Drosophila chk2 plays an important role in a mitotic checkpoint in syncytial embryos

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Received 8 May 2003; accepted 9 May 2003

First published online 23 May 2003

Edited by Jesus Avila

Abstract ***Drosophila*** **chk2 (Dmchk2, also called Dmnk) plays a crucial role in the DNA damage response pathway mediating cell cycle arrest and apoptosis [Xu et al., FEBS Lett. 508 (2001) 394–398; Peters et al., Proc. Natl. Acad. Sci. USA 99 (2002) 11305–11310]. In this study, the role of Dmchk2 in early embryogenesis was investigated. In the absence of Dmchk2 function, abnormal nuclei accumulate in the cortex of the syncytial embryo. We show that the abnormal nuclei result from a failure of chromosome segregation probably due to damaged or incomplete replicated DNA. Importantly, this *Dmchk2* phenotype is partially suppressed by reducing the gene dosage of *polo* or *stg*. As Polo-like kinase was shown to colocalize and coimmunoprecipitate with Chk2 [Tsvetkov et al., J. Biol. Chem. 278 (2003) 8468–8475] in mammals, these observations suggest that polo might be a key target of Dmchk2 in regulating mitotic entry in response to DNA damage or replication block.**

Key words: *Drosophila* Chk2; Polo; Mitotic checkpoint

1. Introduction

Chk1 and Chk2 play important roles in cell cycle arrest in response to damaged or incompletely replicated DNA, and are conserved from yeast to mammals. Chk1 is essential for the G_2 checkpoint in response to DNA damage and replication block, which is regulated by ATR [4,5]. Chk2 is a major effector of Atm, it is phosphorylated in an ATM-dependent manner in response to ionizing radiation and is activated by ATR in response to UV radiation or stalled DNA replication [6–11].

In *Drosophila* embryogenesis, the first 13 cell cycles occur synchronously and rapidly, consisting of alternating S phases and M phases. These rapid cell cycles are controlled entirely by the maternal products. Starting from mitotic cycle 9, the nuclei move to the cortex of the embryo. The last four syncytial divisions occur in the cortical layer and the length of the S phase is progressively increased in each of these divisions [12]. Grp and Mei-41, the *Drosophila* homologues of Chk1 and ATR, are required to delay the onset of mitosis until the completion of DNA replication. Mutations in *grp* or *mei-41* lead to chromosome segregation failures during the later syncytial blastoderm divisions [13–16]. Centrosome inactivation and chromosome condensation defects are responsible for the metaphase delays observed in *grp*-derived embryos [17,18].

DmChk2 protein plays a crucial role in the DNA damage response pathway mediating cell cycle arrest and apoptosis [1,2,19]. It is highly expressed in ovaries and early embryos [20], suggesting a possible function during oogenesis or early embryogenesis. Recently, it was shown that DmChk2 serves as a transducer of the meiotic checkpoint in flies [21]. However, the role of DmChk2 in early embryogenesis is still unclear.

In this report, we analyzed the role of Chk2 during early embryogenesis. Our results suggest an important role of Dmchk2 in preventing the accumulation of abnormal nuclei in the cortex of the developing embryo. We provide evidence that implicate polo as a key target of Dmchk2 in this process. The accumulation of the abnormal nuclei in the cortex of the embryo significantly diminished the zygotic gene expression.

2. Materials and methods

2.1. Fly crosses

All flies were maintained at 25°C. grp^1, $mei\text{-}41^{RT1}$, and $polo^1$ were obtained from the Bloomington stock center. The P element insertion that rescues the barren lethality from our original report turned out to have significant reduced Barren protein level compared to the wild type. To generate new *barren* rescue insertions that have the same Barren levels, flies carrying the *barren* rescue P element insertion on the third chromosome were mobilized by crossing with a stock carrying the transposase (Δ2-3 flies). A new *barren* rescue insertion on the second chromosome, which expresses wild type level of *barren*, was identified. This new *barren* rescue construct was recombined with the $Dmchk2^{E51}$ and was used in all the experiments described here.

2.2. DAPI and antibody staining of embryos

Embryos at indicated times were fixed with formaldehyde/methanol. The nuclei of embryos were visualized by DAPI stain (50 μg/ml). The anti-CNN antibody (from Dr. T. Kaufman) was used to visualize the centrosome. Spindles were visualized by immunostaining with the anti-β-tubulin antibody. Zygotic transcription of *even-skipped* in embryos was examined by anti-Eve antibody staining. In γ-irradiation experiments, 0–2 h embryos were irradiated at 100 rad, and were stained with DAPI after another 30 min at room temperature.

3. Results and discussion

3.1. Phenotypes of Dmchk2 maternal null ($dmchk2^{m-}$) embryos

DmChk2 (Mnk) transcript is highly expressed in ovaries and in early cleavage stage embryos [20,22]. To examine the possible role of Dmchk2 in oogenesis and/or early embryogenesis, we analyzed the fertility of the *Dmchk2* mutants. As

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0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00536-2

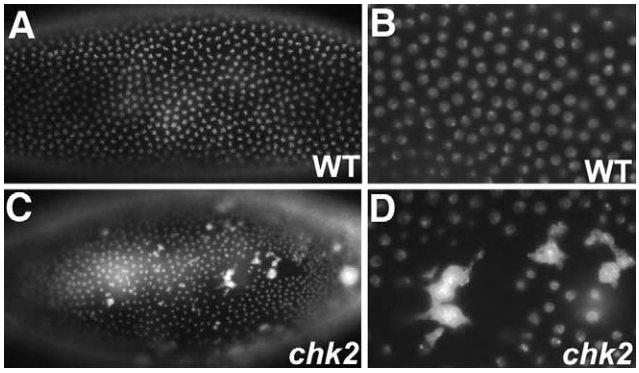

Fig. 1. Embryos from *Dmchk2* null females show abnormal nuclear phenotype. The nuclei of wild type (A,B) and *Dmchk2*$^{m-}$ (C,D) embryos were visualized by DAPI staining. In wild type embryos, the nuclei are evenly distributed (A,B). In contrast, the nucleus distribution near the abnormal nuclei is uneven in *Dmchk2*$^{m-}$ embryos (C,D).

shown in Table 1, *Dmchk2* females are partially sterile, only about 20% or 13% of the embryos hatch when crossed to wild type or *Dmchk2* males, respectively (Table 1). These observations suggest that the maternal contribution of Dmchk2 plays an important role during embryogenesis.

To determine the role of maternal Dmchk2 in early embryogenesis, embryos from *Dmchk2* null females were stained with DAPI to determine the pattern of nuclei. Starting from embryonic nuclear cycle 9, the nuclei migrate towards the cortex of the wild type embryos and are evenly distributed (Fig. 1A,B). In contrast, over 60% of the embryos derived from *Dmchk2* null females (*Dmchk2*$^{m-}$ embryos) exhibited abnormal nuclei of variable sizes (Fig. 1C,D). Such abnormal nuclear phenotypes can be observed as early as in nuclear

Table 1
Reduced fertility of *Dmchk2* mutants

Cross	Embryo hatching (%)
w^{1118} female × w^{1118} male	90.8 ± 0.6
chk2 female × w^{1118} male	20.1 ± 1.9
chk2 female × *chk2* male	13.0 ± 0.2

Virgin females of w^{1118} or *Dmchk2* null mutant were crossed to males as indicated. 0–12 h embryos were collected and percent of embryo hatching was determined. The numbers shown are averages from two independent experiments. At least 600 embryos were scored in each experiment.

cycle 10 embryos (for example, Fig. 2D). Remarkably, the large nuclei remained at the cortex of the embryos and were usually surrounded by areas that lack normal nuclei. Such phenotypes are distinct from the *grp* or the *barr* mutant phenotypes reported earlier [13,15,23].

3.2. *Multiple spindles and centrosomes surrounding the abnormal nuclei in* Dmchk2^{m-} *embryos*

The observed abnormal nuclear phenotype could be caused by a failure to form the proper mitotic structure such as defects in centrosome or spindle formation or could be caused by certain defects on the chromosomes such as damaged or incompletely replicated DNA.

We sought to determine if the abnormal nuclei observed in the *Dmchk2*$^{m-}$ embryos correlated with a failure of spindle formation or centrosome duplication. Embryos were double-stained with DAPI to label the DNA and an antibody against tubulin to determine spindle formation in *Dmchk2*$^{m-}$ embryos. In wild type embryos, there is only one spindle per metaphase nucleus (Fig. 2A–C). In contrast, two or more spindles were observed around the abnormal nuclei (Fig.

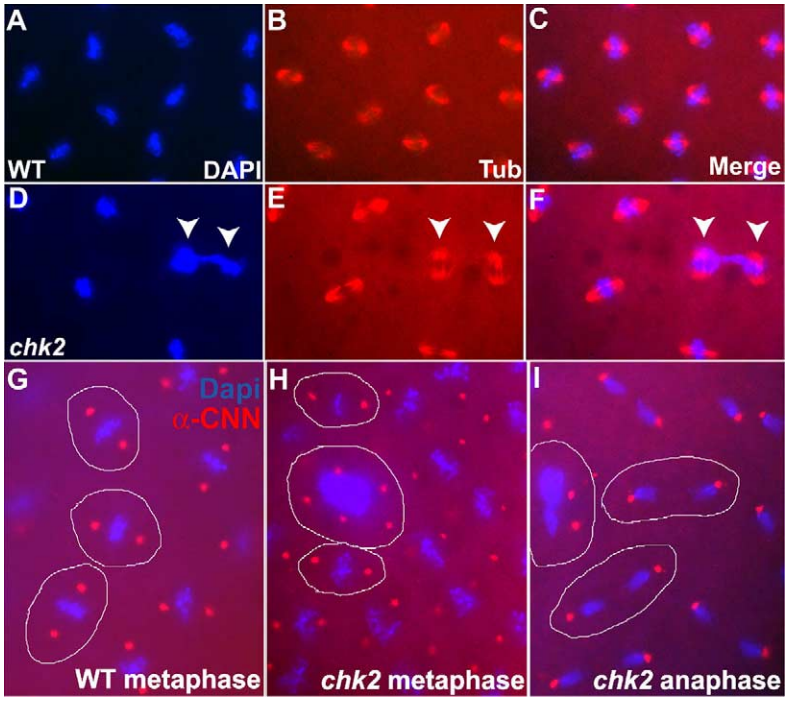

Fig. 2. *Dmchk2*$^{m-}$ embryos show defects in chromosome segregation with multiple spindles and centrosomes surrounding the abnormal nuclei. Wild type (A–C,G) and *Dmchk2*$^{m-}$ embryos (D–F,H,I) were stained with anti-tubulin antibody (A–F) or anti-CNN antibody (G–I). DNA was marked by DAPI stain (blue). Multiple spindles (arrowheads in D–F) and multiple centrosomes were found around the abnormal nuclei (H) in *Dmchk2*$^{m-}$ embryos. During anaphase, chromosomes from the normal nuclei but not the abnormal nuclei separate (I).

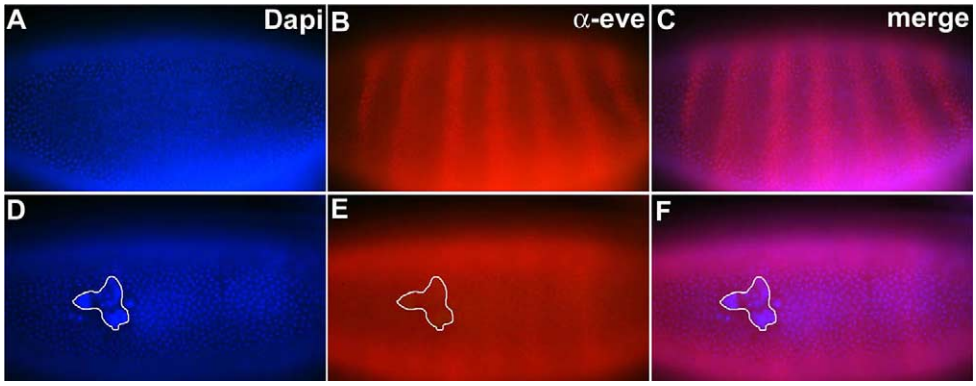

Fig. 3. Embryos with abnormal nuclei reduce the onset of zygotic transcription. Wild type embryos (A–C) and *Dmchk2*$^{m-}$ embryos with abnormal nuclei (D–F) were double-labeled with anti-even-skipped (red) and DAPI (blue). Wild type embryos show the seven stripe pattern of *even-skipped* expression (A–C). In contrast, the expression of *even-skipped* is reduced in *Dmchk2*$^{m-}$ embryos with abnormal nuclei (D–F).

2D–F and data not shown). In addition, an antibody against CNN was used to examine the centrosomes in the *Dmchk2*$^{m-}$ embryos. As shown in Fig. 2G–I, while there are only two centrosomes surrounding each nucleus of the normal nuclei of the *Dmchk2*$^{m-}$ embryos as well as each nucleus of the wild type embryos, there are multiple centrosomes surrounding the abnormal nuclei during metaphase (Fig. 2H). As shown in Fig. 2I, chromosomes from the normal nuclei separate and move to the two poles synchronously during anaphase. However, chromosomes from the abnormal nuclei did not separate (Fig. 2I). We conclude that the chromosome segregation defect observed in the *Dmchk2*$^{m-}$ embryos was not due to a failure to form centrosomes or spindles.

3.3. *A low dose of radiation enhances the phenotype of* Dmchk2^{m-} *embryos*

To test the hypothesis that the chromosome segregation defect observed in the *Dmchk2*$^{m-}$ embryos was linked with some defects on the chromosomes such as damaged or incompletely replicated DNA, we determined the consequence of increased DNA damage induced by a low dose of radiation on the observed *Dmchk2*$^{m-}$ phenotype. Only 4% of the wild type embryos showed abnormal nuclei when treated with 100 rad γ-irradiation ($n = 552$). In contrast, 92% of the *Dmchk2*$^{m-}$ embryos show abnormal nuclei when treated with the same dose of γ-irradiation (increased from 62% to 92%, $n = 567$). These observations suggest that the abnormal nuclear phenotype observed in the *Dmchk2*$^{m-}$ embryos is likely due to certain defects on the chromosomes such as damaged or incompletely replicated DNA, and that Dmchk2 likely plays an important role in regulating mitosis when there is damaged or incompletely replicated DNA. In addition, as abnormal nuclei derived from DNA damage-induced division consistently drop into the interior of the embryo and are removed from the cortex in wild type embryos [24], the observation that large abnormal nuclei can stay in the cortex of the *Dmchk2*$^{m-}$ embryo also suggested a possible function of Dmchk2 in the removal of the abnormal nuclei during early embryogenesis.

Table 2
Suppression of the *Dmchk2*$^{m-}$ abnormal nuclear phenotype by reducing the dosage of *polo*

Female genotype	Embryos with abnormal nuclei (%)
chk2	61.5 ± 1.1
chk2; *polo*^{1}/+	27.0 ± 3.2
chk2; *stg*/+	38.5 ± 7.0
chk2; *cycA*/+	52.2 ± 1.6

Virgin females of the indicated genotype were crossed to w^{1118} males, the percent embryos with abnormal nuclei was scored. The average of at least two independent experiments is shown.

3.4. *Genetic interactions between Dmchk2 and polo, string, and cyclin A*

It has been reported recently that Chk2 colocalizes with Polo-like kinase 1 (Plk1) in the centrosome and midbody in mitosis and coimmunoprecipitates with Plk1 [3]. In addition, fly Chk2 was show to localize to the centrosomes and spindle microtubules [24], which overlaps with the localization of Polo kinase [25]. These results suggest an interesting connection between Dmchk2 and Polo in mitosis. *Polo* is important for all aspects of mitosis including mitosis entry, spindle formation, chromosome segregation and cytokinesis [26,27]. We carried out genetic interaction assays to determine if reducing the dosage of *polo*, *string*, or *cycA* affects the *Dmchk2*$^{m-}$ phenotype. Interestingly, removing one copy of *polo* strongly suppresses the abnormal nuclear phenotype in the *Dmchk2*$^{m-}$ embryos (Table 2). In addition, removing one copy of *stg* also partially suppresses the *Dmchk2*$^{m-}$ phenotype, while removing one copy of *cycA* has only a minor effect (Table 2). In conclusion, these observations suggest Dmchk2 may be required to delay the onset of mitosis when there is DNA damage or incomplete DNA replication, and that Polo might be a key target of Dmchk2 for this DNA damage/incomplete DNA replication-induced cell cycle check. However, it should be pointed out that our results cannot exclude the possibility that Dmchk2 and Polo act in parallel pathways.

3.5. *Abnormal nuclei in the cortex of the embryo blocks zygotic transcription*

The switch from maternal to zygotic control of embryogenesis at the midblastula transition is accompanied by a significant increase in zygotic gene expression [13]. The expression pattern of the pair-rule gene *even-skipped* was examined in wild type and in *Dmchk2*$^{m-}$ embryos. Seven stripes were observed in wild type embryos after mitotic cycle 13 (Fig. 3A–C), the same *eve* expression was also observed in *Dmchk2*$^{m-}$ embryos without abnormal nuclei in the cortex (data not shown). In contrast, *even-skipped* expression was greatly reduced or completely blocked in *Dmchk2*$^{m-}$ embryos with ab-

normal nuclei in the cortex (Fig. 3D–F). These results indicate that there is a sensing mechanism that controls the onset of zygotic expression with the removal of the abnormal nuclei from the cortex of the embryos in early embryogenesis.

Our analysis of the *Dmchk2*$^{m-}$ embryos suggested important roles of Dmchk2 in mitosis when there is damaged or incompletely replicated DNA. We suggest this function of Dmchk2 is mediated at least in part through Polo. In addition, our observation also suggested a possible role of Dmchk2 in the removal of abnormal nuclei from the cortex of syncytial embryos. Removal of the abnormal nuclei from the cortex is essential for the onset of high levels of zygotic expression. Elucidation of the roles of Dmchk2 in these novel biological processes will likely be important for understanding the function of Chk2 as a tumor suppressor.

Acknowledgements: We would like to thank the Bloomington *Drosophila* stock center for providing fly stocks. We thank Dr. Kaufman and Dr. Bellen for providing anti-CNN and anti-Barren antibodies. We would like to thank S. Kron and members of the Du lab for helpful discussions. This work was supported by a research grant from the Ludwig Foundation and an NIH grant (R01-CA80871) to W.D.

References

[1] Xu, J., Xin, S. and Du, W. (2001) FEBS Lett. 508, 394–398.
[2] Peters, M. et al. (2002) Proc. Natl. Acad. Sci. USA 99, 11305–11310.
[3] Tsvetkov, L., Xu, X., Li, J. and Stern, D.F. (2003) J. Biol. Chem. 278, 8468–8475.
[4] Liu, Q. et al. (2000) Genes Dev. 14, 1448–1459.
[5] Takai, H. et al. (2000) Genes Dev. 14, 1439–1447.
[6] Matsuoka, S., Huang, M. and Elledge, S.J. (1998) Science 282, 1893–1897.
[7] Matsuoka, S., Rotman, G., Ogawa, A., Shiloh, Y., Tamai, K. and Elledge, S.J. (2000) Proc. Natl. Acad. Sci. USA 97, 10389–10394.
[8] Bartek, J., Falck, J. and Lukas, J. (2001) Nat. Rev. Mol. Cell Biol. 2, 877–886.
[9] Hirao, A. et al. (2000) Science 287, 1824–1827.
[10] Hirao, A. et al. (2002) Mol. Cell. Biol. 22, 6521–6532.
[11] Takai, H. et al. (2002) EMBO J. 21, 5195–5205.
[12] Foe, V.E., Odell, G.M. and Edgar, B.A. (1993) in: The Development of *Drosophila melanogaster*, Vol. 1 (Bate, M. and Martinez-Arias, A., Eds.), pp. 149–300, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[13] Sibon, O.C., Stevenson, V.A. and Theurkauf, W.E. (1997) Nature 388, 93–97.
[14] Sibon, O.C., Laurencon, A., Hawley, R. and Theurkauf, W.E. (1999) Curr. Biol. 9, 302–312.
[15] Fogarty, P., Kalpin, R.F. and Sullivan, W. (1994) Development 120, 2131–2142.
[16] Fogarty, P., Campbell, S.D., Abu-Shumays, R., Phalle, B.S., Yu, K.R., Uy, G.L., Goldberg, M.L. and Sullivan, W. (1997) Curr. Biol. 7, 418–426.
[17] Sibon, O.C., Kelkar, A., Lemstra, W. and Theurkauf, W.E. (2000) Nat. Cell Biol. 2, 90–95.
[18] Yu, K.R., Saint, R.B. and Sullivan, W. (2000) Nat. Cell Biol. 2, 609–615.
[19] Masrouha, N., Yang, L., Hijal, S., Larochelle, S. and Suter, B. (2003) Genetics 163, 973–982.
[20] Oishi, I., Sugiyama, S., Otani, H., Yamamura, H., Nishida, Y. and Minami, Y. (1998) Mech. Dev. 71, 49–63.
[21] Abdu, U., Brodsky, M. and Schupbach, T. (2002) Curr. Biol. 12, 1645–1651.
[22] Oishi, I. et al. (2001) Mol. Cell. Biol. 21, 1329–1335.
[23] Bhat, M.A., Philp, A.V., Glover, D.M. and Bellen, H.J. (1996) Cell 87, 1103–1114.
[24] Takada, S., Kelkar, A. and Theurkauf, W.E. (2003) Cell 113, 87–99.
[25] Logarinho, E. and Sunkel, C.E. (1998) J. Cell Sci. 111, 2897–2909.
[26] Sunkel, C.E. and Glover, D.M. (1988) J. Cell Sci. 89, 25–38.
[27] Carmena, M., Riparbelli, M.G., Minestrini, G., Tavares, A.M., Adams, R., Callaini, G. and Glover, D.M. (1998) J. Cell Biol. 143, 659–671.