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Cryo-ET Structural Studies of Ty1 Retrotranspon Capsids

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The *Saccharomyces* Ty1 retrotransposon assembles into virus-like particles (VLPs) comprised of the Gag and Gag-Pol proteins, and belongs to the widely-disseminated Ty1/Copia family [1]. Studies of Ty VLP structure and function have provided important insights into the structure, function, and evolution of retrovirus capsids (CA) and eukaryotic retrotransposons [2, 3, 4, 5]. Interestingly, Ty1 VLP assembly is inhibited by a novel self-encoded restriction factor (p22) containing the conserved CA carboxy-terminal-domain. This defense system helps prevent unabated cycles of retrotransposition [2, 3, 4]. Scanning transmission electron microscopy [6] and cryo-electron microscopy (cryo-EM) studies [6,7] of Ty1 VLPs identified significant variation in particle morphology and size. Data from these studies indicated that full-length and C-terminally truncated forms of Ty1 assemble into particles with a range of icosahedral T-numbers.

To address the challenge of particle heterogeneity, we used cryo-electron tomography (cryo-ET) to study the structure of the Ty1 VLPs assembled in the absence of p22. Unlike single-particle cryo-EM, which relies on averaging identical copies of the same subject in various orientations, cryo-ET provides 3D information about a single particle of interest. Sub-volume averaging can be used to align and average identical particles within the volume to further increase the signal-to-noise and ultimate resolution of the subject of interest.

VLPs were purified from a Ty1-less yeast strain overexpressing wildtype full-length Ty1. Negative stain EM was used to determine particle purity and concentration prior to preparation of cryo-EM grids. As expected, we observed significant heterogeneity in particle size and morphology (Figure 1). The significant variation among particles did not allow for sub-volume averaging of full VLPs. Instead, we used sub-volume averaging to determine the structure of sub-regions of the VLPs. Individual VLPs were selected and included for averaging at a number of random orientations and masking was used to focus alignment on a sub-region of the VLP. Subsequent rounds of alignment were performed with limited angular searches and duplicate removal to prevent duplicate orientations from a single particle occurring in the average. Iterative rounds of principal component analysis classification and alignment were used to classify and divide the particles based on the structure and capsomere organization within the masked sub-region. The average from our largest class was a sub-region with apparent T-4 symmetry centered on a pentagonal capsomere (Figure 2A-E).

From the same dataset, we separately obtained an average centered on three hexagonal capsomeres surrounded by additional hexagonal capsomeres (Figure 2F-I). Some of the surrounding capsomeres were less resolved and may include a mix of pentagonal and hexagonal capsomeres at that position in different particles. The absence of clear pentamers in the second average precluded the assignment of a definitive T-number, but suggested a number larger than T-4. These two averages do not account for all positions in the VLPs and there are likely many other possible organizations within the population.

Our data suggest that wildtype Ty1 VLPs are assembled from a combination of hexagonal and pentagonal capsomeres to form heterogenous icosahedral structures. Additional work will be required to determine the organization of these subunits within a complete individual particle prior to analyzing VLPs assembled *in vivo* in the presence of p22. There may also be gaps or other irregularities in the particles contributing to their diverse morphology [8]. Our results suggest the arrangement of capsomeres may include multiple T-numbers within a single particle, and that Ty1 does not assemble into true icosahedral particles with a single defined T-number. This type of VLP organization was recently demonstrated for the distantly related Ty3/Gypsy retrotransposon family [5, 9].

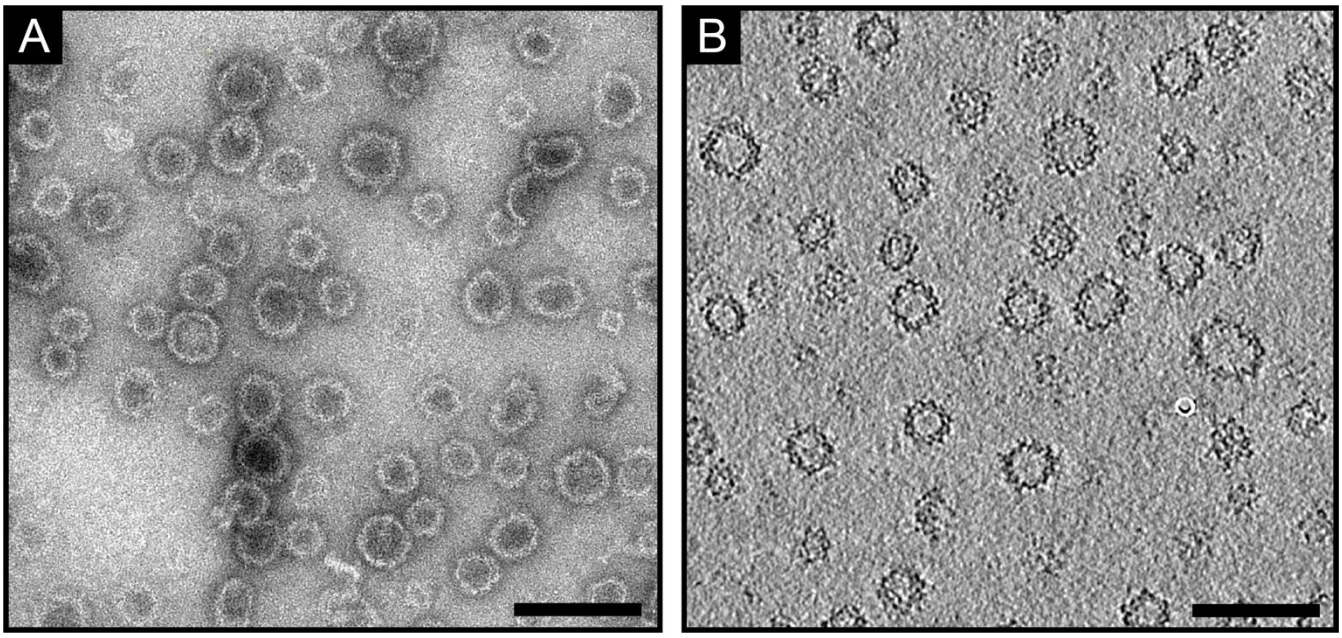

Figure 1. Negative stain and cryo-ET of purified Ty1 VLPs. (A) TEM image of purified yeast Ty1 VLPs prepared by negative staining with 2% uranyl acetate. (B) Z-projection (5.3 nm thick) of slices from reconstructed cryo-tomogram of purified Ty1 VLPs. Scale bars are 100 nm.

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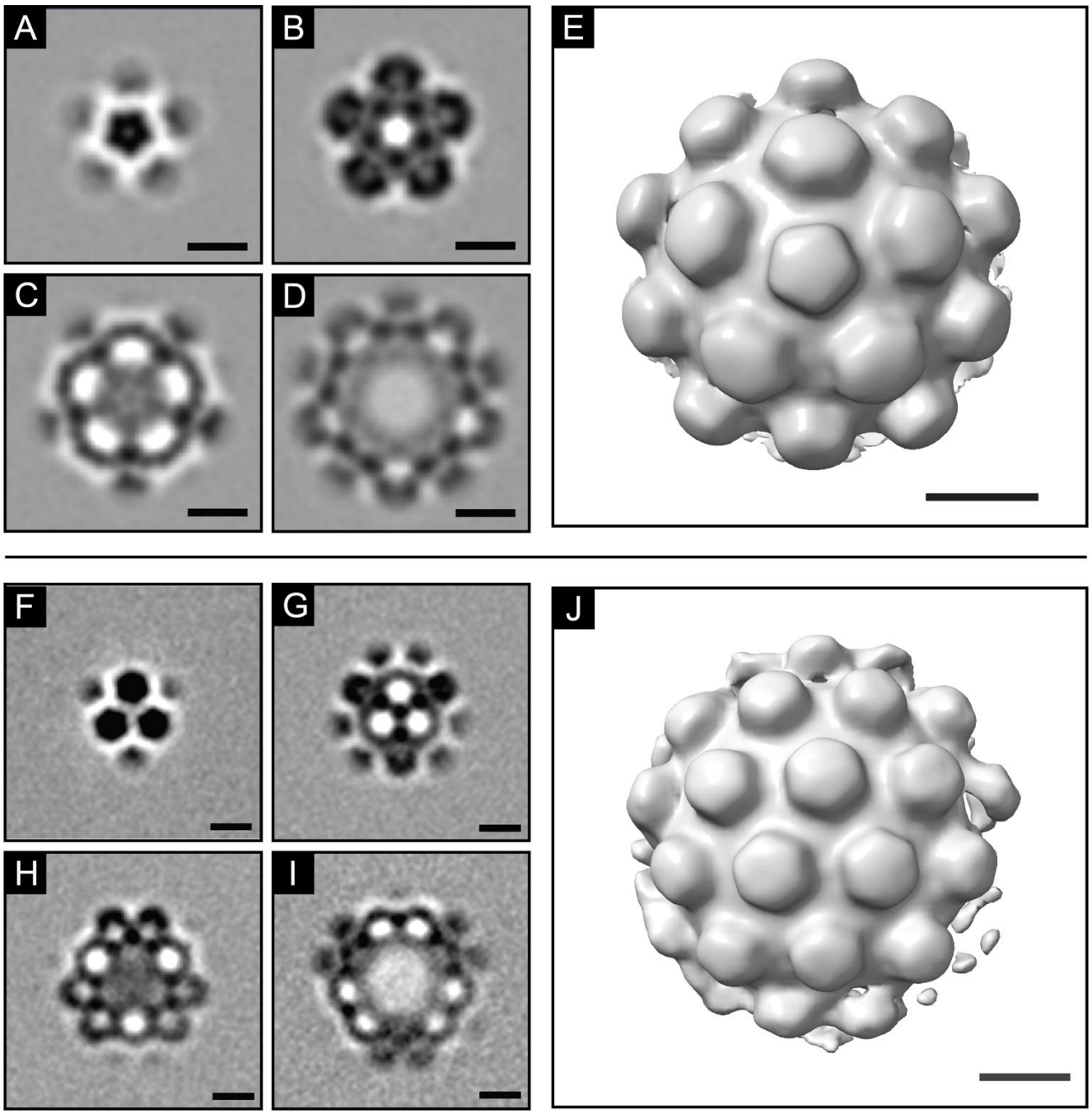

Figure 2. Sub-volume averages from Ty1 VLPs isolated from yeast. (A-D) Individual slices from density maps of a sub-volume average from cryo-ET of Ty1 VLPs. Average includes 39,343 particles after C5 symmetry expansion. (E) Isosurface of sub-volume average in A-D lowpass filtered to 25 Å. (F-I) Individual slices from density maps of a sub-volume average from a separate class of particles than those included in the average in A-E. Average includes 6,939 particles with no symmetry expansion. (J) Isosurface of sub-volume average in F-I lowpass filtered to 25 Å. All scale bars are 10 nm; slices in A-D, F-I are 2.7 nm apart in Z.

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