DOE FINAL PROGRESS REPORT **DE-FGO3-87ER60538**

To: Dr. Kathryn Lee, DOE

From: Prof. R. Scott Hawley, UCD

Part 1. Progress Report for the period 7/1/92 to 2/1/93

1. Overview.

The period covered by this report encompasses a difficult transition period marked by the illness of the original PI, Dr. James Boyd, and his untimely death on October 7, 1993. During this period the work on this contract was supervised by myself.

Despite the enormous dislocation and sadness inherent in these events, the projects have continued to make remarkable progress. Specifically, there have been significant accomplishments in both the understanding of the structure of the *mei-41* gene and elucidation of the role the mei-41 gene product plays in both recombination and repair. We have also continued our genetic and molecular studies of the *mus308* locus and initiated detailed studies of the *mus312* and *mei-9* genes.

2. Structure and function of the *mei-41* gene.

As promised in the preceding progress report (6/1/92-6/1/93), we have completed the sequencing of the rescuing 10kb transforming fragment and of several cDNAs. Of current understanding, based on sequencing of the genomic DNA and cDNAs and on preliminary Northern blot analysis, argues that the two ORFs identified by previous sequencing studies are joined together by splicing to form a single large ORF.

We are now completing the structural analysis of the *mei-41* locus with a major focus on identifying the 5' and 3' end of the transcription unit. We are also constructing in vitro a putative full length cDNA containing

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both of the large ORFs and the necessary upstream and downstream sequences. This putative full length cDNA will be transformed back into Drosophila under the control of an inducible promoter and tested for its ability to rescue the *mei-41* defect. We will also express this protein in E. coli as means of obtaining enough *mei-41* protein to facilitate antibody production.

In addition, studies of meiotic chromosome behavior in *mei-41* oocytes reveals that, unlike most other recombination-defective mutations which appear to prevent exchange, mutants at mei-41 act to prevent the resolution of exchange events. This is to say that mutants at the *mei-41* locus induce the formation of chiasmate bivalents which cannot separate from each other at anaphase I. This finding has allowed us to develop a novel new cytological assay for ordering the defects of various recombination and repair functions within the recombination process.

3. Studies of the *mus308* gene.

We have continued our structural studies of the *mus308* locus along the lines described in the previous. progress report, specifically focusing on the mapping of mutations at this gene onto the molecular map of this locus. We have also initiated a detailed confocal cytological study of the effects of mutations at this locus on the replication and segregation of chromosomes in embryos. These studies are designed to complement biochemical studies of the *mus308* gene product being performed in the Kaguni Lab.

4. Studies of *mei-9* and *mus-312*.

Although mutations at the *mei-9* gene produce perhaps the most curious defects in repair and recombination of any of the repair-defective mutations in Drosophila, work on this locus had been dormant in the Boyd lab for several years. We have restarted this work and placed the project in the hands of a very talented post-doctoral fellow Dr. Jeff Sekelsky. Jeff just arrived from Harvard in November 1993 and has already begun to resolve some of the more confusing puzzles associated with previous work on this locus.

As stated in my previous communications, I view *mus312* as a very likely candidate for a gene required for both chromosome pairing/synapsis and for double strand break repair. We have initiated both a thorough genetic study of this locus and a cytological studies of the meiotic and mitotic defects of mutations at this locus. This effort includes a screen for p-element induced mutation that will facilitate cloning.

5. REVISED SPECIFIC AIMS

My primary objective will be to continue James Boyd's work on two repair deficient mutations in Drosophila melanogaster, namely *mei-9, mei-41*. In addition we will extend this study to include the *mus312* mutation, which likely defines a Drosophila homologue of the yeast rad50 mutation. Specifically we propose to:

(i). Further characterize the molecular processes that underlie both excision repair and meiotic recombination by a molecular analysis of the *mei-9* locus. This work will extend previous studies of Dr. Boyd by employing proven methods of gene localization (specifically the isolation of radiation-induced aberrations with breakpoints in the locus and the creation of new alleles by enhancer-trap mobilization).

(ii). Further characterize the molecular processes that underlie both post-replication repair and meiotic recombination by a molecular analysis of the *mei-41* locus. Dr. Boyd's laboratory has identified a likely candidate for the *mei-41* transcript and we will pursue this molecular analysis. Since *mei-41* is one of only two loci for which mutations alter the morphology of meiotic recombination nodules, we expect the analysis of this locus to provide substantial insights into the mechanisms of meiotic recombination as well as into the processes of post-replication repair.

(iii). Further characterize the molecular processes that underlie both double-strand break repair and meiotic pairing by further characterizing the *mus312* locus at the genetic, cytological, and molecular levels. The *mus312* mutation displays an extreme sensitivity to mutagens that induce double strand breaks in DNA as well as virtually eliminating meiotic recombination in females. These phenotypes strongly resemble those of the yeast RAD50 gene, which is required for both chromosome synapsis and for the repair of double strand breaks. We propose to examine the effects of the *mus312*

mutation on meiotic pairing and recombination by both confocal and electron microscopy. We also propose to isolate new alleles of this locus by a variety of means and to pursue a detailed molecular analysis of the *mus312* gene.

Taken together we expect these studies to provide substantial insights into the both the molecular mechanisms of DNA repair in Drosophila and the role these genes play in normal biological processes such as pairing and recombination.

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Part 2. Progress Report for the period 2/1/93 to 11/1/1994

1. Overview

As stated in previous reports, my primary objective in this effort was to continue James Boyd's work on two repair deficient mutations in Drosophila melanogaster, namely *mei-9, mei-41*. In addition we also proposed to extend this study to include the *mus312* mutation, which likely defines a Drosophila homologue of the yeast rad50 mutation. Specifically we proposed to:

(i). Further characterize the molecular processes that underlie both excision repair and meiotic recombination by a molecular analysis of the *mei-9* locus. Indeed, as described below, Dr. Jeff Sekelsky has cloned the *mei-9* gene and shown it encodes a protein highly homologous to that produced by the yeast RAD1 gene. This is an exciting result for two reasons. First, much is known about the genetics and biochemistry of this protein; and second, RAD 1 is not essential for meiotic recombination in yeast. The latter observation suggests that meiotic recombination in higher organisms (such as flies) may require functions not required for meiotic recombination in yeast. The comparison of the two systems may prove fruitful indeed. Of perhaps more interest to the DOE is the observation that the protein encoded by the *mei-9* gene is probably the Drosophila homolog of human ERCC4/XPF protein which is required for DNA repair in humans.

(ii). Further characterize the molecular processes that underlie both post-replication repair and meiotic recombination by a molecular analysis of the *mei-41* locus. Dr. Anne Santerre has completed our analysis of the *mei-41* transcription unit and found that it encodes a protein highly homologous to that encoded by the yeast MEC1 (also known as ESR1) gene. The MEC1 gene encodes a protein required both for cell cycle regulation in yeast and for some aspects of DNA metabolism. This finding of homology to MEC1 has given us a new set of questions to ask regarding this gene. The *mei-41* gene thus defines the first 'checkpoint' gene to be identified in Drosophila.

(iii). Further characterize the molecular processes that underlie both double-strand break repair and meiotic pairing by further characterizing the *mus312* locus at the genetic, cytological, and molecular levels. The *mus312* mutation displays an extreme sensitivity to mutagens that induce double strand breaks in DNA as well as virtually eliminating meiotic recombination in females. These phenotypes strongly resemble those of the yeast RAD50 gene, which is required for both chromosome synapsis and for the repair of double strand breaks. We have greatly refined the mapping of this gene and are now beginning the studies that will quickly facilitate its cloning.

Taken together we expect these studies to provide substantial insights into the both the molecular mechanisms of DNA repair in Drosophila and the role these genes play in normal biological processes such as pairing, recombination, and cell cycle control. In the sections below we describe our progress on realizing these objectives.

2. Structural and functional analysis of the *mei-9* (RAD1) gene.

The *mei-9* gene is essential for both meiotic recombination in Drosophila females (levels of meiotic recombination are reduced by approximately 90-95% in females homozygous for *mei-9*) and for excision repair in both sexes. Indeed, *mei-9* defines the best studied and best understood of the Drosophila repair and recombination defective mutants.

For these reasons we made cloning *mei-9* our highest priority. We began only with a set of P-element insertions that had previously been isolated in a hybrid dysgenesis screen. We were able to isolate three revertants of one of these alleles by crossing the mutation into a genetic background where the transposable P-elements were induced to excise. By comparing the mutant and revertant DNA on Southern blots, we identified an RFLP caused by the insertion of the P-transposable element in the mutant. This RFLP was absent in the parental strain and disappeared in each of the revertants. The insertion site defined by this RFLP is within a coding region as shown by the isolation of a cDNA using DNA flanking the insertion site as a probe. We have completed sequencing of this cDNA. Database searches with the conceptual translation product have revealed that the MEI-9 protein is a homolog of the S. cerevisiae RAD1.

RAD1, like *mei-9,* is required for excision repair, and is probably the homolog of human ERCC4/XPF. Unlike *mei-9*, however, *rad1* mutants are

not defective in meiotic recombination. Rather, RAD1 is believed to define a RAD52-independent pathway for mitotic recombination/repair, in which a complex between RAD1 and RAD10 (the homolog of human ERCC1) constitutes an endonuclease that cuts DNA near duplex-single strand junctions. These data on the yeast RAD1 enzyme are fully consistent with the fact that in *mei-9* mutant females, recombination intermediates are apparently generated, but not resolved into reciprocal exchanges. What is surprising is that such a function are not required for meiotic recombination in yeast. Taken together, these results suggest that the predominant meiotic recombination pathway in Drosophila is different than that of Saccharomyces.

To extend this study in the coming year we propose to:

-demonstrate a functional significance of the *mei-9* - RAD1 homology. To accomplish this objective we propose reciprocal transformation experiments in which the S. pombe and S. cerevisiae RAD1 genes (the S. pombe homolog of RAD1 is RAD16) are transformed into Drosophila (and vice versa) and then each inserted gene is tested for its ability to rescue the corresponding mutation in the host genome In other words, we propose to ask whether or not the *mei-9* gene of flies can rescue *rad1* or *rad16* mutants in yeast . Testing the ability of the Drosophila *mei-9* gene to complement the ERCC4 defect in human cell lines also represents a long term goal of this effort.

-clone and analyze the Drosophila homolog of the S. cerevisiae RAD10 gene (also known as RAD10 in S. pombe). Since the RAD1 and RAD10 proteins act as a complex in yeast, it is crucial to identify the RAD10 homolog in Drosophila. Fortunately, sequence comparisons between the two yeast genes have given us a set of excellent tools for cloning in Drosophila. Once a putative homolog has been identified, this locus will be subjected to a thorough genetic analysis to identify its role in excision repair and meiotic recombination.

-produce antibodies against the MEI-9 gene product and use those antibodies to immunolocalize this protein in meiotic nuclei. To understand the function of MEI-9 protein in recombination it is crucial to determine when this protein binds to meiotic chromosomes and at which sites (e.g. is it a component of the recombination nodule?).

-study the control of *mei-9* gene expression in mutagen-treated and un-treated cells as a means of understanding the regulation of DNA repair in Drosophila.

3. Structural and Functional Analysis of the mei-41 (MEC1) gene.

The *mei-41* gene was originally defined as a recombination-defective mutant that greatly reduced female fertility. It was subsequently shown to be defective in post-replication repair and also to give rise to a high frequency of chromosome breaks and aberrations in un-treated cells. We have also demonstrated a curious defect in progression of *mei-41* oocytes through the meiotic cell cycle; namely the formation of what appears to be extensive interlocking and 'stickiness' of the chromosomes prior to the end of meiotic prophase.

mei-41 was also cloned by P element tagging prior to Dr. Boyd's death. Indeed, just before his death the lab had succeeded in demonstrating that a 10.4 kb genomic DNA fragment is sufficient to rescue both the mutagen sensitivity and the female sterility phenotypes of *mei-41* mutants. We have sequenced this fragment in it's entirety. The sequence contains two large open reading frames and two smaller ones. Northern blots show that at least the two large ORFs belong to a single transcript of 7.6 kb. Library screens to date have not produced cDNAs that span the putative introns. In addition to continuing such screens, we are using RT-PCR to determine the precise structure of the *mei-41* transcript. Conceptual translation of the ORFs predicts a MEI-41 polypeptide of approximately 2300 amino acid residues.

Database searches with this sequence reveal extensive homologies to the S. cerevisiae gene MEC1 and the S. pombe gene *Rad3*. Both MEC1 and *Rad3* are involved in cell cycle checkpoint controls, including monitoring the completion of S phase and arresting the cell cycle in response to DNA damage. Following the analysis done by Weinert and others in yeast, we interpret the mei-41 defect in terms of both a direct defect in DNA metabolism (specifically in DNA synthesis) and in the ability of the cell to recognize the presence of gapped or un-replicated DNA and to stop the cell from entering mitosis or meiosis.

To extend this study in the coming year we propose to:

-demonstrate a functional significance of the *mei-41* - MEC1 homology. To accomplish this objective we propose reciprocal transformation experiments in which the S. pombe RAD3 and S. cerevisiae MEC1 genes are transformed into Drosophila (and vice versa) and then each inserted gene is tested for its ability to rescue the corresponding mutation in the host genome In other words, we propose to ask whether or not the *mei-41* gene of flies can rescue *rad3* or *mec1* mutants.

-continue our cytological study of the *mei-41* defect in both meiotic and mitotic cells. Specifically we propose to evaluate the effects of irradiation-induced DNA damage on mitotic progression in cultured neuroblasts. The aim of these studies is to determine whether or not *mei-41* cells truly are defective in a checkpoint that would normally cause cells bearing damaged DNA to abort progression through the cell cycle. We also propose to study the effects of the chromatin interlocking that we observe in meiotic prometaphase cells on progression through anaphase. The aim of these studies is to determine whether the interlocking results in chromosome bridging and breaking at anaphase.

-produce antibodies against the MEI-41 gene product and use those antibodies to immunolocalize this protein in meiotic and mitotic nuclei.

-study the control of *mei-41* gene expression in mutagen-treated and un-treated cells as a means of understanding the regulation of DNA repair in Drosophila.

4. Structural and Functional Analysis of the *mus312* gene.

The *mus312* mutation displays an extreme sensitivity to mutagens that induce double strand breaks in DNA as well as virtually eliminating meiotic recombination in females. These phenotypes strongly resemble those of the yeast RAD50 gene, which is required for both chromosome synapsis and for the repair of double strand breaks. We have greatly refined the mapping of this gene and are now beginning the studies that will quickly facilitate its cloning.

To extend this study in the coming year we propose to:

- further refine the cytogenetic mapping of the *mus312* mutation.

- attempt to produce a P-element insertion allele of this gene. Obtaining such a mutation would greatly facilitate cloning.

- determine by serial section electron microscopy whether or not oocytes for *mus312* produce intact synaptonemal complex (SC). The complete absence of meiotic exchange generated this mutation is suggestive of a very early defect in meiotic pairing, similar to that exhibited by c(3)G, mutation that ablates SC formation.

5. Summary

In the last year we have brought two major efforts of the Boyd lab to fruition, namely the molecular studies of the *mei-9* and *mei-41* genes. Fortunately, in both cases the comparison of the putative translation products of these genes with the sequences of known proteins has provided crucial insights into their functions. Even more fortunately, those findings have also raised newer and more exciting questions about the role of these proteins in the processes or repair and cell division. These findings have also given us the tools to seek other genes that play important roles in this process in Drosophila, yeast, and even humans. We expect the next year to be even more eventful and exciting that the current one.

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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).

(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 melanogaste*r. 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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