Annual Progress Report
for the period
June 1, 1989 to September 1, 1990

FG03-87ER60538
DOE/ER/60538--74

1. Overview. The most exciting discovery we have made over the past year derives from an analysis of the interaction between DNA repair and P-element transposition. In that study Dr. Banga has developed a powerful new system for analyzing the repair of DNA double-strand breaks. We have also completed a screen of mutagenized autosomes obtained from two San Francisco laboratories with the recovery of several mutants that will provide the foundation for future efforts to clone repair related genes. At the same time, strong progress has been made in the cloning and characterization of the repair-related genes *mei-41* and *mus209*. Finally, our efforts to clone the *mei-9* gene have uncovered the existence of a unsuspected feature of the system used for transposon-tagging in Drosophila. This new knowledge will aid our future cloning efforts as well as those of others in the field.

2. Cloning of repair genes by transposon tagging. During the previous year, Mrs. Rosenstein, together with others in the laboratory, had completed a screen of 2600 stocks obtained from UC San Francisco for transposon insertions in repair-related genes. Since transposon tagging is rapidly becoming the method of choice for cloning Drosophila genes, she spent much of the current year screening an additional 3,000 stocks from the Rubin lab at Berkeley. Several promising mutants have been recovered from that screen as well. This collection of repair-deficient mutations with potential transposon insertions promises to accelerate the recovery of additional repair-related genes.

3. Transposition as a tool to study the repair of double-strand DNA breaks. At this time last year we had obtained preliminary evidence suggesting that the *mei-41* gene is required for the recovery of chromosomes undergoing P transposition. Since then that conclusion has been strongly substantiated and, together with the Engels laboratory, we have further documented that the repair mechanism which is defective in *mei-41* involves gene conversion. Since the intermediate in that repair process is, in all likelihood, a DNA double-strand break, P transposition can provide a precise means to study the repair of DNA double-strand breaks in a site specific manner. A systematic screen of other Drosophila *mus* mutations has revealed that the *mus302* gene is also required in this repair pathway. This study has, therefore, identified two Drosophila genes that participate in the repair of one class of DNA double-strand breaks. In addition, it has established P transposition as a valuable tool for investigating the mechanism of that process.

4. Characterization of the *mei-41* gene. Having cloned the *mei-41* gene in a chromosomal walk, we are now in the process of identifying a transcription unit and a coding sequence. This has not turned out to be a trivial task because, as we knew from previous genetic studies, this is a relatively large gene for this organism. Accordingly we have initiated a program to subclone the entire *mei-41* region into plasmid vectors to facilitate the transcription analysis. In proceeding bidirectionally from the P insertion sites, which define essential *mei-41* sequences, we have thus far identified two transcription units and a possible third one. This time last year we believed that the transcription unit to the left of the P insertions on the clone map was related to *mei-41*. Since several tests of that hypothesis have failed to confirm that conclusion, it now seems possible that that gene is nested in an intron within the *mei-41* gene. David Binninger has recently used Northern blotting to identify alternate transcribed sequences which show strong promise of being derived from the *mei-41* gene.

DISCLAIMER

5. Cloning of the *mus209* gene. Daryl Henderson has recently completed a thorough genetic analysis of the available *mus209* mutants in preparation for cloning that gene. He has established that this is an essential gene for which hypomorphic mutants are hypersensitive to both x-rays and MMS. His precise cytogenetic localization of that gene has placed it in section 56F of the second chromosome. A stock bearing a P insertion in that section fails to complement EMS-induced *mus209* mutations. Loss of that P-element under dysgenic conditions is accompanied by simultaneous recovery of mutagen resistance. These observations argue strongly that a P insertion is responsible for that mutation. Accordingly Daryl has generated a genomic DNA library from that stock and recovered genomic sequence flanking the P insert. He is, therefore, in a strong position to recover the complete gene from a wild-type genomic library.

6. Cloning of the *mei-9* gene. Our efforts to clone the *mei-9* gene have revolved around three dysgenic induced mutations which we have subjected to standard transposon tagging procedures. Standard dogma, which has been generated in application of this procedure to dozens of Drosophila genes, states that a mutant which simultaneously loses a P element and the mutant phenotype in the presence of transposase can be employed to recover that gene by P-element transposon tagging. Analysis of two of these mutations has recently demonstrated that P-elements may not be the only transposable elements that can be mobilized by P transposase. This conclusion may explain problems we have had with cloning another Drosophila repair-related gene (*mus309*) and has provided new impetus for identifying such an element in another laboratory. This study will greatly improve our efficiency in the future by generally increasing the power of transposon tagging in this organism. Continuing efforts to clone this gene are now being concentrated on the third dysgenic mutation which currently appears to be due to a P element.

Continuation Proposal
Period: October 1, 1990 to September 30, 1991

One of our major goals during the coming twelve months will be to identify and characterize the transcription unit and coding sequence of the *mei-41* gene. Toward that end we will completely subclone the chromosomal walk from lambda clones into plasmid vectors which permit the generation of single strand riboprobes. We have currently subcloned about 14 kb surrounding the P insertion sites, and should complete subcloning of the remaining 35 kb within the next six months. Those clones will be employed to generate probes for Northern analysis to identify each of the transcription units in this region of the genome. Northern analysis of the available mutants should permit an identification of the *mei-41* transcript(s). Expressed sequences from that gene will in turn be employed to screen cDNA libraries in an effort to recover a complete coding sequence. Within the next year we should therefore be in a position to search for homologous human sequences with probes derived from the Drosophila genome. Related procedures will be employed to clone the *mus209* gene once a chromosomal walk in a wild-type genomic library has been completed.

The remaining single dysgenically induced *mei-9* mutation will serve as our initial focus for cloning the *mei-9* gene. Since that mutation has marginal MMS sensitivity, it would be difficult to verify the insertion of a P element by selecting for revertants. Rather we will attempt to make more extreme mutations by selecting for hypersensitive mutations under dysgenic conditions. Generation of such mutations at a reasonable frequency will establish the presence of a transposable element and simultaneously generate aberrations that can be identified molecularly. If this genetic analysis is successful, we will employ a cloned P-element to recover this gene as is being done with the *mus209* gene. As an alternative, we will define the cytological break points of newly available chromosomal aberrations in the *mei-9* chromosomal region. Those deficiencies should help refine the

cytological position of that gene. With that information we will conduct a chromosomal walk between the appropriate break points in analogy with the *mei-41* analysis. Southern analysis of the dysgenically induced mutations and their revertants should establish the position of the *mei-9* gene within that walk. As will be the case with all these genes, final verification will rest upon successful germline transformation and complementation of mutant phenotypes.

As part of our analysis of the *mei-41* genomic region we have recovered a complete cDNA sequence for the transcription unit that lies just to the left of the P insertion sites in the dysgenically induced *mei-41* mutations. Since that gene is likely to reside within the *mei-41* gene, it may be related in function. To identify highly conserved features of that coding sequence we have recovered a homologous genomic clone from the distantly related species *D. virilus*. Those clones are being sequenced to identify critical functional protein domains. Two other laboratories have recently developed PCR procedures for recovering mutations in genes that have been identified in this manner. Mutations recovered with that approach in this gene will be analyzed to establish the biological function of that gene.

Publications

Published within the past year:

Banga, S. S., J. B. Boyd, K. Valerie, P. V. Harris, E. M. Kurz and J. K. de Riel. 1989. denV gene of bacteriophage T4 restores DNA excision repair to *mei-9* and *mus201* mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA., Vol. 86, pp. 3227-3231.

Boyd, J. B., K. Sakaguchi and P. V. Harris. 1989. DNA Metabolizing Enzymes of Drosophila. In: The Eukaryotic Nucleus. Molecular Biochemistry and Macromolecular Assemblies. Edited by P. R. Strauss and S. H. Wilson, Tilford Press. Vol. 1, pp. 293-314.

Oliveri, D. R., P. V. Harris and J. B. Boyd. 1990. X-Ray sensitivity and single-strand DNA break repair in mutagen-sensitive mutants of *Drosophila melanogaster*. Mutat. Res. 235: 25-31.

Yamamoto, A. H., R. K. Brodberg, S. S. Banga, J. B. Boyd and J. M. Mason. 1990. Recovery and characterization of hybrid dysgenesis-induced *mei-9* and *mei-41* alleles of *Drosophila melanogaster*. Mutat. Res. 229: 17-28.

Boyd, J. B., S. S. Banga, A. K. Yamamoto, J. M. Mason, D. R. Oliveri, D. S. Henderson, A. Velazquez, E. Leonhardt and A.W. Rosenstein. 1990. Cloning Drosophila Repair Genes by Transposon Tagging. In: Mutation and the Environment, Part A, Wiley-Liss, Inc., pp. 205-211.

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

In Press

Sakaguchi, K., P. V. Harris, R. Van Kuyk, A. Singson and J. B. Boyd. A mitochondrial nuclease is modified in Drosophila mutants (*mus308*) that are hypersensitive to DNA crosslinking agents. Molec. Gen. Genet.

Submitted

Banga, S. S., A. H. Yamamoto, J. M. Mason and J. B. Boyd.
Molecular Cloning of *mei-41*, a gene that influences both somatic and germline chromosome metabolism of *Drosophila melanogaster*

Banga, S. S., A. Velazquez and J. B. Boyd. Host genes involved in repairing transposon-induced DNA damage in Drosophila.

Sakaguchi, K., P. V. Harris, C. Ryan, M. Buchwald and J. B. Boyd. Alteration of a Nuclease in Fanconi Anemia.

Sakaguchi, K., M. Z. Zdzienicka, P. V. Harris and J. B. Boyd. An Abnormal Nuclease in Chinese Hamster Models of Fanconi Anemia.

ER F 4620.1
(7-85)

U.S. Department of Energy
Grant Application Budget Period Summary
(See Reverse for Definitions and Instructions)

OMB Approval
No. 1910-1400

Please Print or Type

Organization:	Period Covering:	FOR DOE USE ONLY
The Regents of the University of California	From: 2/1/91	Proposal No:
Principal Investigator (P.I.)/Project Director (P.D.): James B. Boyd	To: 1/31/92	Award No.:

A. SENIOR PERSONNEL PI/PD Co PIs, Faculty and Other Senior Associates (List each separately with title, A.6 show number in brackets. Attach separate sheet, if required.)	DOE Funded Person-Mos. Cal.	Acad.	Sumr.	Funds Requested By Applicant $
1. James B. Boyd, P.I., Professor		1.8		-0-
2. Satnam Banga, Assistant Research Geneticist		9.48		31,000
3.				
4.				
5.				
6. (2) TOTAL SENIOR PERSONNEL		11.28		
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (1) POST DOCTORAL ASSOCIATES		12		20,000
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		12		35,975
3. (1) GRADUATE STUDENTS		6		11,000
4. (2) UNDERGRADUATE STUDENTS		3.6		5,950
5. ( ) SECRETARIAL-CLERICAL				
6. ( ) OTHER				
TOTAL SALARIES AND WAGES (A + B)				103,925
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				28,457
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				132,382
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM)				
TOTAL EQUIPMENT				-0-
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				3,000
2. FOREIGN				-0-
F. OTHER DIRECT COSTS				-0-
1. MATERIALS AND SUPPLIES				22,285
2. PUBLICATION COSTS/PAGE CHARGES				-0-
3. CONSULTANT SERVICES				-0-
4. COMPUTER (ADPE) SERVICES				-0-
5. CONTRACTS AND SUBGRANTS				-0-
6. OTHER				-0-
TOTAL OTHER DIRECT COSTS				25,285
G. TOTAL DIRECT COSTS (A THROUGH F)				157,667
H. INDIRECT COSTS (SPECIFY RATE AND BASE)				
TOTAL INDIRECT COSTS				64,959
I. TOTAL DIRECT AND INDIRECT COSTS (G & H)				222,626
J. APPLICANT'S COST SHARING (IF ANY)				-0-
K. TOTAL AMOUNT OF THIS REQUEST (ITEM I LESS ITEM J)				222,626

PI/PD TYPED NAME & SIGNATURE James B. Boyd James B. Boyd	DATE Sept. 19 '90
INST. REP. TYPED NAME & SIGNATURE	DATE