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HIGH-VOLTAGE ELECTRON TOMOGRAPHY OF THE CENTROSOME IN *Caenorhabditis elegans*

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C. elegans is an exciting model system for studying the molecular mechanisms of cell division. Genes required for mitosis can be identified in functional genomic screens using RNA-mediated interference (RNAi). In addition, this organism is well suited for microscopic analysis because the gonad of each hermaphrodite contains two rows of early embryos in various stages of development. We have begun a detailed structural analysis of the centrosome in the early embryo of *C. elegans* by applying a combination of methods, including high pressure freezing and freeze substitution (HPF/FS). Material prepared by HPF/FS is subsequently flat embedded, allowing us to select embryos in whole-mounted worms for analysis by transmission electron microscopy [1]. We are currently applying high-voltage electron tomography [2] to study the 3-D fine structure of the early embryo centrosome in wildtype worms.

The centrosome in *C. elegans* consists of a pair of unusually small centrioles surrounded by the pericentriolar material (PCM), an electron-dense matrix that is responsible for the nucleation of microtubules. To obtain high resolution 3-D structure data, serial 300-400nm thick sections that contain centrosomes were imaged in the high-voltage EM, and serial tilted images were collected about two orthogonal axes. Tomographic reconstructions were then calculated using back projection algorithms and combined using the method of Mastronarde et al. [3]. Examples of 1.4 nm tomographic slices from a cell in anaphase is shown in Figure 1. One centriole (1a, arrow) is shown in cross section; it is formed from 9 singlet microtubules. Individual microtubules (1b,c arrowheads) can be seen radiating out from the electron-dense PCM, which surrounds the centrioles. The microtubules in this tomographic volume can be tracked to illustrate their complex 3-D arrangement at the centrosome.

Our long term goal is to apply these methods to RNAi-treated worms to study mitosis-related structure-function relationships.

References:

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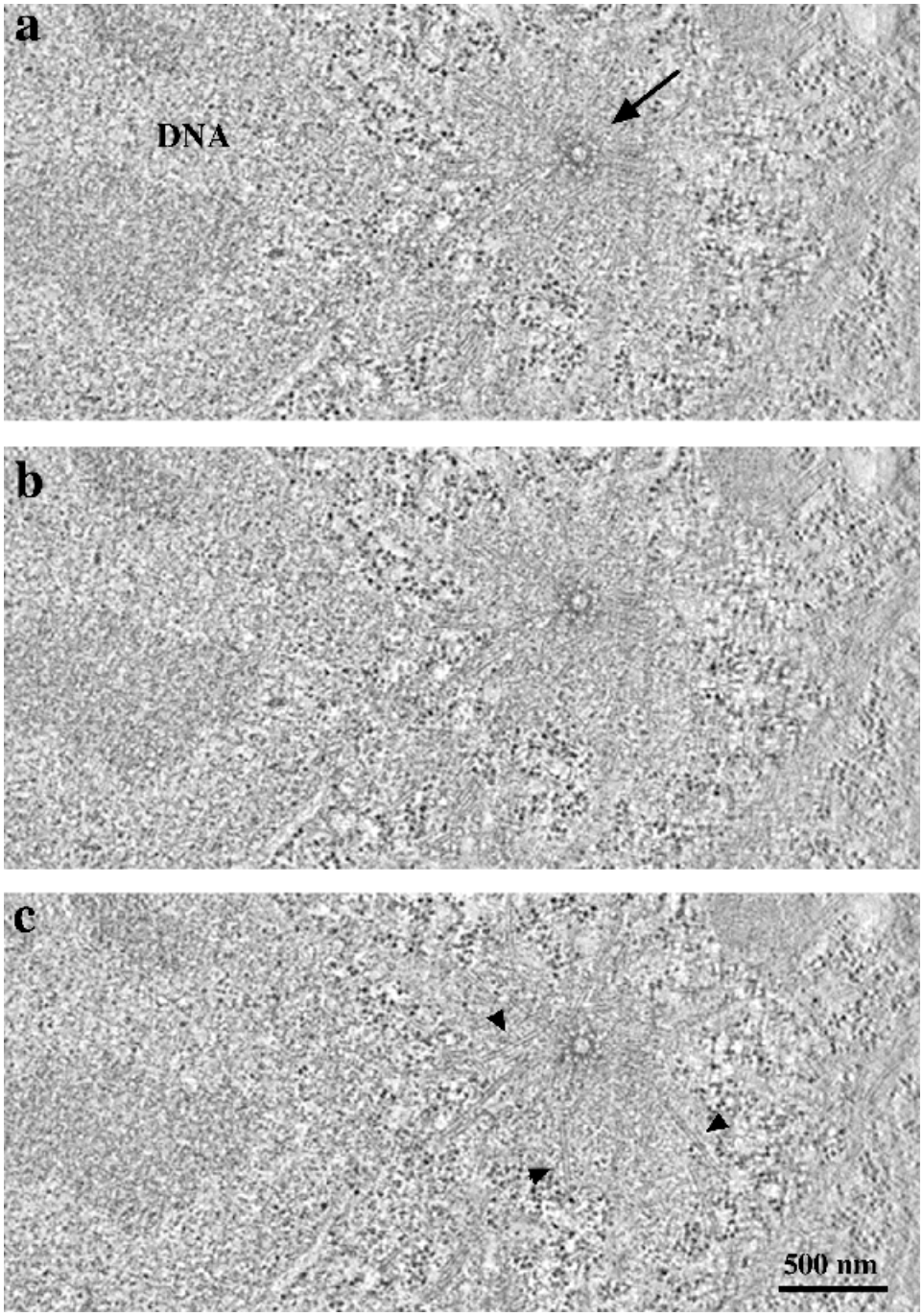

Figure 1. Example of three slices obtained by "stepping" through a tomographic reconstruction. The thickness of each slice is 1.4nm. Portions of the anaphase chromosomes can be seen (DNA). The centrosome (arrow) is formed from nine singlet microtubules. Individual microtubules radiating out from the PCM are marked by arrowheads.

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