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ANNUAL PROGRESS REPORT **DOE DE-FG03-87ER60538**

Part 3. Progress Report for the period 11/1/94 to 1/1/96

Overview

1. Progress on the MEI-41 Protein

We have recently cloned the *mei-41* gene, and showed that its putative translation product is highly homologous to the ATM, MEC1, and RAD3 genes at the level of primary amino acid sequence. That this sequence similarity reflects a functional homology is suggested by three lines of evidence:

(i). As is the case for the ATM gene, loss of function of *mei-41* results in increased sensitivity to X-irradiation. Mutations in the *mei-41* gene exhibit exquisite radiation sensitivity and, as is the case for ATM mutations, *mei-41* mutations also exhibit semidominance in terms of radiosensitivity (Boyd et al.)

(ii). Mutations in the *mei-41* gene also resemble ATM mutations in that they cause high levels of chromosome breakage and genetic instability. In meiotic cells this genetic instability is evidenced by a high frequency of spontaneous deletions and translocations in meiotic cells (Hawley and Tartof). Somatic cells carrying the *mei-41* mutation also show high frequencies of chromosome breakage and instability (Baker et al). Indeed, neuroblasts from the brains of *mei-41* larvae display a high frequency of chromosome breaks and rearrangements and this number is enhanced ten to twenty fold following treatment by X-rays, to the extent that after 220R of irradiation virtually all of the subsequent metaphases can be seen to possess at least one break or rearrangement (Gatti et al.). In addition, the *mei-41* mutations reduce the amount of meiotic recombination (Baker and Carpenter).

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(iii). Like the ATM gene, the wild-type MEI-41 protein also plays a role in mediating the progression of the cell cycle. Indeed, irradiated wild-type larval neuroblasts display a G2 arrest of some 4 hours in length. However, no such arrest is observed in neuroblasts from *mei-41* individuals (Hari and Hawley, unpublished data) and broken or gapped chromosomes are observed in most, if not all, neuroblast metaphases following irradiation (Gatti; Gatti et al.). The failure of *mei-41* cells to arrest in G2 following irradiation offers a simple explanation both for the high frequency of broken chromosomes observed at metaphase and for the extreme radiosensitivity of these animals. As noted above, AT cells show a similar set of defects in halting cell cycle progression following radiation damage.

In addition to its role in a DNA-damage sensitive checkpoint in larval neuroblasts, the MEI-41 protein is also required for a meiotic checkpoint that regulates the metaphase-anaphase transition in female meiosis. In normal Drosophila oocytes metaphase arrest is triggered by tension on one or more bivalents as a consequence of chiasma formation (McKim et al; Jang et al.). In the absence of chiasma formation, this arrest can be over-ridden to allow a premature entry into anaphase. However, in *mei-41* bearing oocytes, arrest cannot be over-ridden, and thus oocytes without chiasmata remain locked at metaphase (Jang and Hawley, unpublished data).

The MEI-41 protein also appears to be required for the syncitial nuclear divisions that take place in pre-blastoderm embryos. In *mei-41* embryos we observe a high fraction of anaphase figures displaying bridges or threads of chromatin between the two chromosome masses, suggestive of an onset of mitotic division prior to full completion of DNA synthesis (Jang and Hawley, unpublished data).

2. Progress on the MEI-9 Protein

In the previous reporting period we demonstrated that the *mei-9* encodes the Drosophila homolog of yeast proteins *Rad1* (S. cerevisiae) and *rad16* (S. pombe) (Sekelsky et al. 1995). Like the MEI-9 protein both the Rad1 and rad16 proteins are required for NER. These proteins are believed to be the yeast homologs of human Xeroderma Pigmentosum complementation group F (XPF) and the mammalian excision repair protein. The Rad1 protein combines with the Rad10 protein to function as an endonuclease capable of nicking DNA at double-strand to single-strand

DNA junctions. In S. pombe the Rad10 homolog is swi10 and in mammalian cells it is the excision repair protein ERCC1. One of the goals of this proposal is to identify the Drosophila homolog of Rad10 and to characterize the phenotype of mutations at this locus.

In NER, the Rad1/Rad10 complex is believed to make an incision 5' to the site of DNA damage, allowing removal of a section of the damaged strand. MEI-9 presumably plays an analogous role in NER in Drosophila. The Rad1 and rad16 proteins also have additional mitotic functions besides their roles in NER. When a double-strand break occurs within a region of non-homology between repeats, RAD1 is required to remove the nonhomologous ends to allow repair through a putative single-strand annealing mechanism. In this annealing pathway, the Rad1/Rad10 endonuclease is thought to cut immediately 5' to the boundary of the single-strand/double-strand junction of a splayed DNA structure, allowing removal of the non-homologous single strand. RAD1 also defines a RAD52-independent pathway for mitotic intrachromosomal repeat. The S. pombe homolog, *rad16,* is also required during mating type switching.

The finding that MEI-9 is homologous to Rad1 and rad16 suggests that the MEI-9 protein may act directly to resolve junctions within recombination intermediates, a role clearly consistent with the exchange defect seen in *mei-9* females. [An in vitro Holliday junction-resolving activity has been reported for Rad1, although the interpretation of this result has been questioned by West (1995), in part because of the lack of an effect of rad1 mutations on meiotic exchange.] Experiments are described below to test the ability of the MEI-9/DmRad10 complex to resolve a series of well-characterized synthetic Holliday intermediates, and thus to characterize its role in the recombination process.

Part 3. Publications Resulting From This Award

Banga, S. S., Shenkar, R., and Boyd, J. B. (1986). Hypersensitivity of Drosophila *mei-41* mutants to hydroxyurea is associated with reduced mitotic chromosome stability. Mutat. Res. **163:** 157-165.

Banga, S. S., Yamamoto, A. H., Mason, J. M., Boyd, J. B. (1995). Molecular cloning of *mei-41*, a gene that influences both somatic and germline chromosome metabolism of *Drosophila melanogaster*. Mol. Gen. Genet. **246:** 148-155.

Yamamoto, A. H., Brodberg, R. K., Banga, S. S., Boyd, J. B., and Mason, J. M. (1990). Recovery and characterization of hybrid dysgenesis-induced *mei-9* and *mei-41* alleles of *Drosophila melanogaster*. Mutat. Res. **29:** 17-28.

Hari, K. L., Santerre, A., Sekelsky, J. J., McKim, K. S., Boyd, J. B., and Hawley, R. S. (1995). The *mei-41* Gene of *D. melanogaster* Is a Structural and Functional Homolog of the Human Ataxia Telangiectasia Gene. Cell. **82:** 815-821.

Sekelsky, J. J., McKim, K. S., Chin, G. M., and Hawley, R. S. (1995). The Drosophila Meiotic Recombination Gene *mei-9* Encodes a Homologue of the Yeast Excision Repair Protein Rad1. Genetics. **141:** 619-627

Banga, S. S., Velazquez, A. and Boyd, J.B. (1991). P transposition in *Drosophila* provides a new tool for analyzing postreplication repair and double-stand break repair. Mutat. Res. **255:** 79-88.

Banga, S. and Boyd, J.B. (1992). Oligonucleotide-directed site-specific mutagenesis in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. **89:** 1735-1739.

Boyd, J. B., Sakaguchi, K., and Harris, V.P. (1990). *mus308* mutants of Drosophila exhibit hypersensitivity of DNA cross-linking agents and are defective in a deoxyribonuclease. Genetics **125:** 813-819.

Harris, P.V., Mazina, O. M., Leonhardt, E. A., Case, R. B., Boyd, J. B. and Burtis, K. C. (1996). Molecular Cloning of *Drosophila mus308*, a Gene Involved in DNA Cross-Link Repair with Homology to Prokaryotic DNA Polymerase I Genes. Mol. & Cell. Bio. **16:** 5764-5771.

Harosh, I., Binninger, D. M., Harris, P. V., Mezzina, M. and Boyd, J.B. (1991). Mechanism of action of deoxyribonuclease II from human lymphoblasts. Eur. J. Biochem. **202:** 479-484.

Leonhardt, E. A., Henderson, D. S., Rinehart, J. E., and Boyd, J. B. (1993). Characterization of the *mus308* gene in *Drosophila melanogaster.* Genetics **133:** 87-96.

Sakaguchi, K., Harris, P. V., Ryan, C., Buchwald, M., and Boyd, J.B. (1991). Altertion of a nuclease in Fanconi anemia. Mutat. Res. **255:** 31-38.

One of the most interesting discoveries that we have made over the past year derives from sequence analysis of cDNAs from the putative *mus308* gene. During the past year we have sequenced cDNAs and genomic clones which we believe comprise the majority of the mus308 gene. The open-reading frame extends for 5.6 kb and spans a region of greater than 7 kb in the genomic DNA. It is comprised of 4 small introns and 1 larger intron containing a nested gene oriented in the opposite direction from *mus308*. This small intronic gene has substantial homology to the Drosophila heat-shock gene Hsp-G2, the function of which is unknown. Transformation of *mus308* embryos with constructs containing the entirety of this small nested gene failed to produce functional complementation of nitrogen mustard sensitivity, and hence this gene is unlikely to have any relationship to *mus308*. However, we have demonstrated that one of our *mus308* mutants contains an insert in the coding region of the intronic gene which eliminates its transcription. Presumably the effect of this insertion on *mus308* function results from disrupted splicing. This is currently under investigation.

The theoretical translation product of this gene contains a DNA polymerase domain near the carboxy terminus and DNA/RNA helicase motifs near the amino terminus. There is currently no precedent in the literature for a single polypeptide containing both of these domains. The protein appears to be a novel DNA repair enzyme which should be fruitful ground for future enzymological analysis. We have identified two ORFs by sequence analysis of the transforming fragment containing the *mei-41* gene and of corresponding cDNAs. ORF 1 includes the P element insertion sites and encodes a peptide of 757 amino acids. ORF 2 starts 900 base pairs downstream of ORF 1 and encodes a peptide of 1037 amino acids. This putative peptide shows homology to the yeast DNA repair genes, rad50 of S. cerevisiae and rad3 of S. pombe.

(Note work on *mus308* was transferred to Dr. Kenneth C. Burtis at the point. His laboratory has continued to study this gene in work supported by the NIH)

December 31,1995 : FINAL PROGRESS REPORT

In the final analysis, this award supported the detailed genetic and molecular analysis of three genes *(mei-9. mei-41*, and *mus308*) whose protein products play critical roles in DNA Repair in Drosophila. In each case the gene was cloned and the protein product was identified. Following the termination of the award on 1/1/96. Subsequent work on *mei-41* was supported by a grant to RSH from the AT Children's Project, Similarly, the continued work on *mei-9* was supported by a grant to RSH from the American Cancer Society. Following Dr. Boyd's death in 1993, subsequent work on *mus308* was supported by a grant to my colleague Dr. Kenneth Burtis from the NIH.

The cloning of *mei-9. mei-41*, and *mus308* represented major advances in the study of DNA Repair in Drosophila. The resulting papers have both opened exciting new areas of inquiry and been widely cited. We believe that this effort represents and enduring legacy of and tribute to the work of Professor Boyd and to his long association with the DOE.

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