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Electrifying rhythms in plant cells

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Abstract

Physiological oscillations (or rhythms) pervade all spatiotemporal scales of biological organization, either because they perform critical functions or simply because they can arise spontaneously and may be difficult to prevent. Regardless of the case, they reflect regulatory relationships between control points of a given system and offer insights as read-outs of the concerted regulation of a myriad of biological processes. Here we review recent advances in understanding ultradian oscillations (period < 24h) in plant cells, with a special focus on single-cell oscillations. Ion channels are at the center stage due to their involvement in electrical/excitabile phenomena associated with oscillations and cell-cell communication. We highlight the importance of quantitative approaches to measure oscillations in appropriate physiological conditions, which are essential strategies to deal with the complexity of biological rhythms. Future development of optogenetics techniques in plants will further boost research on the role of membrane potential in oscillations and waves across multiple cell types.

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Introduction to biological oscillations

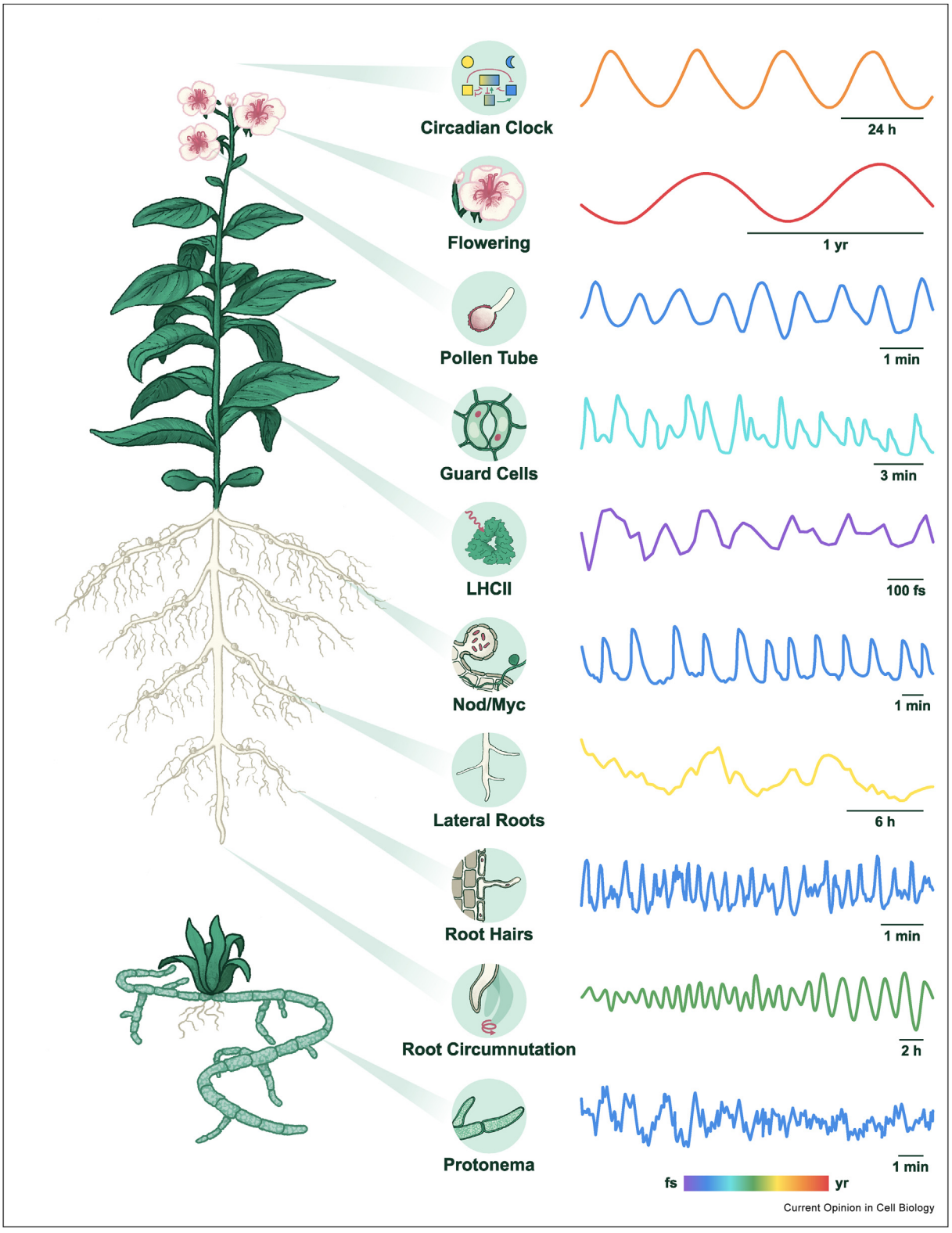
Oscillations are found in virtually all levels of biological organization (Figure 1, Box 1), with reports spanning over 24 orders of magnitude in the temporal scale (10^{-15} to 10^{9} s) and 15 orders of magnitude in the spatial scale (10^{-9} to 10^{6} m). In plants, the boundary for the fastest rhythms is arguably in photosynthesis, where femtosecond electronic-vibrational oscillations named "quantum beats" occur in the light-harvesting complex II (LHCII; Figure 1) across a spatial scale in the nanometer range [1]. At the other end of the scale, important biological rhythms can take years or even over a century to complete, as is the case of bamboo flowering [2] or plant population dynamics [3]. Despite being often complex, biological rhythms can be generated by a simple regulatory motif such as a core negative feedback structure, composing an oscillator (Box 1) [4–6]. The physical nature of a biological oscillator is quite varied, as endogenous rhythms can emerge anywhere in gene regulatory networks, protein interactions, metabolism, ion dynamics, and other cellular processes including cell-cell communication [5–7]. However, detailed and quantitative knowledge of the key relationships generating oscillations is rare. Understanding these control points permits the use of mathematical principles of oscillatory systems to predict and control oscillations, and to investigate their biological functions. Novel experimental techniques are especially promising to allow direct interference with oscillations in single plant cells, such as the optogenetic control of membrane potential (voltage) and specific ions [8,9].

This review will cover advances made in the last few years in understanding endogenous oscillations in terms of their mechanisms, molecular players, and biological functions in plants. We focus on ultradian rhythms (oscillations with period <24 h), with a special emphasis on the role of ion channels in single cells. While represented in Figure 1, circadian rhythms constitute a fascinating field on their own, and will not be reviewed here. They are the best known rhythms in terms of mechanisms, functions, molecular circuitry and other aspects addressed in many recent reviews (e.g. [10]). Given format constraints, this review is limited in scope and references, for which we apologize up front to colleagues who may not have their work covered.

Oscillations in apical-growing cells

Apical- or tip-growing cells, are a peculiar group of cells characterized by growing exclusively at the apex by exocytosis of membrane and cell wall precursors. The best studied examples of this class are protonema cells

Figure 1



Plants generate oscillations in virtually all spatiotemporal scales of biological organization. Archetype of a flowering plant and a moss (drawn on the left) associated with their respective organ/cell/pathway/molecule (zoom on the center) where oscillations of virtually all time scales are generated (traces on the right). The approximate period is shown by the black scale bar, determining the color of the trace (see color code below). From fastest to slowest, it features: femtosecond oscillations in electronic coherence, or "quantum beats," in the light-harvesting complex II (**LHCII**) [63] (detrended with loess); **root**

in mosses, root hairs and pollen tubes in flowering plants [11,12], hyphae or fission yeast in fungi, and developing neurites (comparisons in [13]). Pollen tubes often show synchronized oscillations in many cellular processes, notably in growth rate, vesicle trafficking, actin and ion dynamics [4]. Several ionic species display a rhythmic choreography both in tip-focused gradients of cytosolic concentrations and their fluxes across the apical plasma membrane (Figure 2a from [14]). The time scale of pollen tube oscillations varies with species usually in the range 0.5–5 min, while they can be quite regular - frequently observed in lily or tobacco - or show considerable variation between or within cells, as reported in Arabidopsis Col-0 (Figure 2, [15]).

Apical growing cells display a wide range of oscillatory behaviors, making them a perfect showcase of the inherent complexity of biological oscillations. In the simplest case, regular oscillations occur with a nearly constant period and amplitude potentially across multiple variables, maintaining the pattern across time (Box 1). These can be observed in pollen tube growth rate and cytosolic pH at the tip (Figure 2a). When displayed in phase-space plots (definition in Box 1), the recurrent synchronized changes confine the value of the variables to a roundish shape over time (Figure 2a) reminiscent of a limit-cycle attractor [4,15]. An attractor is a concept in dynamical systems defined by a region in the space of possible states of the system where values tend to gather, corresponding to a regulatory state (or regime), for example, fixed points or cyclic attractors (like the limit-cycle; Box 1) [16,17].

Despite the experimental attempt to measure stable and constant properties like the main period and amplitude of the oscillations, real biological oscillations can change in time, that is, the time series may be non-stationary (Box 1). For example, oscillations may arise or disappear suddenly in time, suggesting a transition into or out of the oscillatory regime (Figure 2b from [15]). Regime transitions (Box 1) are found in pollen tubes, illustrated in Figure 2b, with a cell in approximately "steady-growth" (Regime "a") changing to an oscillatory growth mode, with highly synchronized oscillations with cytosolic Ca^{2+} concentration (Regime "b"), and then going into growth arrest, a regime with high amplitude Ca^{2+} spikes (Regime "c"). These abrupt changes in behavior may be brought by alterations in key control parameters in a so-called bifurcation [16,17], for example, a Hopf bifurcation in the case of the appearance of oscillations (Box 1; [18]).

Drifts in period and amplitude over time characterize yet another mode of non-stationary oscillations (Box 1), where changes occur in a coherent fashion like in the progressive increase of the main period seen in Figure 2c (from [14]). These synchronized oscillations in cytosolic tip pH and growth rate also show multiple coexisting periodic components, besides frequency drift. Finally, oscillations may be irregular, showing erratic changes in periodicity as seen in the filaments of the moss of *Physcomitrium patens* trace in Figure 1 (from [19]). These oscillations are deemed quasiperiodic (Box 1), as their periodic components are not stable in time. Besides this type of variability in time that stems from a single biological oscillator, oscillatory properties can vary widely across cells. For example, some apically growing cells may not oscillate at all, while other can show the full spectrum of oscillatory behavior discussed here. This creates multiple challenges to measure and analyze these potentially complex phenomena, calling for rigorous experimental design and quantitative, more accurate methods.

Technical advances in measuring pollen tube oscillations

Detailed quantitative methods were developed and applied to the analysis of oscillations in Arabidopsis pollen tubes [14,15,20]. The application of these methods to oscillatory behaviors allowed unprecedented precision when using the equimolar reporter CapHensor to simultaneously measure the cytosolic concentration of calcium ($[Ca^{2+}]_{cyt}$) and protons ($[H^+]_{cyt}$ or pH_{cyt}) [20]. When expressed in tobacco pollen tubes, where growth spurts occur with periods of 2–6 min, CapHensor unveiled an increase of $[H^+]_{cyt}$ and $[Ca^{2+}]_{cyt}$ *c.*a. 18 s and c.a. 33 s after growth peaks, respectively (c.a. 15 s apart from each other). The arrest of pollen tube growth elicited a spiking behavior in both $[Ca^{2+}]_{cyt}$ and $[H^+]_{cyt}$ but in opposite phases of each other compared to growing tubes, that is, $[Ca^{2+}]_{cyt}$ peaks occur c.a. 15 s before $[H^+]_{cyt}$ peaks. However, we emphasize that temporal relationships do not

hair $[Ca^{2+}]_{cyt}$ oscillations with period c.a. 30 s [28]; **pollen tube** growth rate oscillations with period c.a. 50 s in Arabidopsis [15]; Apically-growing *Physcomitrium patens* **protonema** tip $[Ca^{2+}]_{cyt}$ oscillations with main periodicity in the 2 min range [19]; microbial symbiosis (**Nod/Myc**) elicited nuclear Ca^{2+} oscillations in root hairs, a nodulation response with periodicity in the minute range [46]; **guard cell** $[Ca^{2+}]_{cyt}$ oscillations in response to a $CaCl_2$ treatment c.a. 3 min [64]; **root circumnutation** with periodic helical movements of tip position with a period c.a. 1.5 h [51]; **lateral root** formation by the so-called "root clock" occur with rhythms generated with periodicity c.a. 6 h, showing here by the activity of an auxin reporter (DR5) [48]; the **circadian clock** is present in most cell types, represented here by gene sets (boxes) peaking at different times of the day (color gradient) in an interlocked transcriptional-translational feedback loops (regulatory interactions). Boxes (from left to right) represent morning (CCA1 and LHY), afternoon (PRRs/RVEs) and evening (LUX, NOX and ELFs) complexes, with a trace of the free-running rhythm of a circadian reporter (CCR2) from [65]; **flowering** related genes show yearly oscillations, here exemplified with the expression of beta-amylase 5 in a natural environment [66]. This by no means a comprehensive list, lacking examples like metabolic oscillations, other types of circadian oscillators, population dynamics and various other ultradian and infradian rhythms. Illustrated by Joana C. Carvalho.

Box 1. Oscillations: Working definitions and features

Oscillation: any quantity that varies in time with a characteristic signature in terms of frequency and/or amplitude, also known as rhythm. This definition encompasses simple oscillations as a sine wave, defined by a single amplitude and period, to more complex cases such as spike trains, where a sequence of pulses occurs and ends suddenly with amplitude and frequency changes in time, possibly with multiple co-existing frequencies.

Oscillator: system responsible for generating the oscillations, that is, their "source," which in terms of regulatory structure is often based on a core negative feedback loop [5]. One of the most studied biological oscillators in plants, the circadian clock, is based on multiple interlocked transcription-translation feedback loops [10].

Regular oscillation: a single or multiple frequencies that remain constant in time (stationary). Examples in Figure 1 circadian clock trace, and Figure 2a cytosolic tip pH.

Non-stationary oscillations: unlike regular oscillations, the frequency and/or amplitude changes in time. The periodic components can exhibit coherent changes in time (e.g. frequency drift or regime transitions) or rather be erratic and yield irregular oscillations (quasiperiodic).

Frequency drift: changes in frequency in time (non-stationary). Example in Figure 2c.

Irregular or quasiperiodic oscillation: Erratic oscillations where frequency changes in time in a complex manner, without any coherent pattern (non-stationary). Example Figure 1 *P. patens* trace.

Regime transition: changes between qualitative distinct dynamical states, as for example going from a steady growth to an oscillatory state (non-stationary). The example in Figure 2b is akin to a system going through a **bifurcation** (see definition below).

Wave-form: the shape of an oscillatory cycle. Compare the waveform of $[Ca^{2+}]_{cyt}$ oscillations when synchronized with growth versus when spiking during growth arrest (Figure 2b).

Synchronization: when two oscillations maintain a constant temporal relationship throughout time (e.g. Figure 2c). This includes when both exactly coincide in time, that is, in-phase synchronization, and any other stable phase relationship.

Phase-space: the space of possible states of a system [16,17]. For example, the plane of possible values of growth rate and $[Ca^{2+}]_{cyt}$ in Figure 2b, considering a system with two variables. Usually depicted by plotting the evolution of two variables in the X and Y axes, in which each point correspond to observed values for the same time (Figure 2b), although time evolution can be displayed in another axis such as growth rate and tip pH in Figure 2a. If the system is stationary, the plot is a simple point; if stably oscillating, a circle, and different shapes for more complex dynamics such as pseudo-chaotic regimens.

Attractor: a set of states that a system tends to adopt over time (e.g. a single point or an attracting orbit in the phase-space like in the case of oscillations). The term "attractor" reflects the observation that these states attract the temporal evolution of the system, where it tends to stabilize at either spontaneously, or after perturbations. These can be simple orbits, such as the limit-cycle (Figure 2a), or complex shapes like the strange attractors produced by chaotic dynamics [16,17]. In terms of biological systems, an attractor is related to the regulatory regime such as maintaining $[Ca^{2+}]_{cyt}$ at a homeostatic set point or oscillating at a particular frequency that can elicit specific responses downstream.

Bifurcation: qualitative change in behavior of a system brought by changing a control parameter over time, frequently producing a shift on its oscillatory properties. Oscillations can arise through a Hopf, infinite period, or homoclinic bifurcation for example [16,17].

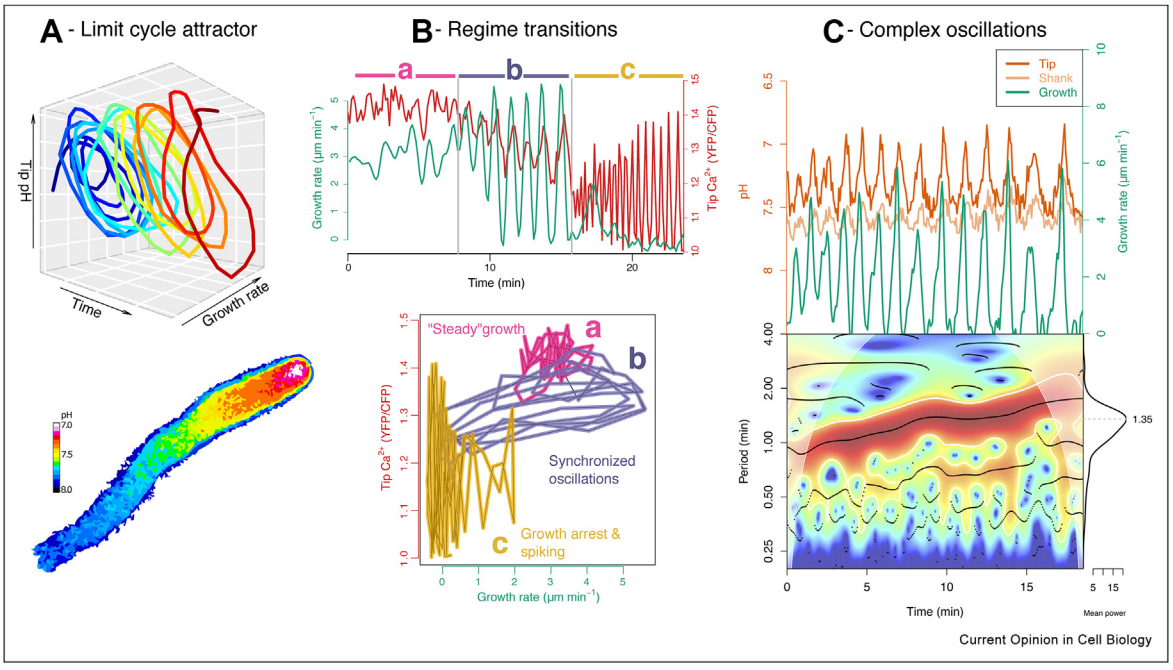
necessarily reflect causality between events [6]. Regarding spatial behavior throughout the tube, $[H^+]_{cyt}$ oscillations occur in phase with the tip, while $[Ca^{2+}]_{cyt}$ oscillations are in anti-phase between tip and shank when measured at c.a. 30 μm distance along the cell length. This probably reflects the differential diffusion rates of these two ions, as H^+ is much faster than Ca^{2+} specially inside a cell. Furthermore, increases in extracellular chloride (Cl^-) concentration was found to promote high amplitude growth oscillations synchronized with $[Ca^{2+}]_{cyt}$ and $[H^+]_{cyt}$. The ER-GCaMP6-210 Ca^{2+} probe allowed to measure oscillations in the endoplasmic reticulum (ER) of pollen tubes, which were found to be synchronized with Ca^{2+} oscillations at the tip albeit with a short delay [21]. Further improving the spatiotemporal resolution to assay tip-focused oscillations and ER dynamics will be critical to establish the contribution of intracellular stores to the oscillations.

Molecular players underlying pollen tube and root hair oscillations

Mutation of the receptor-like kinase ERULUS was reported to lower the frequency of $[Ca^{2+}]_{cyt}$ oscillations when *in vitro* grown pollen tubes were subjected to limited Ca^{2+} growth conditions [22]. The mutation also affects seed-set by impairing pollen tube targeting, suggesting a link between oscillatory $[Ca^{2+}]_{cyt}$ behavior and cell-cell communication during fertilization in flowering plants. The synergid cells are specialized cells within the embryo sac (the female gametophyte of flowering plants) known to secrete the chemoattractants that guide pollen tubes to fertilization of the egg cell. They are located inside the ovule, and show strong $[Ca^{2+}]_{cyt}$ oscillations upon arrival of the pollen tube tip, suggesting that direct physical interaction of these two cells is needed to elicit oscillations, while the amplitude of peak $[Ca^{2+}]_{cyt}$ could play a role on promoting programmed cell death [23,24]. In fact, another member of the CrRLK1L receptor-kinases, FERONIA, has been reported to modulate such oscillations induced upon cell-cell communication [25].

Root hairs (Figure 1) are the other type of tip-growing cells in flowering plants, which also display tip-focused $[Ca^{2+}]_{cyt}$ and $[H^+]_{cyt}$ oscillations associated with

Figure 2



Varying complexity of oscillatory signatures found in pollen tubes. **a**. **Limit cycle attractor**. Top: Synchronized oscillations of tip-pH and growth rate shown in a phase-space plot + time. A limit cycle attractor is evident over time, revealing synchronized oscillations of cytosolic tip pH and growth rate over the course of the cell growth (interval shown c.a. 18 min). Bottom: quantitative spatial profile of cytosolic pH in Arabidopsis pollen tube (after heavy vectorization). Data from [14]. **b**. **Regime transitions**. Top: Time series of pollen tube growth rate and cytosolic Ca^{2+} at the tip. Bottom: phase-space plot showing regime "a" —steady growth; regime "b"—highly synchronized oscillations between growth rate and $[Ca^{2+}]_{cyt}$; regime "c" —growth arrest and $[Ca^{2+}]_{cyt}$ spiking. Data from [15]. **c**. **Complex oscillations**. Top: Time series of an Arabidopsis pollen tube growth rate, cytosolic pH at the tip and shank. Bottom: main periodic components over time found in the synchronized oscillations between tip pH and growth rate, estimated with a cross-wavelet transform. The heatmap indicates the power attributed to each period at every time point, with significant components ($p < 0.01$) circled in white. The power peaks were marked with a black dot (wavelet ridges), evidencing the drift in the main component as a line (frequency drift), as well as the existence of 2 or 3 parallel components (multiple coexisting frequencies). The pale region indicates the cone of influence, a region where estimates are not reliable. The mean power of each component over time is shown with a curve in the right side of the plot. The peak at 1.35 min does not reflect the almost 2 fold drift in period (from 1 to 2 min oscillations), posing challenges for experimental design and analysis. Data from [14].

growth and other cellular processes [26]. Curiously, they also express and have known phenotypes for FERONIA receptor-kinases [26]. These functional and molecular analogies between pollen tubes and root hairs have been explored to tackle one of the biggest challenges in the field: the identification of molecular mechanisms involved in generating these oscillations. One recent example is the association of cyclic nucleotide-gated channels (CNGCs) to Ca^{2+} oscillations. In pollen tubes, CNGC8 and CNGC18 were found to interact at the plasma membrane to form a hetero-tetramer, and further associate with Calmodulin2 (CAM2) [27]. The combined heterologous expression of CNGC8+CNGC18+CAM2 in HEK293T cells can generate Ca^{2+} oscillations, proposed to involve alternating channel gating by CAM2 binding, during the low $[Ca^{2+}]_{cyt}$ phase of the oscillatory cycle, and closure by CAM2 unbinding during the high $[Ca^{2+}]_{cyt}$ phase [27]. Similarly, tip-focused $[Ca^{2+}]_{cyt}$ oscillations were found to depend on CNGC6, CNGC9 and CNGC14 in root hairs [28]. Interestingly, double mutants lacking CNGC14 abolished oscillations in the 30 s range, while the single mutant *cngc9* showed irregular oscillations with slightly longer periods. These phenotypes could be interpreted as effects of the combination of CNGCs subunits forming different heteromers, as revealed in pollen tubes [26]. CNGCs were also shown to behave as hyperpolarized-activated Ca^{2+} channels, suggesting a role of membrane potential in the regulation of oscillations [28].

The pollen tube oscillator: Integrating cellular processes

Oscillations in tip growth rate constitute a challenge in themselves. Winship et al. [29] reported periodic alterations in cell wall tip morphology along an oscillation cycle in lily pollen tubes. They vary from a prolate to an oblate spheroidal tip form, with the curvature peaks preceding growth spurts. These modifications in shape entail changes in tip curvature, indicating variations in

cell wall expansion and transient tensile forces. Modeling of pollen tube cell wall mechanics and hydraulics was used to argue that multiple control points can introduce delays and generate oscillations in tip-growing cells [30]. For example, delay in adjusting an "impermeable callose sleeve" in the pollen tube shank to tip growth can generate large amplitude oscillations in growth rate (c.a. 15 μm min^{-1}) with small amplitude oscillations of turgor and osmotic pressure (c.a. 0.05 MPa). Despite the lack of experimental support for this particular model, these approaches provide a set of very clear conceptual scenarios where regulatory feedbacks in cell wall mechanics and hydraulics can generate oscillations, integrating previous modeling approaches to cell wall mechanics [30].

A key challenge in understanding apical growth in pollen tubes is to determine the relationship between temporal oscillations with the mechanism that establishes the intracellular polarity site. In many systems, intracellular polarity and membrane domains involve the Rho family of small GTPases (in plants, also designated by ROP-Rho of Plants) that can regulate processes like vesicle trafficking, cytoskeletal dynamics and ultimately organize their specific subcellular locations, like during the definition of cell polarity [31]. Various mathematical models around the central role of ROP-GTPases in pollen tube oscillations have been proposed (e.g. [32]), although they did not explicitly account for oscillations. Recently, a model involving ROP1 and Ca^{2+} in pollen tubes was proposed to account for important qualitative characteristics of pollen tube growth, such as apically-focused concentrations and oscillations with ROP1 phase-leading Ca^{2+} [33]. The model is based on a reaction-diffusion mechanism with a two-component "activator-inhibitor" system, where ROP1 activates both itself and Ca^{2+}, while Ca^{2+} inhibits itself and ROP1. Despite its purely mathematical nature, the study draws parallels with dynamic system principles that can explain the spontaneous emergence of oscillatory dynamics (Hopf bifurcation; Box 1) and spatially constrained foci (non-homogeneous solution). These are important steps toward a robust model of the elusive oscillator at the pollen tube tip, but it still requires more detailed validation of the assumptions and predictions, as well as mathematical analysis of the role of time delays.

Despite the tight association of ion dynamics and tip growth, their relationship is complex and not fully understood. Pollen tube growth is powered by electrogenic H^+ extrusion at the shank of the tube, which was found to be largely accomplished by three autoinhibited H^+ ATPases (AHA6, 8 and 9) [14]. The oscillatory signature of $[H^+]_{cyt}$ and extracellular H^+ fluxes are strongly altered in the *aha6/8/9* mutant, showing longer and more irregular periods, with an altered phase relationship with growth. Despite the genetic redundancy in the AHAs family, *aha6/8/9* critically impairs fertilization by causing early pollen tube growth arrest in the transmitting tract. These results suggest a central role of membrane potential, which is in agreement with experiments where membrane hyperpolarization led to Ca^{2+} influx and anionic efflux at the tip [34]. Understanding the role of membrane potential may lead to advances on multiple fronts, calling for further mechanistic studies.

Oscillations in apical-growing moss protonema

Protonema filaments of the moss *P. patens* also show oscillations in tip-focused $[Ca^{2+}]_{cyt}$ together with growth rate and actin dynamics [35]. These oscillations are complex (Figure 1), with multiple co-existing frequencies and time-varying (non-stationary) components (Box 1), also seen in Arabidopsis *in vitro* grown pollen tubes (Figure 2c) [15]. Protonema oscillations were found to be influenced by PIEZO mechanosensitive channels [19], which are permeable to Ca^{2+} and other cations in animal cells. Interestingly, in the moss protonema PIEZO channels are targeted to the vacuolar membrane instead of the plasma membrane, suggesting that this organelle has an important role in $[Ca^{2+}]_{cyt}$ homeostasis, presumably by functioning as a regulated Ca^{2+} store. In consonance, the double mutant *Pp*PIEZO1/2 has weaker oscillations in the 2 min range, demonstrating a role of PIEZO in the generation of the Ca^{2+} signature. Overexpression of *Pp*PIEZO2 in the double mutant restored oscillations in the 2 min range, albeit increasing the average period and also affecting faster oscillations. Tip-focused $[Ca^{2+}]_{cyt}$ in *P. patens* were also found to be altered by chitin oligosaccharides found in potentially pathogenic fungi, disrupting tip-focused actin filaments and increasing the frequency of oscillations at the whole-plant level [36].

Manipulating membrane potential

Membrane potential has long been hypothesized to be an integrator of ion dynamics across the plasma membrane in pollen tubes [37]. Likewise, it has been implicated in emergence phenomena like oscillations and waves capable of encoding information for local and distal responses alike [38]. Recently it became possible to directly manipulate membrane potential in plant cells by optogenetics gated ion channels [39,40]. A blue light–activated potassium channel (BLINK1; [41]) was used to modulate stomatal opening by increasing K^+ fluxes into guard cells [39]. In a critical step for plant biology, enzymes converting β-carotene into retinal were engineered into plant cells, supporting the use of rhodopsin-based optogenetics [8]. Activation of a green light–gated anion channelrhodopsin (*Gt*ACR1) resulted in membrane depolarization caused by anion efflux [8]. The technique allowed the induction of membrane oscillations in a wide range of frequencies, though constant illumination was sufficient to promote stomatal

closure [9]. Future work can further test the hypothesis that $[Ca^{2+}]_{cyt}$/membrane potential oscillations accelerate stomatal closure [42]. In pollen tubes, membrane depolarization-induced at a lateral region of the apex affected growth direction [8], while membrane potential oscillations could be imposed in single tubes [43] although their physiological implications are yet to be investigated. Hypothesis-driven experiments will be facilitated by an expanding optogenetics tool-set for different ions, affecting membrane potential and downstream processes [43].

Biotic interactions

Specific signatures of Ca^{2+} oscillations have also been linked to particular biotic interactions such as symbiosis events (nodulation/mycorrhization; Figure 1) and immune responses, with traveling waves (often associated with electrical signals) in response to wounding and herbivory (reviewed in [44,45]). Nuclear Ca^{2+} oscillations in root hair cells have been implicated in triggering specific developmental responses to microbial symbiosis, which also involve CNGCs [46]. Interestingly, minute-range nuclear Ca^{2+} oscillations in epidermal lateral root cells are elicited by symbiotic organisms through molecules such as chitooligosaccharides (COs) and lipochito-oligosaccharides (LCOs) produced by mycorrhizal fungi, and peptidoglycan (PGN) and LCO present in rhizobia bacteria [47]. However, immunity signaling is concomitantly activated by these molecules individually, being potentially suppressed by their combined action. In *Medicago truncatula*, Ca^{2+} oscillations in response to either CO or PGN were abolished in CO signaling mutants *cerk1* (Chitin elicitor receptor kinase 1), *lyr4* (lysin motif receptor-like kinase 4), *dmi1* (Does not Make Infections) and *dmi2* mutants, while they remained in mutants for LCO signaling *lyk3* (LYSM-CONTAINING RECEPTOR-LIKE KINASE 3) and *nfp* (nod factor perception) [47]. These results suggest that discriminating microbial symbionts and pathogens does not rely simply on the specificity of nuclear Ca^{2+} signatures, but rather on a more complex response elicited by different cell types (trichoblasts and atrichoblasts) to characteristic combinations of molecules, leading to symbiotic or immune responses.

The root clock

Spatial patterning of lateral roots, whereby emergence sites are established periodically (c.a. 6 h) in the main axis of the growing root (Figure 1), is achieved by the so-called root clock [48]. Multiple cells are involved in the process of generating periodic waves of auxin signaling and gene expression supposed to regulate the location and number of lateral root emergence sites. Oscillations in auxins, reported by the synthetic auxin reporter DR5 at lateral root pre-branch sites, were found to be anticipated by a retinal signaling reporter by 5 h [49]. Decreased auxin oscillation amplitude induced by retinal inhibition could be rescued by retinal treatment, whereas mutation of TEMPERATURE INDUCED LIPOCALIN (TIL, a putative retinol-binding protein) decreased oscillation amplitude and retinal sensitivity suggesting it participates in the regulation of the root clock [49]. A novel "reflux-and-growth" mechanism underlying the periodic formation of lateral roots was proposed based on a computational model, yielding auxin oscillations that lead to periodic variations in cell size [50]. The model predicts a relationship between meristematic activity and spatial patterns of prebranch site formation, setting it apart from traditional formalisms employed in modeling morphogenesis.

Other rhythms in plant growth and shape

There are other rhythmic growth processes in plants such as circumnutation, the helicoidal movements of leaves or roots around their main axis (Figure 1). Circumnutation oscillations in the root tip position occur with a period of roughly c.a. 1.5 h in *Oryza sativa* [51], and are drastically reduced in amplitude and regularity in a mutant for histidine kinase-1 (HK1). Ethylene receptor inhibitors mimicked the loss of circumnutation in wild-type plants, while exogenous addition of cytokinins or synthetic membrane-permeable auxin rescued circumnutation in *hk1* mutants. Accordingly, visible oscillations were abolished in a mutant for the auxin importer AUX1, suggesting that circumnutation is regulated by a pathway comprised of ethylene acting on HK1, which promotes cytokinin signaling and auxin transport [51]. This work includes evidence that circumnutation allows object avoidance in growing roots, which should be critical for seedling establishment in rocky soils. Coleoptiles, fast elongating shoots derived from the embryonic shoot meristem, also oscillate in growth rate and curvature with a period c.a. 2 h; when coupled in anti-phase, these oscillations can dampen and yield straight growth of the coleoptile [52]. Oscillations in multiple aspects of plant growth and shape have been argued to provide a form of proprioception (the ability of an organism to sense movement, action and location) whereby plants can adjust their internal processes to respond to environmental fluctuations [52].

Biological functions of oscillations

Biological oscillations can arise from simple regulatory motifs, such as a delayed negative feedback loop [5]. In fact, oscillations may actually be difficult to prevent and rather reflect a byproduct (epiphenomenon) or a pathological state, instead of performing specific biological functions [5,16]. This discussion seems inevitable in any field where oscillations are involved, accompanied by major controversies regarding the identity of the *bona fide* oscillator [6]. Nonetheless, these rhythms are manifestations of underlying regulation, with frequency and amplitude being an emergent property of the

oscillatory network, inheriting powerful properties that are even used in artificial communication systems (e.g. radio) [5,53].

Oscillations can support multiple biological functions such as encoding signals in terms of oscillation frequency, amplitude, waveform and duration (Box1; [16]). In the context of intracellular signaling, while signal encoding can be accomplished by a collection of receptors, channels and transporters, decoding can involve single proteins or entire pathways [16,54]. Ca^{2+} signaling is the best studied field in this regard, with Ca^{2+}-binding proteins acting in both transcriptional and post-translational pathways to decode Ca^{2+}signatures (reviewed in [54—56]). Frequently, Ca^{2+}decoders have an EF-hand motif where Ca^{2+} binds (e.g. calmodulin; CaM) that can activate specific transcriptional responses together with CaM-biding proteins in plant immunity [55]. At the post-translational level, phosphorylation plays a major role in decoding Ca^{2+}signatures such as the Ca^{2+}/CaM-dependent protein kinase (CCaMK), supposed to discriminate Ca^{2+} spiking signatures in symbiosis signaling [55]. In addition, the Ca^{2+}-dependent protein kinases (CPKs), and the calcineurin B-like proteins, when conjugated with their partners CBL-interacting protein kinases (CBLs/CIPKs), are known Ca^{2+} sensors, that can also induce the production of reactive oxygen species (ROS) in response to pathogens [55] and many other processes. Evolutionarily speaking, Ca^{2+}decoders expanded remarkably in plants suggesting the importance of specificity in Ca^{2+} signaling [56].

Specific decoders can avoid crosstalk in the promiscuous usage of the same second messenger (e.g. Ca^{2+}) by multiple pathways, enabling to encode information in oscillatory dynamics even in the presence of simultaneous signals, for example through frequency multiplexing [16]. Incoherent feedforward loops—a common regulatory motif that mixes both activation and inhibition of an output— can distinguish between an oscillatory versus a sustained signal and even allows counting, which could be a mechanism to respond to a specific number of Ca^{2+}spikes [57]. Even a single molecule, such as CaMKII, can act as a decoder of Ca^{2+}spikes due to properties like autophosphorylation that promotes the continued activity of the protein after the Ca^{2+} signal subsides, acting as a molecular memory [16]. In fact, the Venus flytrap has been reported to "count" Ca^{2+}spikes by mounting a response if subsequent stimuli occur closer than a critical time interval, then closing the leaf blades [58].

Despite the general lack of detailed mechanistic knowledge capable of pinpointing the exact role and specificity of oscillatory signatures, multiple advantages to oscillatory signals have been discussed [16]. For instance, oscillatory signals may be more advantageous in terms of resource economy. Reaching a peak concentration involves more resources if the value is maintained than if it is allowed to decrease and reestablish successively. Additionally, oscillations may help to prevent pathological states induced by sustained high levels of a signal, such as deleterious effects of high calcium levels and the formation of insoluble calcium salts [16]. Furthermore, multiple mathematical models suggest that oscillations can confer robustness to noise, prevent desensitization and allow fine-tuning regulation [16].

Although transcriptional regulation is often thought as the downstream effect of oscillatory signals, biological oscillators may perform local signal processing such as ion dynamics and membrane potential at the plasma membrane, and may be involved in morphogenesis and intercellular communication [6]. In the case of the pollen tube, the elusive biological function of their synchronized oscillations may lie in cell-cell communication with the ovary, ovule and embryo sac cells through its journey to fertilization. The "ping-pong" observed in Ca^{2+}spikes during male—female communication could allow for a coordinated response in ion dynamics and guidance, possibly assisting in the spatiotemporal control of sperm cell delivery. The characteristic frequency and amplitude of both synergid and pollen tubes could support resonance between their underlying limit-cycles, allowing the control of burst timing and aiding species specificity (as suggested by emerging results [59]). This hypothesis adds an ion-based component to the current understanding of pollen tube guidance, growth and burst control, which should be further developed and tested.

Conclusions and outlook

Oscillations, waves and many types of pattern formation processes stem from overarching principles of dynamical systems that can manifest themselves in all spatiotemporal scales [60]. In order to tap into the power provided by the mathematical understanding of these principles, approaches capable of precise spatiotemporal quantification are needed. However, there are multiple statistical and sampling challenges due to their inherent complexity. Although biological rhythms can be quite regular, often they show multiple coexisting frequencies and/or variability of oscillatory properties, like period and amplitude, across time. Thus, methods used frequently, like auto- or cross-correlation can have limited resolution or even lead to erroneous interpretations [15]. In addition, even cells from the same type under the same conditions may have distinct oscillatory behavior, which imposes difficulties in establishing the effect of mutations for example. Other than using or developing appropriate statistical methods, experimental design should focus on making physiological regimes comparable. In the case of apical

growing cells, growth rate/regime is still omitted from the analysis all too often even though multiple lines of evidence show its importance for the oscillatory regime [6,15].

In terms of experimental guidelines, it is critical to use an adequate sampling rate and total sampling time. As a minimal dataset, one must plan to capture more than 3 points per oscillation cycle (that is above the Nyquist frequency) and at least 3 complete cycles per replicate. Furthermore, choosing the appropriate sensor and imaging technique demands considering multiple aspects, such as their temporal resolution, dynamic range and sensitivity [61]. Fortunately for the field of ion dynamics, choices of genetically encoded probes are ever-growing, with the option to specifically target the nucleus, ER, apoplast, vacuole and other compartments [61,62], and to assay multiple ions simultaneously [20]. In addition, these technical developments will be critical to establish the role of intracellular stores in the oscillations. Care must be taken when interpreting data from a complex oscillatory system, since there is a tendency to assign the identity of "the oscillator" to any oscillatory variable assayed, especially if preceding other processes [6].

Despite the enormous variability in time scale, amplitude, wave-form and spatial behavior, biological oscillations tend to show some overall distinction in their control. Regular oscillations performed by "clock-like" systems, where the period is robust, allow spatial patterning (root clock) and anticipation of periodic events (circadian clock and flowering time). Tunable oscillations more akin to an analog "radio-like" system—to keep metaphors in the realm of home appliances – include complex time-varying oscillations, like the ones seen in Arabidopsis pollen tubes, *P. patens* caulonema, guard cells and Ca^{2+} signature encoding biotic interactions. Indeed, regulatory motifs of oscillatory systems tend to differ in terms of design and functionality. Roughly speaking they provide either a tunable frequency with robust amplitude (e.g. heartbeat) or a robust periodicity, with greater amplitude variation (e.g. circadian clocks) [53]. While functioning as a window to regulatory dynamics, whether ultradian oscillations perform specific biological functions on their own, or if they are simply a byproduct of the regulatory structure, seems to be a recurrent theme plaguing experimental design and the mechanistic understanding of their biological implications [6]. Fascinating in their manifestations, ultradian oscillations in plant cells often involve a unique "electrifying" mechanistic basis relying on ion channels and probably membrane potential. Future research in plants received a jolt of energy with novel optogenetics tools for direct manipulation and hypothesis testing, promising to power a new era of mechanistic insight.

Conflict of interest

Nothing declared.

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