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The linear and rotational motions of the fission yeast nucleus are governed by the stochastic dynamics of spatially distributed microtubules

Tsz Hin Hui [b,1], Fan Zheng [a,c,1], Yuan Lin [b,*], Chuanhai Fu [a,c,**]

[a] CAS Center for Excellence in Molecular Cell Science, School of Life Sciences, University of Science and Technology of China, Hefei, China
[b] Department of Mechanical Engineering, The University of Hong Kong, Pokfulam, Hong Kong, China
[c] Department of Biochemistry, The University of Hong Kong, Pokfulam, Hong Kong, China

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ABSTRACT

Dynamic nuclei are involved in a wide variety of fundamental biological processes including cell migration, cell division and fertilization. Here, we develop a mathematical model, in combination with live-cell imaging at high temporal resolution, to quantitatively elucidate how the linear and rotational motions of the nucleus are governed by the stochastic dynamics of the microtubule cytoskeleton. Our simulation and experimental results demonstrate that microtubule rescue and catastrophe frequencies are the decisive factors in regulating the nuclear movement. Lower rescue and catastrophe frequencies can lead to significantly larger angular and translational oscillations of the nucleus. In addition, our model also suggests that the stochastic dynamics of individual spatially distributed microtubules works collectively as a restoring force to maintain nuclear centering and hence ensures symmetric cell division, in excellent agreement with direct experimental observations.

1. Introduction

The nucleus is highly dynamic within the cell. For example, the budding yeast nucleus is rotated to be moved toward the bud neck for spindle positioning (Segal and Bloom, 2001). Such rotational and translational motions of the nucleus are also present in one-cell *Caenorhabditis elegans* embryos (Espiritu et al., 2012). In addition, it has been reported that the nucleus of a motile fibroblast is rotationally controlled to align along the migrating axis (Levy and Holzbaur, 2008; Maninova et al., 2013). Therefore, the translational and rotational movements of the nucleus appear to be a general phenomenon important for a wide variety of cellular functions. Thus, it is biologically important to elucidate not only the translational motion of the nucleus but also the rotational motion of the nucleus that is relatively less understood.

It is well-documented that the cytoskeleton plays a critical role in nuclear dynamics. For instance, in addition to being responsible for generating a variety of cellular motility phenomena (Lin, 2009; Lin et al., 2010), actin filaments are essential for the rotational motion of the nucleus in fibroblasts trapped on micro-fabricated patterns (Kumar et al., 2014). However, in many other cell types or organisms, microtubules appear to be the key player instead (Maninova et al., 2014; Starr, 2009). A prime example is the fission yeast *Schizosaccharomyces pombe* whose nucleus displays remarkable oscillations during meiotic prophase, a phenomenon believed to be driven by dynein-dependent pulling forces and requiring the contact of microtubule plus ends with the cell cortex (Vogel et al., 2009). In comparison, in vegetative fission yeast cells, nuclear dynamics is mediated purely by pushing forces generated by growing microtubules at the cell tips, independent of dynein (Daga and Chang, 2005; Daga et al., 2006; Tran et al., 2001). Interestingly, the microtubule pushing forces are asymmetrically regulated at the two cell tips when the nucleus is displaced from the cell center, leading to efficient re-centering of the nucleus (Daga et al., 2006) and thus ensuring proper cytokinesis (Daga and Chang, 2005).

Fission yeast is a rod-shape single-celled organism that grows by tip extension and divides at a defined size (~14 μm in length). The spherical nucleus, with a diameter of ~2 μm which is slightly smaller than the width of the cell (3–4 μm), is positioned at the cell center by 3–4 microtubule bundles in the cytoplasm. These microtubule bundles are organized in an antiparallel fashion, with their overlapped minus ends organized by the conserved microtubule associated protein ase1p (abnormal spindle elongation 1) (Loiodice et al., 2005; Yamashita et al., 2005) and attached either

* Corresponding author. Tel.: +852 2859 7955.
** Corresponding author at: Department of Biochemistry, The University of Hong Kong, Pokfulam, Hong Kong, China. Tel.: +86 551 663607141.
E-mail addresses: ylin@hku.hk (Y. Lin), chuanhai@ustc.edu.cn (C. Fu).
[1] These two authors contribute equally.

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to the spindle pole body (SPB) or to the interphase microtubule organizing centers (iMTOC) on the nuclear envelope (Loiodice et al., 2005) and their plus ends pointing towards the cell tips. Microtubule plus ends are highly dynamic, alternating between growth and shrinkage. The switch from shrinkage to growth is referred to as microtubule "rescue" while the reverse is called microtubule "catastrophe" (Gardner et al., 2013). Hydrolysis of the alpha-tubulin bound GTP plays a key role in promoting microtubule catastrophe (Alushin et al., 2014).

The firm attachment between microtubules and the nucleus allows pushing forces generated at the cell tips to be transmitted back to the nucleus and, subsequently, direct its movement (Tran et al., 2001). The pushing forces are generated by microtubule polymerization, and in turn the forces can affect microtubule dynamics (Dogterom et al., 2005; Foethke et al., 2009). This interplay between microtubule dynamics and the pushing force has been modeled and computationally simulated (Foethke et al., 2009), highlighting the importance of the length-dependent microtubule catastrophe frequency in nuclear re-centering after displacement (Foethke et al., 2009). It is worth noting that, although nuclear rotations have been observed in fission yeast (Tran et al., 2001), such angular motion has never been quantitatively measured and the underlying mechanism remains unclear.

Here, we employed a combination of experimental and theoretical approach to study nuclear dynamics. Specifically, high temporal resolution live-cell imaging was employed to accurately monitor the rotational and translational motions of the nucleus in fission yeast. A mathematical model was then developed to relate the complex nuclear movement with the stochastic dynamics of individual microtubules, showing that lower frequencies of microtubule catastrophe and rescue could lead to significantly larger angular and translational oscillations of the nucleus. In addition, it is suggested that the stochastic dynamics of spatially distributed microtubules can act collectively as a restoring force to maintain nuclear centering. These predictions precisely reflect our experimental observations indicating that the main features of the phenomenon may have been captured by our modeling.

2. Materials and methods

2.1. Yeast strains and plasmids

General yeast genetic approaches were employed to create yeast strains (Forsburg and Rhind, 2006). Yeast culture media were purchased from Formedium (www.formedium.com). All yeast strains in this study are listed in the Supplementary materials (Table A1 in File A1).

2.2. Microscopy

We performed live-cell imaging by spinning-disk confocal microscopy as previously described (Zheng et al., 2014b). All imaging was carried out at 26 °C in a temperature controllable incubator. For high temporal resolution analysis, Z-stack images consisting of 3 planes with a step size of 0.5 μm were acquired every 5 s. For nuclear centering analysis, Z-stack images consisting of 21 planes with a step size of 0.5 μm were acquired every 1 min. Images were analyzed with Metamorph (www.moleculardevices.com) and Image J, and graphs were generated with Kaleidagraph 4.5 (www.synergy.com).

2.3. Measurement of microtubule dynamics

Advanced microscopy techniques have enabled to quantitatively measure microtubule dynamics, in particular the four parameters involved: the growth and shrinkage rates and the rescue and catastrophe frequencies (Tran et al., 2004). Specifically, high temporal resolution imaging was performed to monitor microtubules in cells expressing GFP tagged atb2p, and the resultant movies were used to construct kymographs for the measurement of microtubule dynamics. Microtubule growth and shrinkage rates were determined by measuring the slope of a growing and shrinking microtubule bundle in the kymographs. The frequency of catastrophe was calculated by dividing the number of catastrophe events by the total time of microtubule in growth, and the frequency of rescue was calculated by dividing the number of rescue events by the total time of microtubule in shrinkage, as previously described (Walker et al., 1988).

3. Results

3.1. Microtubules direct the translational and rotational motions of the fission yeast nucleus

Microtubules and the nucleus are connected via the centrosome (the SPB/iMTOC in yeast). To understand how microtubules are involved in regulating nuclear dynamics, we employed live-cell microscopy to monitor the nucleus and microtubules simultaneously. Specifically, we marked in wild type cells the microtubules, the nucleus, and the SPB by fluorescently tagged marker proteins: GFP-atb2p (alpha tubulin 2) (Adachi et al., 1986), cut11p-GFP (spindle pole body docking protein) (West et al., 1998), and sid4p-mTomato (septum initiation defective) (Balasubramanian et al., 1998), respectively, followed by live-cell imaging at 5 s intervals. As shown (Fig. 1A and Movie A1), although the translational movement of the nucleus was not apparent, the SPB rotated remarkably in an oscillatory manner and such rotational motion appeared to be correlated well with microtubule pushing. We referred to these SPB oscillatory movements as the rotational motion of the nucleus because the SPB likely was anchored on the nuclear envelope whose spherical shape remained unchanged during the motion (see the illustration in Fig. 1B). This is further evident when we examined a strain expressing GFP-nup107 (nucleoporin 107; the nuclear envelope marker) (Chen et al., 2004) and mCherry-atb2 (microtubule) (Fig. 1C and Movie A2). Thus, similar to the previous report that microtubule pushing forces contribute to the translational movement of the nucleus (Tran et al., 2001), microtubule pushing forces also contribute to the rotational movement of the nucleus.

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Next, we analyzed the stochastic characteristics of the translational and rotational movements of the nucleus in a quantitative manner. Specifically, translational movements were determined by measuring the displacements of the nuclear centroid, while rotational motions were determined by measuring the angular displacements of the SPB (Fig. 1B). This analysis showed that the average rates of the translational and rotational motions were very small, with ~55% of the translational motions having an average rate between 15 and 20 nm/min (Fig. 1D, red columns), and ~78% of the translational motions having an average rate between 1 and 4°/min (Fig. 1E, red columns). We then examined the persistence of the motions by measuring the time that the nuclei moved in a unidirectional manner. This analysis revealed that ~95% and ~74% of the translational and rotational motions persist for 2–4 min and 0–3 min, respectively (Figs. 1F and G, red columns). Therefore, our results demonstrate that limited but noticeable nuclear movements can be induced by dynamic microtubules.

3.2. Mcp1p modulates microtubule dynamics to regulate nuclear motions

We have previously shown that mcp1p (Meiosis specific Coiled-coil Protein) is a key microtubule destabilizing factor, localizing at the microtubule plus ends to promote microtubule depolymerization (Zheng et al., 2014a). Specifically, the presence of mcp1p at microtubule plus ends increases microtubule catastrophe and rescue frequencies, two critical parameters determining duration of the contact of microtubules with cell tips ((Zheng et al., 2014a) and also see Fig. 2A). To reinforce this conclusion, we overexpressed in mcp1-deletion cells an ectopic mcp1p from the ase1p (abnormal spindle elongation 1) promoter (designated as mcp1Δ+ase1P-mcp1p)