

Annual Progress Report
for the period
September 1, 1990 to July 1, 1991

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1. Characterization of DNA double-strand break repair. At this time last year Dr. Banga had identified two genes in *Drosophila* that are required for the repair of DNA double-strand breaks. During the past year that analysis was completed and has been accepted for publication. The abstract of that study follows: We have developed a genetic screen in *Drosophila* for identifying host repair genes responsible for processing DNA lesions formed during mobilization of P transposable elements. Application of that approach to repair deficient mutants has revealed that the *mei-41* and *mus302* genes are necessary for recovery of P-bearing chromosomes undergoing transposition. Both of these genes are required for normal postreplication repair. Mutants deficient in excision repair, on the other hand, have no detected effect on the repair of transposition-induced lesions. These observations suggest that P element-induced lesions are repaired by a postreplication pathway of DNA repair. The data further support recent studies implicating double-strand DNA breaks as intermediates in P transposition, because the *mei-41* gene has been genetically and cytologically associated with the repair of interrupted chromosomes. Analysis of this system has also revealed a striking stimulation of site-specific gene conversion and recombination by P transposition. This result strongly suggests that postreplication repair in this model eukaryote operates through a conversion/recombination mechanism. Our results also support a recently developed model for a conversion-like mechanism of P transposition (Engels et al., 1990). Involvement of the *mei-41* and *mus302* genes in the repair of P element-induced double-strand breaks and postreplication repair points to a commonality in the mechanisms of these processes.

2. Using injected oligonucleotides as templates to repair double-strand DNA breaks. During the past year Dr. Banga has further exploited that system to develop an efficient technique for performing *in vivo* site-directed mutagenesis in *Drosophila melanogaster*. This procedure involves directed repair of P element-induced DNA lesions following injection of a modified DNA sequence into early embryos. An oligonucleotide of 50 base pairs, whose sequence spans the P element insertion site, mediates base replacement in the endogenous gene. Restriction mapping, DNA sequencing and PCR (polymerase chain reaction) analysis demonstrate that base substitutions present in an injected oligonucleotide are incorporated into genomic sequences flanking a P insertion site in the *white* gene. This analysis suggests that progeny bearing directed mutations are recovered with a frequency of about 0.5×10^{-3} . Because *Drosophila* remains a premier organism for the analysis of eukaryotic gene regulation, this system should find strong application in that analysis as well as in the analysis of DNA recombination, conversion, repair and mutagenesis. That system also opens new approaches for studying the mechanism of double-strand break repair in eukaryotes.

3. Analysis of a gene required for postreplication repair. A year ago Dr. David Binninger had initiated a study in which subclones of the *mei-41* chromosomal region were to be used as Northern probes in an effort to identify the coding sequences of that gene. That study was interrupted when he unexpectedly located a unique academic position which he felt he had to accept. After his departure Dr. Sarla Purohit joined our group to continue that effort. She has recently subcloned a significant portion of our chromosomal walk which has been employed to identify several transcripts from the *mei-41* genomic region. In an attempt to identify the *mei-41* transcript she is currently searching for transcript

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alterations in the *mei-41* mutants. She is also employing these probes to recover cDNA clones from an embryonic library.

Dr. Banga has taken an alternative approach to further establish that the complete *mei-41* gene has been cloned. Two cosmid clones have been isolated from a cosmid library which was constructed in the transformation vector, CaSeR that carries a mini *white* gene as a selectable marker. Restriction mapping of these clones is underway to determine the extent of the genomic inserts and to determine the integrity of the clones. A genomic clone that extends from cTH1 and cTH2 (two transcripts that are not altered in P element-induced *mei-41* mutants) towards the proximal side will be used to transform flies in an effort to rescue the *mei-41* phenotype. We are optimistic that these studies together will provide an identification of the *mei-41* gene and its transcript(s) within the next year.

4. Cloning of a gene required for resistance to DNA cross-linking agents. Edith Leonhardt and Paul Harris are conducting a chromosomal walk which will result in the recovery of the *Drosophila mus308* gene. As we discussed in the previous report, there are a variety of reasons for believing that this gene may be an analogue of the Fanconi anemia A gene in man. Over the past year Edith has performed a detailed cytogenetic analysis of this gene which links it tightly with the *Men* gene. Paul Harris has employed sequence information of the homologous gene in other organisms to recover a portion of the *Drosophila* gene by PCR. That sequence is currently being employed to initiate a chromosomal walk between two deficiency break points that bracket the *mus308* gene. Recovery of this gene should contribute to our understanding of eukaryotic defenses against agents that cross-link DNA and should permit recovery of the homologous human sequence.

5. Cloning of a gene required for excision repair. Cloning of the *mei-9* gene has become a collaborative venture between our laboratory and that of Dr. David Binninger at Florida Atlantic University. While David has been establishing his laboratory Jim Boyd has conducted a cytogenetic analysis of a series of new deficiencies that have been recovered from that region of the genome. Among those a new deficiency has been identified which provides a definitive distal break point for a chromosomal walk. In addition, I have employed *in situ* hybridization to show that our initial chromosomal walk must be extended proximally in order to include the entire *mei-9* gene. As this cytogenetic analysis is being solidified, Dr. Binninger will extend existing chromosomal walks from both the distal and proximal directions to recover the *mei-9* gene. Recovery of this gene will contribute to an improved understanding of the mechanisms of DNA excision repair and recombination in higher organisms.

6. Cloning of a gene required for X-ray resistance. This year Daryl Henderson has extended his cytogenetic characterization of the *mus209* gene to the molecular level. Using the approach of transposon tagging he has recovered genomic sequences flanking a P transposable element that is inserted into that gene. Sequences from the cosmid clones that flank the insertion site have been employed to recover expressed sequences from a cDNA library. The cDNA sequences are in turn being employed to analyze Northern blots carrying mutant RNA in an effort to identify the transcript of that gene. An analysis of this gene is expected to provide insight into a function that is implicated in DNA repair as well as in cell cycle control.

7. Transposon tagging of DNA repair genes. Angela Rosenstein has continued her analysis of the *mus* mutations that we have recovered in a screen of mutagenized stocks obtained from other laboratories. These stocks each contain a single transposon inserted randomly into one of the two major autosomes. Twenty independent *mus* mutations have been recovered and verified from that screen. Those that affect previously unidentified

genes (15) are being subjected to complementation analysis in order to establish the number of new genes represented. Molecular, genetic, and cytological analysis has shown that two mutations in previously identified genes are not due to transposon insertions. This observation is consistent with the observations of others that as many as half of the mutations generated with this approach are secondary mutations unassociated with transposon insertions. A third mutant, however, currently appears to map to the position of its *mus310* mutation by *in situ* hybridization. Continuing analysis of these mutations is providing a rich source of material for future exploitation of this system.

Continuation Proposal
Period: October 1, 1991 to September 30, 1992

We have two primary goals for the coming grant periods. The first of these is to identify and characterize the *mei-41* gene which we have previously cloned. This effort will be the primary responsibility of Dr. Sarla Purohit who has now demonstrated all the technologies necessary to accomplish that goal. She will be assisted in that effort initially by Robert Winters (Masters Student) who is currently investigating the first detectable transcription unit to the left of the P insertion mutations together with the intervening sequences. When he leaves within the next year, he will be replaced by a new graduate student. Sarla will continue to analyze the *mei-41* mutations with Northern and cDNA analysis until the correct transcript has been identified. Sequence analysis of the corresponding cDNA will then be initiated in an effort to establish the function of this gene.

Our second major goal is to recover and characterize the *mus308* gene. This effort will be continued by Edith Leonhardt until she departs within the next 6 months. At that point Boyd and Harris will devote their full attention to the problem. Toward this end, Boyd will attend the Cold Spring Harbor Molecular Biology Laboratory course during the Summer of 1991 in order to assure our familiarity with the latest technologies. The analysis itself will be conducted along the lines outlined in the original research proposal for repair related genes. Since this gene is potentially homologous to the Fanconi anemia A gene in man, we may begin collaborations with human geneticists who are attempting to clone that gene. That effort will initially involve an exchange of probes in an effort to identify any potentially interesting homologies in walks being conducted in *Drosophila*, man and Chinese hamster.

Our collaboration with Dr. David Binninger will continue as he connects two chromosomal walks that span the *mei-9* region. Our contribution will be to provide appropriate stocks, conduct the necessary genetic analysis and monitor the progress of the walk with the aid of the available deficiencies analyzed by *in situ* hybridization. Finally Angela Rosenstein will continue the characterization of the new *mus* mutations in preparation for cloning the identified genes by transposon tagging.

Publications since the previous report:

Boyd, James B., Kengo Sakaguchi, and Paul V. Harris. 1990. *mus308* mutants of *Drosophila* exhibit hypersensitivity to DNA cross-linking agents and are defective in a deoxyribonuclease. *Genetics* 125:813-819.

Sakaguchi, Kengo, Paul V. Harris, Robert van Kuyk, Andrew Singson, and James B. Boyd. 1990. A mitochondrial nuclease is modified in *Drosophila* mutants (*mus308*) that are hypersensitive to DNA crosslinking agents. *Mol. Gen. Genetics* 224:333-340.

Publications in press:

Sakaguchi, K., P. V. Harris, C. Ryan, M. Buchwalk, and J. B. Boyd. Alternation of a nuclease in Fanconi anemia. *Mutation Research*, in press.

Banga, S. S., A. Velazquez, and J. B. Boyd. P transposition in *Drosophila* provides a new tool for analyzing postreplication repair and double-strand break repair. *Mutation Research*, in press.