Short communication

Microtubule dynamics in living cells: direct analysis in the internal cytoplasm

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Microtubules (MTs) are long non-covalent polymers of heterodimeric tubulin that exchange with the soluble pool exclusively from their ends. The two opposite ends of MT are different: one end of the MT (plus end) is more dynamic than the opposite (minus) end. At steady state, plus ends of individual MTs undergo long excursions of growth and shortening. In many animal cells, MTs run from the cell center towards the periphery and form a radial array. Minus ends of MTs are located in the center while plus ends are directed presumably towards the cell margin and display dynamic instability behavior. In the interphase cell, the length of MTs in the radial array is seemingly limited by the cell margin, yet their plus ends are dynamic. How does the cell keep an array of long and dynamic MTs?

To evaluate the formation and turnover of long MTs, it is necessary to follow individual MT not only at the cell periphery, but also deep in the cytoplasm. Direct observation of MTs in the internal cytoplasm is difficult because of the limited resolution of the light microscope. Recently, we developed experimental approaches that allow observation of MT dynamics in the areas where density of MTs is high (Komarova et al., 2002; Vorobjev et al., 1999). Using these approaches, MT dynamics in CHO and NRK cells were found to be different in the internal cytoplasm from those near the cell margin. Nascent MTs grew persistently from the centrosome towards the cell margin and displayed dynamic instability only near the margin (Komarova et al., 2002). Is this behavior common among animal cells? Addressing this question in the present study, we evaluated MT dynamics in the internal cytoplasm of several cultured cell-types (fish keratocytes, PtK1 cells; NIH-3T3 cells and REF fibroblasts) using the following experimental approaches: image processing that enhances contrast of individual MTs; sequential subtraction analysis to follow MTs elongation and growth; and laser bleaching of fluorescence. Cells cultured on the coverslips were microinjected with Cy-3 labeled tubulin and time lapse sequences were obtained on the inverted microscope using a cooled CCD camera as described elsewhere (Vorobjev et al., 1997).

In keratocytes, MT density is very low and growth from the centrosome was analyzed directly after image processing. Nascent MTs grew from the centrosome persistently up to two-third of the cell radius (15–20 µm) and started to oscillate only after reaching cell periphery (outer one-third of cell radius). In PtK and REF cells, growth from the centrosome was analyzed during recovery after photobleaching. A nearly rectangular bleached zone, 5–8 µm wide and 40–50 µm long, was created using an argon laser focused through a cylindrical lens and a 100× objective lens on a Nikon Eclipse inverted microscope as described elsewhere (Keating et al., 1997). To obtain the wide bleached zone, the laser beam was directed onto the objective lens off-center of the main optical axis of the microscope. In the bleached zone, individual MTs could be traced for 1–3 min until the density of fluorescent MTs recovered to the high level, precluding further observation.

MTs in the internal cytoplasm grow and shorten without pause in keratocytes, 3T3 and REF cells. Meanwhile in PtK cells, pauses in the internal cytoplasm were observed frequently. Shortening of MTs in all types of cells was non-processive. In fish keratocyte, length of shortening was 5.7±4.4 µm (one-third of cell radius), and could be approximated by gamma-distribution. Growth periods of MTs from the centrosome were rather long, and about 10% of MTs in 3T3, REF and

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PtK cells reach the cell margin without catastrophe. To quantify persistent growth from the centrosome, MTs were followed from their origin until first catastrophe. The length of uninterrupted growth from the centrosome was 7–10 µm (one-third of cell radius) in PtK cells and 12.2±5.1 µm (half of cell radius) in REF cells.

Analysis of life histories show that transition between phases in the internal cytoplasm was asymmetric: catastrophes were rare, while rescues were frequent. Catastrophe frequency was 0.022 s⁻¹ in PtK1 cells, and 0.011 s⁻¹ in REF and NIH-3T3 cells. In the CHO cells, persistent growth and low frequency of catastrophes resulted in the positive net displacement of the plus ends in the internal cytoplasm. The value of displacement (drift) was about 5 µm min⁻¹ (Komarova et al., 2002).

For analysis of the drift of the MTs in the internal cytoplasm, we obtained histograms of displacements of MT ends during 6 s time intervals. The histograms have a bi-modal distribution (Fig. 1). In fish keratocytes and NIH-3T3 cells, plus ends showed a positive drift of about 3 µm min⁻¹.

Histograms of the instantaneous displacements collected near the cell margin were different—they showed a large maximum corresponding to the zero velocity (pause). The drift near the cell margin was always close to zero, being sometimes slightly positive or negative. These data demonstrate that MT growth is impeded at the cell margin. Besides, in some NIH-3T3 cells, regions where drift of the plus ends was also close to zero were located in the internal cytoplasm. These regions are characterized by: (i) low spatial density of MTs; (ii) high percentage of paused MTs; and (iii) no processive MT growth across the region.

We suggest that the overall behavior of MTs in the interphase cell should be described as ‘dynamic instability with boundary conditions’. Boundary conditions mean that elongation of the plus ends of MTs is effectively limited in the cell. Limitation of MT growth comes from the cell margin and other boundaries located in certain regions of the cytoplasm. Asymmetric transition frequencies and processive growth of the free plus ends of MTs indicate that effective concentration of soluble tubulin in the cytoplasm of living cells is above the steady-state concentration for the plus end. Limitation of MT elongation results in the processive growth of free MTs. Processive growth of free MTs in the cytoplasm is essential for organization of the radial MT array and its rapid remodeling in the cell.

**References**


