The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation

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Mutations in the embryonic *Drosophila* Grapes/Chk1 checkpoint result in an abbreviated interphase, chromosome condensation defects and metaphase delays. To clarify the relationship between these phenotypes, we simultaneously timed multiple nuclear and cytoplasmic events in mutant *grp*-derived embryos. These studies support a model in which *grp* disrupts an S-phase checkpoint, which results in progression into metaphase with incompletely replicated chromosomes. We also show that chromosome condensation is independent of the state of DNA replication in the early embryo. Therefore, *grp* condensation defects are not a direct consequence of entering metaphase with incompletely replicated chromosomes. Rather, initiation of chromosome condensation (ICC) occurs at the normal time in *grp*-derived embryos, but the shortened interval between ICC and metaphase does not provide sufficient time to complete condensation. Our results suggest that these condensation defects, rather than incomplete DNA replication, are responsible for the extensive metaphase delays observed in *grp*-derived embryos. This analysis provides an example of how the loss of a checkpoint can disrupt the timing of multiple events not directly monitored by that checkpoint. These results are likely to apply to vertebrate cells and suggest new strategies for destroying checkpoint-compromised cancer cells.

Checkpoints increase cell-cycle fidelity by providing time for correction or completion of a specific event in the cell cycle[1]. For example, S-phase checkpoints delay entry into mitosis until DNA replication is complete[2]. When this checkpoint is disrupted, cells fail to delay in response to incompletely replicated DNA, and inappropriately proceed into mitosis. It is assumed that the mitotic abnormalities observed in S-phase checkpoint mutants are primarily the result of progressing through mitosis with unreplicated DNA, but that other aspects of the cell cycle proceed normally. But given that the cell cycle consists of a series of parallel pathways, loss of a necessary checkpoint may disrupt the relative timing of multiple pathways. For example, loss of the Grp/Chk 1 checkpoint results in premature entry into metaphase[3,4]. The resulting mitotic defects are presumably a consequence of entering metaphase before completion of S-phase. In addition, other cell-cycle events in parallel pathways distinct from DNA replication also may not be completed before entry into metaphase. This disruption in the relative timing of multiple cell-cycle events may contribute significantly to the phenotype of cell divisions compromised at the S-phase checkpoint.

Here we directly address this issue using the *Drosophila* embryonic checkpoint gene, *grapes* (*grp*). Grp is the *Drosophila* homologue of Chk1, a conserved kinase that is critical for transducing the signal generated from damaged and unreplicated DNA into a cell- cycle arrest[3,5–11]. Grp and Mei41, a *Drosophila* homologue of the conserved checkpoint kinase ATM, may also be required for developmental events that occur at the mid-blastula transition[4]. One model of Chk1 action is that DNA damage activates nuclear localized Chk1, which in turn phosphorylates Cdc25 (refs 6–8). This allows a member of the 14-3-3 protein family to bind to and mediate the export of Cdc25 from the nucleus although there is evidence that this binding also occurs in the absence of Chk1[7,8,12–15]. The net result in fission yeast is separation of the activating Cdc25 phosphatase from its substrate, nuclear-localized Cdc2/cyclinB[13,16]. This model of Chk1 action may not, however, apply in *Drosophila* and other organisms in which Cdc2/cyclinB is cytoplasmic[17,18].

Grp is essential during the late syncytial blastoderm divisions[19]. Specifically it is required for an S-phase checkpoint and the normal lengthening of interphase that occurs at this time[4]. Presumably, the lengthening of interphase cycles is due to progressively slower DNA replication, and Grp is required to prevent entry into mitosis until replication is complete. The extensive mitotic abnormalities observed in Grp-deficient embryos are interpreted as the result of progressing through mitosis with incompletely replicated DNA. However, key aspects of the mutant *grp* phenotype are not in accord with this model. During the late cortical cycles in *grp*-derived embryos, chromosomes fail to condense and align on the metaphase plate and extensive metaphase delays are also observed[3,4,19,20]. In contrast, embryos treated with the S-phase inhibitor aphidicolin condense and align relatively normally on the metaphase plate and do not exhibit metaphase delays[21,22]. This phenotype suggests that factors other than incomplete DNA replication contribute to the *grp* phenotype.

Results

Timing of the cortical nuclear cycles. To address the issues described above and understand the basis of *grp*-induced mitotic abnormalities, we injected rhodamine-labelled tubulin into *Drosophila* embryos transformed with a histone H2A–green fluorescent protein (GFP) construct to follow centrosome, spindle and nuclear envelope dynamics simultaneously[23] (Fig. 1a). Through this analysis we determined the timing and order of the following cell-cycle events: nuclear envelope formation (NEF); initiation of chromosome condensation (ICC); nuclear envelope breakdown (NEB); and anaphase initiation (IA) (Fig. 1a, b, Table 1). By monitoring NEB, we determined the average length of cycles 11, 12 and 13 in wild-type embryos to be 12.5, 17.7 and 24.0 min respectively. The

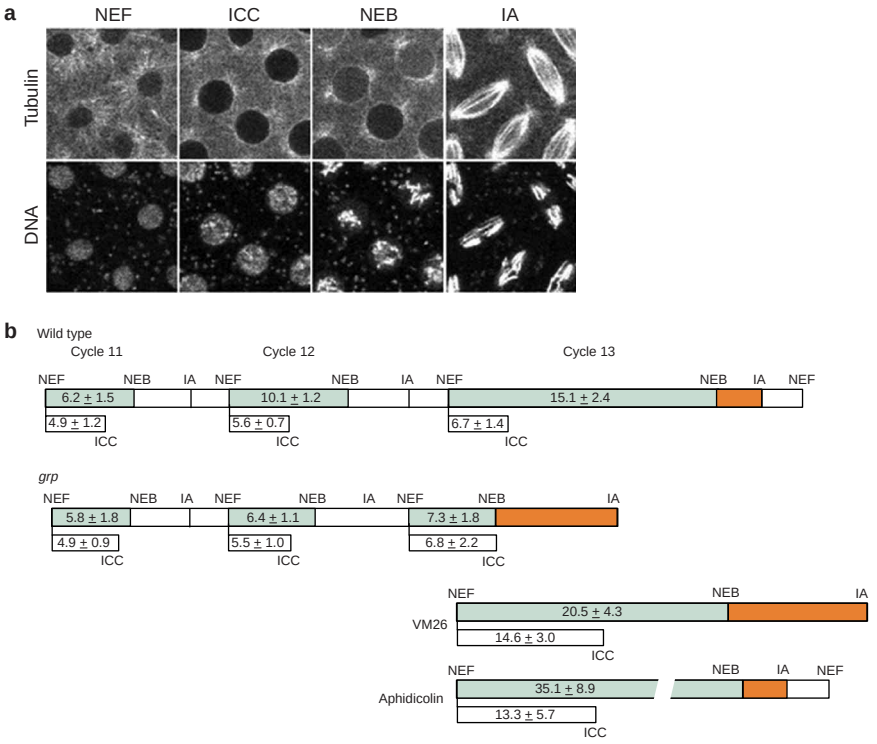

Figure 1 **Timing of the nuclear cycle. a**, Images of a living *Drosophila* embryo bearing the histone–GFP construct injected with fluorescently labelled tubulin. Nuclear envelope formation (NEF) is assayed by the exclusion of labelled tubulin from the nucleus[22]. The appearance of bright histone–GFP spots marks the initiation of chromosome condensation (ICC). Labelled tubulin rushing into the nuclear interior indicates that the nuclear envelope has broken down (NEB)[22]. Separation of sister chromosomes marks anaphase initiation (IA). **b**, Based on the criteria outlined above, the following intervals were timed for wild-type and *grp*-derived embryos during cycles 11 to 13: NEF to NEB, NEB to IA, IA to NEF, and NEB to the ICC. In wild-type embryos, the interval between NEF and NEB increases dramatically as the cortical cycles progress, but the timing of other events in the cell cycle remains relatively constant. In cycle 11 and 12 *grp*-derived embryos the timing of NEF to NEB is disrupted, but not the timing of NEB to IA (which defines metaphase). During cycle 13, spindle assembly proceeds at the normal rate but extensive metaphase delays are observed. During nuclear cycle 13 the topoisomerase inhibitor VH26 disrupts chromosome condensation, causes a slight increase in the time from NEF to NEB and an extensive delay in metaphase (defined as NEB to IA). In contrast, the S-phase inhibitor aphidicolin produces an extensive delay in the time between NEF and NEB but does not produce a delay in metaphase. Orange bars represent the length of cycle-13 metaphase.

lengthening of the cortical nuclear cycles is primarily due to the increase in length between NEF and NEB (6.2, 10.1 and 15.1 min for nuclear cycles 11, 12 and 13 respectively). In contrast, the interval from NEB to anaphase initiation does not dramatically change (3.9, 4.8 and 5.1 min) and there is little change in the interval between anaphase initiation and NEF during these cortical cycles. These results indicate that in normal embryos, once NEB occurs, the events leading to the initiation of anaphase proceed at a relatively constant rate that is independent of the overall length of the nuclear cycle.

The state of chromosme condensation is independent of NEB. Figure 2a presents images of wild-type cycle 10–13 in living embryos at NEB and late metaphase immediately before anaphase. With each successive cortical cycle, the chromosomes are in a greater state of condensation at both of these time points. Initiation of chromosome condensation with respect to NEF is relatively constant for each of the cortical cycles (4.9, 5.6 and 6.7 min after NEF for nuclear cycles 11, 12 and 13 respectively) although the interval from NEF to NEB progressively lengthens (Table 1, Fig. 1b). Consequently, chromosomes have a longer time to condense before NEB and anaphase as the cortical cycles progress.

Chromosome condensation is independent of DNA replication. To determine the relationship between chromosome condensation and the state of DNA replication, we injected normal embryos with aphidicolin, a DNA synthesis inhibitor, during early anaphase and followed chromosome, spindle and nuclear envelope dynamics as they progressed through the next nuclear cycle[22]. The interval between NEF and NEB is dramatically increased in the nuclear cycle following aphidicolin injection. For embryos injected during early anaphase of nuclear cycle 11, aphidicolin increases the NEF–NEB interval at cycle 12 from 10.1 ± 1.2 to 27.4 ± 9.2 min (Table 1, Fig. 1b). Similarly, for embryos injected during early anaphase of nuclear cycle 12, aphidicolin increases the NEF–NEB interval at cycle 13 from 15.1 ± 2.4 to 35.1 ± 8.9 min (Table 1, Fig. 1b). Once NEB occurs in the aphidicolin injected embryos, the nuclei progress at a relatively normal rate through metaphase and the initiation of anaphase (Table 1, Fig. 1b). The specific increase in the interval between NEF and NEB supports a model in which S-phase checkpoints operate in the early embryo to specifically control the timing of NEB.

Figure 3 depicts the state of chromosome condensation in aphidicolin-treated embryos at NEB and late metaphase immediately before anaphase. As described above, embryos were injected with aphidicolin in early anaphase of nuclear cycle 12 and followed through nuclear cycle 13. Initiation of chromosome condensation following NEF in aphidicolin-treated embryos occurs at an equivalent time as in control embryos (data not shown). Chromosome condensation in aphidicolin-treated embryos proceeds normally, and at NEB the chromosomes are much more condensed than at the equivalent stage in untreated control embryos (Fig. 3a). This may be a direct consequence of the aphidicolin-induced delay in NEB allowing more time for chromosome condensation to proceed.

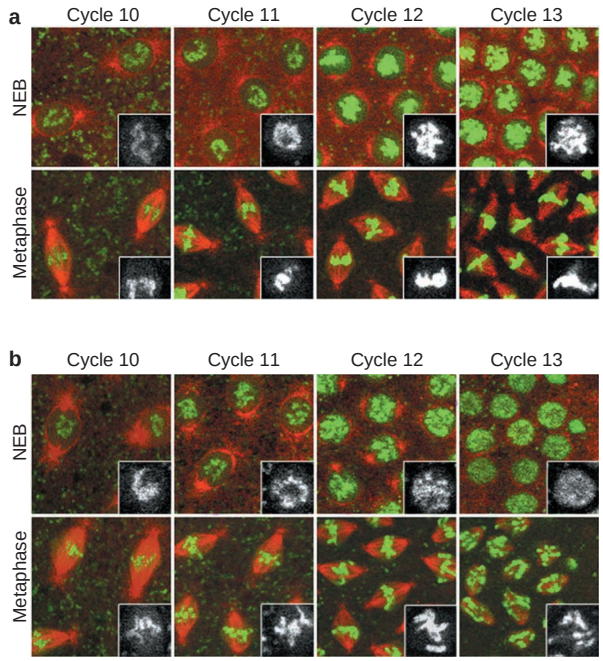

Figure 2 **The state of chromosome condensation during the cortical cycles of normal and *grp*-derived embryos.** Live confocal analysis was used to determine the state of chromosome condensation and alignment (inset) at NEB and immediately before anaphase initiation during the cortical cycles of normal (**a**) and *grp*-derived embryos (**b**). Tubulin is stained red and histone green. During cycles 12 and 13 in wild-type, but not *grp*-derived, embryos the chromosomes are well condensed at NEB. As described, this is a result of premature NEB and entry into metaphase in *grp*$^{-}$ embryos. Consequently, in *grp*-derived embyros the alignment of chromosomes on the metaphase plate is disrupted during these cycles. At cycle 13, the disruption may be severe enough to activate a checkpoint at metaphase.

Table 1 Nuclear cycle timing in *grp*-derived embryos

	NEF→NEB	NEB→IA	IA→NEF	NEF→1CC
Cycle 11				
Wild type	6.2 ± 1.5	3.9 ± 0.8	2.4 ± 0.4	4.9 ± 1.2
grp^{-}	5.8 ± 1.8	4.3 ± 1.2	2.8 ± 0.3	4.9 ± 0.9
Cycle 12				
Wild type	10.1 ± 1.2	4.8 ± 0.4	2.8 ± 0.8	5.6 ± 0.7
grp^{-}	6.4 ± 1.1	5.3 ± 1.5	3.6 ± 0.9	5.5 ± 1.0
Aphdicolin	27.4 ± 9.2	6.1 ± 2.3	–	–
Cycle 13				
Wild type	15.1 ± 2.4	5.1 ± 0.8	3.8 ± 0.6	6.7 ± 1.4
grp	7.3 ± 1.8	10.3 ± 1.6	–	6.8 ± 2.2
Aphdicolin	35.1 ± 8.9	5.6 ± 0.7	3.6 ± 0.7	–
VM26	20.5 ± 4.3	9.3 ± 0.8	–	–

Syncytial nuclear cycles 11, 12 and 13 were followed through confocal microscopy in wild-type and *grp*-derived embryos bearing the histone–GFP construct and were injected with fluorescently labelled tubulin. This procedure enabled the following intervals to be timed (shown in min): NEF to NEB, NEF to ICC, NEB to IA (which defines metaphase), and IA to NEF (see text). For both wild-type and *grp*-derived embryos, $N = 12$. For embryos treated with VM26 or aphidicolin, $N = 6$.

After NEB, nuclei in aphidicolin-treated embryos progress into metaphase at rates equivalent to untreated control embryos (Table 1, Fig. 1b). At metaphase just before anaphase, chromosomes in aphidicolin-treated embryos are much more tightly packaged on the metaphase plate than observed in untreated embryos. Again, this is probably a direct result of aphidicolin-induced delays in NEB allowing more time for chromosome condensation. Significantly, no metaphase delays are observed in the aphidicolin-treated embryos (Table 1, Fig. 1b). As nuclei progress through anaphase, extensive mitotic abnormalities are observed, including chromosome bridging and snap-back of sister telophase nuclei[22]. These defects are likely to be the direct consequence of progressing through anaphase with incompletely replicated chromosomes. These experiments indicate that in the syncytial *Drosophila* embryo chromosome condensation is independent of the state of DNA replication.

Condensation defects elicit metaphase delays. To determine the effect of disruptions in chromosome condensation on the nuclear cycle, we injected normal embryos with the topoisomerase II inhibitor VM26. A number of studies show that VM26 disrupts chromosome condensation[24–26]. Embryos were injected with VM26 during early anaphase of nuclear cycle 12. These embryos progress normally through anaphase and the interval between NEF and NEB in the following nuclear cycle is only slightly increased (Table 1, Fig. 1b). In contrast to aphidicolin-treated embryos, chromosomes are uncondensed at NEB in VM26-treated embryos (Fig. 3a, upper panel). These nuclei progress into metaphase with uncondensed chromosomes and exhibit an extensive metaphase delay (Fig. 3a, lower panel, Fig. 1b, Table 1). These nuclei eventually proceed into anaphase suggesting that this delay may be checkpoint induced.

NEB is premature in *grp*-derived embryos. Previous work demonstrated that the progressive lengthening of interphase during the cortical division cycles does not occur in *grp*-derived embryos[4]. We extended these studies by introducing the histone–GFP construct into the *grp* stock. This enabled us to simultaneously monitor multiple events in the nuclear cycles of *grp*-derived embryos (as in Fig.1a). This analysis shows that NEB occurs prematurely in *grp*-derived embryos. The interval between NEF and NEB for cycles 11, 12 and 13 is 6.2, 10.1 and 15.1 min in wild-type embryos and 6.2, 6.4 and 7.3 min in *grp*-derived embryos (Fig. 1b, Table 1). The interval from NEB to the initiation of anaphase does not, however, differ significantly in *grp*-derived and normal embryos for nuclear cycles 11 and 12 (Fig. 1b, Table 1). During nuclear cycle 13 in *grp*-derived embryos, the interval between NEB and spindle assembly is not altered, but there is an extensive metaphase delay (NEB–IA (which defines metaphase)>5 min; Table 1).

Disruption of chromosome condensation in *grp*-derived embryos. The state of chromosome condensation with respect to other cell-cycle events is disrupted in *grp*-derived embryos. This is not due to defects in the timing of the initiation of chromosome condensation. In normal embryos this occurs 4.9, 5.6, and 6.7 min after NEF for nuclear cycles 11, 12, and 13 respectively. In *grp*-derived embryos, these values are 4.9, 5.5, and 6.8 min, respectively (Fig. 1b, Table 1). The chromosomes are not, however, properly condensed at both NEB and metaphase in these embryos. This is most evident during nuclear cycles 12 and 13 (compare Fig. 2a and b). By nuclear cycle 13, the condensation defects in *grp*-derived embryos may be severe enough to activate a checkpoint that delays progression through metaphase.

These studies provide an explanation of why *grp*-derived embryos, but not aphidicolin-treated embryos, exhibit defects in chromosome condensation and extensive metaphase delays. Although aphidicolin disrupts DNA replication, activation of the

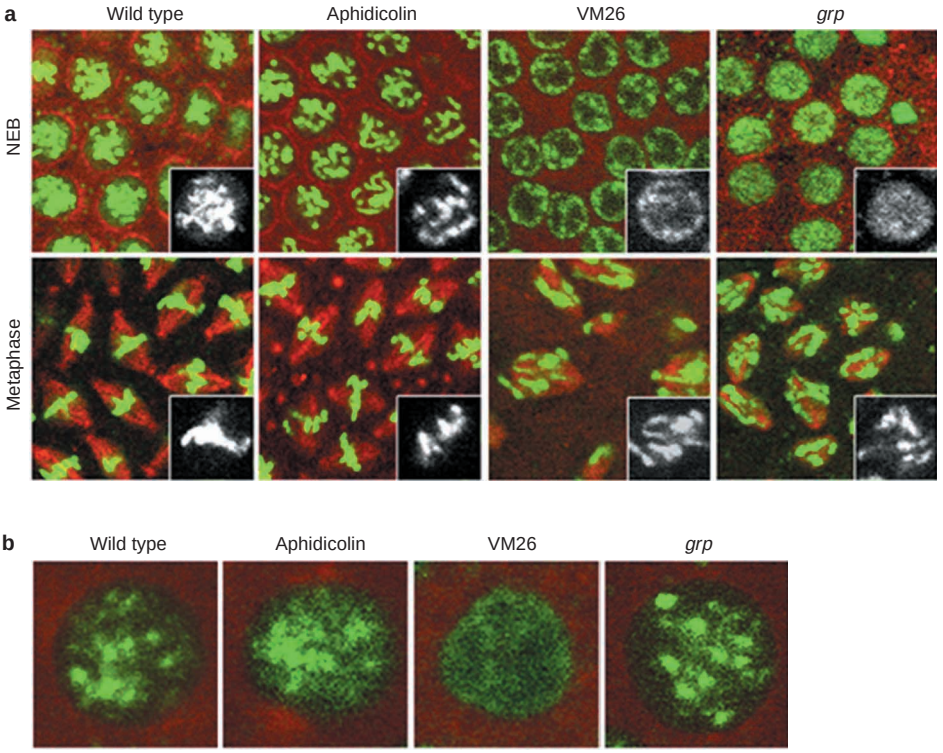

Figure 3 **Comparison of chromosome condensation in wild-type, *grp*-derived, and drug-treated embryos. a**, Live confocal analysis was used to determine the state of chromosome condensation and alignment (inset) at NEB and metaphase (less than 30 s before anaphase initiation) during nuclear cycle 13 in normal, aphidicolin-injected, VM26-injected and *grp*-derived embryos. In contrast to *grp*-derived embryos, NEB is significantly delayed in aphidicolin-injected embryos, providing more time for chromosome condensation. In embryos injected with VM26, condensation is abnormal at NEB as well as at metaphase. **b**, Live confocal analysis was used to determine the state of chromosome condensation approximately 20 min after NEF in cycloheximide-injected normal and *grp*-derived embryos as well as embryos injected with aphidicolin/cycloheximide and VM26/cycloheximide mixtures. Condensation in *grp*-derived and aphidicolin-injected embryos appears similar to that in normal embryos, whereas nuclei in VM26-treated embryos fail to condense.

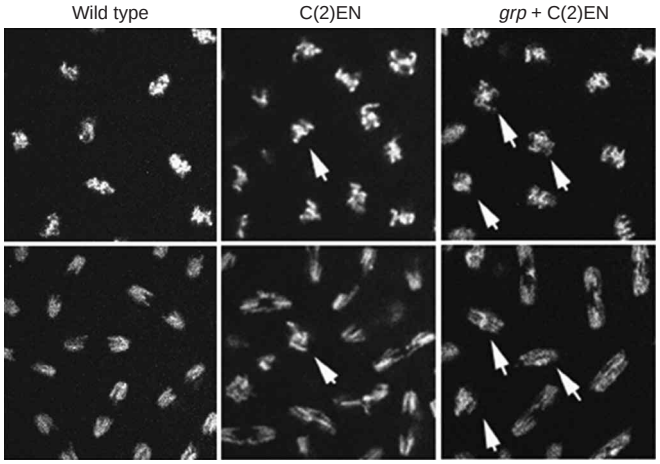

Figure 4 ***grp*-derived embryos are sensitive to chromosome rearrangements.** Time-lapse recordings of wild-type, C(2)EN-bearing and *grp*-derived C(2)EN-bearing nuclei 2 min before initiation of anaphase (top row) and anaphase (bottom row) of nuclear cycle 12 are shown. Chromosome condensation is delayed in the C(2)EN-bearing embryos. The arrows indicate nuclei in which chromosomes are not well condensed and metaphase is delayed. The frequency of improperly condensed chromosomes and metaphase delays is increased in *grp*-derived C(2)EN-bearing embryos.

S-phase checkpoint delays NEB, which in turn provides ample time for chromosome condensation. In contrast, premature NEB and entry into metaphase in *grp*-derived embryos does not provide sufficient time for chromosome condensation.

Delaying NEB ameliorates condensation defects in *grp*-derived embryos. Both wild-type embryos treated with VM26 and *grp*-derived embryos exhibit improperly condensed chromosomes. Whereas VM26 directly effects the topoisomerases involved in chromosome condensation, premature entry into metaphase appears to be the cause of condensation defects in *grp*-derived embryos. To test this, we prevented nuclear envelope breakdown by injecting embryos with the protein synthesis inhibitor cycloheximide. In normal embryos, cycloheximide arrests nuclei with an intact nuclear envelope, but chromosome condensation occurs (Fig. 3b). In embryos injected with both cycloheximide and aphidicolin, condensation also occurs (Fig. 3b). This supports our previous finding that chromosome condensation is independent of the state of DNA replication. In contrast, in embryos doubly injected with VM26 and cycloheximide, chromosome condensation does not occur (Fig.3b). This demonstrates that topoisomerase II activity is necessary for chromosome condensation.

NEB is also inhibited in *grp*-derived embryos injected with cycloheximide. Significantly, chromosome condensation in these embryos occurs to the same extent as in equivalently treated wild-type embryos (Fig. 3b). This supports a model in which Grp is required to prevent premature NEB, providing time for

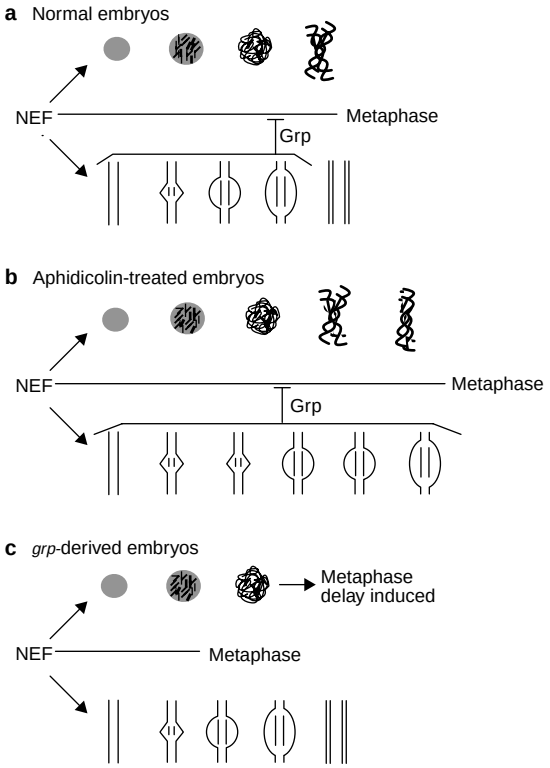

Figure 5 **Model explaining *grp*-induced metaphase delay. a**, In syncytial embryos, chromosome condensation and replication are independent of each other. Although Grp monitors DNA replication to control entry into metaphase (defined as NEB-IA), this also provides time for proper condensation. **b**, Aphidicolin-induced inhibition of replication produces a Grp-mediated delay in entering metaphase. Because this provides more time for condensation, the chromosomes are more tightly packaged on the metaphase plate in aphidicolin-treated embryos. **c**, Loss of Grp results in premature entry into metaphase. This presumably not only results in entry into metaphase with incompletely replicated chromosomes, but also improperly condensed chromosomes. It is the defects in condensation, and not replication, that are responsible for the metaphase delays, presumably through the activation of a checkpoint at metaphase.

condensation, and is not directly involved in the mechanisms driving condensation.

***grp*-derived embryos are sensitive to chromosomal rearrangements.** Our data suggest that premature entry into metaphase in *grp*-derived embryos does not provide sufficient time for proper chromosome condensation. This predicts that these embryos should be particularly sensitive to delays in condensation. To test this, we examined the effects of an abnormally long compound chromosome, C(2)EN in normal and *grp*-derived embryos. In C(2)EN, both second chromosome homologues share a common centromere, creating a chromosome that is twice the length of a normal second chromosome[27]. Previous studies demonstrated that the presence of C(2)EN results in slight but consistent condensation delays, as well as delays in progression through metaphase[28]. The top row of Fig. 4 presents images of normal and C(2)EN-bearing nuclei 2 min before the initiation of cycle 12 anaphase. The C(2)EN-bearing nuclei are not as well condensed as normal nuclei (see arrows). The bottom row of Fig. 4 shows that these nuclei occasionally delay entering anaphase. In live recordings of nuclear cycles 11 and 12 we find that, in normal embryos, 1% (1/121) of nuclei either arrest or delay in metaphase, whereas in C(2)EN-bearing embryos, this is seen in 7% (4/55) of nuclei. Our interpretation is that condensation is delayed in nuclei bearing the longer chromosome. This results in a higher frequency of nuclei entering metaphase with incompletely condensed chromosomes, and a consequent delay in metaphase.

We assayed the effect of the compound chromosome in *grp*-derived embryos. During nuclear cycles 11 and 12 in non C(2)EN and C(2)EN-bearing *grp*-derived embryos, 1% (2/129) and 39% (39/99) of nuclei arrest or delay in metaphase respectively. Normally, *grp*-derived embryos do not exhibit extensive metaphase delays until nuclear cycle 13. This suggests that, in combination with the slightly premature NEB in cycle 12 *grp*-derived embryos, the C(2)EN condensation defects become severe enough to produce metaphase delays.

Discussion

The fact that loss of Grp results in premature entry into metaphase has led to a model in which Grp monitors S-phase to control the timing of metaphase entry[4]. Our data refine this model by demonstrating that in cycle 11 and 12 *grp*-derived embryos, the interval between NEF and NEB is specifically disrupted, but the timing of NEB to initiation of anaphase is not altered. This leads to a model in which Grp is involved in a checkpoint that monitors S-phase to control the timing of NEB. This result, combined with the fact that cycloheximide prevents NEB, indicates that NEB is a key control point that is positively and negatively regulated in the early embryo. During the interval between NEF and NEB, Grp is localized to the nucleus (data not shown) whereas Cdc2/cyclinB is primarily cytoplasmic[18]. In contrast, Chk1 and Cdc2/cyclinB are both localized in the nucleus at the equivalent time in fission yeast[13,29]. Thus, the fission yeast model in which Chk1 kinase activity ultimately results in nuclear export of Cdc25 to separate it physically from Cdc2 does not readily apply to the early *Drosophila* embryo[16]. One possibility is that Grp may be acting to prevent nuclear export of an activator of Cdc2 until replication is complete. *Drosophila* Cdc25 has been reported as primarily localized in the nucleus during interphase of these divisions[30]. It will be interesting to determine whether Grp influences the nuclear-cytoplasmic localization of Cdc25.

The abbreviated interphase of the late cortical cycles in *grp*-derived embryos is likely to result in progression through metaphase with incompletely replicated chromosomes[4]. This interpretation is in accord with the fact that nuclei undergo extensive bridging and snap-backs during anaphase, phenotypes similar to those observed in aphidicolin-treated embryos. The chromosome condensation defects observed in *grp*-derived embryos are, however, not readily explained as a consequence of progressing into metaphase with incompletely replicated chromosomes. Our data indicate that in the syncytial embryo, chromosome condensation is independent of the state of DNA replication. Although aphidicolin-treated embryos exhibit extensive defects in anaphase, chromosome condensation appears normal (Fig. 3a). Instead, we propose that the abbreviated interphase in *grp*-derived embryos does not provide sufficient time for proper chromosome condensation. This model is supported by the observation that the state of chromosome condensation is proportional to the interval between the initiation of condensation and metaphase. For example, as this interval increases from approximately 3 min in nuclear cycle 11 to approximately 10 min in nuclear cycle 13, the chromosomes become more condensed (Fig. 3a). In addition, delaying NEB by injection of aphidicolin or cycloheximide results in highly condensed chromosomes. This suggests that once condensation initiates, it proceeds on a constant rate through entry into metaphase. In *grp*-derived embryos, the interval between the initiation of chromosome condensation and metaphase is dramatically reduced (about 3 min compared to 10 min in normal cycle-13 embryos) and the chromosomes are proportionally undercondensed.

Whereas we find that chromosome congression and condensation occur normally in aphidicolin-treated embryos, a recent study reports that aphidicolin produces defects in chromosome congression[31]. A key technical difference is that we used a histone–GFP transgenic stock to follow chromosome behaviour, while Sibon *et al.*[31] relied on injected OliGreen, a DNA-binding dye. In addition, use of the transgenic line allowed us to inject aphidicolin specifically during anaphase. Because Sibon *et al.* injected a mixture containing apidicolin, rhodamine-labelled tubulin and OliGreen, timing injections with respect to a given stage in the nuclear cycle was not feasible.

A prominent phenotype of *grp*-derived embryos is the metaphase arrest at nuclear cycle 13 (ref. 19). At this time, the nuclei enter metaphase with improperly condensed and presumably incompletely replicated chromosomes. Our analysis supports a model in which failures in condensation, but not replication, elicit the metaphase delay. Treatment of normal embryos with the topoisomerase inhibitor VM26 result in nuclei progressing into metaphase with incompletely condensed chromosomes. These embryos display extensive metaphase delays. In addition, disruptions in chromosome condensation are correlated with metaphase delays in embryos bearing an abnormally long compound chromosome. In both cases, the nuclei eventually progress into anaphase, suggesting that the metaphase delay is checkpoint induced and eventually overridden[32]. In contrast aphidicolin-induced disruption of DNA replication does not produce delays in metaphase.

These studies lead to a model in which Grp is specifically required for monitoring S-phase to control nuclear envelope breakdown. Although Grp does not directly monitor chromosome condensation, by delaying NEB until the completion of S-phase, Grp indirectly provides sufficient time for condensation (Fig. 5). The later NEB occurs, the more condensed the chromosomes. Therefore the abbreviated interphase in *grp*-derived embryos not only results in nuclei entering metaphase with incompletely replicated chromosomes, but also with improperly condensed chromosomes. By nuclear cycle 13, these condensation effects may be severe enough to activate a checkpoint that prevents entry into anaphase.

This model contrasts with a model recently proposed by Sibon *et al.* based on their finding that aphidicolin treatment accurately phenocopies the mitotic defects observed in *grp* mutant embryos[31]. This includes an intriguing centrosome inactivation involving the loss of γ-tubulin. They propose that entry into mitosis with incompletely replicated chromosomes is responsible for inactivation of the centrosomes. This, in turn, produces spindle-assembly defects that prevent chromosome segregation and entry into anaphase. We do not favour this model because we find that the phenotype of *grp*-derived embryos and aphidicolin-treated embryos are not equivalent: cycle 13 *grp*-derived embryos, but not aphidicolin-treated embryos, exhibit condensation defects and extensive metaphase delays. Thus, while entering mitosis with unreplicated DNA may lead to inactivation of the centrosome, it does not necessarily result in metaphase delays. In addition, it is not obvious why centrosome inactivation would produce spindle defects and prevent chromosome segregation. Studies of *cnn*-mutant *Drosophila* embryos, in which centromeres are severely disrupted, and of unfertilised Sciara embryos, which lack centromeres, have shown that spindle formation and chromosome segregation proceed relatively normally in the absence of functional centrosomes[33,34].

These studies show that when a cell-cycle checkpoint is compromised, the relative timing of multiple events in the cell cycle may be disrupted. Consequently, significant aspects of checkpoint-induced phenotypes often may be due to a disruption in the relative timing of events not directly monitored by the checkpoint. Cells that give rise to cancer are often disrupted in cell-cycle checkpoints[35,36]. Therefore, strategies for selectively killing checkpoint-compromised cells may prove effective in treating cancer[37]. Our studies suggest alternative strategies for killing these cells. For example, our analysis of the *grp* phenotype predicts that cells disrupted in an S-phase checkpoint would be particularly sensitive to drugs, mutations and chromosomal rearrangements that inhibit chromosome condensation. □

Methods

Stocks

All experiments were performed with the *grp*[1] allele[38]. The histone–GFP stock is described in Clarkson *et al.* and the compound chromosome 2 stock designated C(2)EN *bw sp* is described in Novitsky *et al*[27]. Microinjections of rhodamine-labelled tubulin (Molecular Probes) and rhodamine-labelled histones (generously provided by Arshad Desai) were performed as described[39]. Aphidicolin (100 μM), VM26 (5 mM) and cycloheximide (24 μg ml^{-1}) injections were as described in Debec *et al.*[22]. Microscopy used a Leitz DMIRB inverted photoscope eqipped with a Leica TCS NT confocal imaging system. Time-lapse recordings and image analysis used the Leica NT TCS software.

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