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 mus-312 mutation, which likely defines a Drosophila homologue of the yeast rad50 mutation. Specifically we proposed to:

1. 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.

2. 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.

3. further characterize the molecular processes that underlie both double-strand break repair and meiotic pairing by further characterizing
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the *mus-312* locus at the genetic, cytological, and molecular levels. The *mus-312* 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.

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 mutant.

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.


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 its 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.


The mus-312 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 mus-312 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 mus-312 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.

4. 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 than the current one.