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Homologous recombination, regulation, and breast cancer emergence

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I. Introduction

Individuals with mutations in the BRCA1 and BRCA2 (Breast Cancer ½, Early Onset) genes are known to be more susceptible to breast cancer emergence. During the life of an organism, DNA damage occurs frequently due to ionizing radiation, free radicals, as well as oxidative species produced by the cells. This damage, if incorrectly repaired, can by itself cause mutations that eventually lead to cancer.

Mutations in the BRCA1 and BRCA2 proteins are particularly detrimental due to their roles in DNA repair and control of the cell cycle. DNA damage can affect a single or both strands causing a double stranded break. Cells use Non Homologous End Joining (NHEJ) and Homologous Recombination (HR) to repair a double stranded DNA break.

Of the two, HR is usually less mutagenic, using the sister chromatid template, instead of simply joining the broken ends as in NHEJ. BRCA1 functions as a recruitment protein for HR, and BRCA2 serves as the engine for strand invasion. When these DNA repair proteins are not functioning correctly, further damage is not repaired correctly, leading to further mutation, genomic instability and cancer emergence.

II. HR And SRS2

RAD51 is the main engine for HR, it is recruited to the site of a double stranded DNA (dsDNA) break by BRCA1. It forms a nucleoprotein filament, coating the leading ends of the break. BRCA 2 later initiates a strand invasion into the neighboring chromatid, which is used as a template for error free repairs.

HR is mediated through a complex and tightly controlled interplay between a fair number of pro, and anti-HR mediator proteins. SRS2 is one such anti-mediator that possesses translocation, and helicase activity. As it moves across Single Stranded DNA (ssDNA) and unwinds dsDNA it removes the RAD 51 filament, and halts HR. (Figure 1)

Figure 1 – SRS2 Activity

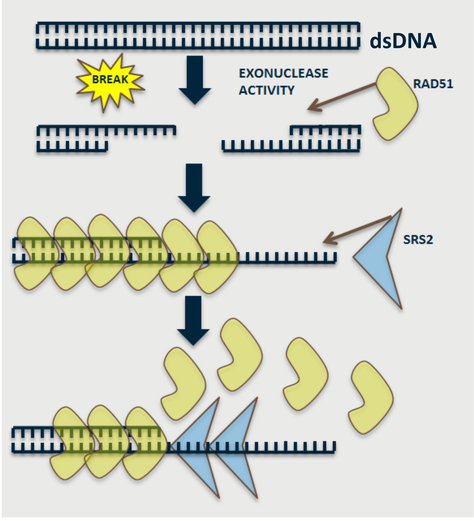

Fig1: Shown above is a condensed schematic of the role of SRS2 in homologous recombination.

III. Research Goals

SRS2 is the main focus of this project, particularly its somewhat curious modes of action, and potential regulatory methods. A crystal structure of SRS2 not yet available, but bacterial orthologues (eg REP) are consistently comprised of four conserved domains: 1A, 1B, 2A, and 2B. SRS2 likely contains these same domains, as well as a C-terminal domain that interacts with RAD51. The 2B domain in particular appears to have regulatory function, undergoing a large conformational shift in REP (Figure 3.) This project focuses on determining this interaction, and the specifics of its regulatory function. Crystallography and enzyme kinetics assays are the two modes of experimentation.

Figure 2 – Dimer Formation

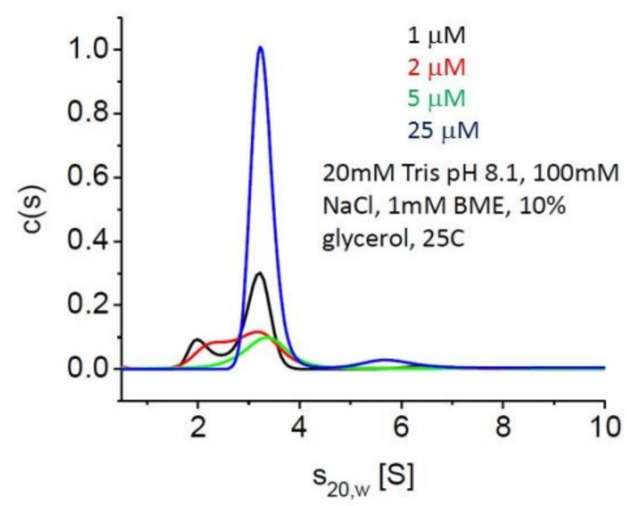

Fig2: Analytical centrifugation showing spontaneous dimer formation in high concentrations of the 2B domain.

Figure 3 – SRS2 2B Domain

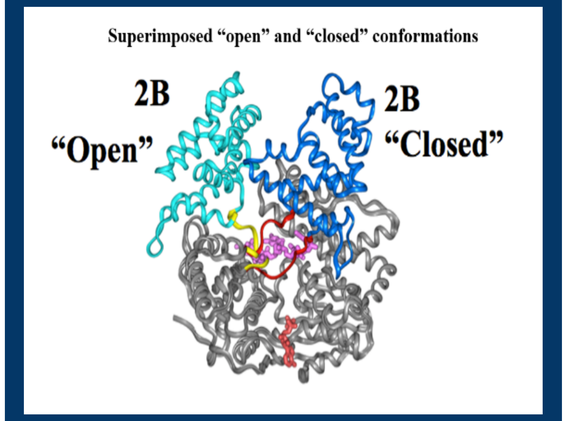

Fig3: Superimposed structure of 2 distinct conformations of the 2B domain in a bacterial orthologue of SRS2.

IV. Current Experiments

- Creation of a Histidine tagged variety of the 2B domain with a cleavable tag. This variety will hopefully produce a diffraction pattern for structure determination.
- Δ2B constructs (versions with a deleted 2B domain.) These constructs are predicted to be hyperactive, and will shed light on SRS2 mediation.
- Site directed mutagenesis of assumed critical residues. Once mutated, their overall importance can be determined.

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Study conducted with funding from a USU Undergraduate Research and Creative Opportunity Grant and lab assistance from the USU Department of Chemistry.

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