doi: 10.1017/S1431927609096196
Microsc Microanal 15(Suppl 2), 2009

Analysis of Simian Virus 40 Chromatin Structure by Cryo-Electron Tomography

P.W. Keller,* O. Ben-nun Shaul,** J. B. Heymann,* D.C. Winkler,* A. Oppenheim, ** and A.C. Steven*

* Laboratory of Structural Biology, NIAMS, NIH, Bethesda, MD, 20892
** Department of Hematology, Hebrew University – Hadassah Medical School, Jerusalem, Israel, 91120

Simian virus 40 (SV40) is a small (~45nm), non-enveloped, DNA virus with an icosahedral capsid. Viral DNA associates with histones in the nucleus of infected cells to form a chromatinized, supercoiled, circular minichromosome. The SV40 capsid proteins assemble around this minichromosome to form new virions. The capsid contains 72 pentamers of VP1 protein arrayed on a T=7*d* icosahedral lattice. Single molecules of VP2/3 protein are associated with the inner surface of all or most VP1 pentamers, potentially linking to the viral chromatin. The icosahedral symmetry of the capsid has allowed its structure to be determined at high resolution by particle averaging and crystallographic methods [1]. However, the organization of the viral chromatin – which is not expected to conform to this symmetry – has not been determined.

To study the chromatin structure of individual SV40 particles, we collected single-axis tilt series of plunge-frozen virus particles across an angular range of -70 to +70 degrees on a 120kV Tecnai T12 TEM equipped with an energy filter operating in zero-loss mode. Images were recorded at 2° increments at a dose of 1 $e^-/Å^2$ per image. Three-dimensional reconstructions of these tilt series were computed using IMOD [2]. Reconstructions were denoised by nonlinear anisotropic diffusion filtering. The average in-plane resolution of the reconstructions was calculated by the NLOO method to be 5 nm [3], sufficient to observe nucleosome structures.

Individual virions were extracted from tomograms for further analysis. Punctate densities, consistent in size and appearance with nucleosomes, are visible inside the capsids (Fig. 1A). The most prominent viral component was the capsid shell, whose T=7*d* icosahedral symmetry was clearly visible in individual subtomograms (Fig. 1B) and was further enhanced by particle averaging (Fig. 1C). As expected, averaging revealed no symmetrically distributed internal densities; rather, it produced a low uniform level of internal density. These findings are consistent with earlier data suggesting that viral chromatin is not icosahedrally ordered [4]. Quantifying the nucleosome densities present in individual particles revealed them to be variable in number, with an average of 19 +/- 2.5 per particle (Fig. 2). The tomograms also suggest the presence of contacts between some internal chromatin densities and the capsid shell. Ongoing efforts are focused on better defining these putative capsid-chromatin links, as well as the organization of encapsidated minichromasomes.

References

[1] T. Stehle et al., *Structure.* 4 (1996) 165-182.
[2] D.N. Mastronarde, *J. Struct. Biol.* 120 (1997) 343-352.
[3] G. Cardone et al., *J. Struct. Biol.* 151 (2005) 117-129.
[4] T.S. Baker et al., *Proc. Natl. Acad. Sci. USA*. 85 (1988) 422-426.

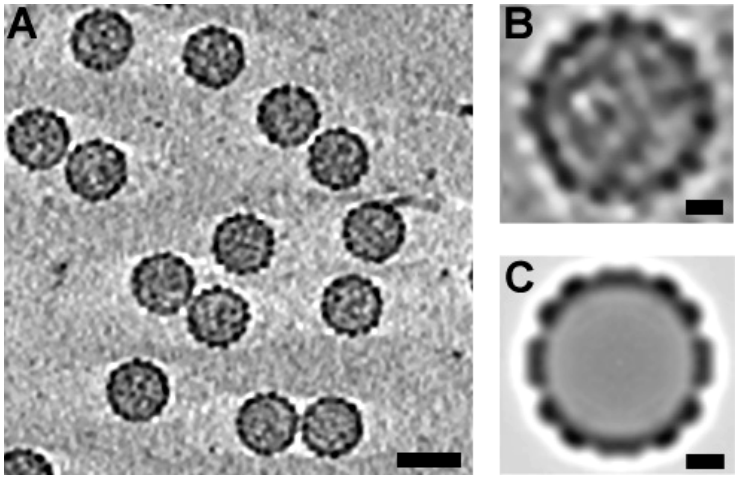

FIG. 1. Central sections through SV40 reconstructions. (A) Z-slice from denoised tomogram, showing several virions containing irregular core densities. Scale bar = 500 Å. (B) Single subtomogram showing capsid enclosing internal nucleosome-associated densities. (C) Symmetrized average of particles extracted from tomograms and aligned viewed along a 2-fold axis. The capsid shell is evident, but the internal density has been smeared to near-uniformity. Scale bars = 100 Å

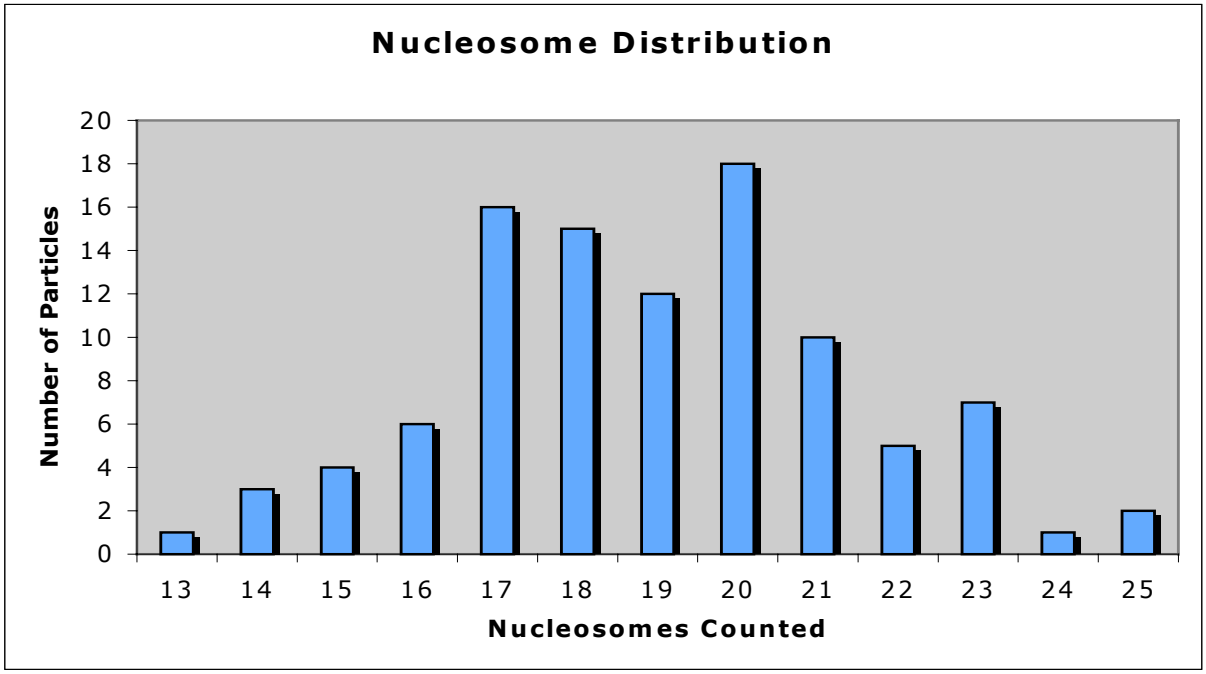

FIG. 2. Quantifying nucleosome distribution: A histogram of assumed nucleosome densities counted by manual analysis of individual extracted virions (n=100). The scored SV40 particles exhibit a variable number of nucleosome densities; the average = 19 (+/- 2.5) nucleosome densities per particle.

https://doi.org/10.1017/S1431927609096196 Published online by Cambridge University Press