

Employing High-Throughput Electron Tomography to Assess the Dynamic State of Kinetochores Microtubules

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Proper chromosome alignment during cell division is essential for the viability of all eukaryotes. In higher eukaryotes, the process begins with the bipolar attachment of replicated chromosomes to the mitotic spindle through a pair of specialized organelles known as sister kinetochores. Fully attached chromosomes then migrate to the spindle equator where they frequently undergo short oscillations toward and away from each spindle pole [1]. Once all chromosomes are equatorially aligned, sister chromatids simultaneously separate and migrate to opposite spindle poles to complete chromosome segregation. These alignment steps involve a unique form of microtubule-based motility wherein the cargo is attached to the plus ends of a bundle of microtubules (Mts), rather than moving along the lateral surface of a single Mt [2,3]. Consequently, chromosome motion away from or towards a spindle pole must be coordinated with transitions between assembly and disassembly of the kinetochore microtubules (kMts). In freestanding Mts, these transitions are abrupt, stochastic, and characterized by three distinct Mt plus-end conformations [4,5]. Electron tomography of high-pressure frozen/freeze-substituted specimens has revealed analogous plus-end conformations for in situ kMts [6]. Here we use high through-put electron tomography to statistically characterize kMt plus-end conformations on equatorially aligned chromosomes. This data is then used to test the hypothesis that kMts bound to the same kinetochore have coordinated dynamic states.

Thus far, we have identified 82 kMts in 18 tomographic reconstructions taken from 12 kinetochores in 3 metaphase PtK₁ cells (Fig. 1). We were able to manually classify 78 of the kMts into three distinct plus-end conformations: blunt, forked, and curled (Fig.2). Blunt and curled roughly correspond to conformations reported for freestanding Mts [5], while forked appears to be an intermediate. We have not observed open sheets in kMts. Curled is the predominate conformation for metaphase kMts (54%), followed by forked (31%), and blunt (15%) (Table 1). Kinetochore #4 shows a relatively large portion of curled conformations (73%) while its sister (#5) has an average distribution. We were also able to characterize kinetochore #9 as predominantly curled (75%) and kinetochore #6 as predominantly forked (56%). Thus, each kinetochore tends to have a definite profile, but we do not observe strict coordination of conformations among kMts on a single kinetochore. Currently we are examining the effects of antimetabolic drugs and stage of mitosis on kMt conformations.

References.

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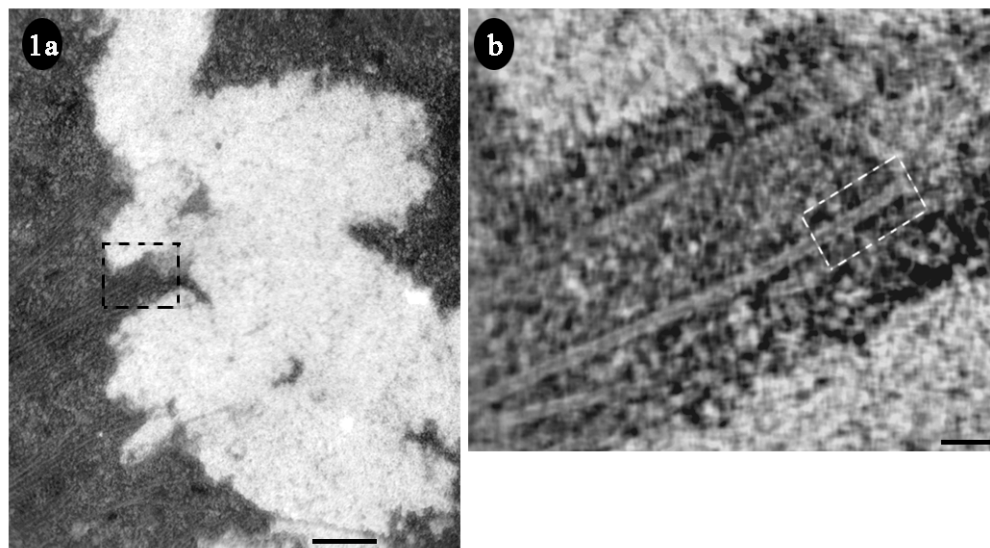


Figure 1. Identifying kMt conformations in situ. (a) Low magnification view of a group of chromosomes from a metaphase PtK₁ cell prepared by high-pressure freezing and freeze-substitution. The specimen was cut into 140 nm thick serial sections and imaged on a Zeiss 910 electron microscope at 120 kV using a fiber-coupled CCD camera. The tomographic reconstructions were computed from dual-axis tilt series collected over a 120 degree angular range using a cosine varying tilt scheme with a 2 degree interval at 0 degrees. (b) A 2 nm thick slice from the tomographic reconstruction of the kinetochore indicated by the dashed box in (a). The kMt indicated by the dashed box in (b) has blunt plus end conformation (see Fig. 2).

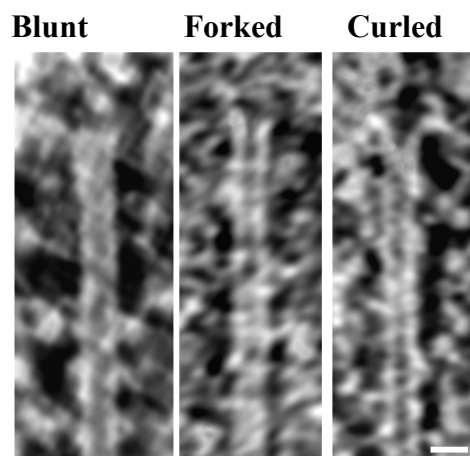


Figure 2. Examples of the three classes of conformations detected for kMt plus-ends in metaphase PtK₁ cells. kMts were extracted from tomographic reconstructions and reoriented vertically, with plus-ends at top. The original orientation of the blunt end kMt is shown Fig 1b.

Table 1. Structural conformations of kMt plus-ends

Kineto- chore #	Total # kMts	% Blunt	% Forked	% Curled
1	2	0	0	100
2	4	25	50	25
3	3	100	0	0
4*	11	9	18	73
5*	11	18	27	55
6	16	0	56	44
7	6	17	33	50
8**	5	40	0	60
9**	8	0	25	75
10	4	0	25	75
11	4	25	50	25
12	4	25	25	50
Total	78	15	31	54

*Kinetochores 4 and 5 are sister kinetochores

**Kinetochores 8 and 9 are sister kinetochores