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A DNA-translocating Snf2 Molecular Motor: *Saccharomyces cerevisiae* Rdh54 Displays Processive Translocation and Extrudes DNA Loops

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We have used total internal reflection fluorescence microscopy (TIRFM) to investigate the characteristics of the yeast homologous recombination factor Rdh54 on DNA. Our results demonstrate translocation of Rdh54 on DNA and extrusion of DNA loops by Rdh54 in an ATP hydrolysis-dependent manner. The translocating Rdh54 was highly processive and displayed a variety of behavior, including variations in translocation rate and distance, pauses, and reversals. We provide evidence that the DNA loops generated encompass an average of 6 kb, and Rdh54 often abruptly releases the extruded DNA. Rdh54 forms a multimeric complex, which we speculate has at least two functionally distinct DNA-binding sites, one of which enables translocation while the other remains anchored to another DNA locale. Our work, together with other recent studies, suggests that translocation-coupled DNA loop extrusion is a common mechanistic feature among the Snf2-family of chromatin-remodeling proteins.

Keywords: Rdh54; homologous recombination; single-molecule; TIRFM; DNA curtain

Introduction

Rdh54 belongs to the Snf2-family of chromatin-remodeling proteins and is required for mitotic and meiotic DNA recombination.[1,2] The Snf2-family of proteins are structurally related to the *Saccharomyces cerevisiae* chromatin-remodeling protein Snf2. These proteins possess seven conserved motifs labeled I, Ia, Ib, II, III, IV and V.[3,4] Motifs I and II are the Walker A and B nucleotide-binding motifs commonly found in ATP-hydrolyzing enzymes. Snf2 proteins are ubiquitous in nature and are involved in many aspects of DNA metabolism, including chromatin remodeling, DNA replication, transcription, translation, and DNA repair.[5] A recent analysis of public databases by Owen-Hughes and colleagues has classified the Snf2 proteins into at least 24 distinct subfamilies.[5] Some of the better characterized Snf2 proteins are the ATPase subunits of complexes such as SWI/SNF, ISWI, RSC, NURF, ACF, CHRAC, INO80, Swr1, NURD, and the DNA repair protein Rad54. *S. cerevisiae* alone has 17 known Snf2 proteins that have important roles in a broad range of biological processes.[5] Although originally regarded as helicases, these proteins appear to be ATP hydrolysis-dependent motors that translocate along duplex DNA.[6–8]

Rad54 is the defining member of one Snf2 subgroup (the Rad54-like subfamily), and is among the best characterized proteins of the Snf2- family.[9,10] *RAD54* was originally identified in *S. cerevisiae* as a member of the *RAD52* epistasis group that is required for the repair of DNA double-strand breaks *via* homologous recombination, and mutations in *RAD54* lead to increased sensitivity to DNA-damaging agents.[2] The crystal structure of zebrafish Rad54 revealed that the protein has a pair of tandemly repeated RecA-like folds, which contain the seven conserved helicase motifs.[11] Similar domains are found in the SF1 DNA helicases PcrA, UvrD, and Rep, and the SF2 proteins RecG, UvrB, eIF4A, and NS3.[4] It is these conserved RecA-like

Abbreviation used: TIRFM, total internal reflection fluorescence microscopy.

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domains that mediate ATP hydrolysis and DNA translocation. Early work with Rad54 had suggested that the protein was a DNA translocase,[8,12] and this prediction was confirmed in a recent single-molecule study, which demonstrated that Rad54 translocates on double-stranded DNA in an ATP hydrolysis-dependent manner.[13] Rad54 appears to utilize its DNA translocase activity to remove Rad51 from duplex DNA[14] and to process various recombination DNA intermediates by branch migration.[15,16]

Rdh54 (*Rad homolog* 54) is a member of the Rad54-like Snf2 subfamily that was identified on the basis of sequence homology with Rad54, and found independently as Tid1 in a yeast two-hybrid screen for proteins that interact with the meiosis-specific recombinase Dmc1.[1,17,18] The role of *RDH54* in homologous recombination was verified by genetic analyses, which revealed that null mutants are defective in meiotic recombination and crossover interference, and show a deficiency in mitotic recombination, DNA repair, and DNA-damage checkpoint adaptation, thus placing *RDH54* within the *RAD52* epistasis group.[1,17–20] Rdh54 and Rad54 are closely related (37% sequence identity and 55% similarity) and appear to be somewhat functionally redundant. Cells can survive in the absence of either of the two proteins; however, *rad54 rdh54* double mutants exhibit growth defects and are more sensitive to DNA-damaging agents than the single mutants. During normal cell growth, Rdh54 is found at kinetochores and may facilitate communication between the DNA damage and spindle checkpoints.[21] Exposure of cells to γ-irradiation causes Rdh54 to partially redistribute to DNA repair centers, which appear as foci comprised of many different DNA repair and checkpoint proteins.[21] *In vitro* experiments have revealed that Rdh54 is a robust ATPase that modifies the topology of DNA, suggesting that the protein could translocate on duplex DNA.[22] Moreover, Rdh54 promotes Rad51-catalyzed strand invasion of duplex DNA,[22] removes Rad51 and Dmc1 from DNA,[23,24] remodels chromatin *in vitro*, and may help establish the accessibility of chromatinized DNA templates during homologous recombination (Y.H. Kwon and P.S., unpublished results).

To begin probing the functions of Snf2 proteins in DNA repair, we sought to develop a system for visualizing the interactions between Rdh54 and duplex DNA at the single-molecule level. Here, we used total internal reflection fluorescence microscopy (TIRFM)[25] and microscale engineered DNA curtains[26] to observe directly the behavior of quantum dot-labeled Rdh54 complexes as they interact with DNA. We show that Rdh54 behaves as an oligomeric complex that exhibits several modes of interaction with DNA, including stationary binding, ATP hydrolysis-driven translocation, changes in velocity, transient pauses, and changes in translocation direction. We have characterized the collisions between two different complexes of Rdh54 traveling in opposite directions on the same DNA molecule. The colliding Rdh54 complexes are unable to pass one another and neither of the colliding partners is displaced. Rdh54 also promotes the extrusion of large DNA loops in a reversible reaction that is coupled to DNA translocation. The DNA loops could be released in an abrupt event consistent with the sudden loss of contact between the DNA and one of the protein complexes. Loop release could occur *via* a slower process that appeared to arise from backtracking or reversal of the Rdh54. The formation and release of these DNA loops implies a molecular architecture for Rdh54 that must include at least two different DNA-binding sites with distinct biochemical activities to accommodate stationary DNA binding as well as active translocation. Our study provides evidence that DNA loop extrusion represents a common mechanism by which Rdh54 and other Snf2 chromatin-remodeling proteins alter DNA topology to influence the outcomes of various DNA transactions.

Results

"High-throughput," single-molecule assay for viewing Rdh54

We have developed a new technology that allows us to assemble "DNA curtains" at defined positions on the surface of a fused silica microfluidic sample chamber (Figure 1(a)).[26] In brief, a fluid lipid bilayer is deposited onto the surface of the sample chamber and DNA molecules are tethered directly to the bilayer *via* a biotin-neutravidin linkage. The tethered DNA molecules are then organized along the leading edges of microscale diffusion barriers by the application of a hydrodynamic force, which also extends the DNA molecules parallel with the surface of the sample chamber and confines them within the detection volume defined by the penetration depth of the evanescent field (Figure 1(a)). This approach allows us to simultaneously visualize up to hundreds of physically aligned DNA molecules in real time within a single field-of-view using TIRFM (Figure 1(b)).

To visualize the behavior of Rdh54, the protein was labeled with an antibody-coupled fluorescent semi-conducting nanocrystal (quantum dot or Qdot). Quantum dots are an ideal fluorophore for single-molecule imaging because they are extremely bright and they do not photo-bleach on timescales relevant for biological measurements. For labeling, Rdh54 was expressed with an N-terminal thioredoxin tag, and the quantum dots were covalently coupled to anti-thioredoxin antibodies (see Experimental Procedures in the Supplementary Data). Thioredoxin-tagged Rdh54 is functional *in vitro* and *in vivo*,[23] and ATPase assays revealed that Rdh54 was active even in the presence of an excess of either antibody or antibody-conjugated quantum dot, indicating that its bulk biochemical properties were not modified by the labeling procedure (Supplementary Data Figure S3).