

Maternal cyclin B levels “Chk” the onset of DNA replication checkpoint control in *Drosophila*

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Summary

In many animals, early development of the embryo is characterized by synchronous, biphasic cell divisions. These cell divisions are controlled by maternally inherited proteins and RNAs. A critical question in developmental biology is how the embryo transitions to a later pattern of asynchronous cell divisions and transfers the prior maternal control of development to the zygotic genome. The most-common model regarding how this transition from maternal to zygotic control is regulated posits that this is a consequence of the limitation of maternal gene products, due to their titration during early cell divisions. Here we discuss a recent article by Crest et al.⁽¹⁾ that instead proposes that the balance of Cyclin-dependent Kinase 1 and Cyclin B (Cdk1-CycB) activity relative to that of the *Drosophila* checkpoint kinase Chk1 determines when asynchronous divisions begin. *BioEssays* 29:949–952, 2007.

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The mid-blastula transition (MBT)

Early stages of embryonic development are characterized by rapid, synchronous cell cycles alternating between S phase and mitosis. Progression through these initial stages of embryogenesis is independent of expression from the zygotic genome and is regulated by maternal stores of mRNA and proteins. Following these initial synchronous cell cycles, zygotic transcription is initiated and the cell cycle acquires gap phases and checkpoint controls. These changes are necessary for gastrulation to occur and embryogenesis to continue. Together these events end the biphasic (S phase & mitosis) and synchronous cell divisions exhibited by the embryo and

transfer the prior maternal control of development to the zygotic genome. The period when these crucial events occur has been termed the mid-blastula transition (MBT).⁽²⁾ Understanding the regulation of this transitional phase is crucial to deciphering the mechanisms controlling early embryogenesis.

The MBT in *Drosophila*

The dramatic changes in developmental events occurring at the blastula stage were first reported in the vertebrate species *Xenopus laevis*,^(2,3) although the MBT appears to be a conserved phenomenon observed in many vertebrate and invertebrate species. *Xenopus* has been widely used as a model system for early development due to the availability of large numbers of easily manipulated eggs. However, studies on the contribution of maternal gene products during embryonic development in *Xenopus* are complicated by the presence of multiple copies of the same gene due to genome duplication events.

Drosophila is an attractive invertebrate model for studies of the MBT due to its rapid embryogenesis and amenability for genetic studies. During *Drosophila* embryogenesis, the nuclei undergo 8 mitotic divisions without accompanying cytokinesis to generate a syncytium of ~256 nuclei that accumulate in the central portion of the embryo.⁽⁴⁾ The nuclei then migrate towards the periphery to form a syncytial blastoderm around the 10th cycle of nuclear division in the zygote. During the final syncytial nuclear divisions at the blastodermal stage (cycles 11–13), there is a prominent increase in interphase duration. During the 14th cycle, cellularization of the blastoderm occurs and asynchronous cell divisions begin. These cell divisions have an interphase length of 60 minutes, which is significantly longer than the interphase length of 15 minutes observed during the 13th cell division.^(1,5) Interestingly, this cellularization of the syncytial blastoderm in *Drosophila* requires zygotic transcription.⁽⁶⁾ Thus, the 14th cycle in the *Drosophila* embryo is associated with an increase in zygotic transcription and prominent lengthening of interphase and is often referred to as the MBT stage. Identifying the maternal molecules that regulate the early cell cycles and MBT in *Drosophila* is crucial to understand embryogenesis.

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Levels of maternal CycB influence the onset of events occurring during blastula transition

Cyclin B (CycB) is an essential maternal protein for cell cycle progression during early embryonic development in invertebrate and vertebrate species. In *Xenopus*, translation of maternal CycB is both necessary and sufficient for progression through the early embryonic cell cycles, which are biphasic and synchronous.⁽⁷⁾ Levels of maternal CycB are regulated both at the level of translation of maternal mRNA and by controlling CycB protein activity. The maternal store of CycB protein accumulates during each interphase and is degraded at the end of mitosis.⁽⁷⁾ The activity of newly synthesized CycB is also regulated in the early embryo by periodic inactivation of its enzymatic binding protein, Cyclin-dependent kinase 1 (Cdk1).⁽⁸⁾

CycB levels also play an important role in regulating early embryonic development in *Drosophila*.^(9–11) Ji et al. reported an inverse relationship between the levels of maternal CycB at the blastodermal stages and the interphase length.⁽¹¹⁾ Wild-type *Drosophila* have two copies of the *cycB* gene. Embryos from mothers with one copy of *cycB* (*one cycB* embryos) begin interphase extension earlier than wild-type embryos, while embryos from mothers with four or six copies of *cycB* (*four cycB* or *six cycB* embryos) have delayed interphase lengthening.

Similarly, CycB levels influence the time of onset of DNA replication checkpoints.⁽¹⁾ Previous studies have shown that the onset of detectable DNA replication checkpoint activity occurs at the blastodermal stage (cycle 12) in fly embryos and is dependent on the levels of the checkpoint regulators, Grapes (homolog of Checkpoint kinase 1 (Chk1) in *Drosophila*) and Ataxia-telangiectasia Rad3-related kinase (ATR).⁽⁵⁾ This DNA replication checkpoint activity was observed much earlier (cycle 10) in *one cycB* embryos and much later in *six cycB* embryos. These results suggest that the levels of maternal CycB influence the timing of MBT-related events such as interphase extension and the onset of DNA replication checkpoints.

Controlling onset of the DNA replication checkpoint

In their recent study, Crest et al. use a genetic screen to identify several maternal products that suppress *six cycB* phenotypes.⁽¹⁾ This screen led to the identification of *dRPA2* (replication protein A) as a modifier for the abnormal mitotic phenotype at cycle 14 in *six cycB* embryos. Reducing *dRPA2* to one functional copy suppressed the ability of *cycB* overexpression (*six cycB*) to delay interphase extension and the onset of DNA replication checkpoint activity. For example, the *dRPA2/six cycB* embryos showed interphase extension times similar to wild-type embryos in late blastodermal stages. Thus, the dose of *dRPA2* in the embryo modulates the ability of Cyclin B protein levels to regulate both interphase lengthening

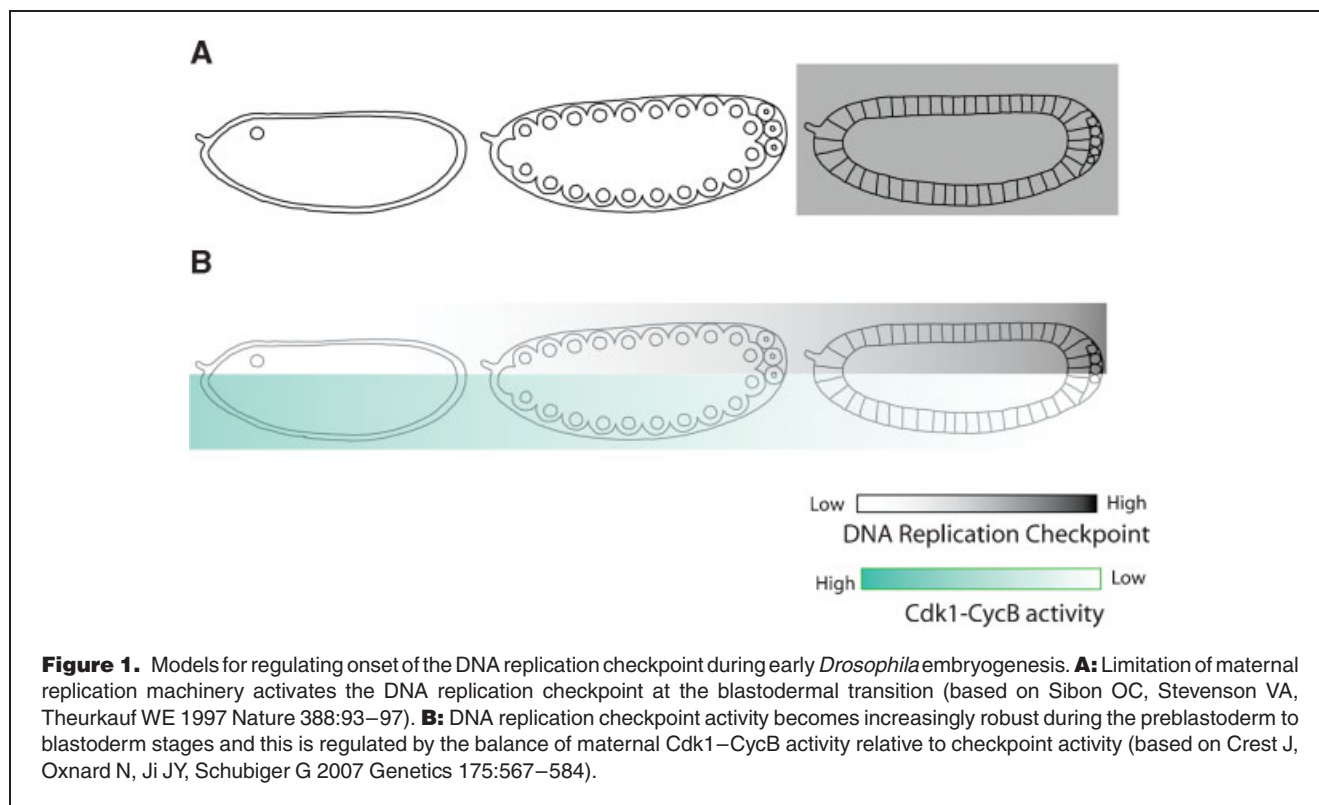
and DNA replication checkpoint controls during the blastula stage.

dRPA2 (Replication protein A) is a part of the RPA complex that stabilizes single-stranded DNA during replication.⁽¹²⁾ How might this activity regulate Cdk1–CycB activity to control blastodermal transition? One possible model proposed by the authors for this phenomenon is that reducing the levels of *dRPA2* results in less RPA-coated ssDNA, which in turn slows down DNA replication and results in the activation of Chk1.^(13,14) Chk1 is part of a pathway that monitors DNA damage at the G₂/M checkpoint in the cell cycle. This pathway is used by cells as a surveillance mechanism to ensure that DNA replication is complete and to monitor genomic integrity before further cell cycle progression occurs.^(15–17) Activated Chk1 leads to cell cycle arrest and inhibition of Cdk1–CycB activity. Thus, decreasing the level of *dRPA2* may override the effects of *cycB* overexpression by activating Chk1. This is consistent with previous studies that demonstrated that the RPA complex regulates Chk1 activity via ATR kinase⁽¹⁶⁾ and that Chk1 inhibits Cdk1–CycB activity through the protein phosphatase, Cdc25.^(15,17)

An alternate model for maternal regulation of the blastoderm cycles and onset of DNA replication checkpoint

Earlier studies suggested that the DNA replication checkpoint is activated at the blastodermal transition. Sibon et al. observed that embryos derived from *grapes* (*chk1* mutant) flies are defective in MBT related events but do not differ from wild-type embryos during the preblastodermal cycles (1–13).⁽⁵⁾ Thus, *Grapes* replication checkpoint activity does not seem to be required for the pre-blastodermal cycles, but is essential for the MBT. As the nuclei divide during the blastodermal stages, this may titrate the maternal DNA replication machinery, thereby inhibiting completion of S phase and activating the DNA replication checkpoint. In agreement with this, nucleo-cytoplasmic ratios and the content of DNA influence MBT timing.^(2,18) Based on these data, Sibon proposed a model for activation of DNA replication checkpoint control during *Drosophila* embryogenesis. According to this model, DNA replication checkpoint activity is activated only after the blastodermal transition due to a critical decrease in the maternal components of the DNA replication machinery.

Based on their current data, Crest et al. suggest an alternate model. In this new model, Chk1 is active all the time, but high levels of Cdk1–CycB activity override Chk1 activity during the early preblastoderm cell cycles. However, at the blastoderm stage, the balance between increasing Chk1 and decreasing Cdk1–CycB activities reaches a critical threshold level. Chk1's activity dominates that of Cdk1–CycB and consequently initiates the onset of replication checkpoint controls. Thus, this model proposes that a change in the



relative levels of Chk1 versus Cdk1–CycB activity, rather than a dilution of the replication machinery, is the rate limiting step in checkpoint activation (Fig. 1).

Temporal regulation of early stages of embryonic development

As the term mid-blastula transition would suggest, this concept suggests that, at the mid-blastula stage, a “transition” occurs wherein gap phases and DNA replication checkpoints are added to the cell cycle simultaneously, resulting in asynchronous cell divisions. Based on the current data and their observations, Crest et al. propose that this “transition” might not occur as a single stage, but is rather a gradual culmination of events occurring through early embryonic development.⁽¹⁹⁾ For example, it has been observed that, during *Drosophila* embryogenesis, the cell cycles are not identical in length during the pre-blastodermal stages (cycles 1–10). Instead, interphase lengthening is observed as early as cycle 7: While the first six cycles occur at the rate of 9 minutes/cycle, the subsequent cycles are lengthened with the 13th cycle (the last stage of the syncytial blastoderm) taking 25 minutes to complete. Similarly, replication checkpoint activity is first detectable after cycle 10 but only gradually attains full activity during subsequent cell cycles. Together, these data suggest that interphase lengthening and addition of checkpoints are not limited to a specific stage of embryo-

genesis and that full checkpoint activity is acquired over several cell cycles.

In summary, Crest and coworkers conclude that the ability to initiate zygotic control during early embryogenesis in *Drosophila* may not be solely due to the dilution of the maternal factors relative to an increasing nuclear content. Rather than an “off to on” transition, instead a changing balance in the relative levels of cell-cycle-promoting versus cell-cycle-restraining checkpoint activities allows embryos to gradually transition from maternal to zygotic control during early development.

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