Activating the DNA damage checkpoint in a developmental context
Tin Tin Su, Jeff Walker and Jason Stumpff

Background: Studies in unicellular systems have established that DNA damage by irradiation invokes a checkpoint that acts to stall cell division. During metazoan development, the modulation of cell division by checkpoints must occur in the context of gastrulation, differential gene expression and changes in cell cycle regulation. To understand the effects of checkpoint activation in a developmental context, we examined the effect of X-rays on post-blastoderm embryos of Drosophila melanogaster.

Results: In Drosophila, DNA damage was previously found to delay anaphase chromosome separation during cleavage cycles that lack a G2 phase. In post-blastoderm cycles that included a G2 phase, we found that irradiation delayed the entry into mitosis. Gastrulation and the developmental program of string (Cdc25) gene expression, which normally regulates the timing of mitosis, occurred normally after irradiation. The radiation-induced delay of mitosis accompanied the exclusion of mitotic cyclins from the nucleus. Furthermore, a mutant form of the mitotic kinase Cdk1 that cannot be inhibited by phosphorylation drove a mitotic cyclin into the nucleus and overcame the delay of mitosis induced by irradiation.

Conclusions: Developmental changes in the cell cycle, for example, the introduction of a G2 phase, dictate the response to checkpoint activation, for example, delaying mitosis instead of or in addition to delaying anaphase. This unprecedented finding suggests that different mechanisms are used at different points during metazoan development to stall cell division in response to checkpoint activation. The delay of mitosis in post-blastoderm embryos is due primarily to inhibitory phosphorylation of Cdk1, whereas nuclear exclusion of a cyclin–Cdk1 complex might play a secondary role. Delaying cell division has little effect on gastrulation and developmentally regulated string gene expression, supporting the view that development generally dictates cell proliferation and not vice versa.

Background
Studies, mainly using unicellular experimental systems, have established that DNA damage can delay the segregation of chromosomes to daughter cells, presumably to allow time for repair (reviewed in [1,2]). Precisely which step in chromosome segregation is affected, however, depends on the organism. In fission yeast and mammalian cells, DNA damage delays the initiation of mitosis and the cell cycle arrests in G2. In budding yeast, DNA damage delays the initiation of sister chromosome separation and anaphase, resulting in arrest in M phase. Arrests in G2 and in M are very different physiological responses. It has been proposed that differential responses to checkpoint activation arise from inherent differences in the way mitosis is regulated [1]. The budding yeast cell cycle lacks a defined G2 period and executes certain mitotic events, such as the duplication and separation of spindle pole bodies, concurrently with S phase [3,4]. Thus, entry into mitosis might not be rigorously regulated in this system, and the regulation of mitotic progression, that is, the metaphase-to-anaphase transition, might therefore be a key response to DNA damage. The cell cycles of fission yeast and cultured mammalian cells, on the other hand, include a well-defined G2 period and the entry into mitosis is clearly regulated in these systems. Likewise, DNA damage leads to a block in G2, probably by using G2/M regulators that operate during an unperturbed cell cycle.

The notion that checkpoints affect cell division differently depending on how the cell cycle is regulated is of particular importance to multicellular organisms. The formation of a metazoan requires that cells proliferate in the context of developmental signals. Perhaps to accommodate developmental needs, the nature of the cell cycle itself, for example, whether it includes a G1 phase and whether S phase is followed by mitosis or another S phase, changes during metazoan development (reviewed in [5]). In Drosophila, embryogenesis begins with 13 nuclear cycles that lack gap phases and occur in a syncytium. During these cycles, mitotic events, such as the separation...
of centrosomes and their migration to opposite sides of the nuclei, occur concurrently with S phase [6], much like in budding yeast. As in budding yeast, DNA damage during syncytial cycles delays not the entry into mitosis but the segregation of sister chromosomes during mitosis [7]. The mechanism for this response remains to be understood. Syncytial cycles are followed by cellularization and maternal-to-zygotic transition of cell cycle control (MZT). Post-blastoderm division cycles 14–16, which take place after MZT, include a G2 period [6].

In addition to a change in the regulation of mitosis, cell divisions after MZT occur according to a strict spatial and temporal program and in response to developmentally regulated gene expression [9,10]. Moreover, these divisions take place in the context of cellular rearrangements that comprise gastrulation. Thus, in contrast to unicellular systems, the modulation of cell division by checkpoints during embryogenesis must occur in the context of cellular differentiation, gastrulation and changes in cell cycle regulation.

We examined the effect of DNA damage by X-rays on post-blastoderm-stage Drosophila embryos with two aims. First, to ascertain whether checkpoint activation affects cell division differently before and after MZT and second, to understand how the activation of DNA checkpoints affects the developmental program of gene expression, cell division and gastrulation in an intact organism. Here we report that, concurrent with MZT and the introduction of a G2 phase to the cell cycle, cells become capable of delaying the entry into mitosis when irradiated. The delay in the entry into mitosis has little effect on gastrulation or gene expression that normally regulates the timing of post-MZT mitoses, but severely alters the temporal order of cell division and gastrulation in an intact organism. A mechanism for the radiation-induced delay of mitosis in cellular embryos is indicated by additional experiments, which should aid future inquiries as to whether this mechanism operates at other points during Drosophila development.

Results and discussion

DNA damage delays the entry into mitosis in cellular embryos.

To examine the effect of DNA damage on the progression of the cell cycle during Drosophila embryogenesis, we exposed embryos of 0–4.5 hours of age to 570 rads of X-rays. At this dose, 40–60% of cellular embryos died and failed to hatch into larvae. This dose therefore corresponds to the half-maximal lethal dose (LD50, data not shown). Irradiated embryos were fixed, stained for DNA and examined for abnormalities. In syncytial embryos, we found that the resulting DNA morphology was as expected from previous studies [7]. In these studies, when syncytial embryos were exposed to X-rays, nuclei entered mitosis normally, but chromosome segregation was delayed. The delay was transient such that nuclei entered the next interphase without completely separating sister chromosomes, resulting in polyploid nuclei (Figure 1b). Polyploidy has been previously confirmed by in situ hybridization [7].

In cellularized embryos of the same irradiated samples, we observed changes in cell cycle indicators that are consistent with a delay in the entry into mitosis. In untreated embryos at these stages, cells divide in stereotypical clusters termed ‘mitotic domains’ [11]. Both the location of a mitotic domain within the embryo and the time at which it goes through mitosis are invariant from embryo to embryo [12]. Thus, the wild-type pattern of mitotic cells at any specific time during this period, as indicated by the extent of gastrulation, is easily recognizable (Figure 1c,e). In irradiated samples, we found embryos in which expected mitotic domains were not in mitosis, as judged by the absence of condensed chromosomes and mitotic figures (Figure 1f). Antibody staining for a mitotic-specific phospho-epitope on histone H3 (PH3; [13,14]), and staining with wheatgerm agglutinin (WGA) to detect nuclear envelope breakdown, confirmed the absence of mitoses in these embryos (Figure 1h). We infer that irradiation delays the entry into mitosis in cellularized embryos, whereas under identical conditions, chromosome segregation is delayed in syncytial embryos (Figure 1g). Treatment of cellularized embryos with a DNA-damaging agent, methyl methane sulfonate, resulted in a similar delay of mitosis (see Supplementary material). Therefore, the observed effect of irradiation on mitosis is probably due to the DNA-damaging activity of X-rays.

The data in Figure 1 are equally consistent with the scenario in which irradiation accelerates gastrulation such that the embryos shown in Figure 1d,j were younger than their non-irradiated counterparts in Figure 1c,i, and hence the embryos shown in Figure 1d,j were younger than their non-irradiated counterparts in Figure 1c,i. The timing of gastrulation of irradiated embryos extended their germ bands similarly. Moreover, irradiated samples, such as those shown in Figure 2b, lacked mitotic cells. To exclude this possibility, we examined the effect of irradiation on gastrulation. We irradiated cellularized embryos in cycle 14 (3–8 hours of age at 25°C), before germ band extension, the major mode of gastrulation at this stage, and fixed after different time intervals (Figure 2). We found that control and irradiated embryos extended their germ bands similarly. However, irradiated embryos, such as those shown in Figure 2b, lacked mitotic domains although these were present in non-irradiated controls, such as those shown in Figure 2a. This finding supports our surmise that control and irradiated embryos at similar stages of gastrulation are of similar developmental age, and that cells of the latter are delayed in entering mitosis.

The delay in mitotic entry was observed after irradiation at higher doses, up to 1140 R, which killed 100% of embryos. Irradiation at any time during interphase 14, that is, regardless of whether cells are in S phase (irradiation of embryos of 2.10–2.50 hours of age) or G2 phase...
(irradiation of embryos of 2.50–3.20 hours of age) at the time of irradiation, delayed the entry into mitosis M14. This delay was not permanent, presumably because repair processes led to recovery. The time at which mitoses resumed in each domain allowed us to estimate the extent of the delay. Domain 1, for example, initiated M14 during embryonic stage 6 in untreated embryos (65–70 minutes into cycle 14 [11]; embryonic staging is according to [12], and is based on the extent of morphogenetic movements). In embryos that were irradiated in G2 of cycle 14 (G2\textsubscript{14}), domain 1 initiated mitosis in stage 8/9 (corresponding to 80–90 minutes into cycle 14 [11]). Thus, we estimate that irradiation in G2\textsubscript{14} lengthened the G2 of domain 1 by 15–20 minutes, from about 30 minutes to 45–50 minutes.

Irradiation alters the temporal order of mitotic domains

Further analyses of irradiated embryos allowed us to address the effect of irradiation not only on cell division, but also on the spatial and temporal program of cell division within each embryo. Cells normally enter and finish the fourteenth embryonic S phase synchronously, experience G2 of varying lengths, and enter M14 in mitotic domains [6] (schematized in Figure 3a; – Radiation). We reasoned that irradiation could either delay the onset of this program to delay mitoses, or allow this program to continue but act downstream of it to prevent its execution. The first scenario predicts that irradiation would delay all mitotic domains equally (+ Radiation 1; Figure 3a). Thus, upon recovery, the temporal order of mitotic domains would approximate the normal order. The second predicts that cells would become competent to divide according to the normal program but would be hindered from doing so; once the hindrance is removed, cells that have become competent would simultaneously enter mitosis, leading to concurrent divisions of early and late mitotic domains (+ Radiation 2; Figure 3a).
Analyses of irradiated embryos that are resuming mitosis helped us distinguish between the two scenarios in Figure 3a. For example, in an irradiated embryo (Figure 3c), domains 1 and 4 are in mitosis whereas in control embryos, these domains have finished mitosis and lack PH3 staining (Figure 3b). In contrast to domains 1 and 4, later-dividing mitotic domains of irradiated embryos entered mitosis with more normal timing. For example, domains 11 and 14 are in mitosis in both irradiated and control embryos (Figure 3b,c). This observation revealed delays in early domains more than in later domains due to apparent increase in mitotic cells at longer times after irradiation (compare Figure 3b to 3c). Thus, the cell cycle checkpoint is activated during development, mitotic index may first decrease (Figure 3j) and then increase (Figure 3c), necessitating caution in the interpretation of results. The finding that later-dividing domains experience a smaller delay than do earlier-dividing domains supports the second scenario in Figure 3a. We propose that irradiation does not interfere with the developmental program of mitotic domains but acts downstream or independently to delay mitoses.

Irradiation has little effect on String expression

The spatial and temporal control of mitotic domains is well understood. The Cdc25 homolog String is a phosphatase that is required to activate the mitotic kinase Cdk1, and is deposited into the egg by the mother. Maternally supplied String mRNA and protein disappear by cycle 13, Cdk1 becomes inhibited by phosphorylation, and the first embryonic G2 appears in cycle 14. Embryonic patterning genes such as snail and even-skipped subsequently direct the zygotic expression of String in a strict spatial and temporal pattern [15]. The accumulation of String then drives the G2/M transition and defines the mitotic domains of the cellular embryo [10]. Although G2-containing cell cycles respond to checkpoint activation by delaying the entry into mitosis, we found that this delay did not occur by limiting zygotic String expression. String mRNA (data not shown) and String protein (Figure 4) accumulated in a pattern that was indistinguishable in control and irradiated embryos. Thus, for example in Figure 4a,b, String was present in cells of domain 5 (arrows) but domains 11 and 14 are in mitosis and show PH3 stain. Western blotting of extracts from control and irradiated embryos confirmed that irradiation did not reduce the level of String protein (see Supplementary material). We conclude that irradiation does not interfere with the developmental program that leads to String expression but rather delays mitosis by another mechanism.

The observation that the delay in the cell cycle seen after irradiation has little effect on gastrulation or string gene expression is consistent with previous findings. For example, string mutant embryos arrest in G2 of cycle 14

Figure 3

The effect of irradiation on the relative timing of mitotic domains. (a) A schematic representation of the timing of mitotic domains (M1–M3) in untreated embryos (– Radiation). Irradiation could either postpone the whole program of mitoses without changing the relative timing of mitotic domains (+ Radiation 2) or postpone earlier domains more than the later ones (+ Radiation 2). The time line is not drawn to scale. (b,c) Mitotic cells in (b) control or (c) irradiated embryos were visualized by PH3 staining. Embryos were fixed and stained as in Figure 1. Mitotic domains are either boxed (domains 1, 4 and 11) or indicated by arrowheads (domain 14). The embryos shown here are riper than those shown in Figure 1–i. (b) In control embryos, domains 1 and 4 have finished dividing and lack PH3 stain but domains 11 and 14 are in mitosis. (c) In contrast, in an irradiated embryo at the same stage, 45 min after irradiation, domains 1–14 are in mitosis and show PH3 stain. Embryos are shown with anterior end to the left and the dorsal side up and towards the viewer.

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![Figure 3](image-url)
and in fuzzy mutant embryos, many epidermal cells arrest in mitosis 16 (M16), and yet both mutants complete gastrulation [9,10,16]. Moreover, cellular differentiation and gene expression usually proceed despite cell cycle arrest. For example, cells in string embryos differentiate as do cells of the Drosophila eye imaginal disc that are arrested in the cell cycle by ectopic expression of a Cdk inhibitor [17,18]. These and our observations support the view that, although developmental signals dictate when and where cells proliferate, the failure to proceed through the cell cycle does not necessarily impede development.

The mechanism for delaying mitosis in cellular embryos

The finding that irradiation leads to two different cell cycle responses — delaying anaphase chromosome segregation or delaying mitosis — in a single organism is unprecedented. Mitotic chromosome segregation and the initiation of mitosis are regulated by different mechanisms. The former requires the proteolysis of proteins, such as PDS1 in budding yeast [19,20] and cyclin A in Drosophila [16], whereas the latter requires the activation of mitotic cyclin-Cdk complexes. We suggest that checkpoint activation by the same dose of radiation under identical conditions must have used different downstream mechanisms in order to delay chromosome segregation in the syncytium and mitosis in the cellularized embryos. Although mechanisms that operate in the syncytium remain elusive, we addressed the mechanisms used by cellularized embryos.

Despite the finding that irradiation did not interfere with String expression (Figure 4), it might have antagonized String activity. Cdk25/0 activates Cdk1 by removing the inhibitory phosphates on Thr14 and Tyr15 [21]. A Cdk1 mutant in which these residues have been mutated (Cdk1AF) bypasses the requirement for String (N. Yakubovich and P.H. O’Farrell, personal communication; see also [22,23]). If the mechanism by which radiation delays mitosis solely involves inhibitory phosphorylation of Cdk1, Cdk1AF should bypass the radiation-induced delay. To test this hypothesis, we expressed Cdk1 or Cdk1AF, in conjunction with a mitotic cyclin (see Materials and methods), from a heat-inducible (hs) promoter during interphase 14. We then asked whether irradiation could delay the onset of mitosis 14 in embryos expressing these transgenes. We found that many cells of heat-shocked embryos that carried hs-Cdk1AF and hs-mitotic cyclin transgenes failed to delay mitosis after irradiation (Figure 5a). This effect was seen with mitotic cyclins A, B or Bs—a truncated version of cyclin B that is resistant to proteolysis [16,24]. In contrast, embryos carrying hs-Cdk1, in combination with the same cyclins, behaved like wild-type embryos and delayed mitosis (Figure 5b). We conclude that Cdk1AF, and not Cdk1, can overcome the radiation-induced delay in mitosis. We infer that inhibitory phosphorylation on Cdk1 is required to delay mitosis in response to DNA damage, in agreement with previous results from fission yeast and vertebrates [25,26].

Interestingly, the ability of Cdk1AF and cyclins to overcome the delay of mitosis in Drosophila was seen only in certain cells of the embryos, and these cells represent mitotic domains, for example, domain 4 (boxed in Figure 4).
Cdk1AF can overcome the radiation-induced delay of mitosis. Embryos were fixed and stained with an antibody against PH3 to detect cells in mitosis. Embryos carrying inducible genes for Cdk1AF and cyclin B or B' Cdk1 and cyclin B' were heat shocked for 20 min during cycle 14 (2.5–3 h old at 25°C), irradiated with 570 R and fixed after 20 min. In Cdk1AF + B' embryos, mitosis is seen in embryos carrying inducible genes for Cdk1AF and cyclin A (c,d). Without heat shock or irradiation, the timing and location of mitoses in these embryos were indistinguishable from those of wild type (compare (c) to Figure 5). And yet cells of the former did not delay mitosis after irradiation (see Materials and methods for additional pertinent information on Cdk1AF + cyclin A embryos and stocks). Embryos carrying inducible Cdk1AF and cyclin A (c,d). Embryos carrying inducible genes for Cdk1AF and cyclin A were irradiated and fixed as in (d) are shown at higher magnification to illustrate that Cdk1AF-containing cells that entered mitosis after irradiation executed all phases of mitosis, although lagging chromosomes and chromosome bridges were frequent (arrowheads and bracket). Such defects were also present in irradiated wild-type embryos that resume mitosis (data not shown), and might contribute to the embryonic lethality seen at the X-ray doses used in our experiments. The embryos in (d) were heat-shocked before irradiation. Bar = 10 µm in (a,b). Embryos are shown with anterior end to the left and the dorsal side up.

Figure 5a-c,d). Cells of mitotic domains are distinguished from their neighbors by their accumulation of String protein (Figure 4a). Although further experiments are required to demonstrate the importance of String, the perfect coincidence of clusters of irradiated cells that entered mitosis in the presence of Cdk1AF and that accumulated String led us to suggest the following. Although cyclin–Cdk1AF activity is not present in sufficient quantities to promote mitosis by itself under our experimental conditions (see Materials and methods), this activity can induce endogenous String to activate endogenous Cdk1 and induce mitosis. A similar feedback mechanism has been proposed for human Cdk1 and Cdc25 [27]. It follows then that endogenous String and Cdk1 might be inhibited by irradiation, but that this inhibition can be overcome by a small amount of Cdk1AF activity.

The same amount of Cdk1AF activity overcame another consequence of irradiation, namely, the nuclear exclusion of a mitotic cyclin. The nuclear accumulation of cyclin–Cdk1 activity is a prerequisite to mitosis [28] and the exclusion of cyclin B1 from the nucleus appears to contribute to the delay in mitosis after irradiation in human cells [29]. In cell-cycle-stage-specific embryos, cyclin A and B remain enriched in the cytoplasm in interphase (Figure 6d) [30,31]. Cyclin A accumulates in the nucleus of cells that initiate mitosis (Figure 6d) [30,32], as does cyclyn B [31]. In irradiated embryos, both cyclins A and B were excluded from nuclei (Figure 6e and data not shown) although their levels remained unchanged (see Supplemental material). In cells that expressed Cdk1AF (with a mitotic cyclin) and entered mitosis even after irradiation, nuclear accumulation of cyclin A was evident (Figure 6f). Thus, a low level of Cdk1 activity, provided by Cdk1AF in our experiments, led to both the nuclear accumulation of a cyclin and the entry into mitosis.

Given these two observations — that Cdk1AF drives the nuclear accumulation of cyclin A and that nuclear accumulation of mitotic cyclins coincides with the entry into mitosis in unperturbed cell cycles — we propose that Cdk1 activity normally drives the nuclear accumulation of cyclin–Cdk1 complexes. Cyclin A remained excluded from nuclei in String mutants, supporting this idea (see Supplemental material). In accordance with this, Cdk1AF, in conjunction with endogenous String, overcame the radiation-induced delay of mitosis because Cdk1AF can start the feedback loop that activates endogenous Cdk1 by endogenous String and Cdk1 activity can drive the nuclear accumulation of cyclin–Cdk1 complexes. These ideas help explain previous observations in human cells. In the latter, although the exclusion of cyclin B1 from nuclei appears to be of some importance to regulating mitotic entry, Cdk1AF can overcome the checkpoint-induced delay of mitosis regardless of whether cyclin B1 or NLS-cyclin B1, which is constitutively localized to the nucleus, is co-expressed [33]. Thus, Cdk1AF in human cells, as in Drosophila, might also drive the nuclear accumulation of cyclin–Cdk complexes and the entry into mitosis by initiating a positive feedback loop for the activation of endogenous Cdk1.
Similar feedback loop of Cdk1, String and cyclin-localization operates to control mitosis in other tissues, such as larval imaginal discs, remains to be seen.

Conclusions
Our study finds that the effect of checkpoint activation on cell division is not rigid but changes concurrently with changes in the regulation of the cell cycle that occur during Drosophila embryogenesis. This situation is reminiscent of the different ways in which the cell cycle in budding or fission yeast responds to the DNA-damage checkpoint. It is well known that molecules such as ATM and Chk1, which sense DNA damage and relay the signal to the cell cycle, are conserved throughout evolution [1]. An essential goal in understanding checkpoints is to understand how conserved checkpoint functions can lead to diverse cell cycle responses. Our observation of these phenotypes suggest that background induction of Cdk1AF + cyclin A can compromise cellular function and viability.

Embryo collection, heat shock and irradiation
Embryos were collected on grape agar plates at 25°C. Embryos were irradiated at 2.2 rad/sec in a TORREX120D X-ray generator (Astrophysics Research, Long Beach, CA) by placing agar plates facing up on shelf 6. The generator was set at 5 mA and 115 kV. Embryos were blocked in PBT + 3% normal goat serum for at least 1 h before staining with antibodies. PBT is PBS [35] + 0.2% Tween.

Staining
Embryos were de-chromonated with bleach and fixed for 20 min in a blyzer of heptane and PBS containing 10% formaldehyde. Fixed embryos were blocked in PBT + 3% normal goat serum for at least 1 h before staining with antibodies. PBT is PBS [35] + 0.2% Tween.

Materials and methods
Fly stocks
Seventy flies were used as wild-type controls in all experiments. Fly stocks carrying heat-inducible transgenes for Cdk1AF [22], cyclin A and cyclin B [34], and cyclin B’ [34] have been described previously. Embryos carrying only hs-Cdk1AF behaved like wild type in our experiments. Thus, Cdk1AF requires the co-expression of a cyclin to be effective. This is consistent with previous observations that Cdk1AF is more potent as a mitosis-inducer when co-expressed with a cyclin [22,23]. Similarly, in human cells, Cdk1AF overcomes the delay of mitosis in irradiated cells only when co-expressed with cyclin B1 [26,30]. It is possible that Cdk1AF must compete with endogenous Cdk1 for endogenous cyclins. Although both cyclin A and B were able to cooperate with Cdk1AF in overcoming the radiation-induced block to mitosis, cyclin A appears to be more potent. Embryos that carried hs-Cdk1AF + hs-cyclin A failed to delay mitosis after irradiation, even without a heat shock (Figure 5d). Embryos with inducible Cdk1 [not AF] and inducible cyclin A, although they carry the same hs-cyclin A transgene insert, succumbed to radiation induced cell cycle delay (data not shown but similar to Figure 5b). Therefore, the ability of hs-Cdk1AF + hs-cyclin A embryos to overcome radiation-induced cell cycle delay is unlikely to be due to the mutual deletion of the cyclin A transgene. Instead, background expression, in the absence of heat shock, might have conferred resistance to cell cycle delay. Consistent with this data, transgenic stocks that carry hs-Cdk1AF in combination with hs-cyclin A or B are highly unstable (that is, they lose transgenes rapidly) and require frequent re-making from amply transgenic lines. Moreover, the hs-Cdk1AF + hs-cyclin A chromosome is hemizygous lethal whereas hs-Cdk1AF, hs-cyclin A and hs-Cdk1 + hs-cyclin A transgenic stocks are viable as homozygotes. Taken together, these phenotypes suggest that background induction of Cdk1AF + cyclin A can compromise cellular function and viability.

Embryo collection, heat shock and irradiation
Embryos were collected on grape agar plates at 30°C. Embryos were aged to reach the desired cell cycle stages by following the time-line in [8] and [11]. Transgenes were induced by floating agar plates in a 37°C water bath. In these experiments, the level of transgene induction (after a 20 min incubation at 37°C) was insufficient to induce mitosis, as judged by nuclear envelope breakdown and chromosome segregation (data not shown, but see Figure 6). Thus, cells in heat-shocked embryos entered mitosis in a normal domain pattern. Although longer heat shocks induced ectopic mitosis, we avoided these conditions because heat shocks of 30 min or longer interfered with the ability to delay mitosis after irradiation regardless of transgenesis.

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Primary antibodies were used at the following dilutions: affinity-purified rabbit polyclonal anti-String [21], 1:10–20; affinity-purified rabbit polyclonal anti-cyclin A [30], 1:100; monoclonal anti-cyclin B [32,33], 1:100; monoclonal anti-Cdk1 [21,36], 1:100; monoclonal anti-cyclin A (F2F6; [32], 1:100; monoclonal anti-cyclin B (F2F6; [32], 1:100.
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Supplementary material


References