have determined the structure of the Rep protein from cryptic plasmid pSTK1 of *Geobacillus stearothermophilus* (*Gst*), and several variants of RepD from *Staphylococcus aureus* (*Sau*), representing the first structural information on this class of initiators.
Cloning and expression of a construct derived from the from pSTK1 Rep yielded a product that relaxed plasmid substrates encoding an inverted repeat sequence from pSTK1, which resembles the replication origin of the pT181 family, and activated the cognate *Gst* PcrA helicase. The crystal structure for the 31 kDa fragment of *Gst* Rep has been solved at 2.3 Å, showing a novel, ring-shaped dimer with a 20Å diameter pore. The inner surface is formed by an 18-stranded β-sheet, while the outer surface is decorated with 18 α-helices. The protein has a novel fold, but the extended sheet exhibits similarities to that in TATA-binding protein (TBP). The active site Tyr179 residues, one from each subunit, lie 26 Å apart across the pore, with a nearby catalytic magnesium ion co-ordinated by three carboxylate side-chains.
Crystal structures for the *Sau* Rep variants RepDE, RepDN and RepDC have been solved by molecular repacement using the *Gst* Rep as a model, and show similar structural features. The implications for the mechanism of rolling circle replication will be discussed in the light of extensive functional data available for *Sau* RepD.

380-Pos Board B149
DNA Replication Initiation Studied at the Single Molecule Level
Hsin-Mei Cheng[1], Philip Gröger[1], Andreas Hartmann[1], Elena M. Seco[2], Silvia Ayora[2], Michael Schlierf[1].
[1]B CUBE – Center for Molecular Bioengineering, TU Dresden, Dresden, Germany, [2]Centro National de Biotechnología, (CNB-CSIC), Madrid, Spain.
DNA replication initiation is mediated by initiator proteins across all domains of life. Initiator proteins form oligomers at replication origin sites and melt the AT-rich region to enable the replisome assembly. The origin melting by the initiator proteins is supposed to be enhanced by the interaction with single-stranded DNA. In this study, we use single-molecule FRET together with hidden Markov modeling to probe the single-stranded DNA binding mechanism of the *Aquifex aeolicus* replication initiator DnaA. We find that in the presence of the ATP-analog ADP•BeF_3, the DnaA oligomer assembly and disassembly occurs one monomer at a time. Our FRET measurements further indicate a curly filament of DnaA monomers on ssDNA that supports the model of negative super-helical strain promoting efficient melting of the AT-rich region. We further test ssDNA binding with an ATP-independent replication initiator G38P from bacteriophage SPP1 and find as well a dynamic assembly and disassembly on ssDNA.

381-Pos Board B150
Impact of Individual PCNA-DNA Contacts on Clamp Loading and Function on DNA
Yayan Zhou.
Wesleyan university, middletown, CT, USA.
Ring-shaped clamp proteins encircle DNA and affect the work of many proteins, notably processive replication by DNA polymerases. Crystal structures of clamps show several cationic residues inside the ring, and in a co-crystal of E. coli β clamp-DNA they directly contact the tilted duplex passing through. To investigate the role of these contacts in reactions involving circular clamps, we examined single arginine/lysine mutants of S. cerevisiae PCNA in RFC-catalyzed loading of the clamp onto primer-template DNA (ptDNA). Previous kinetic analysis has shown that ptDNA entry inside an ATP-activated RFC-PCNA complex accelerates clamp opening and ATP hydrolysis, which is followed by slow PCNA closure around DNA and product dissociation. Here we directly measured multiple steps in the reaction–PCNA opening, ptDNA binding, PCNA closure, phosphate release and complex dissociation to determine if mutation of PCNA residues R14, K20, R80, K146, R149 or K217 to alanine affects the reaction mechanism. Contrary to earlier steady state analysis of these mutants, pre-steady state data show that loss of single cationic residues can alter the rates of all DNA-linked steps in the reaction as well as movement of PCNA on DNA. These results explain an earlier finding that individual arginines and lysines inside human PCNA are essential for pol δ processivity. Mutations in the N-terminal domain have greater impact than in the C-terminal domain, indicating a positional bias in PCNA-DNA contacts that can influence its functions on DNA.

382-Pos Board B151
Simultaneous Interaction of *E. Coli* Single Stranded DNA Binding Protein and Replicativedna Polymerase III Alpha Subunit with Single-Stranded DNA Molecules
Kiran Pant, Susie Nimitpattana, Michelle C. Silva, Jana Sefcikova, Penny J. Beuning, Mark C. Williams.
Northeastern University, Boston, MA, USA.
The *E. coli* single stranded DNA (ssDNA) binding (SSB) protein binds to ssDNA in multiple binding modes and regulates DNA replication via protein-protein interactions. DNA polymerases are enzymes responsible for DNA replication. The alpha subunit of the replicative DNA polymerase III (Pol III) is the DNA polymerase subunit of the 10-subunit bacterial replicase, responsible for DNA replication. It has been shown that replication by the Pol III core is inhibited by SSB and can be relieved by the version of the clamp loader that contains the tau subunit, suggesting that DNA polymerase III is inhibited by SSB unless the replisome complex at the replication fork is fully assembled. Previous work suggests that the mechanism of inhibition is a direct competition between SSB and DNA Pol III core for DNA. SSB inhibits replication by Pol III alpha subunit. We have further shown that there is a direct physical interaction between alpha subunit and SSB. Based on these observations, we hypothesize that the inhibition of Pol III alpha subunit by SSB is due to a specific feedback mechanism facilitated by the direct interaction of the two proteins. To test this hypothesis, we have shown Pol III alpha subunit stabilizes SSB binding to ssDNA using single molecule force-spectroscopy. In the absence of Pol III alpha subunit, SSB stabilizes ssDNA below 20 pN and fully dissociates above 20 pN. However, in the presence of Pol III alpha subunit, SSB does not dissociate above 20 pN and the energy required to dissociate SSB from ssDNA is increased by a factor of two. Therefore, the inhibition of Pol III alpha subunit by SSB is due to a specific interaction between the two proteins.

383-Pos Board B152
Towards Efficient Reconstitution of the Human Mitochondrial DNA Replication Complex
Yufeng Qian, Jessica L. Ziehr, Kenneth A. Johnson.
University of Texas at Austin, Austin, TX, USA.
The minimal human mitochondrial DNA replisome, comprising mitochondrial DNA polymerase (Pol γ), mitochondrial DNA helicase (mtDNA helicase) and mitochondrial single-stranded binding protein (mtSSB) was reconstituted. Assembly of these replication factors on the minicircle template, resembling the replication fork in vivo, supported the rolling-circle DNA replication. Quantitative analysis of the product formed from the minicircle template with the radioactive label on the primer strand or on the incoming ribonucleotides yielded consistent results, which demonstrated the poor efficiency (less than 1% active fraction) of the reconstituted replisome described (Korhonen et al., 2003). We sought to screen conditions to optimize the assembly of the replisome. The optimized assembly of replisome improves the replication efficiency to ~14%. The relative low efficiency may indicate a poor assembly of mtDNA helicase or that the mitochondrial replisome in vivo comprises unidentified essential components. Notably, we found that stimulation of processive DNA synthesis by mtSSB occurs over a narrow range of salt concentration. We obtained more quantitative reconstitution the human mitochondrial replisome using a small synthetic replication fork and measured the kinetics of coupled DNA unwinding and polymerization. Optimal reconstitution was achieved by preincubation of the DNA with the polymerase, helicase and two of the four deoxyribonucleotides. Following the addition of the remaining two nucleotides, 80% of the primers were extended with a half-life of 84 seconds.

384-Pos Board B153
Werner Syndrome Protein Forms a Bidirectional Fork Regression Motor Whose Direction is Switched at DNA Modifications
Jinwoo Lee[1], Sangwoon Yoo[1], Tomasz Kulikowicz[2], Vilhelm A. Bohr[2], Byungchan Ahn[3], Sungchul Hohng[1].
[1]Seoul National University, Seoul, Korea, Republic of, [2]National Institutes of Health, Baltimore, MD, USA, [3]University of Ulsan, Ulsan, Korea, Republic of.
During DNA replication, the replication machinery frequently stalls at various DNA damages. Regression of stalled replication forks by ATP-driven molecular motors is an indispensable process for reactivation of the stalled replication. To elucidate the fork regression mechanism of Werner syndrome protein (WRN), we studied fork regression dynamics of single WRN molecules at model replication forks. Surprisingly, WRN is a bidirectional motor which dynamically switches its branch migration direction at various DNA modifications. Our data suggest that WRN might work as a coordinator of the whole reactivation process of the stalled replication fork

385-Pos Board B154
Probing Rad51-DNA Interactions by Changing DNA Twist
Scott Atwell[1], Ludovic Disseau[1], Alicja Z.Z. Stasiak[2], Andrzej Stasiak[2], Axelle Renodon-Cornière[3], Masayuki Takahashi[3], Jean-Louis Viovy[1], **Giovanni Cappello**[1].
[1]Institute Curie, Paris Cedex 05, France, [2]Centre Intégratif de Génomique, Université de Lausanne, Lausanne, Switzerland, [3]Unité Fonctionnalité et Ingénierie des Protéines, Université de Nantes, Nantes, France.