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Multimerization domains are associated with apparent strand exchange activity in BLM and WRN DNA helicases

Chi-Fu Chen [1], Steven J. Brill *

Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ, United States

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ABSTRACT

BLM and WRN are members of the RecQ family of DNA helicases that act to suppress genome instability and cancer predisposition. In addition to a RecQ helicase domain, each of these proteins contains an N-terminal domain of approximately 500 amino acids (aa) that is incompletely characterized. Previously, we showed that the N-terminus of Sgs1, the yeast ortholog of BLM, contains a physiologically important 200 aa domain ($Sgs1_{103–322}$) that displays single-stranded DNA (ssDNA) binding, strand annealing (SA), and apparent strand-exchange (SE) activities *in vitro*. Here we used a genetic assay to search for heterologous proteins that could functionally replace this domain of Sgs1 *in vivo*. In contrast to Rad59, the oligomeric Rad52 protein provided *in vivo* complementation, suggesting that multimerization is functionally important. An N-terminal domain of WRN was also identified that could replace $Sgs1_{103–322}$ in yeast. This domain, $WRN_{235–526}$, contains a known coiled coil and displays the same SA and SE activities as $Sgs1_{103–322}$. The coiled coil domain of $WRN_{235–526}$ is required for both its *in vivo* activity and its *in vitro* SE activity. Based on this result, a potential coiled coil was identified within $Sgs1_{103–322}$. This 25 amino acid region was similarly essential for wt Sgs1 activity *in vivo* and was replaceable by a heterologous coiled coil. Taken together, the results indicate that a coiled coil and a closely linked apparent SE activity are conserved features of the BLM and WRN DNA helicases.

1. Introduction

Genome integrity relies on the cell's ability to correct a wide variety of DNA lesions. Among the most serious of these lesions are DNA double strand breaks (DSBs) that occur during DNA replication. At a minimum, the faithful repair of such DSBs requires homologous recombination (HR) and the RecQ family of DNA helicases [1]. In humans, the RecQ helicase family consists of RecQ1 and RecQ5, in addition to the proteins responsible for Werner's syndrome (WS; WRN), Bloom's syndrome (BS; BLM) and Rothmund-Thomson syndrome (RTS; RECQ4). All RecQ helicases share a core helicase sequence motif homologous to the RecQ DNA helicase of *E. coli*, and all display 3′–5′ DNA helicase activity *in vitro*. However, WRN, BLM and RECQ4 are further related by the presence of N-terminal domains of approximately 500 amino acids (aa). These N-terminal domains are not fully characterized. Presumably they provide functions that are unique to each protein. However, they may also possess catalytic functions that are conserved within this group of RecQ helicases. The identification of activities associated with the N-terminal domains of these proteins remains an important step in understanding the molecular causes of their respective diseases.

WS is a late-onset progeroid disease in which patients display premature signs of aging, an elevated risk of cancer and cells marked by genomic instability [2–5]. The WRN protein is unique among the RecQ family members in that it contains a 3′–5′ exonuclease domain in its N-terminus [6–10] and the N-terminus contains a coiled-coil domain that is important for proper multimerization *in vivo* [11] (Fig. 1A). BS is associated with low birthweight, immune deficiency and a predisposition to a diverse group of cancers [12,13]. BS cells display an increased rate of sister chromatid exchange (SCE) and an overall increase in genomic instability [14,15]. Thus, loss of these homologous DNA helicases results in some phenotypic similarities. An important difference between WRN and BLM is the role that BLM plays in the "BTR" complex which consists of BLM, Top3α, RMI1, and RMI2 [16–18]. BLM is conserved in most species including the yeast *Saccharomyces cerevisiae* where it is known as Sgs1. Like human BLM,

* Corresponding author at: Department of Molecular Biology and Biochemistry, Rutgers University, 679 Hoes Lane, CABM, Piscataway, NJ 08854, United States. Tel.: +1 732 235 4197; fax: +1 732 445 6186.
E-mail address: brill@cabm.rutgers.edu (S.J. Brill).
[1] Current address: Department of Molecular Biology, Princeton University, Princeton, NJ 08544, United States.

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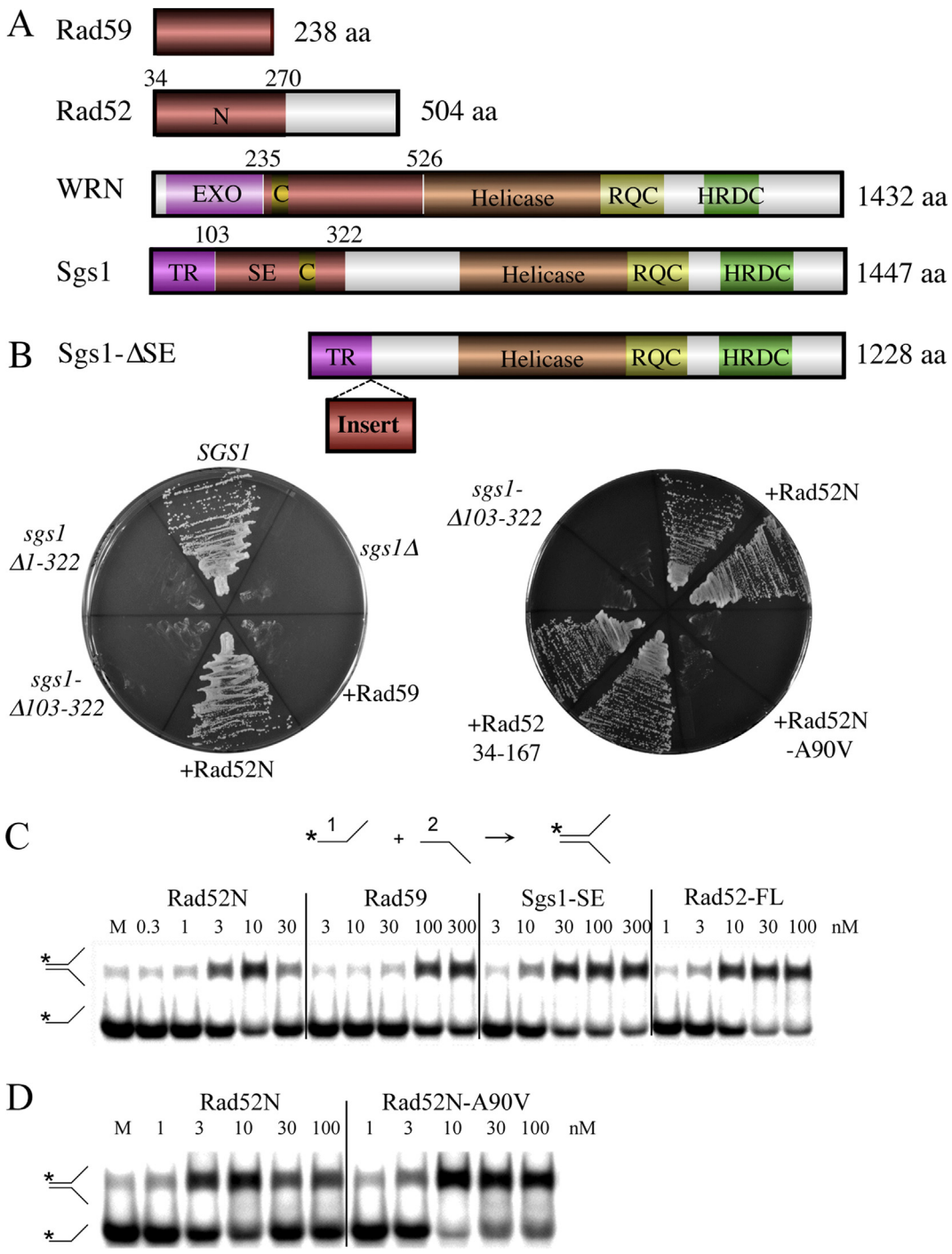

Fig. 1. Complementation of yeast *sgs1Δ* by chimeric Sgs1 fusion proteins. (A) Schematic representations of full-length yeast Rad59, Rad52, Sgs1, and human WRN proteins. Domains: N, N-terminal domain of Rad52; TR, Top3-Rmi1 binding; SE, strand exchange; EXO, 3′–5′ exonuclease domain; C, coiled coil. Note that yeast Rad52 begins at codon 34. (B) Top, The Sgs1-ΔSE protein ($Sgs1_{\Delta 102-322}$) was modified by inserting the red domains from panel (A) at the indicated position to create Sgs1 fusion proteins. Bottom, yeast strain NJY2083 [*sgs1Δ slx4Δ* plus pJM500 (*SGS1/URA3/ADE3/CEN*)] was individually transformed with centromeric *LEU2* plasmids containing the indicated *SGS1* genes, or chimeric alleles expressing Sgs1 fused to the indicated foreign domains (+). To test for synthetic lethality, leucine prototrophs were streaked individually (left) or in duplicate (right) onto plates containing 5-FOA which selects against the balancer plasmid pJM500 [48]. (C) SA reactions contained the indicated concentrations of Rad52N ($Rad52_{34-270}$), Rad59, Sgs1-SE ($Sgs1_{102-322}$), or full-length Rad52 plus 1 nM each of a ^{32}P-labeled 50 nt oligo (#1) and an unlabeled 50 nt oligo (#2) that share 25 nt of perfect complementarity. Following incubation at 37 °C for 5 min, the reactions were stopped as described in Section 2 and the products were resolved by 10% PAGE and subjected to phosphorimager analysis. (D) SA assays were conducted as above using Rad52N and the A90V mutant protein. Asterisks represent positions of ^{32}P-labeling. M, mock reaction without protein.

Sgs1 forms an "STR" complex with its cognate Top3 and Rmi1 subunits [19–26]. The physical interaction between BLM/Sgs1 and the Top3-Rmi1 complex requires a 100 aa domain (TR) at the extreme N-terminus of the helicases (Fig. 1A). BTR and STR act in HR repair pathways where they catalyze a variety of DNA transactions including 5′-end resection and double Holliday Junction dissolution [18,27–33].

In addition to DNA helicase activity, all eukaryotic RecQ family members, regardless of the size of their N-terminal extension, display a ssDNA strand annealing (SA) activity that has not been