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Ultrastructure of Nocodazole-Treated PtK_1 Kinetochore after High-Pressure Freezing and Freeze-Substitution

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Accurate chromosome segregation is critical to the long-term survival of all organisms. In eukarotes, the process is dependent upon a dynamic interaction between microtubules and kinetochores [1, 2]. Recent work has demonstrated that kinetochores in *S. cerevisiae* are composed of over 60 molecular components arranged into at least 14 different complexes [3]. Currently there is only a rudimentary understanding of how these components are arranged at the ultrastructural level. Classical serial section studies of conventionally fixed specimens established the tri-laminar model for vertebrate kinetochores consisting of an inner plate, translucent zone, and an outer plate [4]. Prior to attaching microtubules the outer plate has a robust fibrous corona on its distal surface. Electron tomographic reconstructions indicated a complex pattern of fibrous arrangements in the outer plate that sometimes formed parallel rows and discrete unit substructures [5]. Studies using high-pressure freezing (HPF) and freeze-substitution (FS) revealed that outer plate is a delicate fibrous mat structure composed of a fiber-like element running parallel to the chromatin surface and fibers radiating from the heterochromatin [6]. The mat is wider than the plate observed in conventional preparations, and the translucent middle layer is largely absent. The corona appears as a ribosome-exclusion zone apparently occupied by very fine fibers. These data demonstrate kinetochore sensitivity to general collapse and condensation when using conventional fixation methods.

The initial HPF/FS study of kinetochore structure included preliminary electron tomographic analysis [6]. Here we extend those initial tomographic studies to obtain a more detailed structural map of the unbound kinetochore in PtK_1 cells. Data collection is accomplished by tilting serial 140 nm-thick sections over an angular range of –74° to 72° (Fig1A). Preliminary analysis using three-dimensional windows confirms the complex arrangement of kinetochore fibrous components (Fig 1B). In some sections the pattern appears as a fibrous double-line paralleled to the heterochromatin surface, with the bead-like structures attached (Fig. 2A). Other views indicate a subunit arrangement (Fig. 2B) and fibrous connections to the underlying heterochromatin (Fig. 2C). These results support the previous hypothesis that kinetochores are organized from repetitive subunits [5,7]. A more detailed analysis is being carried out in addition to functional research at the kinetochore molecular level.

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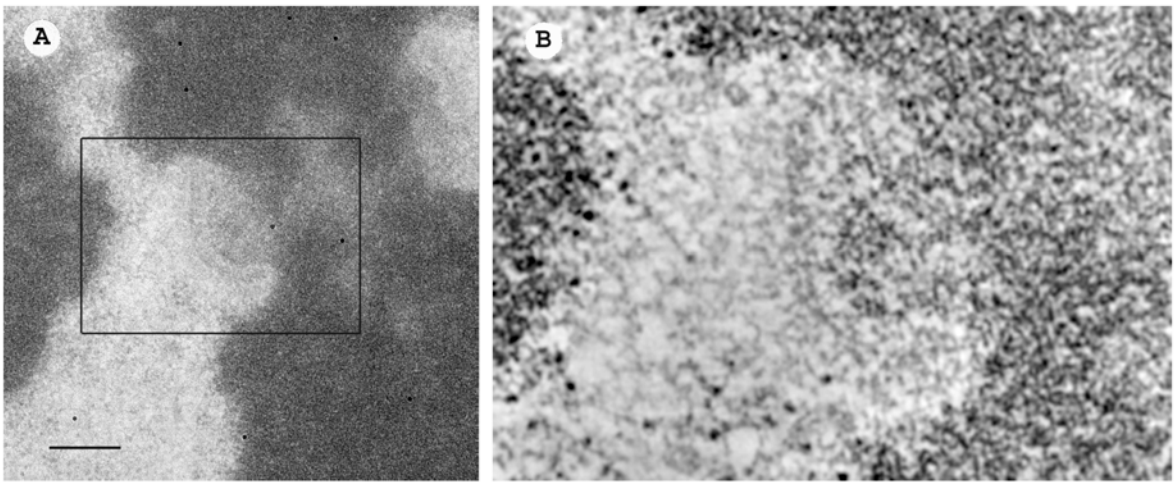

Fig.1. Tomographic reconstructions of nocodazole-treated, HPF/FS kinetochores. (A) Projection image of sister kinetochores located in the primary constriction of the chromosome. Scale bar=400nm. (B) A 1.6 nm-thick slice from the tomographic 3D reconstruction of the boxed area in A. The complexity of structure is evident.

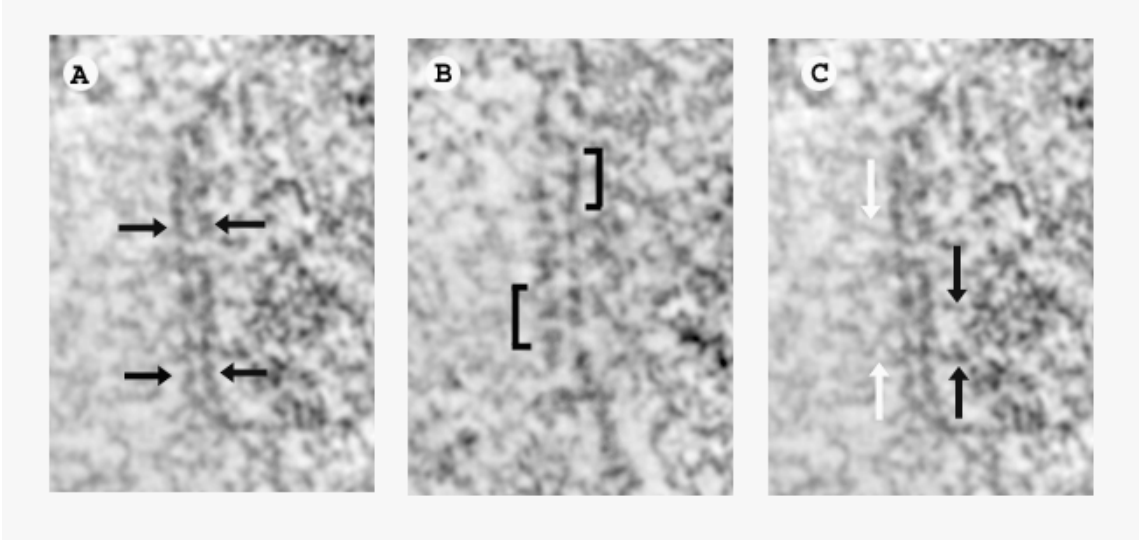

Fig.2. Detailed fine structure at different depths of the tomographic reconstruction shown in Fig.1. (A) The mat appears as a double-string structure with beads attached (arrows). (B) The mat shows repetitive unit blocks (arrows). (C) Fine fibers connect the mat to the underlying heterochromatin (black arrows), while other fibers radiate from the distal surface of the mat (white arrow).

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