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TITLE: Identification of Proteins Required for Repair of Double-Strand Chromosome Breaks, a Predisposing Factor in Breast Cancer

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13. ABSTRACT ***(Maximum 200 Words)***

Defects in repair of double-strand chromosomal breaks (DSB) are critical factors in familial and sporadic breast tumors. In model bacterial systems, such lesions can be ascribed to defects in homologous recombination proteins, which can support chromosomal replication by promoting restart of DNA synthesis when replication forks become arrested. This project has focused on developing a bacterial model for DSB repair by characterizing the enzymatic apparatus needed to initiate DNA replication on recombination intermediates. *Escherichia coli* PriA protein was found to play a critical function in the transition from recombination to DNA replication. PriA specifically binds to forked DNA structures created by recombination or replication fork arrest and promotes the assembly of protein components needed to load the major replicative helicase DnaB onto the template, a critical step in initiation. DnaB requires single-stranded DNA to bind, and this could be created by the helicase action of PriA, an activity that is suppressed by single-stranded binding protein if a duplex opening is already available. These data indicate that PriA can function in the repair of damaged DNA templates by promoting assembly of replication proteins on a wide variety of forked templates, preventing catastrophic loss or alteration of genetic information.

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FOREWORD

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PI - Signature Date

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Introduction:

Genetic defects in breast tumors frequently involve mutations in both oncogenes and tumor suppressor genes. Genes involved in the repair of DNA can be classified as tumor suppressor genes, but thus far only genes required for one type of DNA repair, single-base mismatch repair, have been fully characterized in humans. While defects in these genes appear to play a role in a small number of breast tumors, defects in repair of double strand chromosome breaks (DSBs) are emerging as important factors both in familial and sporadic breast tumors. We have focussed on development of a bacterial model for repair of DSBs by replication coupled to homologous recombination, and such a system will likely provide insight into the mechanism of DSB repair in humans. The reconstituted system for bacteriophage Mu replication by transposition has been an invaluable tool in this process. During Mu transposition, strand exchange catalyzed by the phage-encoded transposase MuA leads to formation of a branched DNA structure with a potential replication fork at either end of the transposing DNA element, similar to the branched intermediates created during homologous recombination. Bacterial proteins including the replicative helicase DnaB and DNA polymerase III holoenzyme then assemble a replisome at one end this substrate and commence semi-discontinuous DNA synthesis from one end to the other. Like replication coupled to recombination on the bacterial chromosome, initiation of bacteriophage Mu replication is independent of the chromosomal initiator protein DnaA, suggesting that bacteriophage Mu may harness the cellular apparatus required for coupling replication with recombination. Our finding that the *Escherichia coli* PriA protein was required for Mu replication by transposition both *in vivo* and *in vitro* supported this hypothesis. Previous to our work, PriA had been hypothesized to couple replication with homologous recombination based on genetic evidence and on the role of PriA in assembly of a primosome for bacteriophage ϕX174 complementary strand synthesis. Our work provided the first definitive biochemical evidence that PriA could couple replication with recombination. This project expands upon these finding with three Specific Aims:

1) Identification of additional *E. coli* proteins required for Mu DNA replication.
2) Analysis of Mu DNA sequences and structures promoting assembly of the primosome.
3) Development of a reconstituted system for identifying proteins required for DSB-induced replication

Report Body

Summary of Research Progress:

Last year, Kimberly Marshall-Batty, a graduate student in our Ph.D. program, decided to join my laboratory, and I was able to obtain funding for her as minority supplement to one of my NIH grants. She was an excellent candidate to continue some of the work of Jessica Jones, who was originally awarded this predoctoral traineeship grant. I inquired to administrators of the Breast Cancer Research Program what to do with unexpended portion of Jessica Jones's grant since I would not be permitted use those funds for Kimberly Batty's stipend or tuition I was advised to nominate Kimberly Batty PI, and this nomination was accepted However, because she was already a funded graduate student, we were not permitted by the army to spend any of the fellowship funds for her. Therefore, I requested an additional one-year extension for this fellowship grant while I searched for another graduate student who could become PI, and this request for the extension was granted by the army. This month I found an appropriate student to become PI of this grant, and I submitted a request to name her PI of this grant. If permission is granted, the student will conduct research with the remaining funds, and she will be submitting a final report in June, 2002. Since none of the research funds was used in the past year, there is no research work funded by this grant to be reported.

Summary of Training:

No student was trained with the funds for this project this year.

Key Research Accomplishments

- None.

Reportable Outcomes

- None.

REVISED STATEMENT OF WORK

I have submitted a request that Stella H. Kim be named PI of this predoctoral grant. Because there has been a lapse since Jessica Jones's graduation and departure from my laboratory, some of the unfinished work has already been accomplished by other members of my laboratory. The identification of additional host factors required for replicative transposition is still an important goal of this project. One factor has already been identified, and we believe there is one more to be identified. Thus, the last year of this project will focus on the fractionation of this factor as indicated in this revised Statement of Work:

Specific Aim 1: Identification of an *E. coli* protein required for Mu DNA replication.

Task 1 (Months 1 and 2): Establish a reaction system of purified proteins as an assay system for the remaining host protein component (MRFα2A) needed to catalyze replicative transposition of bacteriophage Mu.

Task 2 (Month 3): Prepare partially purified MRFα2A by previously described protocols, using heparin agarose and Q-Sepharose chromatography.

Task 3 (Months 4-12): Begin establishing a protocol for purification of MRFα2A by traditional fractionation techniques. Chromatography steps to be tested include Bio-Rex 70, hydroxyapatite, MuA-affinity, and ClpX-affinity (MuA and ClpX already present in sufficient amounts).

Task 4 (Months 10-12): Begin attempts to isolate STC3, the nucleoprotein complex (prereplisome) that is assembled using MRFα2A and MRFα2B. If STC3 free of proteins nonspecifically bound to DNA can be isolated, the specifically bound protein will be isolated by SDS-polyacrylamide gel electrophoresis and identified by N-terminal sequencing and mass spectrometry.