grp (chk1) replication-checkpoint mutations and DNA damage trigger a Chk2-dependent block at the Drosophila midblastula transition

Saeko Takada*, Seongae Kwak, Birgit S. Koppetsch and William E. Theurkauf†

The 13 syncytial cleavage divisions that initiate Drosophila embryogenesis are under maternal genetic control. The switch to zygotic regulation of development at the midblastula transition (MBT) follows mitosis 13, when the cleavage divisions terminate, transcription increases and the blastoderm cellularizes. Embryos mutant for grp, which encodes Checkpoint kinase 1 (Chk1), are DNA-replication-checkpoint defective and fail to cellularize, gastrulate or to initiate high-level zygotic transcription at the MBT. The mnk (also known as loki) gene encodes Checkpoint kinase 2 (Chk2), which functions in DNA-damage signal transduction. We show that mnk grp double-mutant embryos are replication-checkpoint defective but cellularize, gastrulate and activate high levels of zygotic gene expression. We also show that grp mutant embryos accumulate DNA double-strand breaks and that DNA-damaging agents induce a mnk-dependent block to cellularization and zygotic gene expression. We conclude that the DNA-replication checkpoint maintains genome integrity during the cleavage divisions, and that checkpoint mutations lead to DNA damage that induces a novel Chk2-dependent block at the MBT.

KEY WORDS: DNA damage, MBT, Transcription, Cellularization, Drosophila

INTRODUCTION

In most metazoans, embryogenesis is initiated by a series of rapid mitotic divisions that are driven by mRNAs and proteins that accumulate during oogenesis (Foe et al., 1993). The cleavage stage is therefore under maternal genetic control. Embryonic development first requires zygotic gene expression at the midblastula transition (MBT), when the cell cycle slows, gene transcription increases dramatically and a subset of maternal mRNAs are degraded (O’Farrell et al., 2004). There is no growth during the cleavage stage, and the rapid cleavage-stage mitoses thus lead to progressive increases in the ratio of nuclei to cytoplasm (nucleocytoplasmic ratio). Reducing the number of nuclei or DNA content during the cleavage stage leads to additional mitotic divisions prior to the MBT, whereas increasing the DNA content reduces the number of pre-MBT divisions (Edgar et al., 1986; Kane and Kimmel, 1993; Newport and Kirschner, 1982). These observations suggest that titration of a maternally deposited factor by nuclear components triggers the MBT (Newport and Kirschner, 1982). However, the titrated maternal factor has not been identified, and some aspects of the MBT are controlled by a developmental timer that is independent of the cleavage divisions (Bashirullah et al., 1999; Hartley et al., 1996; Hartley et al., 1997). The molecular mechanisms that control the MBT thus remain to be defined.

In Drosophila, the maternally controlled cleavage-stage divisions are syncytial and zygotic control of development begins as the blastoderm cellularizes (Foe et al., 1993). Mutations in the genes grp and mei-41, which, respectively, encode the kinases Chk1 and Atr, which are required for the DNA-replication checkpoint, block cellularization and high-level zygotic gene activation at the Drosophila MBT. The cleavage-stage cell cycle progressively slows during syncytial blastoderm divisions 10 through to 13, but embryos mutant for grp or mei-41 fail to slow the syncytial blastoderm cell cycle and proceed through extra cleavage-stage cycles (Sibon et al., 1999; Sibon et al., 1997). These observations support a model in which maternal DNA-replication factors are titrated during the late syncytial divisions, leading to increases in S-phase length that trigger replication-checkpoint-dependent delays in progression into mitosis (Sibon et al., 1999; Sibon et al., 1997). However, the relationship between checkpoint-dependent cell cycle delays during the late syncytial divisions and the defects in zygotic gene activation and cellularization at the MBT have not been established.

Chk2 is a conserved kinase that functions in DNA-damage signaling. Here, we show that mutations in the mnk (also known as loki – FlyBase) gene, which encodes Chk2, suppress the cellularization, gastrulation and zygotic gene-activation defects associated with grp mutations. However, mnk grp double-mutant embryos lack a functional replication checkpoint and do not show wild-type cleavage-stage cell cycle delays. Progression through the MBT does therefore not require normal increases in cell cycle length during the late cleavage stage, or require Chk1. We also show that grp mutant embryos accumulate DNA double-strand breaks, and that DNA-damaging agents block zygotic gene activation and cellularization in wild-type embryos, but not in mnk mutants. These findings indicate that the crucial developmental function for the replication checkpoint is to maintain genome integrity during the rapid cleavage-stage divisions, and that checkpoint mutations block to the MBT by activating a novel Chk2-dependent pathway that inhibits cellularization and zygotic gene expression.

MATERIALS AND METHODS

Drosophila stocks

The grp‡ and mnk§ alleles are protein nulls (Brodsky et al., 2004; Sibon et al., 1997) (data not shown). w1118 was used as a control because both mutant alleles are in the w1118 background. To obtain grp‡ and mnk§ grp‡
double-mutant embryos, homozygous females were mated to wild-type (Oregon R) males. To obtain mnk-embryos, homozygous-mutant females were mated to homozygous-mutant males.

**Immunofluorescence**

At 2-3 hours of age, embryos were fixed with formaldehyde/methanol and immunostained with mouse anti-α-Spectrin monoclonal antibody 3A9 (1:10 dilution, Developmental Studies Hybridoma Bank) as described (Theurkauf, 1994). DNA was stained with 0.2 μM TOTO3 (Molecular Probes). To cytologically assay for DNA double-strand breaks, 1-3-hour-old gfp embryos were fixed with methanol and immunostained with rabbit anti-phospho-Histone H2A.X (Ser139) (yH2AX) antibody (1:250 dilution, Upstate) as described (Theurkauf, 1994). The embryos were imaged using a Leica TCS-SP inverted scanning confocal microscope. ImageJ was used for image processing (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2006).

**DNA-damage treatment and whole-mount in situ hybridization**

To induce DNA damage, wild-type (w1118) or mnk mutant embryos were bleach dechorionated, rinsed with Trition/NaCl and H2O, and treated in a 1:1 mixture of octane and Robb’s medium (55 mM potassium acetate, 40 mM sodium acetate, 100 mM sucrose, 10 mM MgCl2, 1 mM CaCl2 and 100 mM HEPES, pH 7.4) containing 50 μg/ml bleomycin (Sigma) for 30 minutes. To assay transcription, embryos were then fixed in methanol (Theurkauf, 1994), rehydrated and processed for enhanced fluorescence in situ hybridization. Anti-sense digoxigenin (DIG)-labeled RNA probes were synthesized from cDNA clones or PCR-amplified cDNA fragments using DIG-High Prime following the manufacturer’s instructions (Roche). *rant* and *fushi tarazu* cDNA clones were provided by P. Gergen (Tsai and Gergen, 1995). *slam* (slow as molasses) and sry-α DNA fragments were amplified by PCR using wild-type genomic DNA and gene-specific primers [for *slam*, 5’-CTGTTGATCGGATCTTCTG-3’ and 5’-CTTAA-TACGACTACTATAGGG-3’ (T7 promoter sequence)+5’-AAATCTTGCTC- CATGTCCTGC-3’; for sry-α, 5’-CTCTGACACATTGAGTAC-3’ and 5’-CTAATACGACTACTAAGGG-3’ (T7)+5’-GATTACGCAA- GTAGTCCTGTTG-3’]. Whole-mount in situ hybridization was performed as described (Tautz and Pfeifle, 1989; Cha et al., 2001). Tyramide signal amplification (TSA) was performed following manufacturer’s instructions (Perkin Elmer). Briefly, after the post-hybridization washes, embryos were blocked for 30 minutes in TNB buffer [0.1 M Tris-HCl (pH 7.5), 0.15 M NaCl, 0.5% block-TSA kit], incubated for 2 hours at room temperature with anti-Digoxigenin POD (Roche) at 1:100 dilution, rinsed with TBS, and treated with 3% H2O2 for 5 minutes. The embryos were then incubated with primary antibodies for 2 hours at room temperature, washed with TWE (Twice with TBS, 3 times with wash buffer), incubated with secondary antibodies for 30 minutes and washed with TWE (Twen-20). The membranes were detected with an ECL Plus western blotting detection system (Amersham).

**RESULTS**

**mnk suppresses grp arrest at the MBT**

For all of the following studies, we assayed embryos derived from homozygous single- and double-mutant females that were mated to wild-type males. These embryos were therefore heterozygous for the mutation(s) under investigation. Early embryogenesis is, however, under maternal genetic control; we therefore refer to these embryos by the maternal genotype here.

Embryo mutants for *grp*, which encodes an essential component of the DNA-replication checkpoint, arrest development at the MBT (Sibon et al., 2000; Sibon et al., 1997). The *grp* mutation also causes spindle-assembly defects during the late syncytial divisions (Sibon et al., 2000), and these mitotic defects are suppressed by a null mutation in *mnk* (Takada et al., 2003), which encodes a Chk2 homolog required for DNA-damage signaling (Bartek et al., 2001; Brodsky et al., 2004; Masrouha et al., 2003; Peters et al., 2002). To determine whether the MBT block in *grp* mutants also requires Chk2, we analyzed the development of embryos that lack maternal Chk1 and Chk2. Embryos double-mutant for *mnk* and *grp* (*mnk grp*), like *grp* single mutants, failed to hatch. The *mnk* mutation thus does not suppress the maternal-effect embryonic lethality associated with *grp*.

To determine whether *mnk* suppresses the developmental block at the MBT, embryos were assayed for cellularization, which is the first morphogenetic event that requires zygotic gene expression (Postner and Wieschaus, 1994; Schejter and Wieschaus, 1993; Wieschaus and Sweedon, 1988). During cellularization, the monolayer of cortical nuclei is surrounded by a characteristic hexagonal array of membranes with associated actin filaments. This arrangement is clearly observed in wild-type and *mnk* mutant embryos (Fig. 1A,B). In similarly aged *grp* mutants, by contrast, the syncytial nuclei are uniformly surrounded by a monolayer of cortical nuclei (Fig. 1C, *grp*). These studies, and extensive previous observations, indicate that 100% of *grp*-null mutant embryos fail to cellularize. Strikingly, a significant number of *mnk grp* double-mutant embryos had a uniform monolayer of cortical nuclei surrounded by a hexagonal actin network, indicating that they had initiated or completed cellularization (Fig. 1D, *mnk grp*). The nuclei in the double-mutant embryos were larger than in wild-type controls (Fig. 1D, inset), and this appears to result from chromosome-segregation...
failures during the late syncytial blastoderm divisions (Takada et al., 2003). These initial observations indicated that the mnk mutation at least partially suppresses the cellularization/MBT block associated with the grp mutation.

To evaluate how efficiently mnk suppresses the cellularization defects associated with the grp mutation, we directly analyzed early embryogenesis by time-lapse transmitted light microscopy. In wild-type (w1118) and mnk mutant embryos, cellularization consistently takes place after mitosis 13, when the cell cycle pauses and membrane invaginations surround the cortical nuclei. Completion of cellularization is immediately followed by the morphogenetic movements of gastrulation, which include anterior migration of pole cells (germline cells) (Fig. 1E-H, and see Supplementary Material). The actin cytoskeleton (green) was labeled with an anti-α-Spectrin antibody and nuclei were labeled with TO-TO3 (Molecular Probes; red). Insets are enlarged images of α-Spectrin staining. (E-L) Still images from transmitted light time-lapse recordings of control (w1118), mnk- and grp-null single mutant, and mnk grp double-mutant embryos. Control (E,F), mnk-null mutant (G,H) and mnk grp double-mutant (K,L) embryos cellularize and gastrulate. By contrast, grp-null mutant embryos (I,J) do not cellularize or gastrulate. Arrows indicate the cellularization front; arrowheads indicate pole cells migrating towards the anterior during gastrulation. Scale bars: 100 μm in A-L; 10 μm in insets in A-D.

Table 1. Pre-MBT cell cycle timing and duration from pole-cell migration to cellularization in wild-type and mutant embryos

<table>
<thead>
<tr>
<th>Cell cycle</th>
<th>Wild type (6)</th>
<th>mnk (chk2) (5)</th>
<th>grp (chk1) (5)</th>
<th>mnk grp (9)</th>
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</thead>
<tbody>
<tr>
<td>10 I</td>
<td>4.7±0.43</td>
<td>4.7±0.48</td>
<td>4.7±0.44</td>
<td>4.9±0.51</td>
</tr>
<tr>
<td>M</td>
<td>4.4±0.90</td>
<td>4.0±0.54</td>
<td>4.5±0.75</td>
<td>4.8±0.60</td>
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<tr>
<td>11 I</td>
<td>5.2±0.50</td>
<td>4.4±0.49</td>
<td>4.9±0.75</td>
<td>5.3±0.67</td>
</tr>
<tr>
<td>M</td>
<td>4.3±1.00</td>
<td>4.0±0.49</td>
<td>3.9±0.25</td>
<td>4.5±0.47</td>
</tr>
<tr>
<td>12 I</td>
<td>7.2±0.85</td>
<td>8.4±1.00</td>
<td>6.1±0.16</td>
<td>6.5±0.69</td>
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<tr>
<td>M</td>
<td>4.6±0.41</td>
<td>4.3±0.54</td>
<td>4.8±0.89</td>
<td>5.0±0.37</td>
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<tr>
<td>13 I</td>
<td>13.9±1.42</td>
<td>15.5±2.24</td>
<td>7.1±0.83</td>
<td>9.7±0.93</td>
</tr>
<tr>
<td>M</td>
<td>4.8±0.54</td>
<td>5.0±0.83</td>
<td>8.1±0.80</td>
<td>4.9±0.71</td>
</tr>
<tr>
<td>14 I</td>
<td>NA</td>
<td>NA</td>
<td>10.9±0.62</td>
<td>12.5±1.20</td>
</tr>
<tr>
<td>M</td>
<td>NA</td>
<td>13.8±1.38</td>
<td>6.7±0.89</td>
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Pole-cell migration to cellularization**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Wild type (6)</th>
<th>mnk (chk2) (5)</th>
<th>grp (chk1) (5)</th>
<th>mnk grp (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int</td>
<td>71.0±2.89</td>
<td>68.9±2.42</td>
<td>NA</td>
<td>83.5±3.15</td>
</tr>
<tr>
<td>Mid</td>
<td>104.6±5.15</td>
<td>102.3±4.05</td>
<td>NA</td>
<td>125.7±6.10</td>
</tr>
</tbody>
</table>

**Average durations±s.d. of cell cycle timing are shown. Timing of nuclear envelope formation (NEF) and nuclear envelope breakdown (NEB) were measured from DIC live recordings of embryos. I, interphase from NEF to NEB; M, mitosis from NEB to NEF. Number of embryos analyzed are shown in parentheses.

**Average durations±s.d. of pole-cell migration (pole-bud formation) to cellularization are shown. Number of embryos analyzed are shown in parentheses. Int, initiation of membrane invagination; Mid, invaging membrane reaches to approximately one third of the final cell length; NA, not analyzed.
before cellularization (see Movie 4 in the supplementary material), and chromosome segregation failed during the later syncytial divisions, producing polyploid nuclei (Takada et al., 2003). The polyploidy resulting from division failures during the late cleavage stage may contribute to the gastrulation defects and embryonic lethality in the double mutants. Nonetheless, the mnk mutation dramatically suppressed the developmental arrest associated with the grp mutation, allowing consistent cellularization and gastrulation.

We previously speculated that grp mutants fail to progress through the MBT because the shortened syncytial blastoderm cell cycle times do not allow complete transcription of early zygotic genes (Sibon et al., 1997). However, the cell cycle times in mnk grp double mutants are significantly shorter than in wild type, yet these double mutants cellularize (Table 1). In addition, zygotic expression of a number of genes, including slam and serendipity-α/H9251 (sry-α/H9251), peak very early in interphase 14, at or before the start of cellularization, which is initiated 10 minutes after the completion of mitosis 13 (Foe et al., 1993) (see Movie 1 in the supplementary material). Zygotic gene activation thus requires significantly less than 10 minutes, and interphase 14 in grp mutants averages 11-12 minutes (Sibon et al., 1999; Sibon et al., 1997) (Table 1). The cell cycle timing defects in grp mutants are therefore insufficient to account for the observed block in cellularization and gene expression.

These observations, however, do not eliminate the possibility that signaling through the replication checkpoint is required for transcription and cellularization, and that the mnk mutations restore this signaling. To directly test for replication-checkpoint function in...
mnk grp double mutants, we co-injected embryos with the DNA-polymerase inhibitor aphidicolin and with rhodamine-conjugated tubulin, and analyzed progression into mitosis 11 and 12 by time-lapse confocal microscopy (Sibon et al., 1997). In wild-type embryos, aphidicolin induced an increase in interphase length from 5.4 minutes (s.d. 0.46, n=8) to 14.2 minutes (s.d. 1.5, n=8). By contrast, interphase length in mnk grp double mutants was 4.6 minutes (s.d. 2.7, n=8) under control conditions and 4.9 minutes (s.d. 0.21, n=6) following aphidicolin injection. Therefore, in the absence of Chk2, cellularization does not require a functional replication checkpoint or wild-type cell cycle delays during the syncytial blastoderm stage.

Cellularization is coordinated with a dramatic increase in inhibitory phosphorylation of the mitotic Cdc2 kinase (Edgar et al., 1994). The grp mutation blocks the increase in Cdc2 phosphorylation that normally accompanies interphase 14 and cellularization, and the Chk1 encoded by grp inhibits Cdc25, a dual specificity phosphatase that activates Cdc2 by reversing this modification (Walworth, 2001). These findings suggest that Chk1 activation leads to increases in Cdc2 phosphorylation during interphase 14, and that this modification may be essential to cellularization (Sibon et al., 1997). We therefore assayed mnk grp double-mutant embryos, which lack Chk1, for Cdc2 phosphorylation. As shown in Fig. 2C, Cdc2 phosphorylation is restored in mnk grp mutants, indicating that this process does not require Chk1 or a functional replication checkpoint. Maternal Cdc25 mRNA and protein, which are encoded by string and twine, are normally degraded early in interphase 14, and these processes require zygotic gene expression (Edgar and Datar, 1996; Edgar et al., 1994). The grp mutation could therefore inhibit Cdc2 phosphorylation by activating a Chk2-dependent block to the expression of zygotic factors that trigger the destruction of maternal Cdc25 transcripts and proteins.

To determine whether mnk suppresses the transcriptional defects associated with grp mutations (Sibon et al., 1997), we assayed mutant embryos for the expression of the segmentation genes runt and fushi tarazu (ftz), and for two genes required for cellularization, slam and sry-α (Fig. 2A,B, and data not shown) (Lecuit et al., 2002; Schweisguth et al., 1990). Fluorescence in situ hybridization (FISH) revealed high-level expression of all four genes during interphase 14 in wild-type and mnk single-mutant embryos (Fig. 2A,B, w/118 and mnk). By contrast, runt, ftz (data not shown) and sry-α were not detectable in interphase 14 grp mutants (Fig. 2A,B, grp). The slam transcript was detected in grp mutants, although the mRNA was dispersed and expression appeared to be lower than in wild-type embryos (data not shown). Strikingly, essentially wild-type expression of all four genes was observed in mnk grp double-mutant embryos (Fig. 2A,B, mnk grp, and data not shown). Chk2 is therefore required for the block to zygotic gene activation in grp mutants.

Mutations in the grp locus have been reported to stabilize Cyclin A and accelerate Histone H3 dephosphorylation on mitotic exit during the early cleavage divisions (Su et al., 1999). These defects could reflect a direct role for Chk1 during mitotic exit and in Cyclin A proteolysis, or result from checkpoint failure and Chk2 activation. In an attempt to distinguish between these alternatives, we analyzed Histone H3 dephosphorylation and Cyclin A protein levels in wild-type and mutant embryos. However, using standard immunocytological labeling, we found no significant difference in the kinetics of Histone H3 dephosphorylation in early cleavage-stage wild type or grp mutants (see Fig. S1 in the supplementary material). In addition, we found no significant difference in Cyclin A levels at 0-90 minutes post-egg deposition in wild-type, grp or mnk grp embryos (see Fig. S2A in the supplementary material). We did see an increase in Cyclin A levels in 0-3-hour-old grp embryos (see Fig. S2B in the supplementary material), but this developmental pool included late syncytial blastoderm-stage embryos that were delayed in mitosis due to Chk2 activation (Table 1) (Takada et al., 2003). Significantly, 0-3-hour-old mnk grp double-mutant embryos did not overexpress Cyclin A (see Fig. S2B in the supplementary material). Increased Cyclin A accumulation ingrp mutants thus appears to result from Chk2 activation. These findings, with the observations outlined above, indicate that the primary function of Chk1 is to delay cell cycle progression, thus preventing premature mitosis and DNA damage during the later syncytial blastoderm divisions. The mitotic

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**Fig. 4. DNA damage triggers a Chk2-dependent cellularization block.** (A-H) Control (w/118) and mnk mutant embryos were injected with bleomycin, and cellularization was monitored by time-lapse transmitted light and laser scanning confocal microscopy. Rhodamine-conjugated tubulin was co-injected as a marker for cell cycle stage. Nuclei exclude fluorescent rhodamine-conjugated tubulin and thus appear as dark circles on confocal imaging (Sibon et al., 1997). Recordings were started at the beginning of interphase 14 (0 seconds, 0s). Immediately after injection, nuclei were in a uniform monolayer in both wild-type (w/118) and mnk mutant embryos (A,E). By 1200 seconds (1200s), the nuclear monolayer in w/118 controls was disorganized and some nuclei had dropped into the interior of the embryo (B), and these embryos did not cellularize (C,D). By contrast, all mnk mutant embryos injected with bleomycin consistently maintained a uniform nuclear monolayer and cellularized (E-H). (D,H) Plasma membrane invagination at the cellularization front is indicated (arrows). (D,H) Rhodamine-conjugated tubulin is excluded from interphase nuclei and was used as a cell cycle marker. (Also see Movies 1-5 in the supplementary material.) Scale bar: 50 μm.
and developmental defects associated with grp mutations, by contrast, are a secondary consequence of DNA-damage signaling via Chk2.

To test more directly the hypothesis that the grp mutation leads to DNA damage, we labeled grp mutant embryos for phospho-histone H2Av (γH2Av), a homolog of γH2AX that associates with DNA double-strand breaks (Madigan et al., 2002; Rogakou et al., 1999). As shown in Fig. 3, grp mutant embryos accumulate γH2Av foci (Fig. 3A-F). These findings are consistent with earlier observations indicating that grp mutant embryos accumulate DNA lesions (Fogarty et al., 1997). In a variety of organisms, Chk2 is activated via phosphorylation by Atm or Atr, which leads to oligomerization and auto-phosphorylation. The active phosphorylated form of Chk2 shows reduced electrophoretic mobility by SDS-PAGE (Bartek et al., 2001; Brodsky et al., 2004). In grp mutants, Chk2 showed a modest shift, consistent with kinase activation (Fig. 3G).

Mutations in grp thus lead to both DNA damage and Chk2 phosphorylation, and the MBT block in grp mutants is efficiently suppressed by the mnk mutation. DNA-damage signaling via Chk2 thus appears to induce developmental arrest in grp mutants.

**DNA damage induces a Chk2-dependent block to blastoderm cellularization**

To determine whether DNA damage and Chk2 activation are sufficient to block developmental progression, we assayed cellularization in wild-type and mnk mutant embryos injected with DNA-damaging agents after the final syncytial blastoderm division. For the majority of these studies, embryos were injected with bleomycin, a radiomimetic drug that produces DNA double-strand breaks, and cellularization was followed by time-lapse microscopy (Fig. 4). Bleomycin injection during the final syncytial mitosis (mitosis 13) or during early interphase 14 blocked cellularization in 9 out of the 11 wild-type embryos examined (Fig. 4A-D, and see Movie 5 in the supplementary material). By contrast, 9 out of 9 mnk mutant embryos injected during mitosis 13 or interphase 14 cellularized normally (Fig. 4E-H, and see Movie 6 in the supplementary material). Interestingly,
these embryos did not subsequently gastrulate. In addition, wild-type embryos injected with DNA-damaging agents after initiating membrane invagination went on to complete cellularization, but these embryos also failed to gastrulate (data not shown). DNA damage early in interphase 14 thus triggers a Chk2-dependent block to cellularization. DNA damage during cellularization, by contrast, induces a Chk2-independent block to gastrulation.

To determine the effect of DNA damage on transcriptional activation at the MBT, we analyzed expression of two early zygotic genes, *slam* and *runt*, in wild-type (w^1118) and *mnk* mutant embryos treated with bleomycin. Previous studies have shown that X-ray treatment blocks zygotic *runt* transcription during interphase 14 (Brodsky et al., 2000), and we found that all of the bleomycin-treated wild-type embryos that showed clear cytological indications of DNA damage failed to express *runt* (Fig. 5B). Following bleomycin treatment, a subset of cytologically normal cellular blastoderm-stage embryos were present, and these embryos expressed *runt* at high levels in the normal seven-stripe pattern (see Fig. S3 in the supplementary material). Because bleomycin injection early in interphase 14 consistently blocked cellularization, the embryos that expressed *runt* in a seven-stripe pattern appear to have initiated cellularization when drug treatment was started, or were not efficiently permeabilized and thus did not receive a sufficient dose of inhibitor. Bleomycin-treated *mnk* mutant embryos consistently expressed *runt* at levels comparable to untreated wild-type and *mnk* controls (Fig. 5C,D). Damage-dependent Chk2 activation thus appears to block *runt* transcription. We did detect *slam* transcript in wild-type embryos treated with bleomycin, but transcript localization was severely disrupted (Fig. 5H). In bleomycin-treated *mnk* mutants, *slam* expression and distribution were similar to untreated controls (Fig. 5J). Very similar Chk2-dependent defects are observed in *grp* mutants (Fig. 5E,F,K,L). DNA-damage signaling via Chk2 thus disrupts transcript localization and transcriptional activation of a subset of zygotic genes, and activation of this pathway appears to induce the transcription defects associated with replication-checkpoint mutations.

**DISCUSSION**

Studies in lower eukaryotes initially defined checkpoints as non-essential pathways that delay cell cycle progression in response to external stress (Hartwell and Weinert, 1989). Subsequently, checkpoint mutations in higher eukaryotes were found to induce developmental defects and embryonic lethality (Conn et al., 2004; Kalogeropoulos et al., 2004; Liu et al., 2000; Takai et al., 2000; Petrus et al., 2004; Shimuta and Matsui, 2002). In *Drosophila*, the DNA-replication checkpoint is required to delay the cell cycle during the late cleavage stage, and checkpoint mutants subsequently fail to cellularize or activate zygotic gene expression at the MBT (Sibon et al., 1999; Sibon et al., 1997). These findings suggested that the replication checkpoint has a direct role in metazoan development. Alternatively, the observed developmental defects could be an indirect consequence of checkpoint failure.

Here, we show that a null mutation in *mnk*, which encodes the conserved DNA-damage signaling kinase Chk2, efficiently suppresses the cellularization and zygotic gene-activation defects in *grp*, but does not restore wild-type cell cycle timing or replication-checkpoint function. We therefore conclude that progression through the *Drosophila* MBT does not directly require Chk1 or checkpoint-dependent cell cycle delays. Instead, our data indicate that the essential function for the replication checkpoint is to prevent DNA damage during the syncytial blastoderm divisions, which triggers a Chk2-dependent block to zygotic gene activation and cellularization. Supporting this proposal, DNA-damaging agents trigger a Chk2-dependent block to cellularization and zygotic gene activation, and *grp* mutations accumulate DNA double-strand breaks. Chk2 is likely to have multiple targets during this developmental response to DNA damage; these targets may include transcription factors that control the expression of genes implicated in cell cycle control and cellularization (Grosshans et al., 2003; Postner and Wieschaus, 1994; Schejter and Wieschaus, 1993; Wieschaus and Sweeton, 1988).

Embryos mutant for *grp* or *mei-41* lack a functional replication checkpoint and progress into mitosis prior to S-phase completion, triggering defects in γ-Tubulin localization and microtubule nucleation (Sibon et al., 2000). These mitotic defects are suppressed by *mnk*, raising the possibility that *mnk* suppresses the *grp* mutant developmental block at the MBT by restoring mitotic function. However, *mnk* does not suppress the chromosome-segregation defects associated with *grp* mutants (Takada et al., 2003). More significantly, inducing DNA damage following the final syncytial blastoderm division triggers a Chk2-dependent block to cellularization. DNA damage can therefore induce a Chk2-dependent developmental block that is distinct from the damage and Chk2-dependent block to mitosis.

The studies outlined here support a simple model in which the developmental arrest associated with *grp* mutations results from defects in the established function for this kinase in cell cycle control. The early cleavage-stage divisions have a simplified S-phase/M-phase cell cycle, and we propose that the crucial function of Chk1 is to delay mitosis until DNA replication is complete. In *grp* mutants, progression into mitosis before replication is complete leads to DNA damage, which activates a Chk2-dependent block to developmental progression. Intriguingly, disrupting Chk1 function also leads to early embryonic lethality in frogs, mice and worms (Conn et al., 2004; Kalogeropoulos et al., 2004; Liu et al., 2000; Takai et al., 2000; Kalogeropoulos et al., 2004; Petrus et al., 2004; Shimuta et al., 2002). Chk1 knockdown in *Xenopus* and Chk1 also known as Chek1 – Mouse Genome Informatics) mutations in mouse lead to apoptotic death of the embryo, consistent with a DNA-damage response (Takai et al., 2000; Carter and Sible, 2003; Shimuta et al., 2002). We therefore speculate that Chk1 has a conserved function in maintaining genome integrity during the cleavage stage, and that the early embryonic lethality in checkpoint mutants is a consequence of DNA-damage signaling.

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**Supplementary material**

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/9/1737/DC1

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