Understanding every phase of the retrovirus lifecycle is critical to developing means to treat retroviral infection. This study focuses on the early stages of retroviral assembly and specifically investigates interactions of the Gag structural protein common to all retroviruses. To elucidate the various roles that Gag plays in virus assembly in vivo, Gag-Gag interactions were quantified with fluorescence fluctuation spectroscopy (FFS) on the single molecule level. Experiments focused on the self-associative behavior of HIV and HTLV Gag-YFP and various Gag-YFP mutants in the cytoplasm, which characterize the earliest events initiating viral assembly. The Gag mutants were chosen based on previous studies that confirmed their impact on Gag behavior. HIV and HTLV Gag exhibit differences in oligomerization dependent membrane-targeting involving the myristoyl moiety. In summary, FFS provides in vivo molecular-level information that sheds light on the retroviral assembly pathway. This work is supported by NIH Grant AI81673 and a Cancer Center Cancer Biology Training Grant (T32CA09138).

3414-Pos

A Mechanochemical Model of a Viral DNA Packaging Motor

Jin Yu, Jeffrey Moffitt, Craig Hetherington, Carlos Bustamante, George Oster.
UC Berkeley, Berkeley, CA, USA.

Packaging the genome of a virus into its capsid is a crucial step in viral assembly. The genome of bacteriophage phi29 consists of a linear double-stranded DNA (dsDNA) of about 19,000 base pairs (bps). Packaging a dsDNA this long results in a near-crystalline state inside the ~50 nm length capsid and requires a great deal of energy. The feat is performed by a multimeric molecular motor that derives its energy from ATP hydrolysis and generates forces more than 60 pN. Experimental studies on the phi29 packaging motor have been carried out through single-molecule manipulation techniques using optical tweezers. The DNA packaging proceeds in bursts of four 2.5-bp translocation power strokes upon Pi releases. The translocation is also accompanied by the DNA rotation. From the data we have constructed a mechanochemical framework to explain how this motor packages DNA. The model is built around 'push-and-roll'mechanism that suggests how the motor subunits interact with the DNA and how the DNA passes through the motor ring. We also propose how the five subunits are coordinated around the ring. Our model provides a new perspective on how multimeric ATPases transport nucleic-acids, and it may be applied to other ring motors.

3415-Pos

Swelling and Softening of the CCMV Plant Virus Capsid in Response to pH Shifts

Bodo D. Wilts[1], Iwan A.T. Schaap[1], Mark J. Young[2], Trevor Douglas[2], Charles M. Knobler[3], **Christoph F. Schmidt[1]**.
[1]Third Institute of Physics, Georg August Universität, Gottingen, Germany, [2]Center for Bio-Inspired Nanomaterials, Montana State University, Bozeman, MT, USA, [3]Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA.

Previous research on cowpea chlorotic mottle viruses (CCMV) has revealed a swelling transition and a softening of the protein capsid in response to a pH increase. In this study, we have performed nano-indentation experiments using an atomic force microscope and tested the shell response at low (4.8) up to high pH (7.5) in the absence of divalent ions. We could, for the first time, study the elastic behavior of the swollen virions. Indentations were performed in the reversible linear regime with indentation forces up to 200 pN. The results show a gradual swelling transition of the RNA-filled capsids preceded by a softening of the shell as a function of pH. Control measurements with the empty wt-virus and a salt-stable mutant revealed that the softening is not directly coupled to the swelling of the protein shells. Instead we hypothesize that the softening of the CCMV virions is triggered by pH-dependent opening of bonds within the protein shell which may be necessary, but not sufficient for swelling.

3416-Pos

Dissecting Lambda Terminase: A Viral DNA Packaging Motor

Benjamin T. Andrews[1], Junqing Zhou[1], Jean S. Arens[2], Michael G. Feiss[2], Carlos E. Catalano[1].
[1]University of Washington, Seattle, WA, USA, [2]University of Iowa, Iowa City, IA, USA.

During virus assembly, a single genome is packaged into a preformed capsid by a terminase enzyme. Presently, the mechanistic details of the packaging motor are unknown. Single molecule experiments have demonstrated that viral DNA packaging motors are among the most powerful currently known, capable of packaging dsDNA to over 20 atmospheres within the capsid. Therefore, the mechanistic details of DNA packaging are of interest from both a biological (drug target), and engineering (nanomachine) standpoint. Bacteriophage Lambda (λ) is a model system for the study of dsDNA viruses, including herpesvirus and many bacteriophage. λ terminase is a multifunction enzyme complex with catalytic activities required to (1) recognize the viral genome, (2) prepare the viral genome for packaging, (3) recognize the empty procapsid, and (4) translocate viral DNA into the empty procapsid. The enzyme consists of two proteins, gpA and gpNu1 in a 1:2 ratio known as the heterotrimeric protomer; the motor functions as a tetramer of protomers assembled into a ring complex. Here, we study the λ terminase *in vitro* and characterize macromolecular assembly, genome packaging, ATPase and DNA-cleavage activities of several mutants which are catalytically deficient. These mutants include K76R, which is a mutation in the packaging ATPase site, T194M, which packages at a 10-fold slower rate *in vivo*, and G212S, which stalls during packaging. We characterize protomer self-assembly into the tetrameric motor and we compare the catalytic activities of the mutants relative to wild-type. The mechanistic implications of this work are discussed.

DNA, RNA Structure & Conformation III

3417-Pos

Targeting DNA Hairpin Loops with their Partially Complementary Strands

Hui-Ting Lee, Carolyn Carr, Hollie Siebler, Irine Khutsishvilli, Luis A. Marky.
U. of Nebraska Medical Center, Omaha, NE, USA.

Antisense, antigene and siRNA strategies are currently used to control the expression of genes. To this end, our laboratory is currently mimicking the targeting of mRNA by targeting DNA hairpin loops with their partially complementary strands. We use a combination of differential scanning calorimetry (DSC) and temperature-dependent UV spectroscopy to investigate the reaction of a variety of DNA hairpin loops (single end loops, dumbbell, three-way junction with two loops and a hairpin with a bulge of 5 nucleotides) with single strands that are complementary to the bases in the loop and to one strand of their stem. The resulting reaction products form duplexes with dangling ends, nicks or with a displaced strand. We determine standard thermodynamic profiles for the unfolding of the reactants (hairpin loop) and products (duplex) of each reaction. The DSC and UV melting curves show monophasic transitions for the unfolding of all DNA single hairpin loops (reactants) and biphasic transitions for the unfolding of the double hairpin loops (reactants) and duplex products. The resulting unfolding data is then used to create thermodynamic (Hess) cycles that correspond to each targeting reaction. All eight targeting reactions investigated yielded favorable free energy contributions that were enthalpy driven. These favorable heat contributions result from the formation of base-pair stacks involving the unpaired bases of the loops, indicating that each single strand was able to disrupt the hairpin loop structure. Supported by Grant MCB-0616005 from NSF.

3418-Pos

Effect of Loop-Closing Residues on DNA Hairpin Stability

Nancy C. Stellwagen, Chun Yaw Chang.
University of Iowa, Iowa City, IA, USA.

Structure-prediction algorithms, such as DINAMelt (1), are often used to analyze hairpin formation in single-stranded DNA and RNA oligomers. In general, an oligomer and its complement are predicted to form hairpins with similar stabilities. Here, we have used capillary electrophoresis to analyze hairpin formation in two complementary 26-nucleotide DNA oligomers that exhibit significantly different free solution mobilities at 20°C. The free solution mobility is a useful indicator of the presence or absence of hairpins, because hairpins and random coils containing the same number of nucleotides have different frictional coefficients, leading to different mobilities (2). The two oligomers studied here are predicted to form molecular beacon-like hairpins with 5 base-pair stems and 16-nucleotide loops at 20°C. The oligomer with the higher free solution mobility forms a stable hairpin with melting temperatures that are reasonably well predicted by DINAMelt, especially at high salt concentrations. However, its complement, which migrates more slowly in free solution, exhibits melting temperatures that are significantly lower than predicted by DINAMelt. Stable hairpins are observed at 20°C only in solutions with Na^+ concentrations greater than 200 mM. Since the two oligomers have the same predicted stem sequences, the results suggest that differences in the nucleotides closing the two loops are responsible for the differences in stability of the two hairpins. Hairpin formation in oligomers with different base pairs at the top of the stem and different nucleotides closing the loop are being investigated.

1. N. R. Markham, M. Zuker, Nucleic Acids Res. 2005 33, W577.
2. E. Stellwagen, A. Abdulla, Q. Dong, N. C. Stellwagen, Biochemistry 2007, 46 10931.