Saturday, March 26, Session VIII

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MECHANISM OF REPLICATIVE "ROLLING-CIRCLE" DNA TRANSPOSITION IN EUKARYOTES

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Evolution is driven forward by the acquisition of novel traits which rise as a consequence of changes in the heritable genome. There are many ways how changes in DNA could occur, including recombination, replication errors, inaccurate repair or chemical modifications. One of the most important drivers of such changes is transposition, the protein-catalyzed transfer of discrete mobile DNA elements from one site into another. These „selfish" DNA elements (transposons) often comprise a vast amount of non-coding genome in eukaryotic species and have contributed to their evolution in many ways. Their movement not only disrupts and reorders DNA but it can also provide regulatory sequences that enable the establishment of novel expression patterns providing an evolutionary framework for rapid adaptation in unfavorable environment [1].

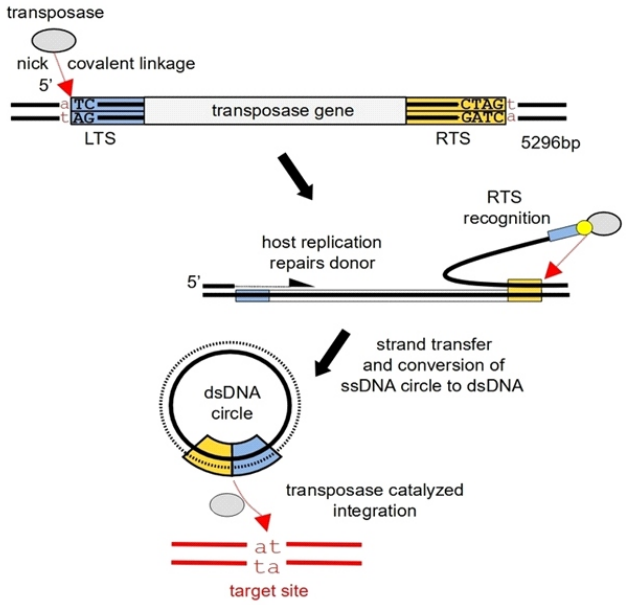

Figure 1. Cartoon schematic of the reconstituted *Helitron* transposon with current model for *Helitron* transposition. *Helitron* is bordered by 150 bp of LTS (left terminal sequence; in blue throughout) and 150 bp of RTS (right terminal sequence; in yellow throughout). Red arrows indicate cleavage or strand transfer reactions. Yellow circle marks a covalent linkage.

Helitrons are ancient DNA transposons that have dramatically reshaped many eukaryotic genomes due to their large numbers and propensity to capture and mobilize host gene fragments. They have contributed to genome diversity with their unusual replicative transposition mechanism that, unlike the better-characterized cut-and-paste DNA transposons, relies on ssDNA intermediates. The current model for the *Helitron* transposition mechanism suggests that the initial nicking at the left transposon end generates free 3'-OH group which primes the DNA replication displacing the leading transposon DNA strand. When the other end is reached, a second cleavage reaction and strand transfer generate an excised circular *Helitron* intermediate. This can then be integrated at a new genomic location (Figure 1). Here, we describe the cryo-EM structure of the monomeric transposase from the recently reconstituted active *Helitron* element [2] covalently bound to the 5'-end of the transposon ssDNA (Figure 2). This represents the first structural view into the molecular architecture and function of *Helitron* transposases, the largest transposases known to date. It reveals how *Helitrons* have solved the problem of uncoordinated reactions on its two transposon ends: the transposase protects the initially cleaved and displaced end by forming a tightly packed assembly that buries covalently bound ssDNA in the core of HUH nuclease and Pif1-like helicase domains with a scaffold formed by other surrounding domains, some with apparently unique protein folds. Despite the lack of evidence for a direct evolutionary link, the architecture is reminiscent of that seen in TraI, a prokaryotic relaxase involved in bacterial horizontal gene transfer. Our work also suggests the structural basis of the interplay between two juxtaposed active site tyrosines that alternate in the roles of the catalytic nucleophiles in the HUH active site; this result is likely generalizable to all proteins that use an HUH Y2 nucleases to initiate replication [3]. We have combined the structural work with *in vitro* biochemical studies that probe the role of the two active site tyrosines as well as *in vivo* transposi-

tion assays that suggested different roles of each tyrosines during the transposition.

This work presents the first three-dimensional insight into a large and important superfamily of eukaryotic transposases [4]. The structure breaks the paradigm that DNA transposases must function as multimers, thus, expanding the conceptual framework for understanding the mechanisms of transpositional DNA rearrangements. It also represents the first structural insight into the coupled actions of an HUH-type nuclease and a helicase – poorly understood arrangement widespread not only in relation to the transposition but also viral replication and propagation of antibiotic resistances. Also, in principle a monomeric transposase opens up possibilities to novel genome targeting strategies simpler than those that have been recently attempted with multimeric transposase systems.

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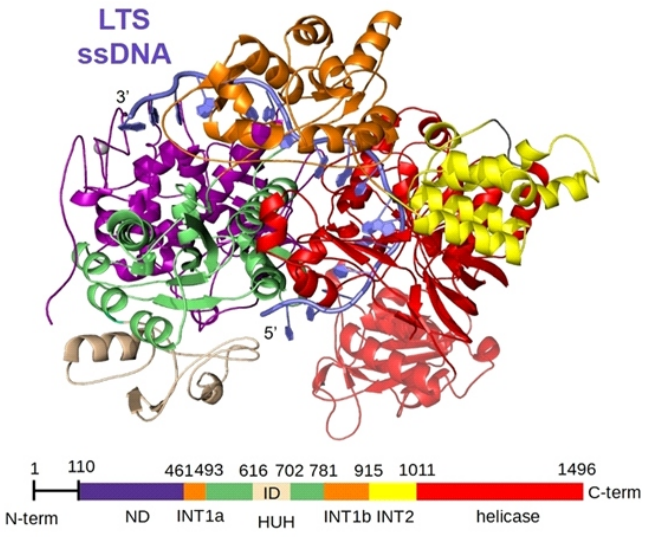

Figure 2. Cartoon view of cryo-EM determined structure of *Helitron* transposase in complex with ssDNA of LTS (in blue) with domains colored as indicated in the schematic below. ND, N-terminal domain; INT1, intermediate domain 1; HUH, catalytic HUH domain with insertion (ID); INT2, intermediate domain 2.

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HOW VIRUSES AND VIRUS-LIKE NANOPARTICLES CAN RELEASE THEIR CARGO/GENOME

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Viruses and virus-like nanoparticles both aim to deliver their content into a cell. Unfortunately, the necessary capsid properties enabling cargo/genome release and the release mechanism itself remains elusive. We combine in vitro cryo-EM experiments with coarse-grained simulations to demonstrate that the cargo/genome can be released in various pathways, including a slow release via small pores in the capsid and a rapid release when the capsid cracks open [1,2,3]. The main capsid property determining the release pathway is the interaction range between capsid subunits. The release success rate depends on the cargo/genome properties, but in general, the rapid release is more successful. These findings indicate how to affect and design the release of cargo/genome from viruses and virus-like nanoparticles.

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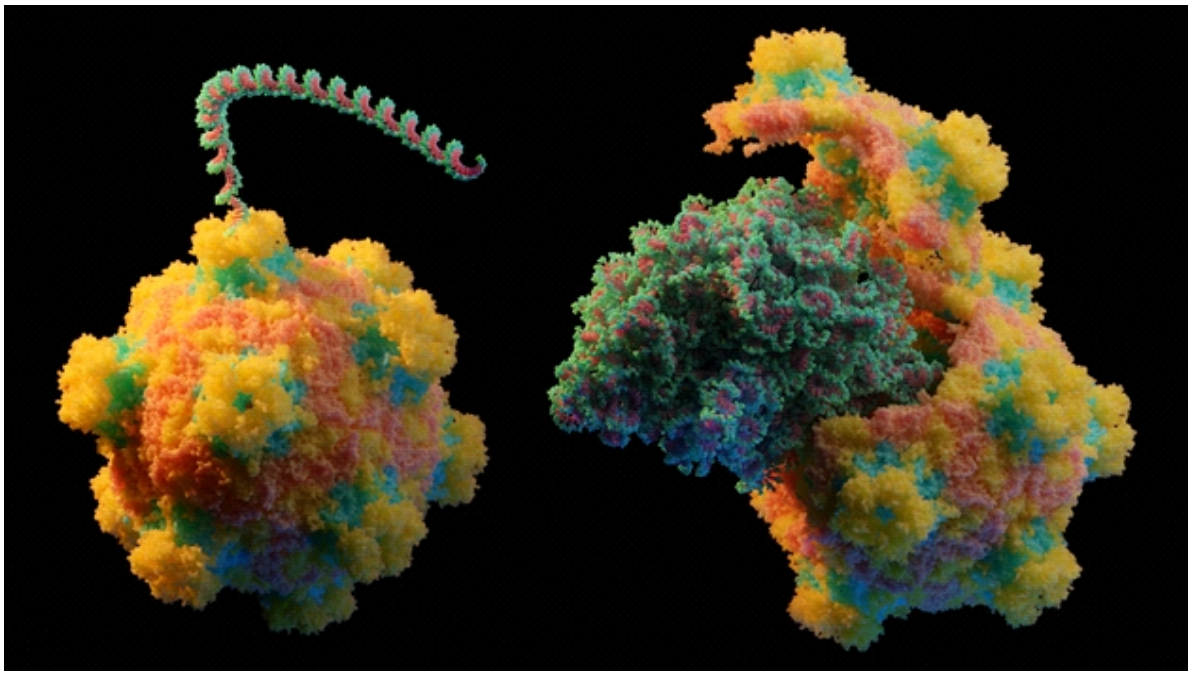

Figure 1. Two types of genome release from non-enveloped RNA virus: a slow release via small pore in the capsid (left) and a rapid release when the capsid cracks open (right).

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STRUCTURE OF TICK-BORNE ENCEPHALITIS VIRUS IMMATURE PARTICLE SOLVED BY CRYO-ELECTRON MICROSCOPY AND SUB-TOMOGRAM AVERAGING

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Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. During the viral life cycle, the immature non-infectious virus undergoes a maturation process. This process includes proteolytic cleavage of prM and major reorganization of the envelope proteins on the viral surface.

To determine the structure of immature TBEV particles, we purified them from infected tissue culture cells and used cryo-electron microscopy for visualization. In comparison with smooth mature TBEV particles, the immature particles have "spiky" surface formed by the E-protein—prM-protein complex. Because of non-icosahedral arrangement of the immature virus surface, single particle analysis methods did not lead to high resolution electrostatic potential maps. To improve the quality of the maps, we employed cryo-electron tomography and sub-tomogram averaging of single "spikes" from the immature particle surface, combined with extensive 3D-classification of the sub-tomograms.

The results show more detailed insight in the viral maturation process which may be targeted by specific antiviral drugs.

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CRYO-EM OF MOUSE RNASE III–RNA COMPLEXES

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The RNase III called Dicer generates small RNAs that post-transcriptionally silence the expression of certain genes, and this regulation is essential for development and normal physiology. Models for small interfering RNA (siRNA) and microRNA (miRNA) processing by vertebrate Dicer have emerged from structural and biochemical studies, yet the active dicing state in Dicer-RNA structures has not been observed and characterized. We used cryo-electron microscopy to determine the structures of mouse Dicer and Dicer^{O}, an oocyte isoform lacking the HEL1 module, alone and in complex with pre-miR-15a RNA. The apo-structure of Dicer showed that HEL1 stabilizes a closed conformation, and its absence promotes helicase opening for substrate engagement into a dicing state. Consequently, Dicer^{O} with pre-miR-15a exclusively forms an active dicing-competent conformation, whereas the structure of Dicer–pre-miR-15a complex captures Dicer in a pre-dicing state. We show that the absence of HEL1 not only activates Dicer but also alters substrate selectivity by facilitating direct substrate loading into the catalytic site and curtailing pre-dicing state, which serves as a selectivity filter for the microRNA precursor architecture.

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CRYO-EM ENSEMBLE REVEALS THE MECHANISM OF +1 RIBOSOMAL FRAME SHIFTING

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To accurately synthesize a protein, the ribosome maintains the mRNA reading frame by decoding and translocating one codon at a time [1]. Change of the reading frame of mRNA during translation, termed frame shifting, provides a strategy to expand the coding repertoire of cells and viruses [2]. The translating ribosome switches to an alternative reading frame, either in the forward (+) or reverse (–) direction, i.e., skipping or re-reading one or more mRNA nucleotides, respectively. For example, +1 frame shifting (+1FS) controls the expression of the essential release factor 2 in bacteria [3] and leads to pathological expression of huntingtin in eukaryotes [4]. How and where in the elongation cycle +1FS occurs remains poorly understood.

Here we address this challenge by using cryo-EM to visualize +1FS on +1FS-prone mRNA sequences. We present cryo-EM structures of 70S complexes, allowing visualization of elongation and translocation by the GTPase elongation factor G (EF-G). Four structures with a?+1FS-prone mRNA reveal that frame shifting takes place during translocation of tRNA and mRNA. The +1FS-prone pre-translocation complex maintains the 0-frame anticodon–codon pairing resembling that in canonical elongation complexes. In the mid-translocation complex with EF-G, the tRNA shifts to the +1-frame near the P site, with bulged nucleotide between the E and P-site codons stabilized by G926 on the 16S rRNA. The ribosome remains frame shifted in the nearly post-translocation state. Our findings reveal that the ribosome is pre-disposed for +1FS before translocation, and that frame shifting is accomplished at an intermediate stage of EF-G catalysed translocation (Fig. 1).

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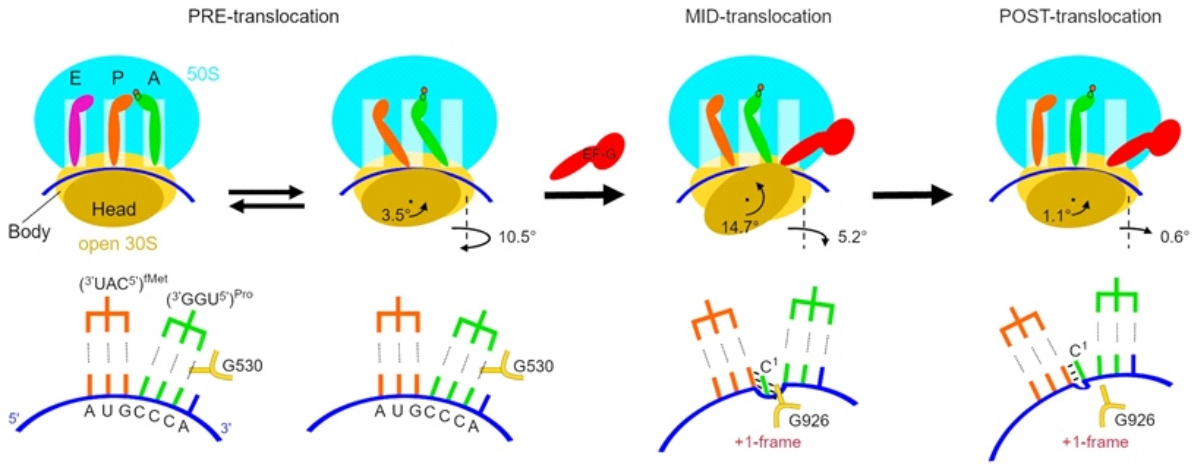

Figure 1. Schematic of ribosomal translocation by EF-G resulting in +1 frame shifting. The second row shows local rearrangements of mRNA-tRNA and positions of the decoding-center nucleotide G530 and P-site nucleotide G926 of the 30S subunit.