Assembly and breakdown of microtubules within the midbody

Tamir, A; Elad, N; Medalia, O

Abstract: In animal cells, cell division concludes with the separation of two daughter cells during a process called cytokinesis. Abscission, the termination of cytokinesis, is performed through formation of the midbody, a vis-à-vis microtubule (MT)-rich structure bridging the daughter cells. Disassembly of the midbody is the final stage of daughter cell separation and occurs in parallel to membrane fusion in this area. To shed light on this process and to better understand MT organization within the dense area of the midbody structure, an integrative fluorescence microscopy and cryo-electron tomography (cryo-ET) approach was taken. These efforts led to a resolving of MT architecture at single-fiber resolution, resulting in a refined model of abscission.

DOI: https://doi.org/10.4161/cib.4.5.16050

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-54364
Published Version

Originally published at:
DOI: https://doi.org/10.4161/cib.4.5.16050
Assembly and breakdown of microtubules within the midbody

Adi Tamir,1 Nadav Elad2 and Ohad Medalia1,3,*

1Department Life Sciences and the National Institute of Biotechnology in the Negev; Ben Gurion University; Beersheva, Israel; 2Center for Human Genetics; KULeuven; and Department for Molecular and Developmental Genetics; VIB, Leuven Belgium; 3Department of Biochemistry; University of Zurich; Zurich Switzerland

In animal cells, cell division concludes with the separation of two daughter cells during a process called cytokinesis. Abscission, the termination of cytokinesis, is performed through formation of the midbody, a vis-à-vis microtubule (MT)-rich structure bridging the daughter cells. Disassembly of the midbody is the final stage of daughter cell separation and occurs in parallel to membrane fusion in this area. To shed light on this process and to better understand MT organization within the dense area of the midbody structure, an integrative fluorescence microscopy and cryo-electron tomography (cryo-ET) approach was taken.1 These efforts led to a resolving of MT architecture at single-fiber resolution, resulting in a refined model of abscission.

Getting Closer to the Live State by Cryo-Electron Tomography

Electron microscopy images of purified midbodies have provided important information on MTs within these structures.2,3 Still, three-dimensional analysis of the hydrated state has proven challenging, due to technical difficulties.4-6 However, with the introduction of cryo-ET, it is now possible to conduct high-resolution analysis of individual pleiomorphic structures, such as intact cells or even midbodies, in a close-to-native state. Hence, to gain detailed insight into MT organization within the midbody, these structures derived from CHO-cells were vitrified and analyzed by cryo-ET, followed by image-processing analysis.

Organizational Groups of MTs within the Midbody

The central domain of the midbody is organized as an anti-parallel array of bundled MTs that form the overlap zone, a highly electron-dense region. Tracking of MTs reveals that within the midbody, these fibers are divided into four morphological groups, depicted in the rendered view presented in Figure 1. The midbody central region contains MTs traversing the overlap region, termed continuous MTs. A second group, the polar MTs, surrounds the continuous MTs and terminates within the overlap region. This group could be further divided into two subgroups, corresponding to the daughter cell from which they originate (Fig. 1 and orange and yellow fibers, respectively). The fourth group represents minus-end capped MTs1 (Fig. 1 and purple fibers).

To identify MT plus-ends, Elad et al. combined the use of fluorescence microscopy with a construct of GFP fused to the MT end-binding protein 1 (EB1). EB1 was thus localized to four foci within the midbody, namely to the two inner foci that surround the overlap region and to the two outer foci found at both midbody ends. This observation supports the MT architecture observed by cryo-ET,1 with the plus ends of the polar MTs constituting the inner foci, and the plus ends of the continuous MTs constituting the outer foci. Further support for this architecture was provided by EM studies performed on cultured mammalian cell sections.7,8 The number of MTs in vertical sections within the overlap region was found to be

Key words: cryo-electron tomography, microtubule, midbody, cytokinesis, three-dimensional imaging

Submitted: 04/23/11
Accepted: 04/25/11
DOI: 10.4161/cib.4.5.16050
*Correspondence to: Ohad Medalia; Email: omedalia@bioc.uzh.ch


552 Communicative & Integrative Biology Volume 4 Issue 5
MT Assembly within the Midbody

Based on the observation that early telophase MTs present only limited degree of overlap, we hypothesized that the continuous MTs present only limited degree of overlap. However, since a third of the MTs are continuous from one polar region to the other, the ratio of the numbers of MTs in the overlap region and reaching either polar region is instead 1.5:1, as predicted by cryo-EM.

Breakdown of the Midbody

Midbody breakdown concludes the structural changes that occur within MT bundles that eventually lead to asymmetric division, wherein the remaining structure is pulled towards one of the daughter cells. A recent study revealed the presence of helical-shaped filaments adjacent to the membrane of one midbody pole. These filaments likely play a role in the asymmetric narrowing of the MT bridge spanning the daughter cells. During midbody breakdown, a general reduction in the number of MTs is observed. Moreover, before final separation, several changes are detected, according to MT type. The surrounding polar MTs lose their interdigitating organization and retract from the overlap region. While the continuous MTs maintain their structural morphology during the longer step of the midbody breakdown, their numbers are reduced. Correspondingly, the inner foci region is barely detected when midbodies are in the final collapse stages.

Midbody breakdown and MT reorganization correlate with the termination of cell division. At this point, those MTs in the middle of the midbody that form the dense middle area are separated from the overlap region, while some of the MTs remain intact and persist until the abscission process ends. Accordingly, various overlap region distances are detected, reflecting the progression of the abscission process. The continuous MTs remain at the later stages of the process, while polar MTs are hardly inter-digitated with the opposing polar MTs at this point. Moreover, the inner foci region is barely detected when midbodies are at the final, collapse stages.

As a result of these observations, we propose a model whereby a continuous MT bundle persists until the final stages of cytokinesis. Consequently, the involvement of continuous MTs in cytokinesis is crucial during separation of the two daughter cells, although these MTs may also play a role in cases where daughter cells remain connected for extended periods. Future studies combining high-resolution imaging with genetic manipulation will shed light on the precise molecular remodeling that occurs during the final stages of cytokinesis.

Acknowledgments

This study was supported by grants from the German-Israeli Cooperation Project (DIP; H.2.2) and by an ERC Starting Grant to O.M. (243047 INCEL).

References


