A) Context determines whether a single ('1') signal will be effective at evoking a response (▲→ no response/response). Two inputs associated with specific individual responses can produce several different responses (depicted as coloured stars) when (B) the duration of their effects compound (summation), (C) the order of their presentation matters (sequentiality; ▲△→response 'x', △▲→ response 'y'), (D) the relative timing, but not necessarily order, of the arrival of each input is critical (coincidence detection; ▲…△/△…▲→ response 'x', △▲ or ▲△→ response 'y') or (E) specific combinations of presented signals (e.g. one of two signals ▲ and △ presented 'n' times) can trigger a number of unique ($\leq 2^n$) responses as an example of associative memory. Strategies for temporal memory of Ca^{2+} signals (such as priming and persistence) are discussed in the main text in the 'Duration' section.

Choreographing Responses

Signal transduction is an engagement of interlinked modules of reactions that adjust cellular behavior to environmental cues. Efficient signaling necessitates ordered temporal execution of these modules and their constituent reactions, each of which may regulate preceding or subsequent events. Kinetic considerations define the speed by which information is relayed between effectors and thereby the timeframe of the overall response, the oscillations in activity of individual components, as well as the impact of positive and negative feedback in determining the sensitivity, range and stability of output from the overall module.

Obviously, different signaling modules execute over different timeframes in different cells: synaptic Ca^{2+} concentration changes occur within microseconds, compared with latencies of up to tens of seconds during agonist-evoked Ca^{2+} signaling in non-excitable cells. The same module can, however, be customized to work over a malleable timeframe, as exemplified by considering the ubiquitous phospholipase Cβ (PLCβ) Ca^{2+} signaling cascade. At one extreme, where this module underpins *Drosophila* visual transduction, responses are executed with minimal latency (<25 ms), an obvious temporal adaptation to the demands of flight. A major contributory factor is the spatial compartmentalization and pre-coupling of signaling components by the scaffolding protein InaD in the rhabdomere, minimizing the need for amplification steps upstream of PLCβ [52]. The tight spatial coupling of this module permits the high temporal fidelity of phototransduction events. Indeed, the latency of the single photon response (time between activation of a rhodopsin molecule and the elementary response to a single photon (the 'quantum bump')), is increased approximately sixfold in mutants lacking such spatial organization through InaD [52]. Scaffolding proteins impact signaling kinetics both generally — by increasing reaction speed and efficiency via compartmentalization of proteins within restricted surfaces that favor productive and privileged interactions — but also specifically, as is becoming increasingly recognized (e.g. InaD [53], Homer-2 [54]), by regulating individual kinetic interactions within Ca^{2+}-signaling modules. In contrast, the coupling of remotely synthesized hormones to phosphoinositide-coupled Ca^{2+} oscillations in the intact liver occurs over a timeframe that is more than 10,000-fold longer and more compatible with metabolic cycling [55]. Here, each protein component is separated by signal relay steps impacted by issues of messenger durability (such as agonist range), GTPase activity (modulated, for example, by regulator of G-protein signaling (RGS) proteins [56]), IP_3 diffusion/metabolism and regionalized Ca^{2+} buffering.

Positive- and negative-feedback regulation of proteins in these modules is critical in shaping the periodicity and kinetics of cytoplasmic Ca^{2+} signals. Feedback regulation ensures that all the relay steps (involving G proteins, IP_3, and Ca^{2+} [57]) as well as interlinked signaling outputs (e.g.

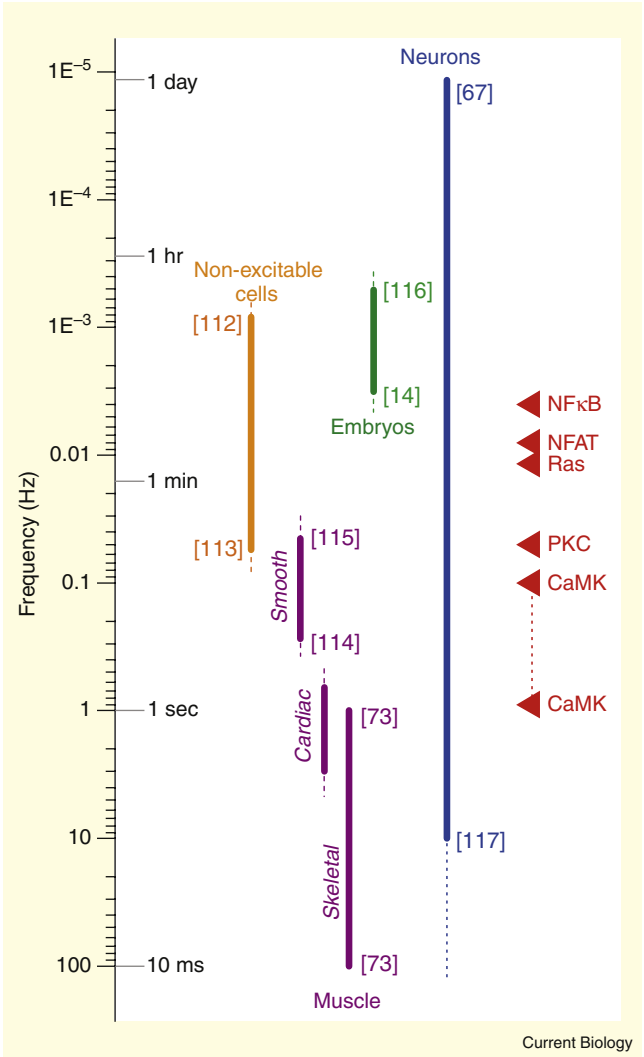

Figure 3. Physiological harmonics: frequency encoding of Ca^{2+} signals.

The ~10,000,000-fold range in the frequency (Hz, left) of repetitive Ca^{2+} changes observed in different cells/tissues, using data from non-excitable cells (orange, [112,113]), muscle (purple, [73,114,115]), embryos (green, [14,116]) and neurons (blue [67,117]). Numbers represent citations of examples that delimit each range. On the right in red are shown estimates of optimal Ca^{2+}-spiking frequencies for half-maximal activation of the indicated proteins: Ras [75], NF-κB [64], NFAT [64], CaMK [63], PKC [33]. These estimates obviously represent single point estimates from a broader *in vivo* range.

phosphorylation and cAMP production [43,58]) can display oscillatory, phase-locked changes in activity. The differential kinetic timecourse of individual feedback steps, even on the same component, is important in ordering reactions, sensing spatial distance and for switching reliably and stably between activity states [59]. For example, many intracellular Ca^{2+} channels exhibit time-dependent regulation by agonists, where the same ligand concentration may increase or decrease Ca^{2+} release depending on the duration of exposure [40,60]. The activity of distinct Ca^{2+} signaling modules is further choreographed by steps that interlink their ordered temporal execution, establishing complex regulatory circuits and crosstalk with other signaling pathways that orchestrate short-term responses while maintaining longer-term homeostatic balance.

Temporal Decoding of Ca^{2+} Signals

Cytoplasmic Ca^{2+} signals relay information to Ca^{2+}-sensitive effectors. Issues of timing — notably the periodicity and duration of Ca^{2+} transients — are crucial in determining how information is decoded into appropriate cellular responses. These parameters are discussed separately below, prefaced with a reminder about their *in vivo* interdependence: the efficiency of frequency encoding of Ca^{2+} signals is impacted by the specific profile of Ca^{2+} spike duration and amplitude, as well as the baseline level of Ca^{2+} over which these signals occur [33,61–65].

Frequency

Repetitive fluctuations in cytosolic Ca^{2+} occur over an exceptionally broad time range (Figure 3). These fluctuations encompass rhythmic changes in baseline Ca^{2+} observed in fungi, plants and animals [66–69], upon which are superimposed stimulation-evoked Ca^{2+} transients that occur episodically as bursts of oscillations or repetitive Ca^{2+} spikes with regulable intermittency [3,70] and cell-specific or agonist-specific profiles [71]. The duration of trains of Ca^{2+} spikes (which can be evoked for hours in experiments) is physiologically significant [72]. The $\sim 10^7$-fold range in periodicity (likely constrained by our inability to resolve exceptionally slow or fast signals without summation) is exemplified at the extremes by the rapid bursts of Ca^{2+} spikes generated within sound-producing muscles (e.g. $\geq$10 ms spike duration in toadfish swimbladder muscle [73]) and the protracted and persisent (e.g. $\leq$24 hour) oscillations in basal $[Ca^{2+}]_{cyt}$ associated with circadian rhythms [67–69]. In this latter case, Ca^{2+} signaling messengers may actually orchestrate the perception of time via entrainment and pacing of the circadian oscillator itself [74]. Therefore, the variable periodicity of Ca^{2+} signals coordinates responses from the cellular to the systems level.

Irrespective of the absolute frequency of Ca^{2+} oscillations, the same key issues hold: what is the mechanistic basis of the oscillator and how is this oscillator read? This latter question may simply entail an understanding of target selection (i.e. how the affinity, kinetics and localization of Ca^{2+}-binding sites delimit which effectors respond) for outputs that directly track Ca^{2+} signals. More elaborately, many effectors have the ability to transduce different frequencies of Ca^{2+} transients into graded levels of activation, a process known as frequency modulation. Over the 30 years or so since repetitive Ca^{2+} oscillations were resolved, considerable effort has been made to address these questions [2,4,57]. Several molecules, and many more processes, have been identified that are optimally regulated by specific Ca^{2+}-spiking frequencies, including: the Ca^{2+}-dependent transcription factors NF-AT [64,65], NF-κB [64], and Oct/OAP [64]; Ras [75]; Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [61,63]; and PKC [33]. Collectively, these data show that different Ca^{2+} sensors are regulated — over distinct ranges (Figure 3) and between distinct limits — by the periodicity of Ca^{2+} transients [58,61,63,64,75].

Considerably less is known, however, about the molecular basis of frequency decoding. Non-linear activation mechanisms in most models are underpinned by elements of coincidence and reinforcement (e.g. interdependent, cooperative or temporally geared reactions where active intermediates persist beyond the transient Ca^{2+} spike) to render integrative behavior. Seemingly subtle kinetic delays are critical in allowing activities to be summated at high Ca^{2+}-

spiking frequencies, as shown, for example, by the short delays (seconds) in PKCγ and DAG association and dissociation as Ca^{2+} rises and falls [33]. The strongest structural insight into frequency decoding is probably provided by CaMKII, and experimental observations that different CaMKII isoforms [63], and notably different splice variants of the same isoform (βCaMKII [61]), display unique *in vitro* sensitivities (~10-fold range) to the frequency of Ca^{2+} oscillations. Specific structural changes that impact initial autophosphorylation rates [61] or residues that determine the dramatic decrease in the rate of calmodulin dissociation from the autophosphorylated holoenzyme are likely to be crucial factors in the kinetic sensitivity of the kinase isoforms [76]. Coupling such biochemical insight with crystal structures [77] will ultimately reveal the conformational mechanics of frequency decoding.

It suffices to conclude that the diversity of responses regulated by Ca^{2+} oscillation frequency emphasizes the utility of frequency encoding as a strategy for relaying information [78]. Ca^{2+} spiking decreases the $[Ca^{2+}]_{cyt}$ threshold for activating responses and preserves signal fidelity and specificity while minimizing the metabolic cost, effector desensitization and cytotoxic risk associated with sustained increases in $[Ca^{2+}]_{cyt}$.

Duration

The duration of Ca^{2+} transients varies considerably: Ca^{2+} signals can be rapid (and likely localized), or more protracted (and likely pervasive). Their individualized duration is determined by the kinetic interplay of Ca^{2+} fluxes that increase, buffer and remove Ca^{2+} ions from the cytosol, the balance between which is regulated physiologically and disturbed pathologically through changes in the functional architecture of Ca^{2+} signaling molecules. The duration of a Ca^{2+} signal therefore depends on the precise molecular complement of Ca^{2+} transporters engaged during a response. Involvement of the endoplasmic reticulum (ER) is particularly crucial: Ca^{2+} signals that evoke regenerative Ca^{2+}-induced Ca^{2+} release via IP_3 receptors (IP_3Rs) or ryanodine receptors (RyRs) trigger propagating Ca^{2+} waves that extend the duration and spatial reach of cytoplasmic Ca^{2+} changes. The consequent ER Ca^{2+} depletion stimulates a homeostatic store-operated Ca^{2+} entry, which, in conjunction with other receptor-operated Ca^{2+} influx pathways, refills the ER with Ca^{2+} via delayed but enduring Ca^{2+} entry currents [79]. Therefore, the involvement of different families of intracellular Ca^{2+} channels in amplifying, and triggering the amplification of, Ca^{2+} signals is a key determinant of Ca^{2+} signal duration.

The time course of a Ca^{2+} signal is important because early and late phases of Ca^{2+} signals are often associated with different responses — sustained Ca^{2+} elevations will reach more targets, and occupy sites of appropriate affinity for longer. For example, transient Ca^{2+} signals are often insufficient to activate transcriptional responses [80] and the duration of more sustained Ca^{2+} signals is important in specifying which transcription factors are most efficiently activated [70,81]. Further examples of Ca^{2+} signals of different durations directing unique cellular responses are as diverse as stomatal opening and closing [62], exocrine function [82], the integrated initiation of Ca^{2+}-dependent events during egg activation [83], positive and negative thymocyte selection [84] and the selective modulation of plasticity in neurons (long-term potentiation (LTP) vs long-term depression (LTD) [29,30]).

In terms of decoding, one would underestimate the versatility of Ca^{2+} as a messenger by assuming that the effective duration of a Ca^{2+} signal was set only by the occupancy of Ca^{2+}-binding sites on Ca^{2+} sensors with singular affinities. Ca^{2+}-dependent effectors exhibit a variety of temporal gearing strategies to ensure that the consequences of transient Ca^{2+} signals persist beyond the duration of the Ca^{2+} signal itself. While cellular Ca^{2+}-binding proteins exhibit a wide range of intrinsic Ca^{2+}-binding affinities, even within specific subfamilies of Ca^{2+} sensors [85–87], these 'basal' affinities can change dramatically on regulatory modification (>25,000-fold [76]), through cooperativity with other Ca^{2+}-binding modules in the same proteins, and on forming interactions with substrate or additional partners. Such mechanisms ensure that ephemeral Ca^{2+} signals have lasting physiological value.

Many Ca^{2+}-triggered interactions are enabled by Ca^{2+}-dependent translocations to cellular membranes [88], such as those mediated by C2 domains (for example, as found within PKC [33,58], Ras regulators [38]), Ca^{2+}/myristoyl switches [86,89], annexin repeats [87]) or via exposure of binding sites for specific target proteins (e.g. CaMKII [90]). After the initial Ca^{2+}-driven membrane translocation, activities may become entirely Ca^{2+} independent ('autonomous') thereby extending the temporal reach of Ca^{2+} signals, as exemplified by the importance of CaMKII autonomy for transcription [91] and plasticity [92]. Localized autonomous CaMKII activity is maintained by autophosphorylation (e.g. on cardiac Ca^{2+} channels [93] and certain targets in the post-synaptic density [94]), or simply by immobilization on the target itself (e.g. for the NMDA receptor subtype NR2B [95] and certain potassium channels [96]). These varied routes to autonomy define whether temporal gearing is controlled by local phosphatase activity, target dissociation, or both. Therefore the extent of temporal amplification through translocation is likely to be unique for different subcellular compartments, as well as for different targets.

Mistiming

When timing goes awry, pathological outcomes ensue. There are many examples of how the temporal profile of cytosolic Ca^{2+} signals is adversely remodeled by disease and specifically by mutations that impact the expression, activity and spatial organization of individual Ca^{2+} homeostatic regulators (see reviews in [97]). In the heart, for example, abnormal Ca^{2+} cycling predisposes to arrhythmias [98] via decreased expression/functionality of Ca^{2+} buffers [99] or mutational dysfunction of voltage-operated Ca^{2+} channels [100], intracellular Ca^{2+} channels (e.g. around 70 mutations in human RyR2 are associated with arrhythmia) or proteins that compartmentalize Ca^{2+} channels and transporters [101]. Protracted Ca^{2+} transients diagnostic of diastolic dysfunction can result from decreased sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) expression or mutational dysfunction of its regulators, thereby impairing recovery from Ca^{2+} loads [102,103]. The timing of mutations is also critical for disease progression, by impairing [104] or persistently potentiating [105,106] Ca^{2+} signaling events at key points in the pathogenic time-line. For example, *PKD1* is a gene mutated in the majority of patients with autosomal dominant polycystic kidney disease. *PKD1* encodes polycystin-1 (TRPP1/polycystin-1), a cell-surface protein that functions as part of a Ca^{2+}-dependent mechanosensitive complex with polycystin-2 during renal development. Loss of polycystin-1 function

results in different pathological outcomes depending on when functionality is impaired. If deleted shortly after birth, kidney cysts form rapidly and mice die within a few weeks, whereas loss of function in adults is associated with a much milder phenotype [104].

Beyond the many 'loss-of-function' mutations that impact global Ca^{2+} cycling, rarer 'gain-of-function' mutations increase the duration of cellular Ca^{2+} fluxes, as exemplified by channel mutants associated with several Ca^{2+} channelopathies where normal channel inactivation kinetics are delayed [107–109]. A dramatic example is the *de novo* Ca^{2+} channel mutation associated with Timothy syndrome (a G406R substitution in the $Ca_v1.2$ channel) where a near complete failure of voltage-dependent channel inactivation precipitates cardiac, neuronal and developmental defects [100]. Many kinetic changes are, however, much subtler, yet still cause disease. For instance, certain mutations (three from more than 50) identified in *ATP2A2*, which encodes the housekeeping SERCA2b isoform, confer relatively normal Ca^{2+} transport and ATP hydrolytic activities (>65% of wild type [110]). These mutants are nonetheless associated with the Darier disease pedigrees [111]. Modulation of SERCA2 affinities in the heart — either to increase or decrease the net Ca^{2+} affinity of sarcoplasmic reticulum Ca^{2+} sequestration — can result in cardiomyopathies [103]. An important principle from such examples is that although different cell types show incredible versatility in their individualized 'Ca^{2+} signatures', there is considerably less tolerance for temporal deviation from differentiated Ca^{2+} profiles without pathological consequences.

Conclusions

The diversity of examples discussed above underscores the many ways in which timing impacts how information is relayed from external stimuli via Ca^{2+} signals into specific physiological outcomes. The broad community of Ca^{2+}-handling proteins that increase, decrease and buffer cytoplasmic Ca^{2+} provide kinetic diversity in assembling signaling modules that are customized for cellular function at any point in time to generate Ca^{2+} signals across a remarkable range of durations and periodicities. The malleable role of Ca^{2+} as a messenger depends on the precise temporal control of the activity of these individual molecules, as well as controlled regulation of the extent of feedback, diversification and gearing between them that rapidly set up and dissipate spatial gradients of activity within cells and tissues. Consequently, quantifying the tolerance of this kinetic architecture to pathological cues is important in elucidating how the temporal dynamics of specific modules within proteins contribute to Ca^{2+}-related pathologies at a systems level.

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