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Loss of *RecQ5* leads to spontaneous mitotic defects and chromosomal aberrations in *Drosophila melanogaster*

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ABSTRACT

RecQ5 belongs to the RecQ DNA helicase family that includes genes causative of Bloom, Werner, and Rothmund-Thomson syndromes. Although no human disease has been genetically linked to a mutation in *RecQ5*, *Drosophila melanogaster RecQ5* is highly expressed in early embryos, suggesting an important role for it in the DNA metabolism of the early embryo. In this present study, we generated *RecQ5* mutants in *D. melanogaster*. Embryos lacking maternally derived RecQ5 contained irregular nuclei in early embryogenesis. These irregular nuclei emerged in nuclear cycle 11–13, lost cell-cycle markers, and were located below the surface monolayer of nuclei. By time-lapse microscopy, these irregular nuclei were observed not to divide, whereas all neighboring nuclei proceeded through normal mitotic division with synchrony. These data suggest that the irregular nuclei exited from the nuclear division cycle. This phenotype is reminiscent of the effect of X-ray irradiation on wild-type embryos and was rescued by expression of *RecQ5*. Thus, the maternal supply of RecQ5 is important for the nuclear cycles in syncytical embryos. Furthermore, the frequencies of spontaneous and induced chromosomal aberrations were increased in *RecQ5* mutant neuroblasts. These data imply that DNA damage accumulates spontaneously in *RecQ5* mutants. Therefore, endogenous genomic damage may be produced in *Drosophila* development, and RecQ5 would be involved in the maintenance of genomic stability by suppressing the accumulation of DNA damage.

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1. Introduction

RecQ helicases are conserved from *Escherichia coli* to humans. In humans, 5 RecQ homologues have been identified, 3 of which are involved in predisposition to cancer, premature aging and/or developmental abnormality diseases such as Bloom and Werner syndromes, which are caused by mutations in the *BLM* and *WRN* genes, respectively, and 3 syndromes, i.e., Rothmund-Thomson, RAPADALINO, and Baller-Gerold syndromes, caused by mutations in the *RecQ4* gene [1–5]. No genetic disorder has been identified that is caused by mutations in *RecQ1* or *RecQ5*. These genes are expressed ubiquitously, whereas *BLM*, *WRN*, and *RecQ4* are expressed in specific tissues [6].

RecQ5 is a well-conserved gene in multicellular organisms. *Caenorhabditis elegans* RecQ5 RNAi reduces the life span of *C. elegans* and increases its sensitivity to ionizing irradiation [7]. RecQ5 is associated with suppression of crossovers and repair of I-SceI-induced double-strand breaks by homologous recombination in mouse ES cells; moreover, mice lacking *RecQ5* have an increased incidence of cancer [8,9].

RECQ5/QE, the longest and nuclear isoform product of the *RecQ5* gene, is maternally provided during early embryogenesis in *Drosophila melanogaster* [10]. *C. elegans RecQ5* is also expressed from earliest embryogenesis, and its expression gradually decreases during late embryogenesis [7]. However, the role of RecQ5 in early embryos is unknown.

The first 13 mitotic cycles in fly embryos are synchronous and occur in a common syncytial cytoplasm [11]. The earliest cycles (cycles 2–9) are very rapid, having a 4–5-min interphase and a 4–5-min phase of mitosis. The final 4 syncytial divisions (cycles 10–13) take place in the cortical monolayer [11]. The first 10 cycles lack feedback regulation to monitor the completion of the S phase [12]. During cycles 11–13, damaged or incompletely replicated DNA triggers centrosome inactivation, leading to defects in spindle assembly and chromosome segregation. The damaged nuclei exit from the nuclear cycles, drop down from the cortex, and are not incorporated into cells that form the embryo proper [13,14]. Therefore, the irregular nuclei harboring damaged DNA might be detected in a later

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stage (cycle 11–13) and can be compared with their neighboring nuclei. Syncytial embryos have advantages for analysis of nuclear dynamics, by allowing judging of cell-cycle progression during synchronous nuclear division and nuclear position fall-out from the cortical monolayer of nuclei.

Here, we generated mutant flies that were deficient in *RecQ5* and examined the cell cycles of embryos from mutant females mated with wild-type flies. As a result, we found that the syncytial cell cycle was stalled in approximately 2% of nuclei in the embryos from mutant flies and that these nuclei dropped down from the cortex. This study suggests that endogenous stress exists in early embryos and that the organism is protected from such stress by redundant defense systems, including RecQ5.

2. Materials and methods

2.1. Generation of RecQ5 mutants

P{GT1}RecQ5^{BG01967}, containing a P-element located in the 5′ end of the *RecQ5* gene, and other strains were obtained from Bloomington *Drosophila* Stock Center. Deletion mutants of *RecQ5* were generated from P{GT1}RecQ5^{BG01967} by using a *Drosophila* technology known as imprecise excision [15]. Offsprings were screened with a PCR-based method using P-element (5′-CTCTCATGGTTCCGTTACGC-3′) and *RecQ5* genomic primers (5′-TGACATCGATAAAGGAAGGTCCAC-3′, 5′-TGTTTCCTGTTCCAACTGCC-3′, Fig. 1A). The stock maintenance and immunoblotting were as described earlier [16].

2.2. X-ray irradiation

Wild-type embryos (0.5–2.0 h old) were exposed to 3 Gy of X-ray irradiation with a Radioflex 350 (Rigaku).

2.3. Embryo-staining procedures

Embryos were collected, dechorinated, fixed, and stained as described previously [17]. They were fixed for 20 min in PBS (phosphate-buffered saline), 1% BSA, 0.05% Triton X-100 and 10% formaldehyde. DNA was stained with 1 μg/ml propidium iodide after treatment with 10 mg/ml RNase (WAKO). Anti-PH3 antibodies (Upstate Biotech) were used at a 1:500 dilution. PCNA was detected with a purified mouse monoclonal antibody, PC-10 (BioLegend, 1:250 dilution). Primary antibodies were probed with FITC-conjugated secondary antibodies (Vector, 1:500 dilution). The stained embryos were observed under a confocal laser-scanning microscope (MRC1024, Biorad, Hercules, CA).

2.4. Live imaging

To follow nuclear division in living syncytial embryos, we used a transgene expressing His2Av-mRFP1 [18]. Embryos were collected, dechorinated, and spread under Halocarbon oil 700 (SIGMA) onto coverslips for subsequent filming with a MODEL 5793 membrane (YSI Inc., OH) as described previously [19]. Time-lapse imaging was performed with a confocal laser-scanning microscope. Images obtained with the confocal

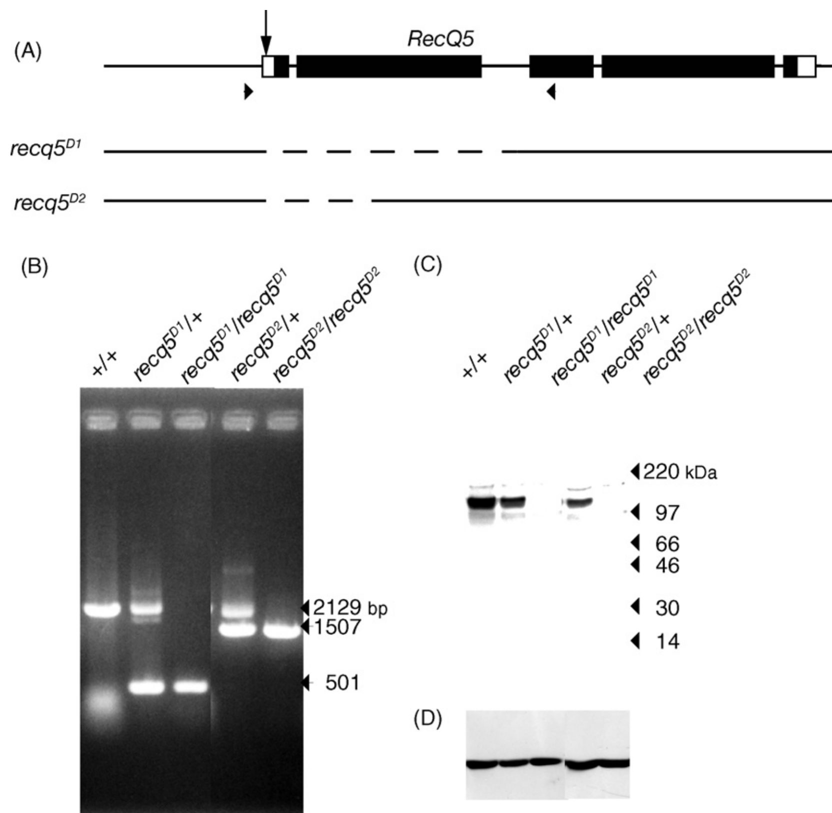

Fig. 1. Generation of *RecQ5* mutants. (A) A diagram of the *RecQ5* genomic locus, showing the site of insertion of the P element (arrow). Coding regions are shown in filled boxes; and untranslated regions, in open boxes. The locations of primers used in the PCR screening are shown by the arrowheads. Two deletion mutants, *recq5^{D1}* and *recq5^{D2}*, are indicated. (B) Results of a PCR screening. PCR analysis using primers (arrowheads in "A"). (C) Immunoblot with anti-RECQ5/QE antibodies. Adult extracts were probed with antibodies specific for RECQ5/QE as described previously [25]. Actin is shown as a loading control (D).

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