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CRYSTAL STRUCTURE OF EXONUCLEASE RECJ
BOUND TO MANGANESE ION

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RecJ is a 5' to 3' exonuclease specific for single-stranded DNA, and is involved in DNA repair and recombination systems. Recently, RecJ has been highlighted as a key enzyme for the replication-recombination relationship. RecJ can interrupt the replication accompanied with RecQ helicase, when replication fork encounters DNA damage. In addition, RecJ has five characteristic motifs. The homologues containing these motifs are ubiquitous in archaea, prokaryotes, and higher eukaryotes. They form a large family of the predicted phosphoesterases, named by DHH family. However, the structure neither RecJ proteins nor the homologues in the DHH family was available. We here describe the crystal structure of RecJ bound to Mn2+ ion essential for its activity. RecJ has a novel fold, in which two domains are interconnected with a long helix to form a central groove. This groove is composed of conserved residues and positively charged, which may be involved in DNA binding. Mn2+ is coordinated by amino acid residues in the motifs characteristic to the DHH family.

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THE WEAK INTERDOMAIN COUPLING OBSERVED IN
THE 70 kDa SUBUNIT OF HUMAN REPLICATION
PROTEIN A is UNAFFECTED BY ssDNA BINDING

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Replication protein A (RPA) is a heterotrimeric protein that is essential for eukaryotic DNA metabolism. Using heteronuclear NMR methods, we have investigated the domain interactions and ssDNA binding of a fragment from the 70-kDa subunit of human RPA (hRPA70). This fragment contains an N-terminal domain (NTD), which is important for hRPA70/protein interactions, connected to a single stranded DNA binding domain (SSBI) by a flexible linker (hRPA70,326). High correlation coefficients were observed when the amide 'H and r5N chemical shifts of the NTD and SSB1 in hRPA70,326 were compared with two smaller fragments that corresponded to the individual domains, indicating weak interdomain coupling. We also examined the structure of hRPA70t.326 after the addition of three different ssDNA substrates, which induced specific amide 'H and/or t5N chemical shift changes in both the NTD and SSB1. A similar analysis of an hRPA70 fragment containing the NTD and the flexible linker (hRPA701,.68) revealed the NTD interacts directly with ssDNA. Based on this relationship, and other available data, we propose a model where binding between the NTD and ssDNA interferes with hRPA70/protein interactions.

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A NEW DETECTION SYSTEM FOR GENOTOXIC
SUBSTANCES USING EXCINUCLEASE UvrAB WITH
FLUORESCENCE CORRELATION SPECTROSCOPY

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The bacterial UvrABC excinuclease system specifically recognizes various types of chemically induced DNA damage, such as dimerization of nucleotides, base modifications and intercalation. The A2B-heterotrimer of UvrA and UvrB actively scans genomic DNA while hydrolyzing ATP. UvrB forms a highly specific complex with DNA exclusively at the damaged sites that can be used as a marker for primary DNA alterations.
Previously, a detection assay for DNA alterations induced by genotoxic substances was developed. This assay functions similar to an ELISA system with a comparable detection sensitivity.
To further characterize the mechanism of DNA repair, the single molecule technique of fluorescence correlation spectroscopy can be employed. Labelled with Alexa 546, the sole cysteine residue in UvrB allows the observation of changes in diffusion time when UvrB complexes with damaged DNA. The introduction of a second label with Alexa 633 may allow for conformational changes to be measured.
This work may provide a new highly sensitive detection system for DNA alterations on a single molecule scale that can be used in high throughput screening for genotoxic effects ofnew drugs.

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COOPERATIVITY AND OTHER PROPERTIES OF THE DNA BINDING ACTIVITY OF HUMAN 06-ALKYLGUANINE-DNA ALKYLTRANSFERASE (AGT)

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AGT is a small, monomeric protein which is responsible for the repair of potentially mutagenic and cytotoxic alkyl and haloalkyl adducts of DNA at the 06-position of guanine and, to a lesser degree, at the 04-position of thymine. While some details of the stoichiometric and irreversible alkyltransfer process have been elucidated, comparatively little is known of the mechanisms by which AGT binds DNA, recognizes repairable lesions, and dissociates to undergo degradation. Employing electrophoretic mobility assays and analytical ultracentrifugation, we have found the AGT-DNA interaction to be highly cooperative with Xo well in excess of 100. The AGT binding site size varies from over 9.5 base pairs with linearized plasmid DNA to a mere 4 base pairs with short oligonucleotides. And although modifications such as alkylation and mutation result in appropriate changes in binding affinities, the magnitude of the affinity differences is far too small to allow AGT to partition effectively to lesions embedded in the human genome. We conclude, therefore, that since cooperativity strongly influences the in vitro behavior of the protein, it may also influence the in vivo functions of AGT-a protein whose wellstudied homologs in other species lack a cooperative mode of macromolecular assembly.

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ANALYSIS OF DNA DOUBLE-STRAND BREAK REPAIR CAPABILITIES IN PROKARYOTES: ROLE OF DNA FRAGMENT DIFFUSION.

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Eubacteria of the genus Deinococcus have the ability to mend up to one hundred double-strand DNA breaks per chromosome. This ability is remarkable since most prokaryotes can mend no more than two or three double strand DNA breaks per chromosome by

RecA-mediated homologous recombination. Although several novel pathways have been suggested to explain this observation, the analysis of the complete genome of these organisms did not reveal their presence. Because RecA-mediated strand exchange requires that two homologous chromosomal fragments come in close proximity to each other, favorable encounters between diffusing homologous DNA fragments is likely to play a dominant role in the process of DNA double strand break repair. To explore this phenomenon we performed Brownian Dynamics Simulations (BDS) of the individual fragments resulting from DNA double strand breaks. The effect of cell size, genome size, genome copies, temperature and fragment size on the DNA repair capabilities were assessed. To assess the role of diffusion in the overall rate of DNA double-strand break repair, the results of the simulations were compared with experimentally observed time courses of the DNA double-strand break repair process in Deinococcus radiodurans.