study, we demonstrate that the nonspecific ssDNA binding of MutL can be involved in subsequent loading of UvrD helicases onto the ssDNA in a manner independent of ATP hydrolysis of MutL.

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Extremely-Low-Frequency Magnetic Field Induces DNA Double Strand Breaks in Human Cells

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For several decades, we have been exposed to chronic environmental extremely-low-frequency magnetic fields (ELF-MF) from various electric appliances that range the power frequencies of 50-60 Hz. In this study, we aimed to investigate the potential effect and genotoxicity of ELF-MF on human cells. When human cervical cancer cell line (HeLa) and human fibroblast cells (IMR90) were exposed to a 60 Hz magnetic field at intensities of 7-35 militesla (mT) continuously or intermittently, there was no change in cell viability by MTT assays. However, we observed severe double strand breaks (DSBs) in chromosomes of HeLa and IMR90 cells exposed to 60 Hz MF of 7 mT for 10-30 min. The phosphorylated H2AX (γ-H2AX), an obvious DNA double strand break marker, was detected in the chromosomes of these cells by immunofluorescence microscopy and western blots. In addition, ATM and Chk1 kinases in the DNA damage checkpoint pathway were activated in these cells. These results strongly suggest that continuous exposures of human cells to 60-Hz ELF-MF cause genomic instability that may lead to carcinogenesis. This possibility could produce human health issues associated with exposure to ELF-MFs in the occupational and public environments.

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Specificity of *E.coli* SSB Protein Binding To the Chi Subunit of DNA Pol III He and Pria Helicase in the Presence and Absence of ssDNA

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The homotetrameric E.coli single stranded DNA binding (SSB) protein, is a key protein involved in replication, recombination and repair. Its unstructured C-terminal domains (SSB-Ct), which are not required for ssDNA binding, provide the binding site for at least 14 accessory proteins and serve to target these proteins to regions of DNA, where they function (Shereda et al., 2008, *Crit Rev Biochem Mol Biol*, **43**, 289). Here, we present a thermodynamic study of SSB interactions with two such proteins, the Chi subunit of DNA Pol III holoenzyme and the PriA helicase, using Isothermal Titration Calorimetry (ITC). Both proteins interact with SSB via the last 9 amino acids of SSB-Ct with similar moderate affinities and stoichiometries of approximately 4 molecules per SSB tetramer. However, these affinities are somewhat weaker than for PriA and Chi interactions with the corresponding Ct peptide. We ascribe this to an inhibitory effect of the SSB core, which may compete for the binding to SSB-Ct. We find that dT_{70} prebound to the SSB core (forming 1:1 complex) eliminates this inhibitory effect for Chi protein. However for PriA, a much greater binding enhancement (>10 fold) is observed. We discuss a possible origin of this specificity for PriA and the role it may play at initial stages of DNA processing (supported by NIH Grant GM30498).

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Conformational Dynamics of Single RecBCD Molecules

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RecBCD is a multifunctional enzyme possessing both helicase and nuclease activities. It harnesses the energy of ATP hydrolysis to processively unwind DNA. We used an optical-trapping assay featuring one base-pair stability to investigate the mechanism of RecBCD unwinding. Records of RecBCD motion at 6 pN of applied load showed fluctuations [4.1 $\pm$ 0.1 bp, (mean $\pm$ std. err.; freq. bandwidth = 0.1-10 Hz)] substantially above the control records with DNA alone. These fluctuations persisted when the enzyme's forward motion was stopped by removing ATP. Records of RecBCD bound to blunt-end DNA in the absence of ATP showed reduced dynamics (2.4 $\pm$ 0.2 bp), indicating the primary origin of the fluctuations was not due to anchoring via RecBCD. Prior biochemical studies showed that unwinding activity is preceded by an initiation phase consisting of several kinetic steps that generates a 10-nt, 5′-tailed substrate inside the RecBCD-DNA complex that engages RecD's helicase domain. This work also showed that binding to a forked 3′-$(dT)_6$ and 5′-$(dT)_6$ DNA substrate is kinetically equivalent to binding to a blunt-end DNA, while a 3′-$(dT)_6$ and 5′-$(dT)_{10}$substrate bypasses initiation. We found that records of RecBCD bound to these tailed DNA substrates showed fluctuations that quantitatively mirrored our records of RecBCD bound to blunt-end DNA and stopped within a long DNA substrate, respectively. Thus, the onset of large fluctuations in the RecBCD-DNA complex was coincident with that of unwinding activity. The magnitude and frequency of fluctuations increased when the DNA sequence immediately in front of the forked substrate was changed from GC to AT base pairs, consistent with RecBCD transiently translocating along the DNA without ATP hydrolysis. A tightly bound state with reduced dynamics (2.7 $\pm$ 0.1 bp) was observed with ADP-BeF_2. These findings support a ratchet model for RecBCD movement.

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Biochemical Analysis of RuvA-RuvB Complex Formation During Branch Migration of Holliday Junction DNA

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Escherichia coli RuvA-RuvB protein complex promotes Holliday junction branch migration during homologous recombination and recombination repair. RuvA forms tetramer and the two tetramers sandwich planer Holliday junction. RuvB is a member of AAA+ ATPase superfamily and forms a hexameric ring, which acts as a motor protein. The two rings flank the junction by interacting RuvA octameric core and promote branch migration by pumping out DNA duplex through their central cavities. Two models are conceived to explain how the DNA double helices are pulled out through the cavities of the rings. (i) RuvB hexameric rings rotate against RuvA octameric core and the duplexes are moved by interacting with inner surfaces of the rotating RuvB rings. (ii) RuvB hexamric rings are fixed to the RuvA octameric core and the duplexes are moved by interaction with RuvB subunits which undergo sequential conformational changes. Previously, we showed that I150T-RuvB mutant was defective in interaction with RuvA. Here, we show the detailed analysis of the heterooligomer composed of wild type and the mutant I150T RuvB proteins in vitro to clarify which mechanism is employed for the RuvA-RuvB directed branch migration of Holliday junction. In this study, we would like to discuss how RuvA-RuvB promote branch migration of Holliday junction.

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Atomic Force Microscopy Shows that Chi Sequences and SSB Proteins Prevent DNA Reannealing Behind the Translocating AddAB Helicase-Nuclease

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Recombinational repair of DNA breaks requires processing of a DNA end to a 3′-ssDNA overhang. In B.subtilis, this task is done by the helicase-nuclease AddAB which generates ssDNA overhangs terminated at a recombination hotspot (Chi) sequence. This is a substrate for the formation of a RecA nucleoprotein filament that searches for a homologous donor molecule and catalyses DNA strand exchange to promote repair. In this study, we have used AFM to visualize the products of reactions including AddAB and double-stranded DNA molecules. AFM images consistently showed a remaining population of apparently unprocessed dsDNA molecules. The fraction of unprocessed molecules dropped upon addition of increasing concentrations of SSB protein or larger amounts of AddAB protein. Moreover, a larger fraction of DNA molecules were processed to ssDNA when DNA substrates contained the regulatory Chi sequence. Our results are consistent with a model in which the DNA strands reanneal behind the translocating AddAB enzyme. This effect is suppressed by destabilizing the interaction between DNA strands via binding of SSB or multiple AddAB motors, or by the interaction between AddAB and Chi during translocation.