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REVIEW

Cell cycle control during early embryogenesis

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ABSTRACT

Understanding the mechanisms of embryonic cell cycles is a central goal of developmental biology, as the regulation of the cell cycle must be closely coordinated with other events during early embryogenesis. Quantitative imaging approaches have recently begun to reveal how the cell cycle oscillator is controlled in space and time, and how it is integrated with mechanical signals to drive morphogenesis. Here, we discuss how the *Drosophila* embryo has served as an excellent model for addressing the molecular and physical mechanisms of embryonic cell cycles, with comparisons to other model systems to highlight conserved and species-specific mechanisms. We describe how the rapid cleavage divisions characteristic of most metazoan embryos require chemical waves and cytoplasmic flows to coordinate morphogenesis across the large expanse of the embryo. We also outline how, in the late cleavage divisions, the cell cycle is inter-regulated with the activation of gene expression to ensure a reliable maternal-to-zygotic transition. Finally, we discuss how precise transcriptional regulation of the timing of mitosis ensures that tissue morphogenesis and cell proliferation are tightly controlled during gastrulation.

KEY WORDS: Cell cycle, Cytoplasmic flows, Signaling waves, Gastrulation, Maternal-to-zygotic transition

Introduction

In many species, early development initiates with rapid and almost synchronous mitotic divisions that slow over time as the embryo approaches the key developmental transition to gastrulation (Foe et al., 1993; O'Farrell, 2015). These early divisions are much faster than somatic divisions in adult tissues and do not contain gap phases of the cell cycle (Farrell and O'Farrell, 2014); the cell cycle simply oscillates between DNA synthesis and mitosis. This happens because the supply of maternal mRNAs deposited in the oocyte during gametogenesis allows the cell cycle oscillator to run essentially as an unperturbed biochemical clock (Murray and Kirschner, 1989). In *Drosophila*, these early pre-gastrulation divisions are nuclear divisions that take place in a syncytium, i.e. a large multinucleated cell, whereas in vertebrates these rapid divisions include separation of the cytoplasm.

The large size of embryos and speed of divisions impose a need for coordination across several hundred micrometers over timescales of a few minutes or less. Thus, the regulation of these early cleavage divisions requires specialized mechanisms. Long-range coordination is achieved through gradients or traveling waves of the cell cycle oscillator. Moreover, the cell cycle-dependent regulation of cytoskeletal dynamics generates physical forces, which further facilitate embryo-wide collective dynamics.

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In most metazoans, there is a transition prior to gastrulation from synchronous and rapid cell divisions to patterned divisions, which often corresponds with the establishment of cell fate specification domains (Foe, 1989; Kane et al., 1992; Arora and Nüsslein-Volhard, 1992; Murakami et al., 2004). This transition and remodeling of the cell cycle coincides with the activation of zygotic transcription (Newport and Kirschner, 1982; Lasko, 2013; Farrell and O'Farrell, 2014; Yartseva and Giraldez, 2015; Yuan et al., 2016; Jukam et al., 2017). In many species, the timing of these events is linked to the changing nuclear-to-cytoplasmic (N/C) ratio that naturally occurs when cells divide without growth (Newport and Kirschner, 1982; Edgar et al., 1986). Thus, both before and after gastrulation, the dynamics of cell cycle regulation are key to early transitions in embryonic development.

Here, we will review recent progress that dissects the spatial regulation of the cell cycle oscillator and how biochemical and mechanical signals are integrated to ensure proper coordination of early development. We will also discuss recent progress on how embryos control the number of cleavage divisions and how transcription can ensure the precise timing of the cell cycle during gastrulation. This Review will focus primarily on recent insights from the *Drosophila* model system, where quantitative live-imaging tools have enabled the measurement of cell cycle dynamics with high spatiotemporal precision. However, we will also discuss how mechanisms similar to those observed in the fly embryo have been uncovered in other models, including frog, fish, worm, mouse and starfish embryos.

Molecular mechanisms of cell cycle control in the pre-gastrulation embryo

The cell cycle machinery is highly conserved and has been extensively reviewed elsewhere (Heim et al., 2017; Morgan, 2006). Here, we briefly introduce the key molecular mechanisms pertinent to this Review. Cyclin-dependent kinases (Cdks) drive progression through the mitotic cell cycle (Heim et al., 2017). Activation of Cdks requires the binding of a cyclin, and different cyclins bind and activate different Cdks at each cell cycle stage (Nigg, 1995; Jeffrey et al., 1995). The oscillating concentration of cyclins throughout the cell cycle is the initial driver of mitotic periodicity (Glotzer et al., 1991; Morgan, 1997; Murray, 2004). A central control mechanism of the oscillations is cyclin degradation at mitotic exit (Holloway et al., 1993; Parry and O'Farrell, 2001). In the vast cytoplasm of developing embryos, this degradation initiates near dividing spindles and chromosomes, and can drive either local or global downregulation of Cdk1 activity (Evans et al., 1983; van der Velden and Lohka, 1994; Edgar et al., 1994a; Su et al., 1998; Huang and Raff, 1999; Ban et al., 2007; Bischof et al., 2017). This dependence primarily on autonomous, oscillatory Cdk1 activity for cell cycle progression is unique to the simple cell cycles in the early embryo, which lack gap phases, whereas in somatic cells the cell cycle can be paused at the G1/S or G2/M transitions (Elledge, 1996; Morgan, 1997).

In addition to changes in Cdk1 activity driven by oscillations in cyclin concentration, feedback mechanisms are also important for

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the regulation of Cdk1 activity. At mitotic entry, two inhibitory phosphorylation events, mediated by the kinases Wee1 and Myt1 (Campbell et al., 1995; Mueller et al., 1995a,b), shape Cdk1 activity (Fig. 1Ai). Wee1 tyrosine kinase phosphorylates Cdk1 at the Y15 residue (Parker and Piwnica-Worms, 1992), whereas Myt1 can phosphorylate both Y15 and T14 residues (Kornbluth et al., 1994; Mueller et al., 1995a). Cdc25 phosphatases antagonize the action of Wee1 and Myt1 by removing Y15 and T14 inhibitory phosphate

Fig. 1. Cdk1 activity oscillates in the cell cycle through a network of positive- and negative-feedback loops. (A) Cdk1 activity is regulated by negative phosphorylation, positive phosphorylation and feedback from APC/C and phosphatases. Pink-labeled enzymes and phosphorylations indicate negative feedback on Cdk1 activity; cyan-labeled enzymes and phosphorylations indicate positive feedback on Cdk1 activity. (i) Inhibitory phosphorylation of Cdk1 is regulated by the kinases Wee1 and Myt1, which phosphorylate Cdk1 at T14 and Y15. The phosphatase Cdc25 counteracts the T14/Y15 phosphorylation and is itself regulated by the kinase Chk1. (ii) Cyclin B binding to Cdk1 results in an activating phosphorylation event at T161. The APC/C protein complex is activated by Cdk1 and provides negative feedback by resetting cyclin B levels. (iii) Phosphatases play an important role in antagonizing Cdk1 activity directly and on Cdk1 substrates. Cdk1 activates the phosphatase PP1, which then removes phosphorylation from other Cdk1 substrates. The phosphatase PP2A, when associated with the B55 regulatory subunit, acts as a direct inhibitor of Cdk1 by removing the T161 activating phosphorylation. Cdk1 promotes its own activity in a positive-feedback loop by phosphorylating Gwl, which inhibits PP2A-B55 through activation of ENSA. (B) Feedback loops lead to changes in the Cdk1 phosphorylation state and thus oscillations of Cdk1 activity within the cell cycle. During the early divisions of the embryo, the cell cycle oscillates between mitosis (M) and DNA synthesis (S), without gap phases. The ratio of active Cdk1 to PP1 is high (cyan line) as nuclei undergo mitosis, and drops as nuclei exit mitosis and enter S phase. The steep slope of the switch from inactive to active Cdk1 is reflective of measures of Cdk1 and Chk1 activity from Deneke et al. (2016), and illustrates the bistability of Cdk1 activity in which Cdk1 quickly switches between two stable states.

groups from Cdk1 (Kumagai and Dunphy, 1992; Morgan, 1997). Cdk1 participates in a positive-feedback loop by inactivating Wee1 and activating Cdc25 (Mueller et al., 1995b; Kumagai and Dunphy, 1992; Hoffmann et al., 1993). This positive-feedback loop is important for the regulation of mitotic entry (Solomon et al., 1990; Sha et al., 2003; Pomerening et al., 2003; Stumpff et al., 2004; Tsai et al., 2014) and integrates inputs from an additional layer of regulation – the DNA replication checkpoint – to ensure that nuclei do not enter mitosis before completion of DNA replication. A major effector of this checkpoint is the protein kinase Chk1, which controls Cdk1 activity by activating Wee1 and inhibiting Cdc25 (Fig. 1A-B) (Tang et al., 1993; Peng et al., 1997; Sanchez et al., 1997; Lee et al., 2001; Shimuta et al., 2002; Uto et al., 2004).

Following mitosis, a negative-feedback mechanism resets Cdk1 activity through Cdk1-dependent activation of the anaphase-promoting complex/cyclosome (APC/C) (Pines, 2011) (Fig. 1Aii). The APC/C is a large complex of proteins that has E3-ubiquitin ligase activity and thus promotes ubiquitylation and degradation of cyclins, leading to reduced Cdk1 activity (King et al., 1995; Irniger et al., 1995). Mitotic exit is also facilitated by phosphatases that remove mitotic phosphorylation from Cdk1 targets (Fig. 1Aiii). Protein phosphatase 2A (PP2A) associates with the regulatory subunit B55, forming a holoenzyme that is a major Cdk1 antagonist (Mochida et al., 2009). Protein phosphatase 1 (PP1) directly removes mitotic phosphorylation laid down by Cdk1 and it additionally facilitates the activation of PP2A (Wu et al., 2009; Grallert et al., 2015; Heim et al., 2017). Both PP2A and PP1 undergo cell cycle-dependent oscillations in their activity towards mitotic targets. Specifically, both phosphatases have low activity at the onset of mitosis and their activity rapidly increases at mitotic exit (Mochida et al., 2009; Wu et al., 2009) (Fig. 1B). Several feedback mechanisms contribute to this. For PP1, direct phosphorylation by Cdk1 and self-dephosphorylation, as well as regulation of co-factors (inhibitor 1 and inhibitor 2), have been implicated (Wu et al., 2009). PP2A activity is also regulated by feedback from Cdk1. The kinase greatwall (Gwl) inhibits PP2A-B55 by phosphorylating endosulfine alpha (ENSA), which binds and inhibits PP2A (Mochida et al., 2010). Gwl is in turn phosphorylated and activated by Cdk1, so that phosphatase activity remains low while Cdk1 activity is high (Hégarat et al., 2014). Collectively, the positive- and negative-feedback loops highlighted above provide avenues by which the cell cycle oscillator can be programmed during embryogenesis.
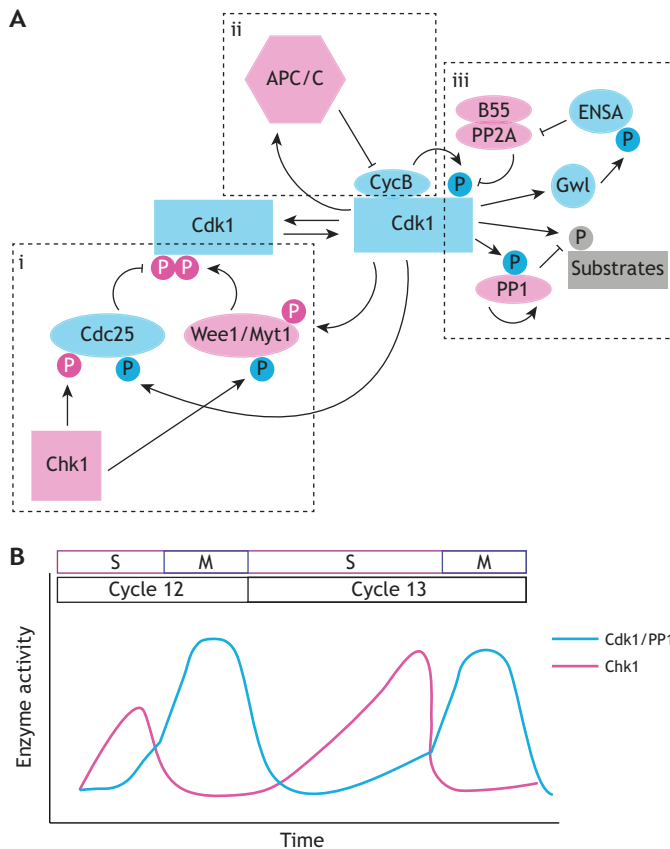
Traveling waves coordinate the rapid cell cycle events in developing embryos
Trigger waves: an early model for propagating mitotic waves in the embryo
Coordinated mitotic events characterize the early stages of embryogenesis of *Drosophila*, *Xenopus* and other systems. These events are coordinated across the large distance of the embryo, a phenomenon that cannot be achieved by simple diffusion (see Box 1). The early divisions of the *Drosophila* embryo occur within minutes across the 500 μm syncytium (Foe and Alberts, 1983). However, simple diffusion of active Cdk1 would take hours to traverse this distance (Box 1). To overcome this problem, it was theorized that the combination of diffusion and bistability of the cell cycle oscillator could generate trigger waves of Cdk1 activity that would synchronize mitosis across the large embryo (Novak and Tyson, 1993, Box 1). Trigger waves have several desirable features for propagating a robust biological signal across the expanse of the embryo. Specifically, they do not lose amplitude as they travel and they can transfer signals much more rapidly than by simple diffusion

Box 1. Bistability and diffusion

Bistability

Bistability describes the property of dynamic systems with two stable (equilibrium) points, e.g. a low activity and high activity state. When the activity of the system lies between the two stable states, the activity will either increase or decrease to reach a stable equilibrium point. Whether an activity value will evolve toward the low or high activity state depends upon a threshold for the system. Values below the threshold will proceed toward the low steady state, whereas values above the threshold will proceed toward the high steady state. Thus, bistability is usually characterized by rapid transitions between the two states. The cell cycle oscillator is characterized by a series of bistable transitions, where the low and high net activity of Cdk1 and opposing phosphatases represent the two states (Morgan, 2006). In biological systems such as the cell cycle control network, bistability usually arises from nonlinear positive feedback (Ferrell and Ha, 2014).

Diffusion

All molecules inside a cell are subject to random thermal motion. Such motion can be described by the diffusion equation, in which the molecule of interest is characterized by a diffusion constant D. In first approximation, this constant is dependent on properties of the medium and the molecule. Typical values for the intracellular diffusion constant of proteins are of the order $D \approx 1\text{-}10 \; \mu m^2 \; s^{-1}$. A major property of diffusion is that the time (t) needed for molecules to spread across a given distance (l) scales as the square of distance, $t \approx l^2 \; D^{-1}$. Although diffusion can easily spread signals in several seconds across distances on the order of 10 μm, the time needed to spread such signal across 100 μm is 100 times as long. Thus, for rapid communication (within a few minutes) across a large expanse such as the 500 μm *Drosophila* embryo, simple diffusion is insufficient. Alternative mechanisms, such as fluid flows, direct transport, reaction-diffusion-generated gradients and waves must be adopted. These strategies have the additional advantage that the transported signal does not become dampened during spreading, as is typically observed for signals spreading by simple diffusion.

Box 2. Reaction-diffusion systems

Long-range order and rapid signal communication can be established in reaction-diffusion systems. Often in cellular systems, the reaction terms assume the form of protein synthesis and degradation, and non-linear feedback (positive and negative). In this Review, we discuss two mechanisms by which reaction-diffusion systems can generate wave-like spreading of biochemical activity: trigger waves and sweep waves.

Trigger waves

Trigger waves arise from the coupling of diffusion and bistability. As nuclei in the embryo approach mitosis, high Cdk1 activity (mitotic state) in a region causes some of the active Cdk1 molecules to diffuse to neighboring regions, which are in the low state (interphase). In this region, positive feedback can cause Cdk1 to further increase and nuclei to enter the mitotic state. This process will continue until the entire embryo enters the mitotic state. In the *Drosophila* embryo, experimental and modeling arguments suggest that the speed of a Cdk1 trigger wave would be about 0.4 μm s^{-1}, which implies that it would take about 10 min for a wave to travel across the entire embryo. Yet all nuclei divide within 1-2 min, arguing that a more rapid mechanism is at play. This mechanism was shown to be sweep waves.

Sweep waves

Sweep waves arise when bistability is transient and the transition from a bistable system to a system with only one stable state (e.g. high Cdk1 activity) happens on a much faster timescale than that needed for a wave to propagate. In this case, even if a bistable wave is initiated in a region of the embryo, this wave would not be able to travel far before the activity in the entire system switches towards the high state. To understand how this switch to the high state gives rise to waves, one needs to understand what happens when Cdk1 activity is low during interphase. Using both theoretical and experimental analyses, we showed that the early reaction-diffusion dynamic generates Cdk1 activity gradients that extend for about 100-150 μm. Late in interphase, when bistability is lost, Cdk1 activity rises towards the high stable state at a uniform rate across the entire embryo. The uniform rise of Cdk1 gradients causes wave-like mitotic entry as different regions across the gradient enter mitosis at slightly different times. Thus, the speed of the mitotic wave is inversely proportional to the steepness of the gradient and directly proportional to the rate at which the system moves away from bistability, a theoretical prediction that we confirmed experimentally. Moreover, if the Cdk1 gradients are not very steep and the drive is rapid, very fast mitotic waves will be observed.

(Gelens et al., 2014; Deneke and Di Talia, 2018). In *Drosophila* embryos and *Xenopus* egg extracts, imaging studies of mitotic waves and their regulation have uncovered how Cdk1 activity and simple diffusion couple to move signals quickly across the embryo.

The trigger wave model proposes that mitotic signaling is propagated as a bistable wave (Box 2). Observing nuclear membrane breakdown in *Xenopus* egg extract loaded into long, thin Teflon tubes, it was found that mitosis traveled down the tube at a constant velocity, which is consistent with a wave (Chang and Ferrell, 2013). Partitioning the tube caused nuclear breakdown to decouple on the two sides, suggesting that coordination relies on physical coupling. Inhibition of Wee1 activity changed the speed of the mitotic wave front, supporting a role for Cdk1 in mitotic waves (Chang and Ferrell, 2013). Thus, these experimental data are consistent with mitotic waves being described as trigger waves.

Cdk1 activity spreads through *Drosophila* embryos via sweep waves

Although the above experiments and the bistable nature of mitosis had argued that a bistable wave could explain the dynamics of mitotic waves in early embryos, direct observation of Cdk1 activity waves was crucial to elucidate their biological and physical mechanism (Deneke et al., 2016; Vergassola et al., 2018). Strikingly, observation of Cdk1 activity *in vivo* demonstrated a novel mechanism of wave-like propagation, a sweep wave (Deneke et al., 2016; Vergassola et al., 2018), that can travel across the embryo even more quickly than a trigger wave (Box 2). This observation was achieved in the *Drosophila* embryo using a fluorescence resonance energy transfer (FRET) sensor that reports on the phosphorylation state of a Cdk1 target (Gavet and Pines,

2010; Deneke et al., 2016, 2019). Analysis of the spatial profiles of Cdk1 activity demonstrated that they lack the crucial property expected for bistable trigger waves, i.e. a wavefront, in which a high state of Cdk1 activity connects to regions of low Cdk1 activity (Murray, 1989; van Saarloos, 1998). Instead, at the onset of mitosis, gradients of Cdk1 activity that rapidly rise uniformly are observed (Vergassola et al., 2018) (Fig. 2A, Box 2). This phenomenon allows the *Drosophila* embryonic divisions to progress across the embryo even more rapidly than is theoretically possible by a trigger wave under the same parameters. In fact, weakening the positive-feedback loops that promote Cdk1 activation transforms sweep waves into trigger waves, which leads to slower mitotic waves (Vergassola et al., 2018) (Fig. 2A).

The generation of sweep waves relies on the fact that early reaction-diffusion Cdk1 dynamics result in the establishment of signaling gradients that can span regions of about 100-150 μm (Box 2). Late in interphase, the rapid accumulation of cyclins drives loss of bistability – the only stable state becomes high Cdk1 activity (mitosis) – and thus Cdk1 activity rises at a uniform rate across the embryo. This causes a global uniform increase of the gradients of Cdk1 activity and a wave-like pattern of mitotic entry (Vergassola et al., 2018) (Fig. 2A). After mitotic entry, the other mitotic events follow in a clock-like manner; thus, waves of mitotic exit are also
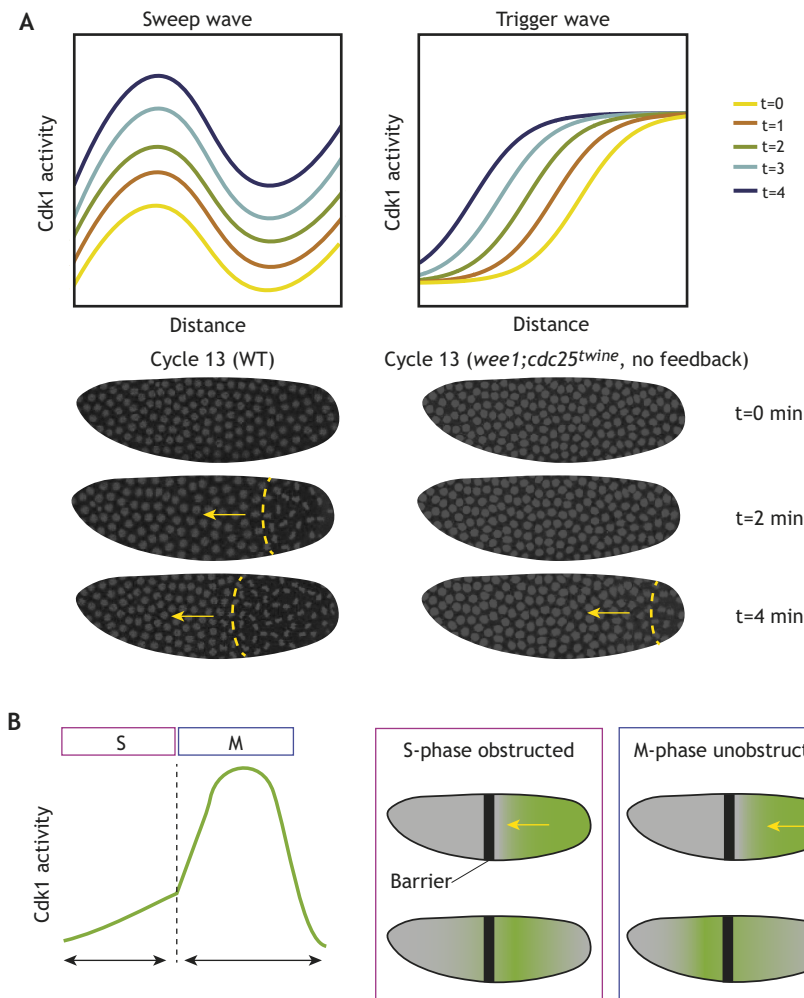
Fig. 2. Time-dependent sweep waves of Cdk1 activity grow synchronously across the *Drosophila* embryo to drive faster entry into mitosis. (A) Theoretical Cdk1 activity curves in the *Drosophila* embryo illustrate the differences between sweep waves and trigger waves. Sweep waves preserve the spatial gradient of Cdk1 activity across the embryo while increasing the overall level of Cdk1 activity. The levels of Cdk1 activity at the left and right ends of each curve sweep upward with time. Unlike sweep waves, trigger waves arise from the invasion of a metastable state by a stable state. The left and right ends of the curves (the metastable and stable points of the bistable system) are roughly unchanged with time. The embryos pictured below right lack any feedback on Cdk1 activity due to mutations in *wee1* and in the *Drosophila cdc25* homolog, *cdc25^twine*, leading to a slower sharp wave front that propagates across the embryo. Images reproduced, with permission, from Vergassola et al. (2018). (B) In *Drosophila* embryos, the cell cycle propagates as a wave (green) that relies on diffusion during S phase. A barrier (black) placed during interphase blocks wave propagation. After mitosis is initiated by high Cdk1 activity, the events of mitosis proceed as a wave that is pre-patterned by the early Cdk1 levels and thus propagates through the barrier. Adapted, with permission, from Deneke et al. (2016).

observed (Deneke et al., 2016; Vergassola et al., 2018). Consistently, it was demonstrated that insertion of an impenetrable barrier in early interphase in *Drosophila* embryos causes mitosis to decouple in the two sides, but insertion in late interphase or early mitosis does not (Deneke et al., 2016) (Fig. 2B).

A qualitative difference between sweep and trigger waves is in the dependency of the wave speed on the rate of cyclin accumulation. For trigger waves, one expects a very small dependency on that rate, whereas the speed of sweep waves shows a strong dependency (Vergassola et al., 2018) (Box 2). This is seen in *Drosophila* embryos, where there is a decrease in the speed of Cdk1 waves when cyclin A and cyclin B levels are reduced by heterozygous mutations (Vergassola et al., 2018). Moreover, the wave speed is quantitatively captured by the theory of sweep waves in both wild-type and mutant embryos (Vergassola et al., 2018). Recent experimental results in *Xenopus* extracts might be consistent with a transition from sweep to trigger waves as the cell cycle slows down. Seeding a frog egg extract with fewer nuclei resulted in initially fast cycles that progressively slowed down (Chang and Ferrell, 2013). The speed of the mitotic waves initially demonstrated a strong dependency on cell cycle duration (Nolet et al., 2020), but eventually mitotic waves slowed and achieved speeds independent of cell cycle duration (Nolet et al., 2020; Afanzar et al., 2020). Collectively, these observations suggest that there are two mechanisms for mitotic waves and that the dominating one is determined by how rapidly the system progresses through the cell cycle.

Waves in *Xenopus* embryos are controlled by cell-autonomous clocks
In *Xenopus* extracts and *Drosophila* embryos, simple diffusion can propagate signals across the shared cytoplasm. However, in the intact *Xenopus* embryo, cytokinesis physically separates the cytoplasm of neighboring cells (Singal and Sanders, 1974; Keller, 1991), yet waves of cell division across multiple cells are still observed (Anderson et al., 2017). Using temperature gradients, it was investigated whether the waves observed from the second cell cycle onwards required diffusion or whether they were regulated by cell-autonomous clocks ticking at different periods (Anderson et al., 2017). Remarkably, these experiments showed that mitotic waves are due to autonomous clocks ticking at different rates, such that cells on the 'cold' side of the embryo and cells on the 'warm' side of the embryo never caught up with one another through 10 rounds of cell division. Instead, the two sides of the embryo maintained their own periodicity of mitosis (Anderson et al., 2017). Understanding what determines the periodicity of cell-autonomous cycling will reveal interesting insights into the developmental mechanisms of cell cycle control. Intriguingly, embryos that were forced to desynchronize with temperature gradients were viable (Anderson et al., 2017). Such remarkable robustness was previously observed in *Drosophila* embryos, where temperature gradients can similarly desynchronize the cell cycle, yet patterning seems robustly restored at gastrulation (Lucchetta et al., 2005). How embryos can compensate for significant delays in the timing of mitosis remains an unanswered question. It would be intriguing to find out whether there are developmental

checkpoints able to resynchronize developmental pathways or whether the embryo is able to deal with the timing differences gradually by compensatory mechanisms.

Contractility and cytoplasmic flow couple cell cycle oscillations with mechanics in the embryo

In addition to chemical mechanisms that coordinate mitotic divisions across space, the mechanical properties of the early embryo must be tightly controlled to bring about proper morphogenesis. This is achieved by inter-regulation of the cell cycle oscillator and the cytoskeleton, and ensures the positioning of organelles, e.g. nuclei in the *Drosophila* embryo, and the separation of yolk from cytoplasm in zebrafish.

The cell cycle regulates cortical actomyosin to reposition nuclei in *Drosophila*

In the early *Drosophila* embryo, the first six nuclear divisions occur in the center of the embryo (Foe and Alberts, 1983). The first three nuclear divisions are characterized by little nuclear movement. From nuclear cycle 4 to 6, the nuclei gradually spread across the anterior-posterior axis of the embryo. Following this phase, also known as axial expansion, the nuclei progressively migrate to the cortex from nuclear cycle 7 to the end of nuclear cycle 10 (Foe and Alberts, 1983). Early time-lapse imaging studies suggested that axial expansion is controlled by dissolution of an F actin network in the bulk cytoplasm, which initiates cytoplasmic flows that push nuclei along the anterior-posterior axis (von Dassow and Schubiger, 1994). However, it was later proposed that cycles of myosin II activity at the cortex might produce cytoplasmic flows and drive nuclear movements (Royou et al., 2002). Indeed, oscillations of myosin II activity were observed at the cortex of early *Drosophila* embryos starting at the 4th mitotic division, and these oscillations were shown to depend on Cdk1 activity (Royou et al., 2002). However, as cyclin degradation is restricted to a very small region near the nuclei (Edgar et al., 1994a; Su et al., 1998), this raised the

question of how the cell cycle oscillator could control cortical contractions many micrometers away. Such coordination would not be required for the bulk actin model, which could be controlled by cell cycle oscillations restricted around the chromosomes.

Distinguishing whether bulk or cortical actomyosin drives nuclear spreading was made difficult by the fact that most genetic and pharmacological perturbations would affect actomyosin globally across the embryo. How the cell cycle and actomyosin dynamics are integrated to drive nuclear spreading required novel imaging approaches that were introduced by our group (Deneke et al., 2019). Using a FRET biosensor, we measured the ratio of Cdk1 and PP1 activity directly *in vivo*. Although cyclin degradation is restricted to a region of a few micrometers around the chromosomes, the oscillation of Cdk1/PP1 activity extends to a region of about 50 μm, which is sufficient to couple nuclear and cortical dynamics. PP1 is a major player in the spreading of the oscillation and in the recruitment of myosin II at the cortex (Deneke et al., 2019). Using an optogenetic approach to alter actomyosin contractility specifically at the cortex, we also demonstrated that cortical contractions drive the flow of cytoplasm within the embryo (cytoplasmic streaming), which in turn drives nuclear positioning. More generally, the mechanism by which nuclear spreading is accomplished in the *Drosophila* embryo is an excellent example of a self-organized system. The cytoplasmic flows responsible for nuclear spreading are driven by the presence of myosin gradients. Myosin gradients are established by the localized oscillations between Cdk1 and phosphatase activity, and are present only when nuclei are unevenly distributed across the embryo (Fig. 3). As soon as nuclei are uniformly positioned across the embryo, myosin oscillations, although still present, are uniform and unable to generate flow. Thus, the embryo generates only the number of cortical contractions required for uniform positioning. This mechanism likely allows the embryo to correct for variability in initial nuclear positioning and to achieve a uniform nuclear distribution across the embryo. This uniformity ensures that the
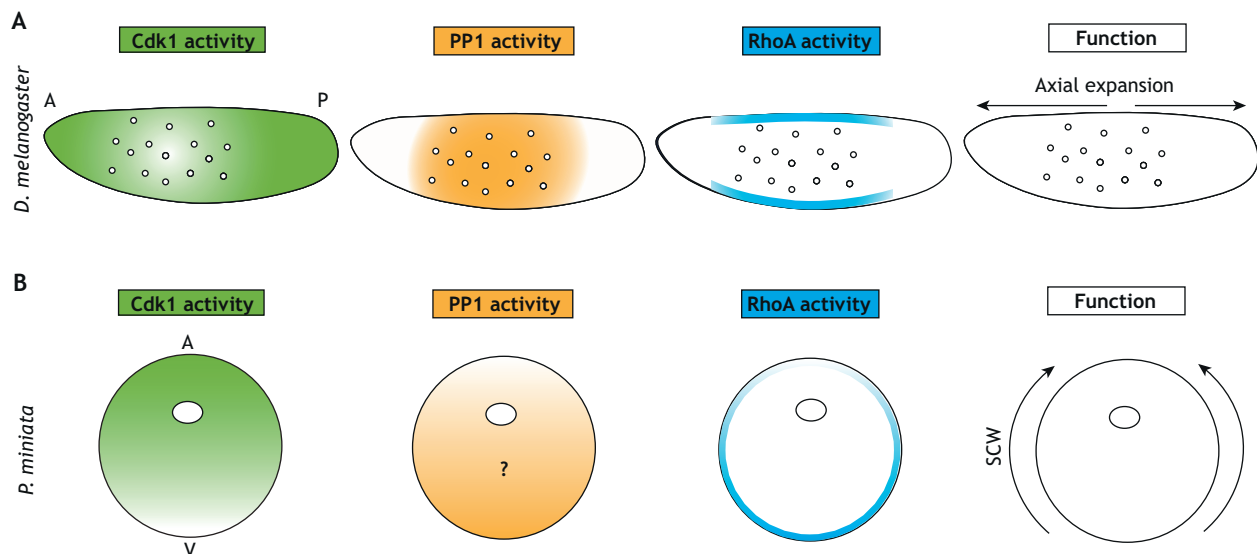

Fig. 3. Summary of known activities of Cdk1, PP1 and RhoA in fly and starfish embryos. (A) In the fly embryo, a self-organized system positions nuclei across the embryo syncytia. Cdk1 activity is low around nuclei following mitosis, which leads to local increases in PP1 phosphatase. PP1 can activate RhoA at the cortex, thus coupling nuclear position with activity at the cortex. RhoA then promotes contraction at the cortex and mixing of the cytoplasm to spread nuclei along the anterior-posterior (A-P) axis. (B) A similar self-organizing system might exist in starfish oocytes, which have a gradient of Cdk1 activity that negatively correlates with RhoA activity. The role of PP1 in starfish embryos remains unknown. Cdk1 and RhoA gradients promote contraction at the surface and the propagation of surface contraction waves (SCWs) from the vegetal (V) to animal pole (A).

correct number of nuclear divisions precedes gastrulation. Further study of the *Drosophila* embryo will shed light on the molecular mechanisms that couple the cell cycle with actomyosin contractility, as well as elucidate the physical mechanisms by which cortical contractions generate cytoplasmic streaming. More importantly, investigating whether similar mechanisms work in insect species laying embryos of different sizes and shapes will provide interesting evolutionary insights, alternative mechanisms (Donoughe et al., 2021 preprint), and possibly a framework for how mechanical forces and cytoplasmic flow can scale in embryogenesis.

Cdk1 generates surface contraction waves via RhoA in starfish oocytes

Cell cycle-dependent regulation of cortical contractility has also been studied in starfish oocytes, where it was shown that surface contraction waves (SCWs) are linked to Cdk1 activity (Bement et al., 2015). It was subsequently proposed that SCWs are regulated by a gradient of active Cdk1 that opposes RhoA activity (Fig. 3B) (Bischof et al., 2017). In this model, the gradient of Cdk1 activity results in RhoA activation at the vegetal pole. Feedback mechanisms on RhoA activity then generate a traveling wave. Inhibition of Cdk1 at a specific point in the embryo through local drug application causes RhoA activity to accumulate at that point and change the direction of the SCW. RhoA ultimately activates myosin II, causing waves of cortical contraction (Bischof et al., 2017). In these studies, the levels of cyclin B were taken as a proxy of Cdk1 activity, and moving gradients, similar to those explained by sweep waves (Vergassola et al., 2018), were proposed to position RhoA waves (Bischof et al., 2017; Wigbers et al., 2021). However, the bistable regulation of Cdk1 argues for non-linearity in the relationship between cyclin B and Cdk1 activity. Furthermore, the activities of PP1 and PP2A might contribute to the spatial control of the phosphorylation status of mitotic targets (Mochida et al., 2016; Rata et al., 2018; Kamenz et al., 2020; Deneke et al., 2019). Notably, SCWs have also been observed in *Xenopus* embryos, originating at the animal pole and moving laterally down the embryo (Rankin and Kirschner, 1997). Propagation of SCWs coincides with mitosis (Rankin and Kirschner, 1997), and it was proposed that they are directly driven by wave-like activation and inactivation of Cdk1, at least during the first cell cycle (Chang and Ferrell, 2013). In the future, the use of cell-cycle biosensors coupled with perturbations of the cell cycle and cytoskeleton will be crucial to reveal how SCWs are controlled by Cdk1 and mitotic phosphatase activities.

Ooplasmic reorganization in zebrafish occurs through cell cycle-dependent regulation of bulk actin polymerization

Coordination between Cdk1 activity and actin polymerization in the cytoplasm also plays important roles in early embryos. *Xenopus* egg extracts under a coverslip undergo periodic contraction as actin polymerizes and myosin promotes contraction of the filamentous network. This process is mostly active in mitotic extracts, supporting the idea that the cell cycle affects actin dynamics (Field et al., 2011). Recently, this coordination has been shown to play a central role in ooplasmic separation during the first division of zebrafish embryos. Soon after fertilization, there is a directional separation of yolk granules and ooplasm in the embryo, such that yolk granules move ventrally (Beams et al., 1985; Leung et al., 2000; Shamipour et al., 2019). Previous work in zebrafish and other models suggested that ooplasm flow was likely driven by cortical actomyosin contractions (Leung et al., 2000; Munro et al., 2004; Prodon et al., 2008; Klughammer et al., 2018). Indeed, cortical actomyosin disassembly has been observed in the animal pole of

zebrafish oocytes, where it was thought to allow streaming of ooplasm into an expanded animal pole (Beams et al., 1985; Fuentes et al., 2018). However, the actomyosin cortex is dispensable for cytoplasmic flow in the fish oocyte (Shamipour et al., 2019). Rather, cell cycle-mediated bursts of bulk actin polymerization drive ooplasm reorganization. Strikingly, actin polymerization proceeds in a wave throughout the time of the first cell division of the zygote. The periodicity of these waves is dependent on Cdk1 activity, with Wee1 inhibition giving rise to faster waves of actin polymerization and Chk1 inhibition slowing the wave. Thus, these experiments led to a model in which bulk actomyosin dynamics controlled by the Cdk1 wave drive ooplasmic segregation (Shamipour et al., 2019). Notably, the positive regulation of bulk actomyosin contractility in mitosis contrasts with regulation at the cortex, where contractility is inhibited at mitotic entry and rises at mitotic exit. This differential regulation is not yet fully elucidated on a mechanistic level. However, it predicts that actomyosin-dependent flows would be observed at different phases of the cell cycle: at interphase for cortical contractions and at mitotic entry for bulk contractions. This prediction is confirmed by observations in *Drosophila* and zebrafish embryos (Deneke et al., 2019; Shamipour et al., 2019).

Microtubule-dependent regulation of nuclear movements

In addition to actomyosin-dependent cortical dynamics, microtubules also play a role in the spatial distribution of nuclei in *Drosophila* embryos and *Xenopus* extracts (Baker et al., 1993; Nguyen et al., 2014; Cheng and Ferrell, 2019; Mitchison, 2020; Pelletier et al., 2020; Deshpande et al., 2019). In fly embryos, microtubules are important in controlling the migration of nuclei to the surface (Baker et al., 1993) following actomyosin-driven axial expansion and in regulating internuclear distance (Telley et al., 2012; Deshpande et al., 2019; de-Carvalho et al., 2020 preprint). Similar processes have been observed and well characterized in *Xenopus* extracts (Nguyen et al., 2014; Field and Mitchison, 2018; Pelletier et al., 2020). Importantly, these microtubule-driven processes are also linked to the cell cycle. For example, in *Drosophila* embryos, nuclear migration happens in three cycles (nuclear cycles 7 to 9), in which nuclear movements towards the cortex are restricted to mitotic exit/early interphase (Foe and Alberts, 1983). In the future, it will be important to dissect how the cell cycle oscillator and microtubule dynamics are integrated to ensure that nuclei move towards the cortex at the correct time and in the correct direction.

Cell cycle slowing at the mid-blastula transition promotes embryonic patterning

The mid-blastula transition (MBT) is a distinct stage of embryonic development in most organisms characterized by slowing of the cell cycle and ultimately the inclusion of gap phases. This lengthening of the cell cycle likely gives embryos the time necessary to turn on zygotic transcripts, so the MBT also coincides with a transition from maternal to zygotic control of mRNA products (Fig. 4A). Recent studies have focused on identifying the mechanisms that control this crucial developmental transition by initiating these dramatic changes in transcription and cell cycle control.

Increased Chk1 activity mediates cell cycle slowing at the MBT

Slowing of the cell cycle at the MBT is pronounced and often follows a more gradual slowing of pre-MBT cycles that is observed, e.g. in cycles 10-13 of *Drosophila* embryogenesis (Sibon et al., 1997; Farrell et al., 2012) and less so in cycles 10-12 in *Xenopus* (Howe et al., 1995). The timing of cell cycle slowing is tied to DNA content. In *Drosophila*, one of the clearest illustrations of this
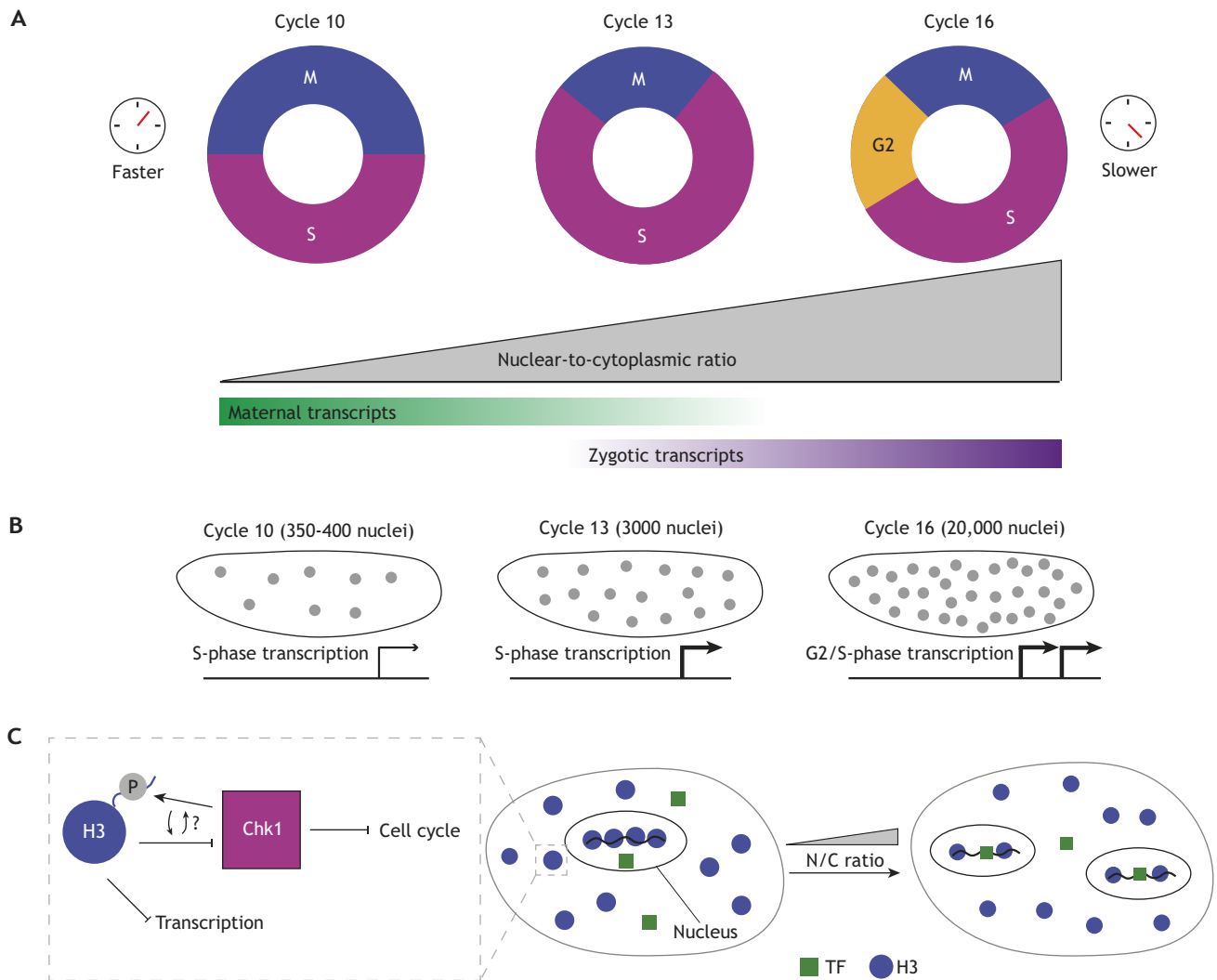
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Fig. 4. Convergence of multiple events at the *Drosophila* mid-blastula transition. (A) The cell cycle is remodeled at the mid-blastula transition (MBT) through the lengthening of S phase and then introduction of G2. This slowing of the cell cycle coincides with a transition from maternal to zygotic transcription. (B) Transcriptional output from zygotic nuclei also coincides with the increase in nuclear density. The expression of S-phase and, later, G2-phase genes progressively increases with time due to increased numbers of nuclei and to an increase in the transcription-competent cell cycle length. (C) We illustrate the embryo compartment and the nuclei inside before and after a nuclear division to illustrate titration as the nuclear-to-cytoplasmic (N/C) ratio changes. A titration model has been proposed as a mechanism by which nuclear events, such as DNA content and transcription, might correlate to cell cycle remodeling at the MBT. Early on, the binding of histones (blue) prevents the access of transcription factors to DNA, preventing the expression of zygotic genes. An increase in the N/C ratio leads to less competition between histones and transcription factors (green), promoting the expression of zygotic genes. Moreover, cells spend an increased time in the transcription-competent phases of the cell cycle. Histone proteins themselves may also feedback on Chk1, which inhibits cell cycle progression (left box). Cooperatively, these changes allow the embryo to undergo this crucial moment in development.

concept comes from studies of haploid embryos, which undergo an additional division before cellularization to reach the appropriate DNA content for the MBT (Edgar et al., 1986). The lengthening of the cell cycles preceding the MBT is due to slower DNA replication. The duration of S phase in the last cycle preceding the MBT (cycle 13) is more than three times longer than the duration in cycle 10 (14 versus 4 min), whereas the duration of M phase itself is constant. This cell cycle lengthening is mediated by the DNA replication checkpoint and requires the activity of the checkpoint kinase Chk1. The molecular regulation of Chk1 activity and its impact on Cdk1 have been demonstrated and investigated in both *Xenopus* (Lee et al., 2001; Shimuta et al., 2002; Petrus et al., 2004) and *Drosophila* (Sibon et al., 1997; Su et al., 1999; Takada et al., 2007; Royou et al., 2008; Fasulo et al., 2012). Chk1 activity likely increases in response to increased DNA content in the embryo. In *Drosophila* embryos, it

has been shown that increasing Chk1 activity causes Cdk1 activity to rise progressively more slowly during interphase of nuclear cycles 12 and 13 (Fig. 1B), thus delaying the cell cycle to correctly couple DNA replication and mitosis (Deneke et al., 2016). Negative feedback from Cdk1 to Chk1 activity, which has been observed in cell culture (Shiromizu et al., 2006; Enomoto et al., 2009; Xu et al., 2012), might further sharpen and control the transition between S phase and M phase (Yuan et al., 2012; Deneke et al., 2016). This early remodeling of the cell cycle due to S-phase lengthening is followed by a more dramatic slowing at the MBT with the inclusion of gap phases. Cell cycle remodeling at the *Drosophila* MBT is controlled by downregulating maternal Cdc25 to pause the cell cycle in G2 (Farrell et al., 2012). This downregulation is mainly controlled post-translationally through mechanisms targeting Cdc25 for degradation (Farrell and O'Farrell, 2013; Di Talia et al., 2013).

Maternal *cdc25* mRNA degradation (Edgar and Datar, 1996) ensures that pulses of zygotic Cdc25 transcription can then drive entry into mitosis in the subsequent cycles (Edgar and O'Farrell, 1990). Regulation of Cdc25 levels is also required for cell cycle slowing in zebrafish and in frogs (Shimuta et al., 2002; Dalle Nogare et al., 2009).

Titration of histone proteins and/or of the DNA replication machinery has been suggested as a mechanism to regulate the cell cycle at the MBT (Collart et al., 2013; Pérez-Montero et al., 2013; Amodeo et al., 2015; Joseph et al., 2017; Liu et al., 2019; Djabrayan et al., 2019). Recently, a molecular mechanism has been proposed by which titration of histones might be linked to activation of Chk1 (Shindo and Amodeo, 2021). The authors show that the tail of histone H3 is a direct competitive inhibitor of Chk1 activity. This model can explain why Chk1 activity is low in earlier cycles, in which maternally deposited H3 is present in excess, and higher in later cycles, when histones have been sufficiently titrated to allow Chk1 activity to rise (Fig. 4C). In zebrafish, recent work has shown that histone proteins directly compete on chromatin with transcription factors necessary for zygotic transcription. Thus, the dilution of histone protein can also time the onset of zygotic transcription (Joseph et al., 2017), a hallmark feature of the MBT together with cell cycle remodeling and degradation of several maternal mRNAs.

Cell cycle control of zygotic gene activation

Titration of factors involved in both DNA replication or zygotic gene activation (Almouzni and Wolffe, 1995; Collart et al., 2013; Amodeo et al., 2015; Joseph et al., 2017) could explain how DNA content impinges on all MBT processes. A complication in this analysis is that the cell cycle and zygotic gene activation are strongly co-regulated. The early cleavage divisions are not conducive to gene expression, as transcription aborts during mitosis and the short interphase only leaves time to transcribe a few short mRNAs (Heyn et al., 2014; Jukam et al., 2017). Thus, lengthening of the cell cycle favors zygotic gene activation. In turn, transcription can promote activation of the DNA replication checkpoint (Sibon et al., 1997; Blythe and Wieschaus, 2015) and zygotic gene products can inhibit the rapid cell cycle (Edgar et al., 1994b), further supporting transcription. These feedback mechanisms are likely to ensure a robust coordination of all the MBT processes, but they have made dissecting the mechanisms driving the control of the MBT difficult. For example, it was recently argued that slowing of the cell cycle is not merely a central feature of the MBT but is the driving process of this developmental transition in *Drosophila* embryos. Arresting the cell cycle as early as cycle 12 through inhibition of Cdk1 activity triggers the initiation of several MBT processes, namely zygotic gene expression and gastrulation, even when DNA content is below the amount usually needed for the MBT (Strong et al., 2020). Even genes previously identified as dependent upon the embryo reaching a critical N/C ratio were transcribed in Cdk1-inhibited embryos arrested in interphase 12. These and other experiments led the authors to argue that rapid cell cycles are a major inhibitor of the MBT (Strong et al., 2020).

Quantitative arguments support the role of the cell cycle in inhibiting transcription. For example, in cycle 13 embryos there are eight times more nuclei than in cell cycle 10 embryos. Moreover, interphase, i.e. the period compatible with transcription, is at least three times longer (Foe and Alberts, 1983; Farrell and O'Farrell, 2014). Thus, in principle, the embryo would have increased its overall transcriptional capacity by about 25-fold just by nuclear proliferation and cell cycle remodeling (Fig. 4B). Therefore, gradual

inhibition of Cdk1 by Chk1 might be the initiating and controlling event of the MBT, ultimately allowing sufficient time for a significant upregulation of zygotic genome activation, and consequent cell cycle remodeling and morphogenetic processes. Live imaging of transcription also revealed a crucial role for the cell cycle in regulating gene expression, while also arguing for effects independent of cell cycle control (Syed et al., 2021). Imaging of zygotic transcription at single-cell resolution in *Xenopus* embryos revealed significant and systematic differences in the timing of zygotic gene activation. This argued that cell size, which dictates the N/C ratio in individual cells, controls zygotic genome activation (Chen et al., 2019). The importance of the N/C ratio in *Xenopus* embryos is further supported by observations in embryonic chimeras of *Xenopus laevis* and *Xenopus tropicalis*, which result in embryos with different DNA content (Jukam et al., 2021 preprint). The local nature of the sensing of the N/C ratio and its impact on cell cycle control was also suggested in *Drosophila*, where altering the mechanisms of nuclear positioning can generate embryos with different N/C ratios across the anterior-posterior axis. These different ratios are likely the cause for different cell cycle durations along the embryo (Deneke et al., 2019). Importantly, in zebrafish, cell cycle control and the N/C ratio can be decoupled from zygotic transcription and, instead, genome activation is timed from fertilization through the translation of chromatin modifiers from maternal mRNAs and the deposition of epigenetic marks (Chan et al., 2019).

The MBT is a complex transition point for the early embryo that involves multiple hallmark phenomena, most importantly the slowing of the cell cycle and a significant increase in zygotic transcription (Fig. 4A). Quantitative approaches coupled with classical embryological approaches are likely to reveal the mechanisms by which embryos reproducibly initiate these events and how these processes feedback on one another.

Cell cycle control during gastrulation

The remodeling of the cell cycle during gastrulation is most clearly illustrated in *Drosophila* when cells first begin to divide asynchronously. After cellularization in the *Drosophila* embryo, 25 mitotic domains emerge that have local synchrony of cell division but are asynchronous with other neighboring domains in the embryo (Foe, 1989). In these domains, mitosis often begins in few cells and spreads in a wave-like pattern to the boundary of that domain (Foe, 1989). Disruption of the timing of these mitotic patterns can have consequences for embryo viability (Edgar and O'Farrell, 1989). It has been proposed that mitotic domains map to specific fates later in development and may facilitate the establishment of cell populations that show a similar response to differentiation signals (Edgar et al., 1994a; Cambridge et al., 1997). Specifically, mitotic domains must avoid conflicts between cell division and cytoskeletal rearrangements that drive morphogenesis. This is illustrated by the control of mesoderm specification, where a delay is introduced in the cell cycle machinery to ensure that cell divisions always follow the apical constriction process that drives invagination (Grosshans and Wieschaus, 2000).

The spatiotemporal pattern of cell divisions during gastrulation in *Drosophila* is controlled by the transcriptional activation of *string* (*stg*), one of the two Cdc25 phosphatases present in *Drosophila* (Edgar and O'Farrell, 1989, 1990) (Fig. 5A). Quantitative imaging experiments revealed that almost all cells across the embryo are equally sensitive to Cdc25 levels and that the rapid accumulation of Cdc25 can drive mitotic entry independently of Cdk1-positive feedback (Di Talia and Wieschaus, 2012). At the MBT, both
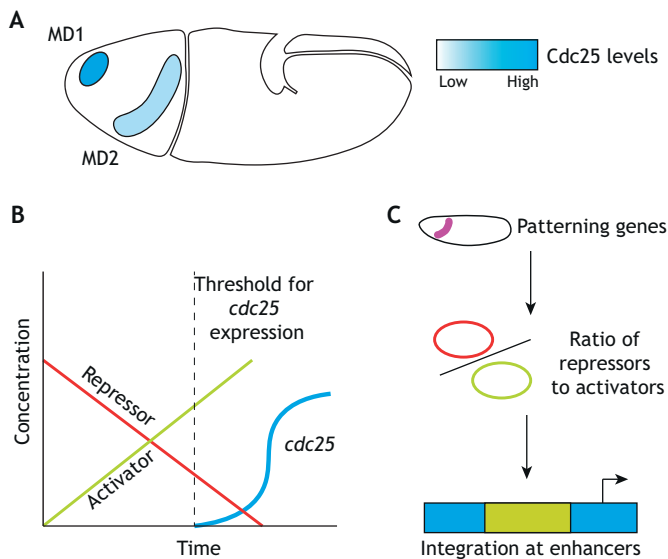
Fig. 5. Differential levels in Cdc25 protein result in spatial asynchrony of mitotic divisions in *Drosophila*. (A) The timing of Cdc25 transcription correlates with the speed of division in mitotic domains (MDs) in *Drosophila*. MD1 and MD2 are the first two of 25 groups of cells to divide during the asynchronous 14th mitosis in *Drosophila* embryos. The onset of mitosis correlates to levels of Cdc25 in each mitotic domain. (B) A ratio of activators and repressors to initiate new *cdc25* transcription controls the timing of mitosis in gastrulating fly embryos. (C) Using multiple activators and repressors whose expression is responsive to patterning genes allows for robustness and reproducibility of the highly stereotyped divisions that give rise to the proper body plan in *Drosophila*.

maternal String and Twine (the other Cdc25 phosphatase) are degraded, and thus pulses of Cdc25 transcription become rate-limiting to initiate mitotic divisions 14-16 (Edgar et al., 1994b). The developmental patterning genes that control differentiation and fate specification, rather than input from the cell cycle, promote *string* transcriptional activation (Edgar et al., 1994a). This was supported by the observation that homozygous mutants in patterning genes lead to spatial changes in mitotic domains or even the loss of entire domains (Edgar et al., 1994a). However, this approach did not provide clear insights into the mechanisms encoding the timing of *stg* expression. These came from a different genetic approach that used heterozygous deficiencies to perform whole-genome screening for regulators of the timing of division in mitotic domains 1 and 2 (Momen-Roknabadi et al., 2016). The authors found that not only are mitotic domains established by spatially restricted transcriptional regulatory networks, but within a domain, the timing of division is tuned by a subset of those same transcriptional regulators. It was proposed that a balance between inhibitory and activating transcription factors controls mitotic precision in each region of the embryo (Momen-Roknabadi et al., 2016) (Fig. 5B,C). Using a combination of inhibitors and activators to time division might reduce the impact of fluctuations in the levels of the transcriptional regulators. However, both theoretical and experimental work is still needed to address this idea and to elucidate how transcriptional networks can achieve both rapid and precise control of cell cycle timing during gastrulation.

How asynchronous divisions are timed during morphogenesis has also been investigated in the chordate ascidian (sea squirt) *Ciona* (Ogura and Sasakura, 2016). In *Ciona*, the 11th mitosis, which occurs after gastrulation, is the first asynchronous mitosis in embryonic development and spreads in a wave across the embryo.

Interestingly, asynchrony is already observed in S phase during cell cycle 10. As in cell cycle 11, anterior cells progress more slowly through S phase than posterior cells. However, there is a reciprocal compensation in G2 so that mitosis in cell cycle 10 remains synchronous, whereas in cycle 11 there is no G2 compensation, leading to asynchronous mitosis across the embryo. As in the mitotic domains of the *Drosophila* gastrula, asynchrony in *Ciona* also depends on the regulation of Cdc25. Two developmentally regulated transcription factors, ci-AP-2 and ci-GATAb, were shown to promote Cdc25 transcription, and loss of these two proteins in cell cycle 10 can disrupt mitotic synchrony (Ogura and Sasakura, 2016). It will be of great interest to determine whether, similar to *Drosophila*, the combinatorial action of activators and repressors is responsible for timing divisions. Understanding whether similar strategies are used in these different embryos to obtain precise transcriptional control of mitotic timing has the potential to reveal general insights on how precise timing is achieved during morphogenesis.

Asynchronous cell divisions are not only observed during gastrulation. In the roundworm *C. elegans,* division is asynchronous as early as the two-cell stage. In this case, the two cells (AB and P_1) are of different sizes, with AB being larger and reproducibly dividing earlier than P_1 (Sulston et al., 1983; Schierenberg and Wood, 1985; Encalada et al., 2000; Brauchle et al., 2003). Regulation does not occur at the level of transcription, as it takes place before zygotic transcription is active. Rather, the PAR proteins, which regulate anterior-posterior symmetry, partition more Polo kinase PLK-1, another positive regulator of mitosis known to contribute to the control of the mitotic switch (Archambault and Glover, 2009), to the AB cell (Rivers et al., 2008; Budirahardja and Gönczy, 2008). Loss of function in *chk-1* and *wee-1* in *C. elegans* diminishes the delay between the AB and P_1 cells, suggesting that the feedback on Cdk1 activity through inhibitory phosphorylations is likely important (Brauchle et al., 2003; Michael, 2016). It has been proposed that differences in the nuclear-cytoplasmic ratio might contribute to cell cycle slowing in *C. elegans* (Brauchle et al., 2003), similar to what is observed in other systems. How polarity cues might play into cell cycle timing in other systems has not been well studied. Comparisons across species will uncover both general principles and species-specific mechanisms of the regulation of cell cycle timing.

Future directions and open questions
The recent use of quantitative imaging tools to dissect the dynamics and molecular mechanisms that couple cell division, morphogenesis, actomyosin dynamics and cytoplasmic flows in developing embryos has given us a better understanding of the coordinated processes that shape early embryogenesis from flies to vertebrates. Here, we have focused on the ways in which molecular mechanisms that control the cell cycle act locally and globally to coordinate development. Developing biochemical sensors of Cdk1 activity in diverse model systems will likely uncover the mechanisms by which embryos coordinate the cell cycle and subsequent physical or morphological events across the expanse of an embryo. These tools have been useful in the *Drosophila* embryo, and directly measuring Cdk1 activity *in vivo* in vertebrates may also provide new insights as these embryos have different geometries and perhaps different spatial regulation of the cell cycle. The syncytial nature of the early fly embryo is very different from the cellular nature of fish embryos, for example. Therefore, although the molecular mechanisms across model systems are likely to be conserved, how each species uses these mechanisms for their own unique biology and body plan organization

will be an interesting avenue for future work. Moreover, using imaging biosensors and quantitative approaches will uncover how waves and flows contribute to collective decision making in other biological systems (Hubaud et al., 2017; Sonnen et al., 2018; Streichan et al., 2018; Jörg et al., 2019; Saadaoui et al., 2020; De Simone et al., 2021).

In mammals, the series of rapid divisions before gastrulation is slightly delayed in comparison with other vertebrate embryos due the need to establish extra-embryonic tissues soon after fertilization (O'Farrell et al., 2004). However, the rapid divisions of the pre-gastrulating epiblast cells in mammalian embryos share similar cell cycle dynamics with non-mammalian embryos, as gap phases in epiblast cell cycles are shortened or absent (O'Farrell et al., 2004). New technology that enables imaging of mammalian embryos *ex vivo* or in reconstituted systems (Shahbazi et al., 2016; Deglincerti et al., 2016; McDole et al., 2018; Zheng et al., 2019; Minn et al., 2020; Kohrman et al., 2021) provides developmental biologists with the opportunity to address how cell cycle machinery might coordinate developmental events in mammalian embryos. It will be interesting to see whether mechanisms similar to ones described in this Review are at play in the rapid cell divisions that precede gastrulation and in the transition to asynchronous divisions after gastrulation (Mac Auley et al., 1993). Most importantly, there may be previously unidentified feedback mechanisms involving cell cycle machinery that are crucial for gastrulating mammalian embryos.

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