A Hypomorphic Mutation Reveals a Stringent Requirement for the ATM Checkpoint Protein in Telomere Protection During Early Cell Division in Drosophila

Patrizia Morciano,* Yi Zhang,* Giovanni Cenci,† and Yikang S. Rong*,†
*Laboratory of Biochemistry and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, and †Sapienza, University of Rome, 00185 Rome, Italy

ABSTRACT Using Drosophila as a model system, we identified a stringent requirement for the conserved function of Ataxia Telangiectasia Mutated (ATM) in telomere protection during early embryonic development. Animals homozygous for a hypomorphic mutation in atm develop normally with minimal telomere dysfunction. However, mutant females produce inviable embryos that succumb to mitotic failure caused by covalent fusions of telomeric DNA. Interestingly, although the atm mutation encodes a premature stop codon, it must not have eliminated the production of the mutant protein, and the mutant protein retains kinase activity upon DNA damage. Moreover, although the embryonic phenotype of this mutation resembles that of hypomorphic mutations in the MRN complex, the function of MRN appears normal in the atm embryos. In contrast, there is a prominent reduction of the level of HipHop, an essential member of the Drosophila capping complex. How ATM functions in telomere protection remains poorly understood. The amenability of Drosophila embryos to molecular and biochemical investigations ensures that this newly identified mutation will facilitate future studies of ATM in telomere maintenance.

KEYWORDS ATM kinase Drosophila hypomorphic mutation MRN complex maternal lethal telomere protection

The Ataxia Telangiectasia Mutated (ATM) protein is best known for its role in sensing and repairing DNA damage (reviewed in Lee and Paull 2007). However, ATM is also important for telomere maintenance. The yeast ATM homolog Tel1 was first discovered for its role in maintaining telomere length (Lustig and Petes 1986). Although the function of ATM at telomeres is conserved through evolution, the underlying mechanism is not well understood. It has been suggested that the fundamental role of ATM at telomeres is in end processing, similar to its function at DSBs, but independent of the telomerase function (Ritchie et al. 1999; Gao et al. 2010a). This finding is consistent with results from ATM studies in Drosophila, which use telomerase-independent mechanisms to maintain the essential functions of telomeres (reviewed in Rong 2008a).

We along with others have shown that the ATM protein, which is encoded by the telomere fusion (tefu) gene in Drosophila, is essential for preventing telomere fusion in proliferating tissues (Bi et al. 2004; Oikemus et al. 2004; Silva et al. 2004; Song et al. 2004). In addition, ATM functions in the same telomere-protecting pathway as the conserved Mre11-Rad50-Nbs (MRN) complex, which is partially redundant with the pathway controlled by the ATM-related ATR protein (Bi et al. 2005; Ciapponi et al. 2006; Oikemus et al. 2006). Moreover, this pathway relationship is conserved in yeast (Moser et al. 2009) and quite possibly in plants (Vespa et al. 2005) and higher eukaryotes.

One possible function of ATM at Drosophila telomeres is to facilitate the recruitment of capping proteins. Although the binding of the HOAP capping protein to telomeres appears normal in atm mutants, as assayed by immunostaining of mitotic chromosomes (Bi et al. 2004; Rong 2008b), loading of HP1/Orc-associated Protein (HOAP) to atm-mutant telomeres might be defective after all, as binding can be further eroded by the loss of ATR. Although the loss of ATR alone does not impair telomere protection, it abolishes HOAP loading to telomeres in the atm mutant and exacerbates telomere uncapping in the atm atr double mutant to a degree similar to that in the single
caravaggio (cav) mutant that encodes a defective HOAP protein (Cenci et al. 2003; Bi et al. 2005; Rong 2008b). In addition, the level of the HipHop capping protein and its localization to telomeres are moderately reduced in an atm mutant (Gao et al. 2010b), consistent with the fact that telomeres in atm mutants have a suboptimal level of protection.

Recently, we discovered a stringent requirement for the MRN complex in telomere protection during early development (Gao et al. 2009). Hypomorphic mutations of mre11 and nbs support viability, but mutant females are unable to produce viable progeny due to rampant mitotic failure during the earliest cell cycles in embryos. Here, we characterized a hypomorphic mutation in Drosophila atm that has a similar maternal lethal phenotype. We show that loss of maternal ATM function leads to telomere fusion in the embryos. At the cellular level, this hypomorphic mutation displays features of telomere dysfunction similar to those caused by severe loss of function mutations of the capping machinery. This mutation will facilitate the future elucidation of telomere protection mechanisms by ATM, particularly due to the amenability of Drosophila embryos to molecular and biochemical investigations.

MATERIALS AND METHODS

Drosophila stocks and genetics

The tefuZIII-5190 stock and its corresponding parental stock were obtained from the Zuker Collection (Koundakjian et al. 2004). The tefu+ allele has been described previously (Gong et al. 2005). The atm− allele was obtained from the Bloomington stock center. The stock that contains the atm+ transgene was provided by Dr. Shigla Campbell at the University of Alberta. The mre11Δ587 and nbs1Δ single mutants, as well as the atm atr double mutant have been described previously (Bi et al. 2004; Bi et al. 2005).

To measure the effect of tefuZIII-5190 on viability, heterozygous stocks of tefuZIII-5190 and tefu+ were crossed with the TM6B chromosome, were crossed. Progeny were scored as heterozygous or trans-heterozygous for tefu+. The expected ratio between these two classes is 2:1. The observed ratio was 1.4:1 (n = 797). The transheterozygous females were mated with their wild-type siblings to produce the embryos used in phenotypic analyses.

Molecular biology

Between 0.2 and 1 μg of genomic DNA from embryos was used in a 50- to 100-μL polymerase chain reaction (PCR) to recover telomere fusion junction essentially as previously described (Gao et al. 2009). Five primers were designed from the orf region of the HeT-A element (Supporting Information, Table S1). The combinations: 1 + 4, 2 + 4, 3 + 4, and 3 + 5 were used for junction isolation. PCR products were cloned using TOPO TA cloning and subsequently sequenced.

Genomic DNA from tefuZIII-5190 homozygotes and its parental stock were used as the template in PCRs to amplify 13 fragments covering the tefu genomic region. PCR products were cloned using TOPO TA cloning, and independent clones of a particular fragment were sequenced to identify nucleotide differences between the two stocks. The primers are listed in Table S1.

Cytology and Western blotting

Mitotic chromosome preparations from larvae and embryos were made as previously described (Gao et al. 2009). 4,6-diamidino-2-phenylindole (DAPI) and antibody staining of embryos were performed as described previously (Gao et al. 2009). Embryo extracts were obtained from embryos as previously described (Gao et al. 2010b). Antibodies against HipHop and HOAP were described previously (Gao et al. 2010b). The antibodies against MRN have been previously described (Gao et al. 2009). The anti-Giotto antibody (Giansanti et al. 2006) was provided by M. Giansanti (SAPIENZA, University of Rome).

To detect damage-induced phosphorylation of H2AvD, larvae were irradiated with 1000 rads. At the indicated time points, proliferating tissues (brains and imaginal discs) were dissected and extracts were generated. A polyclonal antibody raised against a phosphorylated peptide from H2AvD was used to detect H2AvD and its phosphorylated form as previously described (Madigan et al. 2002). Due to depletion of this antibody stock, we later used an affinity purified rabbit antibody against the phosphorylated form of H2AvD (Rockland Inc., Gilbertsville, PA).

RESULTS AND DISCUSSION

A hypomorphic mutant of atm/tefu causes maternal lethality

In a genetic screen for cell cycle mutants, Rickmyre et al. (2007) identified the ZIII-5190 stock from the Zuker collection as harboring a potential mutation in the tefu gene by the fact that ZIII-5190 failed to complement a small chromosomal deletion for female fertility, which eliminates part of tefu. We repeated the complementation test with our tefu+ allele that specifically affects tefu (Bi et al. 2004). As we showed before, the tefu+ allele causes pupal lethality as cells in proliferating tissues suffer genome instability caused by telomere fusion. In contrast, we recovered tefuZIII-5190/tefu+ trans-heterozygotes at a Mendelian ratio (see Materials and Methods), indicating that the tefuZIII-5190 allele minimally affects viability. Consistently, a near background level of telomere fusion, as measured by examining mitotic chromosome preparations (average 0.06 fusion, n = 337), was found in tefuZIII-5190 larval neuroblasts, which is significantly lower than the average of three fusions reported earlier for tefu+ neuroblasts (Bi et al. 2004).

Females that are homozygous for tefuZIII-5190 or trans-heterozygous for tefuZIII-5190 and tefu+ produced a normal amount of eggs; however, none of more than 10,000 embryos counted hatched regardless of the genotypes of the mated males. DAPI-staining revealed significant development of these embryos (Figure 1), indicating that the embryos were fertilized and embryonic lethality was caused by the defective maternal contribution from the tefu mutant allele. The tefuZIII-5190 allele also failed to complement another pupal lethal allele, atm6 (Silva et al. 2004). Heterozygous tefuZIII-5190/atm6 females also did not produce viable offspring, but this defect could be rescued by a wild-type tefu transgene. Taken collectively, our genetic analyses strongly suggest that the tefuZIII-5190 allele is a partial loss-of-function allele of tefu that causes maternal effect lethality.

Telomere fusion causes mitotic failure in embryos from mutant females

We observed nuclear patterns indicative of mitotic failure in DAPI-stained embryos produced by females that are tefuZIII-5190/tefu+, hereafter referred to as maternal-tefuZIII-5190/tefu+ (m-tefu5190) embryos (Figure 1, B and C). First, we observed chromosome bridges between segregating nuclei. Second, we observed multilobed nuclei, possibly resulting from a second round of mitosis after failed chromosome segregation. Finally, we observed large areas in the embryos that are free of nuclei, indicating cell-cycle defects due to “sinking” of abnormal nuclei to the interior after exiting the cell cycle, leaving nuclear-free areas at the surface (Raff and Glover 1988).
ATM prevents telomere fusion in the dividing tissues of larvae; therefore, we hypothesized that the mitotic failure observed in m-tefu⁵¹⁹⁰ embryos could be due to telomere uncapping. By using a recently developed protocol for producing mitotic chromosome preparations from embryos (Gao et al. 2009), we obtained convincing cytological evidence that m-tefu⁵¹⁹⁰ embryos experience telomere fusions (Figure 1, E−G).

To investigate whether the “fusions” observed cytologically indeed represent covalent attachment of chromosome ends, we used a recently developed PCR protocol to detect fusion junctions (Gao et al. 2009). This protocol is based on the fact that telomeric transposons in Drosophila are arranged as directed repeats such that the use of two transposon-derived primers oriented in the direction of the telomere is unlikely to support productive amplification of DNA samples from wild type flies. The same pair of primers, however, would amplify telomere fusion junctions on the DNA templates from uncapping mutants (Figure 2A). Using four different combinations of primer pairs (Table S1), we generated abundant PCR products of various sizes from m-tefu⁵¹⁹⁰ DNA, but not from wild type embryonic DNA (Figure 2B). Sequencing of 15 independent clones of potential fusion junctions identified signatures of nonhomologous end joining of telomeric transposons in a head-to-head fashion in all of the clones (one is shown in Figure 2C, the others in Table 1). In summary, our results indicate that maternal lethality of m-tefu⁵¹⁹⁰ embryos is caused by end-to-end fusions that impede chromosome segregation during cell division.

**Figure 1** Mitotic defects in m-tefu⁵¹⁹⁰ embryos. (A−B) DAPI-staining of 0−2 hr wild-type (wt) and m-tefu⁵¹⁹⁰ (tefu) embryos. Although wt embryos show a uniform distribution of nuclei on the cortex, m-tefu⁵¹⁹⁰ embryos exhibit an aberrant pattern and the presence of large nuclei-free areas (arrowhead). (C) A close-up of panel B. Nuclei connected by chromatid bridges (arrow) and multilobed nuclei (asterisk) are abundant in m-tefu⁵¹⁹⁰ embryos. (D−G) Mitotic chromosome preparations from wt and m-tefu⁵¹⁹⁰ (tefu) embryos. Whereas all wild-type chromosomes are individually distinct (D), metaphase chromosomes from m-tefu⁵¹⁹⁰ exhibit telomeric fusions that give rise to chromosome chains (E), anaphase bridges (F), and hyperploid cells (G).

**Figure 2** Telomere fusions in m-tefu⁵¹⁹⁰ are covalent DNA linkages. (A) Schematic of the PCR procedure used to recover telomere fusion junctions. Telomeric HeT-A retro-transposons are depicted as block arrows in gray. They attach to chromosomal DNA (in black) unidirectionally. Black and white arrowheads denote a pair of telomere-facing primers. They anneal to multiple positions along the HeT-A arrays. The top diagram denotes the wild-type situation in which the PCRs are not expected to be productive. The middle diagram depicts a telomere fusion in which PCR with some primer pairs will lead to productive amplifications. (B) A picture of a DNA gel electrophoresis showing PCR products obtained using wild type (+) or m-tefu⁵¹⁹⁰ (−) DNA templates. The primer combinations are listed at the top. m: marker DNA with sizes in kb. (C) Sequence of a fusion junction from m-tefu⁵¹⁹⁰. The nucleotide numbers are from GenBank entry U06920.2. Three strands (in the 5' to 3' direction) are shown, with the actual sequences connected through a fusion (underlined). The rest of the sequences are those predicted from U06920.2. The top sequence is from a telomere that fused with another telomere (bottom sequence), giving rise to the fusion product denoted in the middle sequence. The fusion was created by the use of an overlapping “GT” (in bold) microhomology for repair.

**The tefuZIII-5190 mutation is a premature stop codon**

We set out to identify the DNA lesion responsible for the mutation in tefuZIII-5190 to better understand the molecular mechanism underlying the uncapping phenotype. We amplified via PCR, cloned, and sequenced 13 overlapping fragments covering the entire tefu coding region and approximately 800 bp of the 5' and 3' UTR from tefuZIII-5190 homozygous and the parental wild-type stocks. We identified (1) a nonsense mutation (G to A) that changes a Tryptophan (TGG) at the predicted position of 356 to a STOP (TGA), (2) three synonymous changes, and (3) a single C to A change in one of the introns. We thought it most likely that the W356* mutation is responsible for the tefuZIII-5190 mutation. To further explore this possibility, we recovered a cDNA fragment by using primers that span the W356* mutation in a downstream intron to recover PCR products exclusively from mRNA but not from genomic DNA. Sequencing of this fragment identified the W356* mutation in tefuZIII-5190 but not in wild-type, suggesting that the mutation is part of the tefu transcript in the mutant.

The hypomorphic nature of tefuZIII-5190 seems incompatible with the nature of the mutation (a premature stop codon). In addition, the W54* mutation in the atm allele causes a lethal allele but lies further...
to the N-terminus than W356/C3 (Pedersen et al. 2010). The hypomorphic nature of W356/C3 suggests that it does not result in the elimination of the ATM protein and that perhaps a downstream START codon is utilized. Currently available reagents did not allow us to further address this issue.

The tefuIII-5190 mutation does not disrupt MRN localization

The cellular defects in m-tefu5190 embryos phenocopy those in embryos produced by mothers with a hypomorphic mutation in either mre11 or nbs (Gao et al. 2009). In those mutants, the nuclear localization of Mre11 and Rad50 is defective due to depletion of the maternal Nbs protein. Given the fact that ATM and MRN function in the same pathway to prevent telomere fusion in larval tissues, we investigated whether telomere uncapping in m-tefu5190 embryos is caused by defective MRN function. We observed no obvious reduction in the total level of Mre11 or Nbs proteins in the mutant embryos (Figure 3A). Moreover, Rad50 localization to chromatin appears normal (Figure 3B). Therefore, the tefuIII-5190 mutation does not seem to affect MRN function.

Integrity of the telomere capping complex in m-tefu5190 embryos

The uncapping phenotype in m-tefu5190 embryos suggests defects in telomere capping complexes. We along with others have shown that HOAP and HipHop are constitutive components of a telomere capping complex (Rashkova et al. 2002; Cenci et al. 2003; Gao et al. 2010b). We also showed that loss of ATM function does not prevent the telomeric binding of HOAP, nor does it affect its steady-state level in larval neuroblasts (Bi et al. 2004; Rong 2008b; Gao et al. 2010b). In contrast, we observed a reduction of loading of HipHop to telomeres in the tefuIII-5190 mutants, accompanied by a significant drop in HipHop level (Gao et al. 2010b). In m-tefu5190 embryos, similar to atm-null larvae, we did not observe a reduction of HOAP by Western blot (Figure 3C). However, we did detect a significant reduction in HipHop level in m-tefu5190 embryos (Figure 3C), again consistent with results from atm-null larvae. Therefore, loss of ATM function consistently reduces the abundance of HipHop protein, possibly because inefficient loading of HipHop to telomeres leads to its destabilization. This proposition is consistent with our immunostaining results that show the lack of HipHop signal on telomeres in most m-tefu5190 nuclei (Figure 3D). However, we did observe HipHop foci in some nuclei (Figure 3D), suggesting that the effect of loss of ATM on HipHop loading is partial, similar to the situation in larval neuroblasts.

One of the most interesting aspects of this study is our discovery that cells from different stages of development can react very differently to the same genetic mutation. We envision that HipHop needs to be loaded onto newly replicated telomeres for their protection, and this loading requires the function of ATM. Perhaps,

Table 1 Telomere fusion junctions

| Telomere 1 | Telomere 2 | Junction | ID sequence |
|------------|------------|----------|-------------|
| AGTAATTTTGTGACTGACGCA | GCTCACTCATGGCAGGAGA | AGTAATTTTGTGACTGACGCA | X68130.1 |
| CTGTAGGGGATCTGCATTA | CTATGCGGGAGATTGCTTC | CTGTAGGGGATCTGCATTA | U06920.2 |
| CATATTTTGGTATAGGCGGA | ACTACCAAACTATTG | CATATTTTGGTATAGGCGGA | U06920.2 |
| GTGCTTTTTGCGCATTAT | GTGCTTTTTGCGCATTAT | GTGCTTTTTGCGCATTAT | U06920.2 |
| TAACTTGAAGCAGCCATTTA | CTCACTGGCTTC | TAACTTGAAGCAGCCATTTA | U06920.2 |
| TGATGAGAGCTGGATTGACGCA | AACACCAAACTATTG | TGATGAGAGCTGGATTGACGCA | U06920.2 |
| TAGGAGGATTAATCCGGATGGTG | TGGAGAATTTTGGTAACTGG | TAGGAGGATTAATCCGGATGGTG | X68130.1 |
| TATGCGGCCCTGCTGGCTTC | TATGCGGCCCTGCTGGCTTC | TATGCGGCCCTGCTGGCTTC | U06920.2 |
| GGAAGGTAAGCTGGAGATGCTTC | GGAAGGTAAGCTGGAGATGCTTC | GGAAGGTAAGCTGGAGATGCTTC | U06920.2 |
| GGAAGGTAAGCTGGAGATGCTTC | GGAAGGTAAGCTGGAGATGCTTC | GGAAGGTAAGCTGGAGATGCTTC | U06920.2 |
ATM is a protein kinase, and its kinase activity is critical for telomere maintenance in yeast (Mallory and Petes 2000). To investigate whether the reduction of kinase activity is the underlying defect in tefuZIII-S190 mutants, we used DNA damage-induced phosphorylation of the H2AX variant (H2AvD in Drosophila, Madigan et al. 2002) as an in vivo readout for ATM kinase activity. It has been previously shown that ATM is important for H2AvD phosphorylation induced by DNA damage (Joyce et al. 2011). Using proliferating cells from third instar larvae, we found that H2AvD phosphorylation (P-H2AvD) largely depends on ATM and the MRN complex, as greatly reduced levels of P-H2AvD induced by X-ray irradiation were observed in single mutants of these genes (Figure 4A). In addition, we found that most if not all of the H2AvD phosphorylation activities can be attributed to the ATM and its related ATR kinases, as a double mutant essentially abolishes P-H2AvD (Figure 4B).

When tefuZIII-S190 mutant larvae were irradiated, we observed a robust H2AvD phosphorylation, similar to the response from wild-type cells (Figure 4C). This result suggests that ATM encoded by the tefuZIII-S190 allele retains its ability to modify H2AvD upon DNA damage, and that loss of kinase activity might not be the underlying cause for telomere uncapping in m- tefu2190 embryos.

We considered measuring P-H2AvD in embryos since the terminal phenotype of tefuZIII-S190 is embryonic lethal. However, damage-induced P-H2AvD happens on chromatin, and the high maternal deposition of free histones would make the results difficult to interpret. In addition, m- tefu2190 embryos likely experience DNA damage due to telomere instability, which could further complicate the situation.

We don’t believe that the seemingly normal P-H2AvD level induced by X-ray is due to a preponderance of the maternal ATM in tefuZIII-S190 larvae. Using telomere fusion as an indicator, we found that the maternal ATM function is lost before the third instar stage. To estimate the timing of the loss of ATM function during development, we took advantage of the fact that telomere fusions in the tefuZP25 mutant result in chromosome bridges during mitosis (Bi et al. 2004). These bridges can be detected by staining mitotic chromatin for phosphorylated histone H3. We staged homozygous mutants as embryos, first, second, and third instar larvae. Using telomere fusion as an indicator, we found that the maternal ATM function is lost before the third instar stage.

As we and others have proposed, ATM is essential for telomeric processing. Given the speed of the cell cycle (10–20 min) in early embryos, efficient telomere processing would be more stringently required, such that even a partial loss of ATM could have a strong effect over a few divisions. On the contrary, cell cycles in somatic tissues are much longer, such that a partial loss of function might be much better tolerated.

**Figure 3** Integrity of the telomeric complexes in m- tefu2190 embryos. (A) Western blot analysis of Nbs and Mre11 in wildtype (+) and m- tefu2190 (−) embryonic extracts. Tubulin is probed as a loading control and molecular marker weights (kD) are indicated to the left. (B) Localization of Rad50 in tefuZIII-S190. Grayscale pictures show DAPI-stained DNA or antibody-stained Rad50. (C) Western blot analysis of the levels of Hiphop and HOAP in m- tefu2190 (−) embryos. (D) Localization of Hiphop in tefuZIII-S190. Grayscale pictures show DAPI-stained DNA or antibody-stained HipHop. In wild-type, HipHop forms nuclear foci. In the m- tefu5190 mutant, nuclei lacking of HipHop signals are shown on the left. On the right are two nuclei, one of which shows HipHop foci. The Giotto protein was used as a loading control.

**Figure 4** Damage-induced H2AvD phosphorylation is normal in tefuZIII-S190 mutants. ATM is a protein kinase, and its kinase activity is critical for telomere maintenance in yeast (Mallory and Petes 2000). To investigate whether the reduction of kinase activity is the underlying defect in tefuZIII-S190 mutants, we used DNA damage-induced phosphorylation of the H2AX variant (H2AvD in Drosophila, Madigan et al. 2002) as an in vivo readout for ATM kinase activity. It has been previously shown that ATM is important for H2AvD phosphorylation induced by DNA damage (Joyce et al. 2011). Using proliferating cells from third instar larvae, we found that H2AvD phosphorylation (P-H2AvD) largely depends on ATM and the MRN complex, as greatly reduced levels of P-H2AvD induced by X-ray irradiation were observed in single mutants of these genes (Figure 4A). In addition, we found that most if not all of the H2AvD phosphorylation activities can be attributed to the ATM and its related ATR kinases, as a double mutant essentially abolishes P-H2AvD (Figure 4B), as we and others have proposed, ATM is essential for telomeric processing. Given the speed of the cell cycle (10–20 min) in early embryos, efficient telomere processing would be more stringently required, such that even a partial loss of ATM could have a strong effect over a few divisions. On the contrary, cell cycles in somatic tissues are much longer, such that a partial loss of function might be much better tolerated.

DNA damage induced kinase activity is normal in tefuZIII-S190 mutants

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16.1% of the mitotic nuclei (n = 1043) from eight second instar animals, which is similar to the frequency from late third instar tefuZIII-5190 larvae (20%, Bi et al. 2004). This led us to conclude that the loss of maternal ATM function likely occurs between the first and second instar stages.

H2AvD phosphorylation is severely compromised in tefu loss of function mutants, yet a normal kinase function in tefuZIII-5190 does not prevent telomere fusion. To reconcile these observations, we suggest that the damage-induced kinase activation of ATM is distinct from its kinase function at telomeres, possibly due to different ATM targets at telomeres vs. damage sites. If true, this predicts that the N-terminal portion of ATM deleted in tefuZIII-5190 might be responsible for interacting with a telomere-specific ATM target.

Here we have characterized a hypomorphic mutation in the conserved ATM checkpoint protein in Drosophila that specifically disrupts telomere capping during early embryonic cell divisions. The mitotic segregation defects are very similar to those observed in embryos that are genetically devoid of maternal ATM (Silva et al. 2004).

One long-standing question regarding ATM function in telomere maintenance concerns the identity of the targets of its kinase activity. Recently, the Ccq1 protein has been identified as an ATM target that is important for telomere protection in fission yeast (Moser et al. 2011). The availability of a maternal lethal atm mutation could facilitate the identification of similar targets in Drosophila. First, a large amount of mutant embryos is easy to collect and makes biochemical purification an attractive approach. Second, the synchrony of the early cell cycles simplifies both cytological and molecular characterizations. Finally, the hypomorphic nature of the tefuZIII-5190 allele should permit screening of enhancer/suppressor mutations, facilitating the genetic identification of new members of the ATM pathway.

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