as part of the CRISPR defense system. Crystallographic study of this protein illustrates a tetrameric ring structure. Interestingly, monomeric Csn2 is not observed in our solution scattering study. In 2 mM or higher Ca^{2+} concentrations when Csn2 has strong DNA-binding affinity, our SAXS data agree with the calculated scattering from the crystalline tetramer and consequently elucidate the importance of Ca^{2+} ions in its ring structure formation and biological function. When the [Ca^{2+}] is lowered or completely chelated by EGTA, a conformational change followed by higher order oligomerization was observed through direct comparison of the normalized scattering profiles. Analysis of the SAXS data by R_g and P(r) and shape reconstruction shows that the conformational change is consistent with a pore elongation in 1.5 mM Ca^{2+}. These biochemical and biophysical results lead us to propose a physiological model for the functioning of the Csn2 protein upon phage invasion.

1297-Pos Board B189

Phospholipase C Beta reduces the Binding and Cleavage of Oligonucleotides by Component 3 Promoter of RISC

Finly Philip, Suzanne Scarlata.
Stony Brook University, Stony Brook, NY, USA.

Phospholipase C beta1 (PLCβ1), a key component in the classical pathway of G protein signaling system, relays information from G-proteins to downstream effectors upon stimulation of G-protein coupled receptors. Recently we found a novel role for PLCβ1 in gene regulation through TRAX, a protein implicated in RNA interference pathway. TRAX in complex with its protein partner translin, a DNA and RNA binding protein, has nuclease activity. We found that in cells, PLCβ1 selectively reverses the siRNA-mediated down-regulation of certain housekeeping genes such as GAPDH and LDH, but not of Cyclophilin A or Hsp90. Over-expression of TRAX inhibited the PLCβ1-mediated rescue of down-regulation of GAPDH and LDH. Our data in HEK and Hela cells indicate that PLCβ1 might play a role in TRAX-mediated RNA-induced silencing complex (RISC) activities of metabolic proteins. We have carried out in-vitro studies to characterize the biochemical properties of TRAX and translin, and the effect of PLCβ1 on binding and rate of hydrolysis of oligonucleotides by TRAX and TRAX-translin octameric complex, Component 3 promoter of RISC (C3PO). From acrylamide as well as agarose native gel electrophoresis and anisotropy measurements, we observed that translin, TRAX and C3PO exist in multiple oligomeric states that bind DNA and RNA. We were able to determine the cleavage rates for ssRNA by TRAX, translin and C3PO. We found that PLCβ1 reduces the rate of hydrolysis by C3PO through decreasing the affinity of a weak binding site of RNA on C3PO complex. The effect of PLCβ1 is seen at higher concentration of C3PO suggesting a role for PLCβ1 at localized elevated levels of C3PO, as in RISC.

1298-Pos Board B190

Time Dependence of Partitoning between Polymerization and Editing Sites by Klenow Polymerase

Hiromi S. Brown, Vince J. LiCata.
Louisiana State University, Baton Rouge, LA, USA.

Klenow polymerase is the large fragment of DNA polymerase I from *E. coli*. Klenow possesses 5'-3' polymerase and intrinsic 3'-5' exonuclease activities with two distinct active sites that are located ~30 Å apart. During DNA replication, proofreading activity enhances replication fidelity by excising misincorporated nucleotides from the 3' end of primer strand. The first step of the proofreading process is sending the 3'-primer terminus to the proofreading site, which requires separation of the primer terminus from the template strand. Here we examined the binding of Klenow to matched and mismatched primed-template DNA (pt-DNA) by monitoring the steady state fluorescence intensity change of a single 2-aminopurine base site-specifically substituted in the template strand within the duplex part of pt-DNA. The changes in fluorescence intensity allow us to follow shuttling of the primer terminus between the polymerization and proofreading sites.
We have found that the rate of partitioning of the primer between the two active sites depends on: 1) the number of mismatched bases at the primer-template junction, 2) the presence or absence of divalent ions, and 3) the type of divalent ions. Magnesium and calcium ions have opposite effects on the direction of the shift between the pol and exo sites. Substitution of the normal phosphodiester linkage between the last two bases of the primer strand with a non-hydrolysable phosphorothioate linkage also has significant effects on the partitioning between sites.

1299-Pos Board B191

Unraveling the Mechanism of the Primosome Assembly Process

Michal R. Szymanski, Maria J. Jezewska, Wlodek M. Bujalowski.
UTMB, Galveston, TX, USA.

E. Coli primosome is a multiprotein-DNA complex present at the crossroads of DNA replication and repair where it is used to restart stalled replication fork at the damage sites. The assembly and function of the primosome is controlled by synchronized action of seven proteins, PriA, PriB, DnaT, PriC, DnaB, DnaC, DnaG, and nucleic acid. The presence of two helicases: PriA and DnaB, ensures bidirectional mechanical translocation and/or unwinding while DnaG primase synthesizes oligoribonucleotide primers used by DNA polymerase III holoenzyme.
In spite of intensive studies on primosome structure and function over the last two decades, many basic aspects of the primosome assembly process remain ambiguous. Fundamental characteristics, such as the stoichiometry and interactions within the primosome are not well understood. As the result, the molecular mechanism of the assembly process of the primosome is still lacking.
Here, using fluorescence intensity, fluorescence anisotropy titration, fluorescence resonance energy transfer and analytical ultracentrifugation methods, we present direct quantitative analysis of the initial steps in primosome assembly, involving PriA, PriB and DnaT proteins and the minimal primosome assembly site (PAS). The obtained results provide a quantitative framework for initiation of primosome formation and elucidation of further steps in the assembly process.

1300-Pos Board B192

Hoogsteen or not Hoogsteen? Iodine-125 Radioprobing of the P53-Induced DNA Deformations

Victor Zhurkin, Valery Karamychev, Difei Wang, Sharlyn Mazur,
Ettore Appella, Ronald Neumann, Igor Panyutin.
NIH, Bethesda, MD, USA.

Radioprobing is suitable for tracing the DNA and RNA trajectories in nucleoprotein complexes in solution. The method is based on analysis of the single-strand breaks produced by decay of iodine-125 incorporated in the C5 position of cytosine.
Two recently crystallized p53-DNA complexes have different conformations of the CATG motifs: one with the Hoogsteen A:T pairs (Shakked et al., 2010) and the other with the Watson-Crick pairs (Chen et al., 2010). The two complexes differ in the sequence of the central YYY|RRR junction: the first one has the C|G step and the second one has the T|A step.
Thus, it is interesting to apply the radioprobing method to the two DNA sequences used in crystallography, to see if the local changes (T|A to C|G) in the center of the p53 response element would produce significant distortions in the CATG motifs. To this aim, the iodine-containing cytosine, C*, was incorporated in the duplexes containing p53-binding sites, in one of the two CATG motifs, and the frequencies of DNA breaks were analyzed. Frequencies of breaks negatively correlate with the iodine-sugar distances, therefore, one can evaluate the changes in DNA conformation upon binding to a protein. The radioprobing distances thus obtained proved to be consistent with the Watson-Crick structure observed by Chen et al. That is, our data testify against formation of the Hoogsteen A:T base pairs – the C*ATG motifs retain canonical B-DNA conformation in both DNA sequences.
The most significant changes in the break frequency distributions were detected in the central segment of the p53 binding site, YYY|RRR, which is consistent with an increase in DNA twisting in this region and local DNA bending and sliding. We interpret these p53-induced DNA deformations in the context of p53 binding to nucleosomal DNA.

1301-Pos Board B193

DNA Looping Induced by Tubular Confinement

Robert Riehn, Maedeh Heidarpour-Roushan.
North Carolina State University, Raleigh, NC, USA.

DNA looping is essential for the function and maintenance of genetic information. At short lengths the cyclization rate is limited by the semiflexible properties of DNA, while at long distances it is limited by the rapidly declining probability of colocation of the anchoring sequences of the loop. We propose that a facilitated diffusion process increases the rate of cyclization for large loops. The maze-like nuclear environment then becomes a major impediment for the required colocation.
We have investigated the kinetic evolution of DNA loops (48500 bp) induced by T4 ligase inside a nanofabricated channel system with a channel cross-section of 100x100 nm2, and a few micron channel length. We found that addition of the ligase profoundly alters the behavior of DNA. In particular, ligase acts to stabilize hairpin geometries in which the extended forward and backward arms of the hairpin scan past each other. From the linear density of DNA inside the channel, we deduce that the effective excluded volume vanishes upon addition of T4 ligase and ATP.