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Temperature-Induced Uncoupling of Cell Cycle Regulators

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The early stages of development involve complex sequences of morphological changes that are both reproducible from embryo to embryo and often robust to environmental variability. To investigate the relationship between reproducibility and robustness we examined cell cycle progression in early *Drosophila* embryos at different temperatures. Our experiments show that while the subdivision of cell cycle steps is conserved across a wide range of temperatures (5-35°C), the relative duration of individual steps varies with temperature. We find that the transition into prometaphase is delayed at lower temperatures relative to other cell cycle events, arguing that it has a different mechanism of regulation. Using an *in vivo* biosensor, we quantified the ratio of activities of the major mitotic kinase, Cdk1 and one of the major mitotic phosphatases PP1. Comparing activation profile with cell cycle transition times at different temperatures indicates that in early fly embryos activation of Cdk1 drives entry into prometaphase but is not required for earlier cell cycle events. In fact, chromosome condensation can still occur when Cdk1 activity is inhibited pharmacologically. These results demonstrate that different kinases are rate-limiting for different steps of mitosis, arguing that robust inter-regulation may be needed for rapid and ordered mitosis.

Introduction

Development is composed of reproducible sequences of events. In the early fly embryos for example, the initial cleavage divisions involve alterations between interphase and mitosis. These alternations occur simultaneously with changes in the organization of actin and microtubule cytoskeleton, the morphology of the cell surface, nuclear import and the location and behavior of chromosomes (Rabinowitz, 1941; Foe and Alberts, 1983; O'Farrell, Stumpff and Su, 2004; Farrell and O'Farrell, 2014). All these cell cycle dependent oscillations coincide with other morphological and biochemical changes that occur on longer time scales over the thirteen cleavage divisions. These include and involve complex patterns of translational control, RNA degradation, transcriptional initiation and the stage specific formation of organelles like nucleoli (McKnight and Miller, 1976; Zalokar, 1976; Lu *et al.*, 2009; Falahati *et al.*, 2016). Although each individual system involves large number of proteins and enzymatic processes, the timing of progressions are thought to depend on rate limiting components. In normal development, these multiple sequences appear to run in parallel, and while sequences are clearly coupled with each other, it is not clear how distinct processes are coordinated. The mechanisms that underlie this coordination are unclear even in fairly straight forward linear processes like cell cycle progression. Although overall progression is thought to depend on the

cyclin dependent kinase Cdk1 (Pomerening, Sontag and Ferrell, 2003; Morgan, 2007; Ferree and Di Talia, 2018), specific transitions involve other kinases (such as Aurora B and Polo) that appear to target specific processes (Adams *et al.*, 2001; Giet and Glover, 2001; McClelland and O'Farrell, 2008). How these activities and rate constants are coordinated remains unclear.

Development must be robust over a range of environmental conditions. This is particularly important for ectotherms like flies where embryos develop outside the body of the mother and therefore are especially sensitive to ambient temperature, while the sequence of each developmental process must maintain its normal order over tens of degrees. Although some developmental processes like liquid-liquid phase transitions are enhanced by colder temperatures (Falahati and Wieschaus, 2017; Dignon *et al.*, 2019; Falahati and Haji-Akbari, 2019), most developmental processes are enzymatically driven and their rates increase as temperatures are raised. For simple biochemical reactions this behavior is well described by the Arrhenius equation, where the rate depends on the reciprocal of the temperature. The Arrhenius equation has even been applied and shown to describe complex biological processes like yeast growth rates (RICHARDS, 1928) and plant's nonphotosynthetic metabolism (CRIDDLE *et al.*, 1994) and root respiration (Earnshaw, 1981). A study of 1072 thermal responses of 112 physiological and ecological traits across 309 species showed that 87% of these traits fit well with the Arrhenius model, albeit with varying degrees of scaling coefficients, as is expected for processes that are regulated by different mechanisms (Dell, Pawar and Savage, 2011).

In the following experiments, we examine the temperature sensitivity of the early cleavage divisions in *Drosophila*, and find a remarkable robustness in cell cycle progression, with normal morphological transitions and successful nuclear division at temperatures as low as 5°C. In these experiments we use confocal microscopy and various fluorescent markers to track nuclear morphology, centriole replication, nuclear membrane break down and chromosome condensation and movement to the metaphase plate. Although all these processes occur normally and successfully, we find that the relative duration of some cell cycle transition are prolonged relative to others. We interpret the differential delays in a model based on the Arrhenius relationship. In this model, cell cycle transition are governed by different limiting enzymes with different activation energies and therefore different temperature dependent kinetics. One consequence of the model is that entry into prophase must be driven by a different kinase than its exit into prometaphase. We test this possibility by following the ratio of Cdk1/PP1 activity and also by pharmacologically inhibiting Cdk1 and show that while chromosome condensation can occur in the absence of a detectable Cdk1 activity, the start of prometaphase as indicated by chromosome congression to the metaphase plate coincides with and requires Cdk1 activity.

Results

The first 13 divisions in *Drosophila* embryos occur synchronously through rapid rounds of DNA synthesis (S-phase) and nuclear mitosis (Rabinowitz, 1941; Farrell and O'Farrell, 2014). After their ninth division when the nuclei have moved to the surface of the embryo, progression through the cell cycle can be easily followed using fluorescently tagged proteins for nuclear and

cytoplasmic components. To follow these mitoses during different temperature regimes, we transferred embryos laid at room temperature to a microfluidic device that allows confocal imaging at a constant temperature between 5-35°C (Fig. S1) (Lucchetta *et al.*, 2005; Falahati and Wieschaus, 2017). Under these conditions and at temperatures from 5°C to room temperature, development is morphologically normal to at least the gastrula stage. 68% of the nuclear cycle 11 (NC11) embryos that are shifted to 7°C degrees hatch ($n = 37$). At high temperatures (e. g., 35°C) defects in chromosome segregation are observed and later development is abnormal (Fig. S4), consistent with previous reports of induction of heat-shock mechanisms at this temperature (Dura, 1981).

For more detailed analysis of individual components, we focus on NC11 due to the ease of imaging. While lowering the temperature to a minimum of 5°C increases the duration of NC11 (from 10m:30s $\pm$ 15s at 22°C to 92min $\pm$ 12min at temperatures 5-7.5°C, Fig 1A, Table S1), it does not change the examined events characteristic of each cell cycle step. In particular, as depicted in Fig. 1B and movie1 for two embryos developing at 22 and 7°C, there are no detectable differences in the large-scale chromatin structure and chromosomal movements, as visualized by fluorescently-tagged Histone H2A variant (H2Av). Interestingly, we also found no detectable irregularities in centrosome cycle at low temperatures. To visualize the microtubule organization, we imaged a microtubule associated protein (MAP), Jupiter-GFP (Karpova *et al.*, 2006), and quantified the distances of the centriole pairs (Fig. 1B and S2, movie2). Similar to systems with cold stabilizing MAPs (Denarier *et al.*, 1998), no reduction is detectable in the density of microtubules in fly embryos at 8°C. In addition, between 22 and 5°C, a conserved pattern is observed for the changes in the nuclear envelop integrity at different steps of cell cycle (Fig. 1B, S3A), visualized by following the nuclear localization of fluorescently-tagged Fibrillarin (Falahati *et al.*, 2016). Finally, the foci of a replication machinery protein, PCNA (Shermoen, McClelland and O'Farrell, 2010), are observable during interphase at both temperatures, and become undetectable prior to late prophase (Fig. 1B, S3B).

In addition to the qualitative similarities, we examined the changes in the length of NC steps. We find that although progress through the cell cycle shows the normal sequence, the relative fraction of interphase spent in each subphase varies with temperature. The most striking deviation from perfect scaling is observed in the relative lengthening of interval between prophase entry and the exit into prometaphase. At room temperature (22°C, $n = 6$), the duration (1.3 $\pm$.3 minutes) represents 12% ($\pm$ 2%) of the total length of NC11, whereas at 6°C (between 5 and 7.5 mins, $n=7$), its duration (23 $\pm$ 4 minutes) represents 25% ($\pm$ 2%) of NC11 length.

The kinetics of simple chemical reactions are often well described by the Arrhenius equation in which the rate of the reaction depends on temperature and the activation energy (Arrhenius, 1915). In the simplest formulation,

$$k = Ae^{\frac{-E_a}{k_b T}} \quad (1)$$

where A , E_a and k_b are the pre-exponential factor, activation energy, and Boltzmann constant, respectively. This equation is based on the transition-state theory, which describes the dependence of the rate of a reaction on the number of molecules with energies higher than E_a , or the activation energy. The number of molecules with such high energy in turn depends on the temperature, as described by the Maxwell-Boltzmann distribution. Biological processes can be tested for whether they follow this relationship by plotting the log of their rate against the reciprocal of the temperature. In such plots, Arrhenius-like behaviors show linear relationships with slopes proportional to the activation energy of the relevant enzymatic process.

To test whether an Arrhenius type model can be used to describe the temperature dependence of cell cycle progression, we calculated rates for individual processes as the reciprocal of their durations. When the log of the measured rates is plotted as a function of temperature, the rates of all NC steps between 5-22°C fit well with the Arrhenius equation ($r^2 \geq 0.82$, Fig. 2A-B). At 35°C, rates are significantly lower and deviate from the linearity observed over the remainder of the temperature range, consistent with the morphological abnormalities and lethality observed at that temperature (Fig. S4). The temperature dependencies of the *Drosophila* cleavage stages are similar to the Arrhenius-type behaviors reported for the rate of completion of the first embryonic cycle of *C. elegans* at this temperature range (Begasse *et al.*, 2015), and also for the total developmental time of *Drosophila* species between 17.5 and 27.5°C (Kuntz and Eisen, 2014).

Because temperature dependencies are approximately linear in *Drosophila* between 5-22°C, the slopes can be used to calculate apparent “activation energies” associated with each process (Fig. 2C). The E_a values obtained are within the range of other reported biological reactions (RAVEN and GEIDER, 1988; Gillooly *et al.*, 2001; Dell, Pawar and Savage, 2011). Interestingly, the slopes fall into distinct classes, with very similar slopes and calculated activation energies for interphase, prometaphase, anaphase and telophase. The Arrhenius plot of the prophase temperature-response has a slope that is significantly steeper ($P < 0.009$ with t -test), suggesting that exit from prophase and initiation of prometaphase may be determined by a regulatory enzyme with an E_a higher than the regulators of other cell cycle steps. The prolonged prophase at low temperatures has morphological consequences, resulting in hypercondensed chromosomes that decorate the nuclear envelope, while awaiting the delayed chromosome congression (Fig. 1B, movie1). The delay in prophase exit is associated with a delayed breakdown of the nuclear membrane, as visualized by redistribution of the nuclear protein, RFP-Fibrillarin (Fig. 1B, S3A). The duration of metaphase is also prolonged at lower temperatures, although it has a different slope from that observed for prophase, or for the other processes of the cell cycle, suggest that its regulation may also be distinct from that of other cell cycle steps. For the purpose of this paper, we will focus on the regulation of prophase.

To characterize the enzyme driving the entry into prometaphase, we employed a Förster resonance energy transfer (FRET)-based sensor to monitor the activity of Cdk1 in embryos

developing at different temperatures (Gavet and Pines, 2010; Deneke *et al.*, 2016, 2019). This sensor measures the ratio of Cdk1 to PP1 activity (Cdk1/PP1 activity, hereafter) (Deneke *et al.*, 2019). As depicted in Fig. 3A and S5, two main differences are observable between NC11 embryos developing at 22 and 7°C. First, the Cdk1/PP1 sensor shows a slower down-regulation of the FRET signal at 7°C, which suggests slower dephosphorylation of the sensor by Anaphase Promoting Complex and/or PP1. Determining this temperature-sensitive step can be the topic of future studies. Interestingly, at 7°C, the increase in Cdk1/PP1 activity is only detectable later in prophase or at the onset of prometaphase, but no increase in the FRET signal is detectable at the onset of prophase. Although exit from prophase is coupled to a rapid increase in the Cdk1 activity, entry into prophase, that is the beginning of chromosome condensation, occurs when Cdk1 activity is much lower than that observed for prophase entry at higher temperatures. Since the FRET sensor measures the balance between phosphorylation by Cdk1 and dephosphorylation by PP1, our results at 7°C demonstrate a prolonged period during which PP1 activity is high and Cdk1 is low (Fig. 3A), arguing for a delayed activation of Cdk1. Remarkably, nuclei were still able to enter prophase despite the low Cdk1 activity, an observation which was confirmed by examining cell cycle progression when Cdk1 activity was inhibited by the small molecule inhibitor roscovitine. Injection of roscovitine blocks exit from prophase, but had no effect on entry. Chromosomes initiate condensation (Fig. 3C, movie3) and ultimately show the same hypercondensation observed during the prolonged prophase at low temperatures (Fig. 3C). Together, these results indicate that at NC11, the fast activation of Cdk1 is necessary for entry into prometaphase, while earlier cell cycle events such as chromosome condensation can occur independently of such Cdk1 activation.

The altered cell cycle dynamics observed at 7°C raises one main question: What causes the high sensitivity of Cdk1 to temperature? It is unlikely that the delayed Cdk1 activation is caused by activation of cell cycle checkpoints, given that mutant embryos lacking the checkpoint components *grapes* and *loki* (*chk1* and *chk2*) still exhibit chromosome hypercondensation at 7°C (Fig. 3C) and given previous reports that the inhibitory Tyr15 phosphorylation associated with checkpoint activation is not detectable in NC11 embryos at normal temperatures (B. A. Edgar *et al.*, 1994). We confirm that the phosphorylation remains undetectable at 7°C (Fig. 3D). Another possible explanation might be a delayed accumulation of Cyclin B (CycB) levels at low temperatures. At 22°C, mean nuclear intensity of CycB measured using a GFP-CycB reporter increases monotonically from the onset of interphase, and sharply drops at anaphase (Fig. 3B, S6A). At 7°C, the basic pattern is similar, although the monotonic increase is only detectable in prometaphase. At both temperatures, the kinetics of CycB accumulation parallels the rise in Cdk1 activity (Fig. S6B). The linear relationship between the nuclear accumulation of CycB and Cdk1 activity at both T's suggests that in our experiments CycB is rate limiting for the activation of Cdk1, and that a slower CycB synthesis rate or delayed nuclear import might underlie the unique temperature dependence of prophase duration in the Arrhenius plots. To further assess the role of CycB as the limiting factor for Cdk1 activation, we used the FRET sensor to compare the Cdk1/PP1 activity in NC11 of embryos from WT versus *CycA*^{+/-}*CycB*^{+/-} females (Fig. S6C). Two main features are noticeable: 1. The increase in Cdk1/PP1 activity is slower/delayed when *CycA* and *CycB* levels are reduced, and 2. the length of NC11 in embryos with reduced *CycA/CycB* mRNA levels is longer than that of the wildtype. Consistent with these results, previous reports

have also shown that increasing the maternal level of CycB decreases the length of NC11 (Stiffler et al., 1999). Such a suggestion would be consistent with the central role for CycB described in previous reports (B. a. Edgar *et al.*, 1994; Pomerening, Sontag and Ferrell, 2003; Gavet and Pines, 2010). A general feature of this model is that the hypothetical activation energy measured in our Arrhenius plot would govern a rate limiting step in the CycB accumulation process, rather than a temperature sensitive feature of the Cdk1 enzyme itself.

Discussion

Understanding how sequences of cellular transitions are regulated remains a fundamental, yet poorly understood, biological question. During cell cycle progression, for example, it remains unclear whether the various morphological transitions between interphase and mitosis reflect the accumulation pattern of a single rate limiting regulator, or whether each step is associated with its own distinct regulatory components. The complex feedbacks that characterize genetic networks (Morgan, 2007; Lindqvist, Rodríguez-bravo and Medema, 2009; Heim, Rymarczyk and Mayer, 2017) make it difficult to define roles of single components using null mutations and has led to conflicting conclusions on the relative importance of the various kinases involved. Here, we use subtle perturbations in temperature as an alternative to distinguish the mechanisms regulating different transitions. Our analysis of these temperature effects suggest that different enzymes are characterized by different scaling behaviors with respect to temperature, and thus, measuring the dependence of the timing of cellular events on temperature can be used to reveal underlying regulatory dynamics.

Cell cycle transitions in early *D. melanogaster* embryos proceed through a strict sequence of events - DNA replication and centrosome doubling are followed by chromatin condensation, movement of the chromosomes to a single metaphase plate and their eventual separation at mitosis that can be easily scored in living embryos. The timely order with which these events occur is pivotal for the successful transfer of genetic material to daughter cells. This is particularly important for the embryos of ectotherms such as fly, that despite the changes in the ambient temperature, are able to maintain this order. This robustness to the environmental changes is surprising as the rate biochemical reactions is expected to be affected differently with temperature. Yet, we observed that fly embryos are able to complete cell cycle in an orderly fashion over a large temperature range (5-22°C). Several regulatory mechanisms have been linked to an autonomous oscillator centered on the activity of Cdk1 to control the order of cell cycle (Morgan, 2007). However, the presence of feedbacks between Cdk1 and other mitotic kinases such as Polo and Aurora B makes it difficult to determine whether individual cell cycle events are triggered at specific levels of activity of Cdk1 or whether other mitotic kinases, coupled to Cdk1 play more direct roles in regulating mitotic processes. We reasoned that precise measurements of the temperature dependency of mitotic processes might reveal novel insights.

Our application of the Arrhenius equation assumes that the rate of a multi-reaction system can be approximated by a single rate-limiting step. In the case of cell cycle, examples of such a step include phosphorylation by a mitotic kinase, protein synthesis leading to the production of a

key enzyme or substrate, or active redistribution of the key enzyme in mitotic regulation. While this reductionist model does not explain all aspects of complex biological systems, it can be used as a tool to suggest the presence of different regulatory mechanisms when different Arrhenius dependencies are observed. Based on this, if Cdk1 is rate-limiting for all mitotic processes, we expect that these processes will show a similar dependency on temperature. In contrast, if different mitotic kinases contribute to the regulation of the timing of mitotic processes, it is likely that we would observe different Arrhenius dependencies. We exploit this differential sensitivity to uncouple cell cycle processes and evaluate the contribution of Cdk1 in regulation of the minimal cell cycles of early *Drosophila* embryos. The use of this method is warranted as the sequence of events in this system remains unchanged across a temperature range of 5-22°C, and similar to many other biological traits (Dell, Pawar and Savage, 2011), the rates of different cell cycle steps fit well with the Arrhenius model.

The sensitivity of Cdk1 activation to low temperatures correlates well with a delay in the accumulation of CycB in the nucleus, suggesting that nuclear accumulation of CycB is rate-limiting for the activation of Cdk1. This slower increase in the nuclear CycB could be due to a delay in translation, which is an energetically costly process. Alternatively, the nuclear import of CycB could be more sensitive to temperature, and therefore causing its slower accumulation. Since the nuclear accumulation of other proteins such as Fibrillarin is not affected, this possibility would indicate a specific mechanism for the nuclear import of CycB. Future studies are needed to examine these possibilities.

By combining this temperature-based method with optical biosensors and pharmacological approaches, we studied the regulatory mechanisms of cell cycle in fly embryos. Our results indicate that entry into prometaphase is regulated by Cdk1. However, earlier cell cycle events such as chromosome condensation and centrosome cycle can occur independently of detectable Cdk1 activity, potentially by relying on other mitotic kinases such as Aurora and Polo (Adams *et al.*, 2001; Giet and Glover, 2001; McClelland and O'Farrell, 2008). Consistent with this model, experiments in fly embryos using RNAi against mitotic cyclins show that the centriole cycle still continues in reduced Cdk1 activity (Novak *et al.*, 2016). Together, these results suggest that the role of Cdk1 as the master regulator of cell cycle might not be as general as previously assumed.

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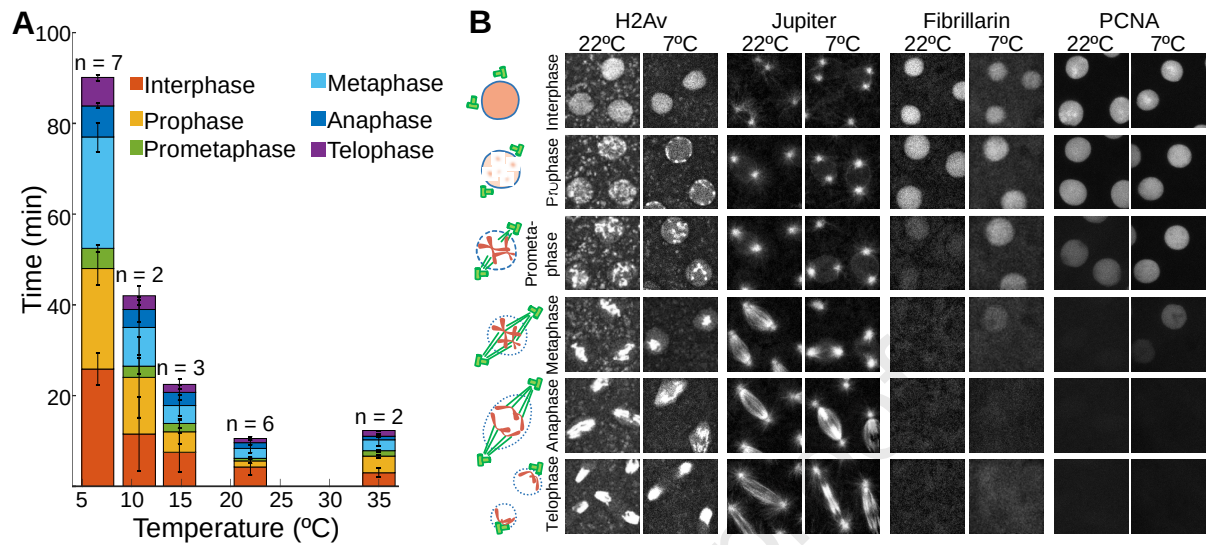
Figures:

Fig. 1: Effect of temperature on cell cycle events. **A** The total length of different steps of cell cycle were calculated based on the subcellular dynamics of H2Av. **B** The following fluorescently-tagged proteins were used as a proxy for different cell cycle events: H2Av for cell cycle steps; Jupiter for centrosome cycle; the nuclear protein, Fibrillarin, for nuclear envelope breakdown and reformation; and PCNA for DNA replication. Images are $25 \times 25 \mu\text{m}$.

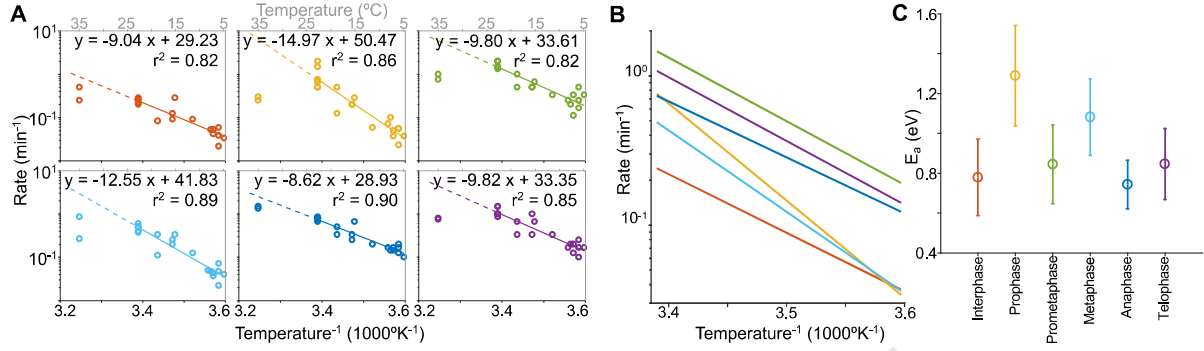


Fig. 2: Arrhenius-dependence of cell cycle steps. **A** and **B** The Arrhenius plot for each of the cell cycle steps is depicted, with a fitted line to the linear region (5–22°C). Each dot represents the rate for a single embryo, and 18 embryos are at the linear region. The rate is shown in logarithmic scale. A comparison of the fitted lines is shown in **B**. **C** shows the calculated E_a for each cell cycle step. Error bars: 95% confidence intervals.

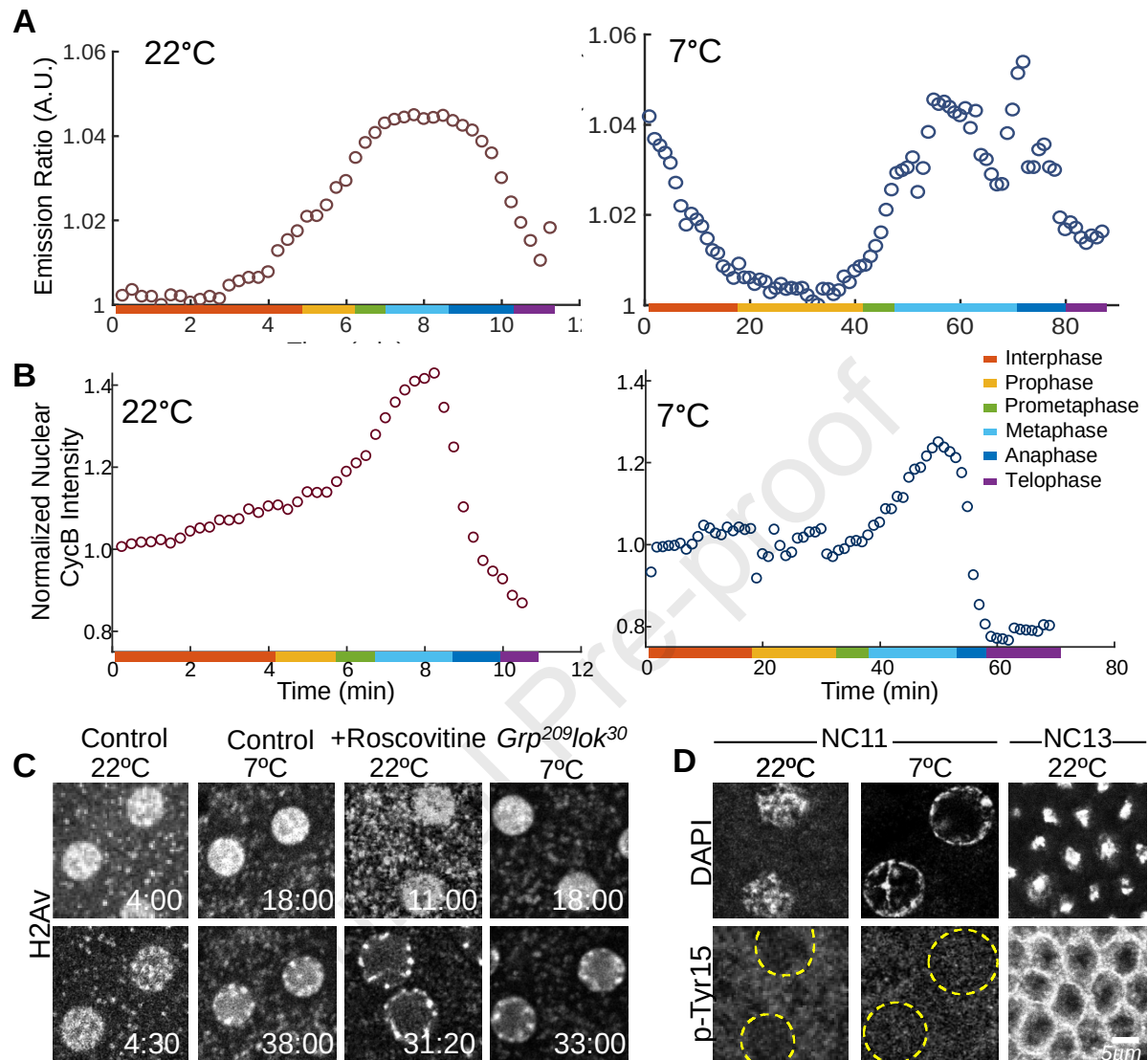


Fig. 3: Cdk1 activity drives exit but not entry into prophase. **A** Cdk1 activity at 22 and 7°C is quantified using a FRET-based biosensor. **B** Normalized nuclear concentrations of CycB throughout NC11 at 22 and 7°C. Shaded areas show prophase. **C** Single plane images of WT untreated embryos (controls), WT embryos treated with roscovitine, and check-point mutant embryos (*Grp*^{209lok30}), during early and late prophase at different temperatures. **D** Cdk1's inhibitory p-Tyr15 is not detectable at any temperatures at NC11, but can be detected in NC13 embryos. Times in min:sec.

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Methods

Fabrication and utilization of the microfluidic device. The microfluidic device used here was fabricated as described previously (Lucchetta *et al.*, 2005; Falahati and Wieschaus, 2017). Fig. S1 shows the schematic of the device. It is comprised of two parts: The PDMS channel which is placed on top of a PDMS-coated cover glass. The embryos are mounted on the coated coverglass by placing a drop of heptane glue on top of the embryos. The excess heptane is swiftly wiped out. Approximately 20 embryos were mounted each time. After assembling the two parts, three pairs of small magnets were placed across the channels to lock the device in place and prevent leakage. A syringe pump was used to control the inward flow of water into the channels, and a second pump was placed at the outlet to apply suction and direct the flow through the channels. Since coordinating the speed of the two pumps is difficult, the suction pump was placed at full speed and an inlet for air was added to the channel close to the connection of the suction pump to compensate for the extra volume and prevent discontinuity in the flow of water passing through the embryos. The temperature of the fluid is measured by two thermometers placed at 0.5cm upstream the embryos. The temperatures were kept at $\pm 1^\circ\text{C}$ of the reported values.

Staging of live embryos. The staging of live embryos was done using fluorescently-tagged H2Av. The beginning of interphase was denoted when the chromosomes decondense and histone is homogeneously distributed in the nucleus. The start of prophase is noted by reappearance of inhomogeneities in the distribution of histone, indicative of the beginning of chromosome condensation. Prometaphase starts with chromosomes migration toward the metaphase plane. The beginning of metaphase is when all chromosomes reach the metaphase plane. The separation of sister chromatids toward opposing poles determines the duration of anaphase, and chromosome decondensation occurs at telophase. The rate of each step shown in Fig. 1 is defined as the inverse of the time required for the completion of that particular step.

Transgenic lines. Flies expressing Cdk1 FRET sensor (Deneke *et al.*, 2016), PCNA-EGFP (Blythe and Wieschaus, 2016), and RFP-Fibrillarin (Falahati *et al.*, 2016) were described previously. CycB-GFP fly stock is from Bloomington *Drosophila* Stock Center (BDSC 51568).

Confocal imaging and image analysis. Embryos were imaged either using 63 $\times$ HCX PL APO CS 1.4 NA oil-immersion objective on a Leica SP5 laser-scanning confocal microscope equipped with GaAsP 'HyD' detectors, or Leica SP8 confocal microscope, 20 $\times$ /0.75 numerical aperture oil-immersion objective. All image analyses were performed with ImageJ (Rasband WS, ImageJ; National Institutes of Health; (1997-2008)) and MATLAB (MathWorks, Natick, MA) routines.

To measure the changes in nuclear concentration of CycB, embryos coexpressing CycB-GFP and H2Av-RFP were imaged. The maximum projected images were used for quantification, and a nuclear mask was generated by applying a Gaussian filter and thresholding the H2Av-RFP signal. The mask was then used to measure the average nuclear intensity of CycB-GFP. This average intensity is then divided by its initial value in each cell cycle to normalize.

To measure the distance between two centrosomes, embryos coexpressing H2Av-RFP and Jupiter-GFP were imaged at different temperatures. An ImageJ macro was developed to automatically segment the field of view to detect the area surrounding each nucleus. This segmentation was manually evaluated to fix the false positive/negatives. Then in each segmented area, the local maxima of the Jupiter-GFP signal were detected automatically and reconfirmed manually. Finally, the distance between the two centrosomes in each image was measured.

PCNA foci where quantified by measuring the local inhomogeneity of the GFP signal in the nucleus over time, as described previously (Falahati *et al.*, 2016). Maximum projected images were used and the homogeneity in the pixel intensities were measure by a customized GLCM method. To reduce the noise, the neighboring 5x5 pixels were averaged. The homogeneity was measured using MATLAB's built-in function with the following equation:

$$Homogeneity = \sum_{i,j} \frac{p(i,j)}{1 + |i - j|}$$

where i and j are the centers of intensity bins, and $p(i,j)$ is the probability of two particular intensities being neighbors.

For FRET measurements, the fluorescent intensity of CFP and YFP were measured by averaging over the part of the embryo that is in the field of view at different conditions and different time-points, and the ratio of YFP/CFP is calculated.

Fixation and immunostaining at different temperatures. The newly laid embryos were collected at 22°C for 90min and then incubated either at 22°C or at a cooling device set at 7°C for 30min. For embryos incubated at 7°C, the fixation process was carried out at 4°C, using pre-cooled fixative. The primary antibody used is rabbit Cdc2 phosphor-Tyr15 (IMG-668, IMGENEX).

Injection of inhibitors. All the embryos were collected without using the halocarbon oil (this is to make sure that embryos are not protected from desiccation because of the oil), dechorionated in 50% bleach for 1 minute, desiccated in a chamber filled with Drierite

(desiccant) for 8 minutes, and the drug was injected into the middle of the embryo using the Eppendorf Femtojet microinjector.

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Highlights

- Cell cycle processes are conserved in early *Drosophila* embryos across a wide temperature range.
- The duration of individual cell cycle steps is consistent with an Arrhenius model.
- Prophase is the process most sensitive to temperature and likely controlled by a different regulatory mechanism.
- Pharmacological approaches and FRET sensor show that Cdk1 drives entry into prometaphase, but not prophase.