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Organization of early frog embryos by chemical waves emanating from centrosomes

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The large cells in early vertebrate development face an extreme physical challenge in organizing their cytoplasm. For example, amphibian embryos have to divide cytoplasm that spans hundreds of micrometres every 30 min according to a precise geometry, a remarkable accomplishment given the extreme difference between molecular and cellular scales in this system. How do the biochemical reactions occurring at the molecular scale lead to this emergent behaviour of the cell as a whole? Based on recent findings, we propose that the centrosome plays a crucial role by initiating two autocatalytic reactions that travel across the large cytoplasm as chemical waves. Waves of mitotic entry and exit propagate out from centrosomes using the Cdk1 oscillator to coordinate the timing of cell division. Waves of microtubule-stimulated microtubule nucleation propagate out to assemble large asters that position spindles for the following mitosis and establish cleavage plane geometry. By initiating these chemical waves, the centrosome rapidly organizes the large cytoplasm during the short embryonic cell cycle, which would be impossible using more conventional mechanisms such as diffusion or nucleation by structural templating. Large embryo cells provide valuable insights to how cells control chemical waves, which may be a general principle for cytoplasmic organization.

1. Introduction

Physical extremes in biology are interesting. They may reveal special mechanisms, or underappreciated aspects of widespread mechanisms. Here, we address the challenges faced by cells—frog zygotes and early blastomeres—that are extremely large, but need to divide rapidly. *Xenopus laevis* eggs are 1.2 mm in diameter, and they divide every 30 min. These numbers represent extraordinary challenges in terms of spatial and temporal organization: How can a millimetre-scale cell be spatially patterned by nanometre-scale molecules? How can a cell that would take a protein hours to cross by diffusion alone generate the precisely controlled timing required for cell cycle progression on a scale of minutes? We argue, based on observations by others [1] and our group [2–4], that these spatial and temporal organizational challenges may be solved by a common class of biophysical mechanism: chemical reaction waves, also called 'trigger waves' [1]. Furthermore, the centrosome plays a key role in organizing these waves, and can be re-imagined as a wave-initiating site.

A chemical reaction wave is a special case in kinetic organization of dissipative dynamical systems. It requires an excitable medium that locally amplifies a chemical state so the state can propagate through the medium faster than diffusion. Familiar examples include the Belousov–Zhabotinsky reaction [5], action potential in neurons [6], calcium waves during fertilization [7] and cAMP waves in a population of *Dictyostelium* cells [8,9]. Microtubule organization and cell cycle progression are not normally considered as chemical waves, apart from a few notable exceptions [10–13]. We will argue that this is an interesting way to think about their spatio-temporal organization in frog zygotes, and that chemical waves are perhaps the only way the huge zygote cell can self-organize

in space and time. By extension, we believe chemical waves may be much more prevalent inside cells than has been thought, and may play important roles in organizing small [14–16] as well as large cells.

The centrosome plays a special role in our hypothesis as an *initiator*. Chemical waves can occur spontaneously in excitable media, but in many cases a defined initiation site provides a trigger or catalyst to initiate waves. As the wave propagates outwards, it carries spatial information on the location of the initiator to distant sites. In this way, we believe that the centrosome informs the millimetre-sized frog egg of the location of its centre. Chemical waves allow this spatial information transfer to occur in minutes, even in the millimetre-scale cytoplasm. It would take many hours by diffusion alone, and might be impractical by microtubule polymerization alone. The centrosome was defined in the nineteenth century as the centre of the cell largely based on observation of large embryo cells [17,18]. In the 1980s, its role as a microtubule nucleation template was established [19]. Since then it has been viewed as organizing cells primarily by templating minus ends using γ-tubulin complexes. The centrosome has also been implicated in cell cycle control. We propose that the centrosome coordinates space and time in extremely large cells by initiating chemical waves of cell cycle progression [1] and microtubule organization (this review). We write to provoke discussion, with the warning that some of our ideas are quite speculative. In the spirit of this volume, we hope to generate feedback and new experiments. Even if only partially correct, the idea that the centrosome functions as a chemical wave initiator is an interesting new way to think about spatio-temporal organization of cells.

2. Chemical waves: the basics

Pattern formation by reaction–diffusion systems has foundations in physics and chemical engineering, but has broad implications to epidemiology, ecology and cell biology (reviewed in [20]). It represents a general class of mathematical models that explains how patterns arise from collective interactions occurring at much smaller scales. Turing famously analysed a pair of interacting, diffusible chemical species and derived the conditions that give rise to spontaneous emergence of apparently stable, periodic patterns, providing a hypothetical conceptual basis for morphogenesis long before any morphogens had been identified [21]. Here, following the early works of Luther [22], Fisher [23] and Kolmogorov & Petrovskii [24], we use the example of a simple one component system that conveys the essence of how reaction–diffusion systems generate transient waves inside cells. Imagine that the cytoplasm exists anywhere between the 'on' and 'off' state of some biochemical activity, in our case, mitotic Cdk1 kinase activity and microtubule assembly (figure 1*a*), and that this activity can vary over time and space. The medium may support transient waves if the following two requirements are met: (i) an autocatalytic reaction exists which promotes the 'on' state and (ii) reactant transport occurs, for example by molecular diffusion, but other transport processes could substitute. A prototypical example of an autocatalytic reaction is the logistic growth model, in which a chemical species increases exponentially and saturates. Starting with an initial concentration restricted in space, growth (rate α (1/min)) in the absence of any transport will predict this local region to increase ('on') while the rest of the system remains 'off' (figure 1*b*). With a similar initial condition, diffusion (D ($\mu m^2\,min^{-1}$)) alone will predict the gradual homogenization of reactants (figure 1*c*). However, when growth and diffusion occur simultaneously and satisfy quantitative conditions, the system gives rise to a moving front that travels at a linear speed of order $\sqrt{4D\alpha}$ ($\mu m\,min^{-1}$) [22–24] (figure 1*d*). As the front passes through a given location, the state of the cytoplasm is converted from the 'off' to the 'on' state. At the microscopic level, this could be understood as a cascade of active molecules diffusing into adjacent space to induce more activity. With more complex oscillatory reactions, this mechanism may result in pulsing or spiral patterns. Importantly, this phenomenon is observed in a continuous medium of arbitrary size and shape. To describe the dynamic patterns that emerge in reaction–diffusion systems in cells, we will use the term 'chemical waves' to emphasize the biochemical nature of the cytoplasm.

Owing to their ability to rapidly communicate information across space without a predefined blueprint, chemical waves have great potential as the physical basis supporting cellular physiology. This may be particularly useful for large cells, where simple diffusion of signalling molecules would be too slow. For example, a typical protein with a cytoplasmic diffusion coefficient of $D = \sim 500\ \mu m^2\ min^{-1}$ [25,26] would take hours to travel from the centre to the periphery of a frog egg with radius 600 μm (time $t = L^2/6D = 120$ min). By contrast, cell cycle waves traverse similar distances in minutes [1,27] and calcium waves in tens of seconds [7]. This can be understood by assuming an autocatalytic reaction of rate approximately $1\ min^{-1}$, resulting in a chemical wave of speed $\sqrt{4D\alpha} = \sqrt{4 \times 500 \times 1} = 45\ (\mu m\ min^{-1})$, which will travel the same distance in approximately 13 min. Despite their rapid, adaptable properties, chemical waves possess potential drawbacks. In addition to requiring particular biochemistry and energy dissipation, excitable systems are prone to spontaneous waves triggered by fluctuations in local concentrations. This presents a challenge in adopting chemical waves for highly coordinated physiological processes such as cell division, since spontaneous initiation would defeat the purpose of a spatial organizing system. One strategy to mitigate this issue is to implement a strong, nonlinear negative feedback into the system, which increases the threshold for activation. Another non-exclusive strategy is to robustly and quickly initiate the reaction at a controlled location and allow the chemical wave to sweep through large space before any spontaneous waves occur. In this scenario, the initiator's role is analogous to that of the conductor in an orchestra who instructs the correct timing of biochemical reactions with a common signal that spreads across the cell. For both cell cycle progression and microtubule organization, we argue that the centrosome acts as the initiator for these chemical waves, albeit by poorly understood mechanisms. In the following sections, we discuss how cell cycle progression and microtubule aster organization are coordinated in the early frog embryo. To evaluate critically the hypothesis that chemical waves emanating from centrosomes form the physical basis of these phenomena, we ask whether the systems exhibit two key characteristics of chemical waves: (i) the cytoplasm supports autocatalytic reactions and (ii) these reactions rapidly propagate activity through

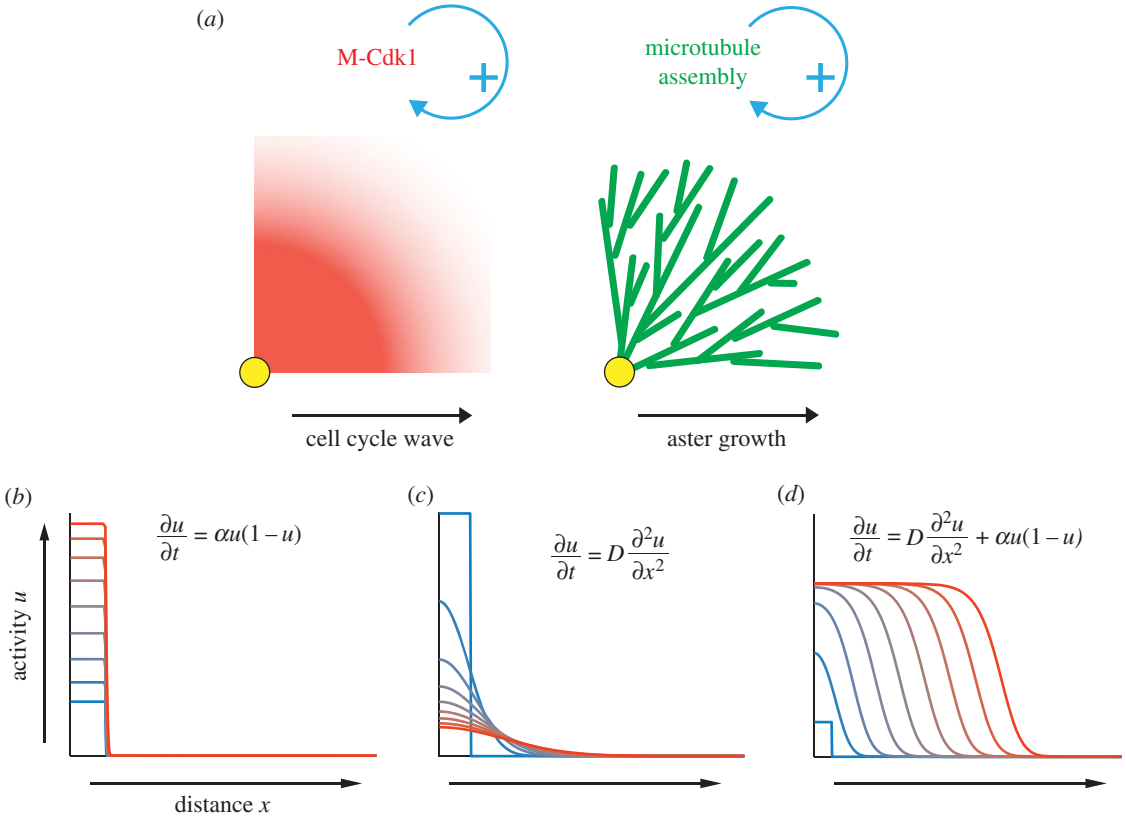

Figure 1. Chemical waves initiated by the centrosome. (*a*) In our hypothesis, the centrosome (yellow circle) triggers two types of autocatalytic reactions that spread radially outward through the cytoplasm. Cell cycle waves are mediated by mitotic Cdk1 feedback regulation, while aster growth is mediated by microtubule-stimulated microtubule assembly. (*b–d*) Requirements of the cytoplasm to support chemical waves. Curves show spatio-temporal dynamics of biochemical activity according to the equations shown, which are non-unique examples of each situation. (*b*) Growth, or an autocatalytic reaction, with saturation results in local amplification of activity. (*c*) Diffusion results in the homogenization of activity. (*d*) When growth and diffusion are coupled, a propagating front or a chemical wave, may be observed. Equations are shown for the one-dimensional case, but the long time-scale prediction of a propagating wavefront is remarkably robust for higher dimensions, radial geometry and a range of initial conditions. Note that the logistic growth term in (*d*) represents a broad class of reactions with positive feedback, including the growth phase of excitable/bistable kinetics.

space without dilution. We also ask if, and how, the centrosome functions as an initiator.

3. Cell cycle progression as a chemical wave

The discovery of surface contraction waves (SCWs) in large amphibian zygotes is perhaps the earliest observation of cell cycle states propagating through the cytoplasm. In time-lapse images of fertilized eggs made in the 1970s, Hara observed a travelling wave of cortical contraction, visualized by movement of pigment granules. These SCWs occurred just before the first cleavage and proceeded as circular waves from the animal to the vegetal pole [27,28] (figure 2*a*). SCWs consist of two distinct waves: SCWa was the result of the relaxation of the cortical tension, whereas the succeeding SCWb was a stiffening of the cortex [30]. The two sets of waves occurred periodically corresponding to the period of normal cleavage cycle in developing embryos [27], suggesting that they were a manifestation of cell cycle control of cytoskeletal behaviour [27,31,32]. Subsequent research demonstrated that increase and decrease in mitotic Cdk1 activity was responsible for the onset of SCWa and SCWb [11,33], suggesting that SCWs are mitotic entry and exit waves, respectively. SCWs that travel across the millimetre cytoplasm in early amphibian embryos are a dramatic example of cell cycle regulation in space. Cell cycle waves have also been reported in other large cells [34–36]. They fulfill one important criterion for chemical waves in that they travel at near constant velocity without dilution of activity, but these data alone do not establish that the egg is an excitable medium for cell cycle progression.

What is the molecular basis of the cell cycle waves travelling across large amphibian eggs? The biochemical clock that generates sustained cycles of division is centred on cyclin B-Cdk1 kinase activity (reviewed in [37,38]). This system oscillates due to continuous synthesis and periodic destruction of cyclin B, and harbours positive feedback of kinase activation by the Cdc25C phosphatase and Wee1A/Myt1 kinase. The potential for chemical waves of mitotic activation in this system had been noted [11,39]. In a recent report, Chang and Ferrell proved that waves can indeed occur by combining *in silico* and *in vitro* experiments [1]. Using computer simulations to model the spatial changes in cell cycle state, they showed that a locally elevated activity of Cdk1 should propagate through a large cytoplasm as a chemical wave. The propagation speed of such waves is predicted by the kinetics of the cell cycle oscillator and diffusion of proteins and was estimated to be 40–120 μm min^{-1}, which is in similar order with the speed of SCWs at approximately 60 μm min^{-1} observed *in vivo*. Next, the propagation of cell cycle waves was reconstituted in a cell-free system by filling Teflon tubes with cycling egg extract supplemented with sperm nuclei. To monitor cell cycle progression, times of nuclear envelope assembly and breakdown were recorded (figure 2*b*). After the first

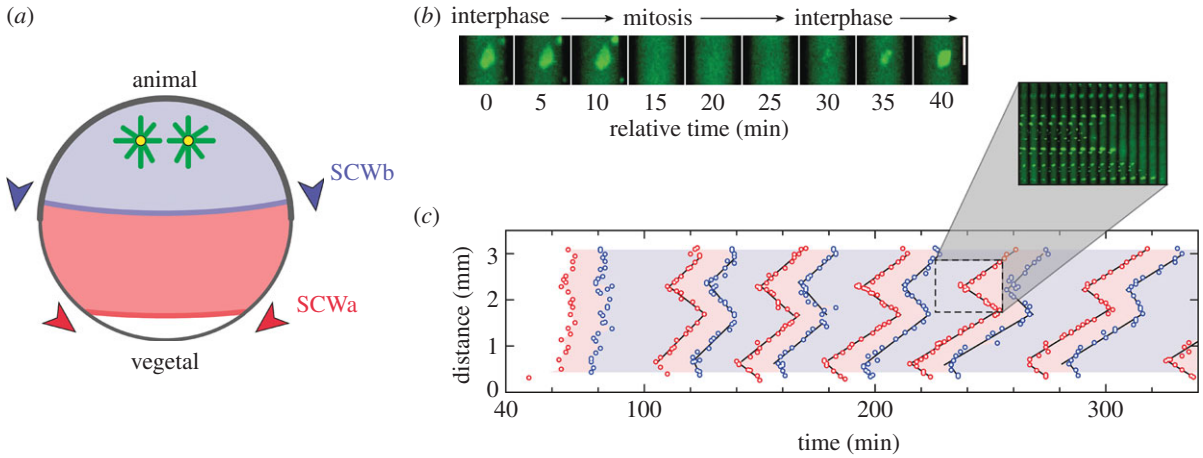

Figure 2. Cell cycle waves in large cytoplasm. (*a*) In fertilized frog embryos, SCWs travel from the animal to the vegetal pole. Regions of high (pink) and low (purple) mitotic Cdk1 activity coexist in the common cytoplasm. Centrosomes (yellow) reside in the animal half of the embryo until the eight-cell stage. Illustration adapted from [29]. (*b*,*c*) Reconstitution of cell cycle waves in cycling *Xenopus* egg extract filled in a Teflon tube. Reproduced with permission from *Nature* [1]. Copyright © 2013 Macmillan Publishers Ltd. (*b*) Cell cycle state is monitored by nuclear envelope dynamics with GFP fused to nuclear localization sequence. (*c*) Spatial dynamics of cell cycle inside tube of length 3 mm. Nuclear envelope breakdown (red points) and reformation (blue points) indicate whether the cytoplasm is in mitosis (pink) or interphase (purple). The sloped lines indicate cell cycle waves and their velocities.

synchronous cycle, the centre of the tube first started to enter mitosis and this spread laterally across the entire tube at approximately 60 μm min^{-1} (figure 2*c*). Multiple pairs of mitotic entry and exit waves were observed in the same reaction over time. Inhibition of Wee1A/Myt1 accelerated the wave speeds in a dose-dependent manner underscoring the fact that the strength of autocatalysis imposed on Cdk1 activity is an important parameter for the speed of these waves. As the extract contained multiple nuclei and centrosomes in these experiments, it is difficult to draw conclusions on how their presence affected the kinetics of cell cycle progression. However, the striking finding from Chang and Ferrell's work was that the cytoplasmic oscillator responsible for cell cycle progression is sufficient to generate chemical waves that temporally organize a large cytoplasm.

SCWs are initiated in the animal half of the zygote, suggesting the presence of an initiation site. Accumulating evidence suggests that the centrosome initiates the mitotic entry wave. Injection of purified centrosomes accelerates mitotic entry in frog and starfish oocytes [40,41] consistent with an initiator role. Spindle pole bodies, the centrosome equivalent in fission yeast, are known to promote entry into mitosis [42–44]. In somatic cells, active cyclin B-Cdk1 concentrates on the centrosome in early prophase [45], and centrosomal recruitment of the Cdc25C phosphatase may be responsible for this initial activation [46]. The centrosome could also act indirectly, by positioning the nucleus, which then acts as the proximal trigger of mitotic entry waves. The nucleus is thought to amplify mitotic Cdk1 activity through compartmentalization [47,48]. In this view, the centrosome and nucleus may together establish the perinuclear region as a robust initiation site for the mitotic entry wave [1]. Finally, we note that the nucleus and the animal half cytoplasm initiate SCW in the absence of centrosomes [27,40]. Therefore, it is likely that the additive effects of the centrosome, nucleus and the animal half cytoplasm ensure the robust initiation of the cell cycle in the animal half of the zygote.

In embryos [30] and egg extract [1], a mitotic exit wave follows the mitotic entry wave and has a similar velocity (figure 2*c*). Whether mitotic exit is a self-sustaining autocatalytic reaction that can generate waves, and if so what determines their velocity, remain to be determined. Feedback between Cdk1 and the anaphase-promoting complex/cyclosome (APC/C) through XErp1 [38,49,50] presents a potential autocatalytic mechanism for a mitotic exit, but it is not clear why this different reaction would propagate with the same velocity as the entry wave. An alternative is that the exit wave is a consequence of local changes in the cytoplasm that follow the mitotic entry wave with a fixed time delay [33], and therefore does not need to be autocatalytic or capable of wave propagation on its own. In either model, the centrosome may influence the kinetics. In human cells and early fly embryos, cyclin B-GFP localizes on spindles and starts to disappear first near the spindle poles in anaphase [51–53]. Thus, during mitotic exit, APC/C activity may be initiated at centrosomes and then propagate outwards.

4. Radial organization of microtubules as a chemical wave

The cell cycle wave discussed above presumably exists to synchronize the cell cycle across a large cell, rather than transmit positional information. We propose that microtubule asters also grow by a chemical wave mechanism in large embryo cells and, in this case, the goal is spatial organization and correct positioning of cleavage planes. In frog eggs, microtubule asters are small in mitosis due to high Cdk1 activity which limits microtubule growth [54,55] (figure 3*b*). When Cdk1 activity decreases following fertilization, or anaphase onset, asters grow out rapidly from centrosomes. The sperm aster grows to fill the whole cell (figure 3*a*), whereas anaphase asters fill half the cell, since they do not interpenetrate across the mid-plane (figure 3*c*,*d*) [3,4,56]. The outer edge of the aster expands at approximately 30 μm min^{-1} in *Xenopus* zygotes, and approximately 15 μm min^{-1} in zebrafish first mitosis [2,3]. This rapid growth is essential for the aster to fill the whole cell in time to position the centrosomes for the following mitosis and also to trigger cleavage furrow ingression following anaphase. Ingression initiates when, and

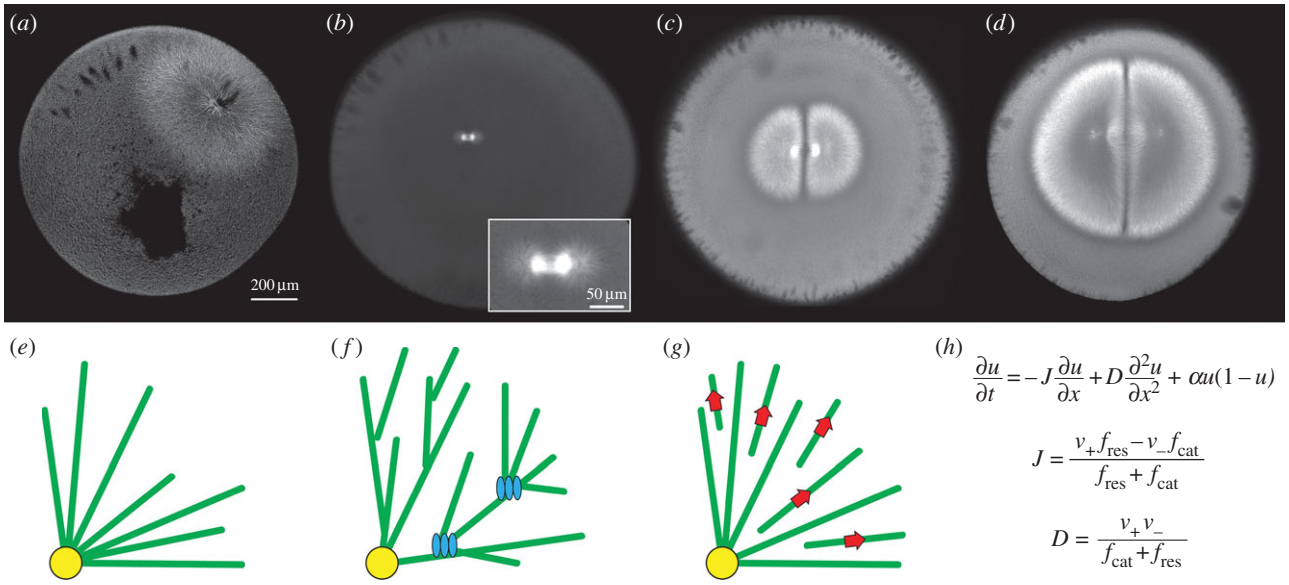

Figure 3. Growth of microtubule asters in the large interphase cytoplasm of frog zygotes. (*a–d*) Growth and interaction of asters in the first division in *Xenopus laevis*. Fertilized eggs were fixed, stained for tubulin and imaged from the animal pole by a confocal microscope as described [2,3]. (*a*) Sperm aster during the interphase following fertilization. The sperm aster eventually covers the entire cytoplasm. (*b*) Metaphase of first mitosis (spindle magnified in inset). Aster size is limited at spindle poles. (*c*) Anaphase–telophase of first mitosis. Aster growth and interaction between asters originating from the same spindle. (*d*) Later telophase. Note the dense, bushy appearance of microtubules at the aster periphery, low microtubule density in the interaction zone. (*e–g*) Models for aster growth in large cells. (*e*) Conventional radial elongation model. Microtubules polymerize outward from centrosomes (yellow). Microtubule density at the aster periphery decreases. (*f*) Nucleation away from the centrosome may occur on pre-existing microtubules or Golgi membranes (blue stacks). (*g*) Release and outward transport. Minus ends are released from the centrosomal nucleation site and microtubules slide outward (red arrows). (*h*) Reaction–diffusion model of microtubule aster expansion. v_+, v_- are rates of polymerization and depolymerization. f_{cat} and f_{res} are catastrophe and rescue frequencies of the microtubule plus end.

where, the overlap zone between the two asters expands to touch the cortex. Presumably, the aster–aster interaction results from the collective action of microtubule-associated proteins that specifically modulate the dynamics of antiparallel bundles [4] and is beyond the scope of the chemical wave model presented in this paper. In addition to growing rapidly, we believe it is important that microtubules in the aster are not spatially diluted as the asters grow. Initiating a furrow presumably requires signalling from some sufficient density of microtubules, and images in frog and fish embryos suggest that the aster retains an approximately constant microtubule density at its periphery as it expands (figure 3*d*). Propagation at a constant, rapid rate and lack of dilution during propagation are two of the hallmarks of chemical waves.

The standard model for aster growth, which we call the radial elongation model, is illustrated in figure 3*e*. Microtubules are nucleated with their minus ends anchored at the centrosome, and addition of GTP tubulin at plus ends promotes aster growth. In this geometry, the density of microtubules must decrease with radial distance r (density scales as approx. $1/r$ in two dimensions or $1/r^2$ in three dimensions). However, immunofluorescence images from frog embryos show that the microtubule network at the aster periphery has a bushy appearance, and its density appears to be constant, or even increase, with aster radius (figure 3*d*). Movies from zebrafish provided a similar appearance [3]. Given this microtubule distribution, it seems highly unlikely that all microtubule minus ends are anchored at the centrosome. We propose instead that microtubule nucleation occurs within the growing aster away from the centrosome [56], and that nucleation is stimulated by pre-existing microtubules (figure 3*f*). In support of this hypothesis, there is accumulating evidence for microtubule nucleation at non-centrosomal sites associated with pre-existing microtubules. In the cortex of higher plant cells, microtubules nucleate from γ-tubulin ring complexes attached to the side of other microtubules [57,58]. A similar self-amplifying microtubule nucleation process was demonstrated in *Xenopus* egg extracts arrested in meiotic metaphase, by a poorly understood process requiring augmin/HAUS complex [59]. The morphology of interphase aster growth is consistent with parallel nucleation templated from the walls of existing microtubules, but other hypotheses cannot be excluded at present. For example, microtubules might activate a kinase that locally promotes nucleation, as is seen for Aurora B kinase activity in meiotic metaphase [60,61]. The Golgi apparatus nucleates microtubules in interphase somatic cells [62–64], including neurons [65] and muscle cells [66]. Pre-existing microtubule could accumulate Golgi membranes and thus enhance nucleation. Whatever the molecular mechanism, several observations suggest the interphase cytoplasm may be an excitable medium for microtubule assembly. Electrically activated eggs, where no centrosome is introduced, form asters [67,68] and furrow [69]. Enucleated, activated eggs also form microtubule asters spontaneously [68]. Thus, microtubules eventually form, and perhaps expand as asters, in the absence of centrosomes or nuclei. Even following normal fertilization, microtubules assemble at the vegetal cortex to promote rotation of the cytoplasm relative to the cortex, and this population can form spontaneously [70]. Thus, the interphase cytoplasm does fulfill one property for an excitable medium: spontaneous formation of microtubules when the initiation site is not present.

Self-amplification of microtubules should prevent dilution of microtubule density in radial geometries. An interesting question is whether this has a kinetic contribution; in other words, does this accelerate aster expansion? Though we note that the rates of microtubule polymerization and that of aster

expansion are of comparable magnitude, microtubules in interphase asters evidently depolymerize ([3] and K. Ishihara & T.J. Mitchison 2013, unpublished data). In one model of dynamic instability [71], the transitions of microtubule plus ends between the growing and shrinking states have been modelled as a biased random walk at long time-scales [55,72]. The net polymerization rate (J) is simply the weighted average of the polymerization and depolymerization, where the weighting depends on catastrophe and rescue rates (figure 3*h*). Though the time-averaged net polymerization rate J is thought to take a positive value in interphase [55], it is considerably smaller than the instantaneous polymerization rate of growing plus ends (v_+). This raises the question of how the aster expands at a rate much faster than the net polymerization rate J. The chemical wave hypothesis offers a potential explanation to this apparent discrepancy. In the biased random walk model, the net polymerization rate J contributes through an advective term (also known as bias in direction), whereas the random walk behaviour, characterized by the coefficient D, is present in the diffusive term (figure 3*h*). It is important to note that the diffusive term does *not* represent a molecule diffusing through the cytoplasm, but rather the stochastic nature of microtubule plus end positions. When a reaction term corresponding to microtubule nucleation is introduced, the coupling of diffusive process with growth (nucleation rate alpha) predicts the overall aster expansion velocity V as $V = J + \sqrt{4D\alpha}$. Therefore, the chemical wave model offers a quantitative explanation to how microtubule-stimulated microtubule nucleation and dynamic instability may synergistically contribute to the aster expansion rate. An aster could expand to cell-spanning dimension in this model even if J was negative, as it is in mitosis. D and/or α would have to be correspondingly larger, so the net velocity of aster expansion V was positive.

Applying the prototypical reaction–diffusion equation above was more a conceptual starting point than a truly accurate description of aster growth. Many questions remain regarding the apparently simple process of how a large embryo aster grows. Though centrosomes are obvious candidates as initiators for microtubule waves, it is unknown whether their capability for microtubule nucleation is locally restricted or reaches longer distances [73,74]. Cytoplasmic dynein is thought to exert outward force on astral microtubules [3], so it is possible that minus ends are released and glide outwards (figure 3*g*). We have so far assumed the minus ends formed away from centrosomes are stable, presumably capped by γ-tubulin ring complex [75,76] or the CAMSAP/Patronin family of proteins [77–79], but they may well be free to depolymerize. The reaction–diffusion model presented above predicts that the aster expansion rate increases indefinitely with nucleation rate, but this seems physically implausible given that microtubules polymerize at finite speed. Reaction-telegraph equations that combine the process of growth and *persistent* random walk [80,81] may be better suited for predicting such physical bounds. In any case, we believe that the key to understanding aster growth lies both in the identification the key molecular factors and rigorous evaluation of quantitative models under the framework of chemical waves.

Finally, are the cell cycle and microtubule assembly waves that propagate from centrosomes independent, or are they coordinated in some way? During mitosis, high Cdk1 activity regulates a complex network of microtubule-associated proteins, promoting catastrophes and bounding microtubule length [54,55]. Passage of the mitotic exit wave removes this constraint, and may be sufficient to convert the cytoplasm from non-excitable to excitable for microtubule assembly. Alternatively, aster growth may require time-dependent activation of molecular factors during interphase. In either case, the microtubule polymerization wave depends on prior passage of the mitotic exit wave. It might be possible to co-image the two waves to gain insights into their coordination. In the early zebrafish embryo, actin-associated vesicles change motility behaviour as the zygote proceeds from mitosis to interphase, and particles closer to the centrosome are affected earlier than more distal ones [3,32], suggesting that they are responding to a mitotic exit wave emanating from centrosomes. Importantly, this motility change wave precedes the aster growth wave. In frog eggs, cell cycle waves travel at approximately 60 $\mu m\ min^{-1}$, which is faster than the estimated aster growth rate of approximately 30 $\mu m\ min^{-1}$. Therefore, a plausible scenario is one in which a preceding mitotic exit wave primes the cytoplasm to support aster growth.

We know much less about interphase aster disassembly at the onset of mitosis. This also needs to occur rapidly, and must relate in time to the mitotic entry wave. In some movies, aster disassembly appears to propagate outwards from the centrosome, suggesting disassembly might also occur as a chemical wave initiated at centrosomes. Whether this wave is the mitotic entry wave, or some downstream biochemistry that depends on mitotic entry, is an interesting question for future study.

5. Perspective and future directions

Over the past few decades, the centrosome's role in cell cycle control and microtubule organization has been well established. Our proposal is to expand this view and reimagine the centrosome as a chemical wave initiator in the context of large dividing cells. Further investigation of cell cycle waves calls for imaging the cell cycle in space and time at the molecular level. The behaviours of fluorescent probes such as cyclin B-GFP [51–53], Cdk1 activity FRET probes [82], and cell cycle-dependent protein–protein interactions [83] should be studied in relation to centrosome position and microtubules. Using *Xenopus* egg extract, we have recently developed a system that reconstitutes the growth and interaction of large microtubule asters [4] and permits high spatio-temporal imaging of microtubule dynamics during aster growth. Candidate factors for microtubule nucleation away from the centrosome can be perturbed by immunodepletion. Another interesting direction of research concerns the cross talk between the chemical waves of cell cycle and microtubule. Observations made in cell-free systems should ultimately be tested *in vivo*. Microinjection and live imaging performed on the large transparent zebrafish embryos have already advanced our understanding of large asters [3,84]. The complementary advantages offered by *Xenopus* extracts and zebrafish zygotes will advance our understanding of cell cycle waves and aster growth. Furthermore, it is tempting to speculate whether centrosomes initiate other kinds of chemical waves [73,74].

Chemical waves might be the only physically plausible way for large cells to organize their cytoplasm rapidly. Are chemical waves a special property of large cells, or is it an intrinsic

capability of the cellular cytoplasm that is underappreciated in smaller cells? Increasing evidence suggests that cytoplasmic patterning by reaction–diffusion mechanisms is widespread in a variety of cells [85]. In the medium-sized *Caenorhabditis elegans* embryo, cell polarity is thought arise from a diffusion–advection–reaction mechanism [16]. Actin polymerization waves are initiated at the cell periphery and underlie neutrophil chemotaxis [15,86]. The membrane-bound MinCD proteins oscillate between the poles in tiny bacterial cells [87,88], and their spontaneous surface waves have recently been reconstituted in bulk solution [14,89]. The early *Drosophila* embryo has been suggested to produce metachronous mitotic waves by coupling biochemical and mechanical excitability [90]. In all cases, localized initiation is a key design element that allows spatial propagation of information by chemical waves. Studying how large embryos divide with this renewed interest on the centrosome as the initiator will help us understand how chemical wave mechanisms are implemented and conserved to support basic physiological functions across different cell types and organisms.

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