Cytokinesis defect in BY-2 cells caused by ATP-competitive kinase inhibitors

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Abbreviations: AFs, actin filaments; BY-2, Bright Yellow-2; DMSO, dimethylsulfoxide; GFP, green fluorescent protein; 5-ITu, 5-iodotubercidin; MTs, mictrotubules.

Abstract

Cytokinesis is last but not least in cell division as it completes the formation of the two cells. The main role in cell plate orientation and expansion have been assigned to microtubules and kinesin proteins. However, recently we reported severe cytokinesis defect in BY-2 cells not accompanied by changes in microtubules dynamics. Here we also confirmed that distribution of kinesin NACK1 is not the cause of cytokinesis defect. We further explored inhibition of the cell plate expansion by ATP-competitive inhibitors. Two different inhibitors, 5-Iodotubercidin and ML-7 resulted in a very similar phenotype, which indicates that they target same protein cascade. Interestingly, in our previous study we showed that 5-Iodotubercidin treatment affects concentration of actin filaments on the cell plate, while ML-7 is inhibitor of myosin light chain kinase. Although not directly, it indicates importance of actomyosin complex in plant cytokinesis.

Cytokinesis in plants is essentially a process of building a new cell wall that separates two daughter cells. It is achieved by phragmoplast-guided vesicle transport to the division plane and vesicle fusion that forms a precursor of the cell wall. Dynamic changes in phragmoplast are essential for proper cell plate formation and expansion; however, our understanding of what triggers and controls those changes is far from complete.

Phragmoplast consists from microtubules (MTs), actin filaments (AFs) and associated proteins. MTs act as a driving force for vesicle trafficking and cell plate expansion. Kinesin proteins interact with MTs, providing spatial information for cell plate guidance or affecting their stability. Kinesin POK1 (phragmoplast-orientating kinesin-12) together with TAN and RANGAP1 is a part of pre-prophase band and during mitosis is localized to the cortical division zone. Knockouts of any protein from POK1, TAN and RANGAP1 results in cytokinesis defects[1,2,3]. It is considered, that in late cytokinesis POK1 is associated with peripheral MTs and provides directions for cell plate expansion[1]. Another kinesin NACK1 (kinesin-7 HINKEL) is an upstream member or NACK1/PQR pathway which regulates MTs turnover and promotes cell plate expansion[4].

We recently reported a cytokinesis defect, characterized by inhibition of the late cell plate expansion and disturbed AFs dynamics, in BY-2 cells[5] which occurs after treatment with ATP-competitive inhibitor of mitotic kinase Haspin, 5-Iodotubercidin (5-ITu), at final concentration 1 μM. Motor proteins, such as kinesin NACK1, play important role in cytokinesis in plant cells, however factors required for NACK1 localization to the cell plate are still unknown. 5-ITu could affect yet unknown pathway, important for NACK1 concentration on the cell plate, therefor we checked distribution of NACK1-GFP in BY-2 cells by live-imaging (Fig. 1). In control cells,

NACK1-GFP signal was restricted to the plate and dissipated shortly after cell plate fused with lateral cell borders (Fig. 1; DMSO). In cells treated with 5-ITu, NACK1-GFP was also concentrated on the cell plate; however, the cell plate did not complete expansion (Fig. 1; 5-ITu). NACK1-GFP signal gradually faded from the incomplete cell plate.

Kinesin NACK1 is an upstream member of the NACK-PQR pathway that promotes cell plate expansion in plant cells[4]. In our experiments, 5-ITu treatment did not affect initial setting of NACK1 on the cell plate, suggesting that NACK1 function was intact. It is consistent with previous results, where we demonstrated that localization and phosphorylation of the downstream member of NACK-PQR pathway, MAP65, did not change after 5-ITu treatment[5]. Although NACK1 faded from the cell plate that did not complete expansion, we suggest it was not the reason, but the outcome of cytokinesis defect.

Phragmoplast research have focused mostly on microtubules role in cytokinesis, thus, actin function remained ambiguous. Actin disruption with drugs delays cell plate expansion and causes tilted cell plates[6], but the underlying mechanisms were yet to be discovered. Recently actomyosin-driven phragmoplast guidance was suggested in moss[6]. Indeed, actomyosin complex is essential for division via contractile ring in animal and yeast cells[7]. In BY-2 cells, myosin proteins have been reported to co-localize to the phragmoplast[8,9,10,11] and further investigation of actomyosin complex in cytokinesis appears highly promising.

Cytokinesis defect in BY-2 cells induced by 5-ITu was characterized by inhibition of the cell plate expansion and changes in AFs dynamics, which were no longer concentrated on the cell plate under 5-ITu treatment[5]. Interestingly, similar incomplete cell plate formation was observed in stamen hair cells of *Tradescantia*

93 *virginiana* L treated with ML-7, specific ATP-competitive inhibitor of the myosin light
94 chain kinase[12]. It should be noted, that myosin light chain kinase homologues were
95 not discovered in plants so far, therefor proteins affected by ML-7 treatment are
96 unknown.
97 We tested the ML-7 effect on the cytokinesis in BY-2 cells expressing cell plate
98 marker GFP-KNOLLE. Indeed, we observed some cells that could not complete cell
99 plate expansion, similarly to 5-ITu treatment (Fig. 2A). In line with previous
100 publications[12], only high concentrations of ML-7 (200 μM) could cause cytokinesis
101 defect, however 1 μM 5-ITu treatment was sufficient to demonstrate same
102 phenotype (Fig. 2B). It indicates that 5-ITu and ML-7 affect same protein cascade,
103 involved into cell plate expansion in plant cells.
104 Both 5-ITu and ML-7 are ATP-competitive inhibitors of the kinase proteins. ATP
105 molecule is one of the universal energy carriers inside the cell and multiple proteins
106 have ATP-binding site. During cytokinesis, motor proteins will use ATP energy to
107 promote vesicle transport and cell plate expansion. Kinesins are associated with MT,
108 which a considered to play a major role in plant cytokinesis (Fig. 3). Although 5-ITu
109 treatment did cause sever cell plate expansion defect, the MT dynamics, vesicle
110 transport[5] and localization of kinesin NACK1 (Fig. 1) were not affected. On the
111 contrary, AFs were no longer concentrated on the cell plate. Myosin proteins are
112 known for actin-based motility and are indispensable from contractile ring in animal
113 cells. However, importance of actomyosin in plant cytokinesis only started to
114 emerge[8]. Potentially, ML-7 can bind to myosins ATP-binding site, and prevent it from
115 moving. In future studies, it can be confirmed by *in vitro* motility assay and by
116 observing the cytokinesis phenotype using non-competitive inhibitors of myosin
117 proteins[13]. Another explanation for cell plate expansion defect is inhibition of

phosphorylation pathway, which directly or indirectly affected AFs dynamics. 5-ITu is considered a specific inhibitor of Haspin kinase[14,15], however Haspin substrates in cytokinesis are still unknown. We suggest that phosphorylation analysis of 5-ITu treated cells, such as mass-spectrometry phosphoproteome studies, will provide valuable insights in this area.

Disclosure of interest

No potential conflicts of interest were disclosed.

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References

[1] Lipka E, Herrmann A, Mueller S. Mechanisms of plant cell division. WIREs Dev Biol. 2015. doi:10.1002/wdev.186.

[2] Walker KL, Muller S, Moss D, Ehrhardt DW, Smith LG. Arabidopsis TANGLED identifies the division plane throughout mitosis and cytokinesis. Curr Biol 2007, 17:1827–1836.

[3] Xu XM, Zhao Q, Rodrigo-Peiris T, Brkljacic J, He CS, Muller S, Meier I. RanGAP1 is a continuous marker of the Arabidopsis cell division plane. Proc Natl Acad Sci USA 2008, 105:18637–18642.

[4] Sasabe M, Machida Y. Regulation of organization and function of microtubules by the mitogen-activated protein kinase cascade during plant cytokinesis. Cytoskeleton. 2012; 69:913-918. doi:10.1002/cm.21072.

[5] Kozgunova E, Suzuki T, Ito M, Higashiyama T, Kurihara D. Haspin has Multiple Functions in the Plant Cell Division Regulatory Network. PCP. 2016; 57:848-861. doi:10.1093/pcp/pcw030.

[6] Kojo, K.H., Higaki, T., Kutsuna, N., Yoshida, Y., Yasuhara, H. and Hasezawa, S. Roles of cortical actin microfilament patterning in division plane orientation in plants. PCP. 2013; 54:1491-1503.

[7] Cheffings TH, Burroughs NJ, Balasubramanian MK. Actomyosin Ring Formation and Tension Generation in Eukaryotic Cytokinesis. Curr Biol. 2016 Aug 8;26(15):R719-37. doi: 10.1016/j.cub.2016.06.071.

[8] Wu SZ, Bezanilla M. Myosin VIII associates with microtubule ends and together with actin plays a role in guiding plant cell division. Elife. 2014; 3:1-20. doi:10.7554/eLife.03498.

[9] Haraguchi T, Tominaga M, Matsumoto R, Sato K, Nakano A, Yamamoto K, Ito K. Molecular Characterization and Subcellular Localization of Arabidopsis Class VIII Myosin, ATM1. J Biol Chem. 2014; 289(18):12343-12355. doi:10.1074/jbc.M113.521716.

[10] Yokota E, Ueda S, Tamura K, Orii H, Uchi S, Sonobe S, Hara-Nishimura I, Shimmen T. An isoform of myosin XI is responsible for the translocation of endoplasmic reticulum in tobacco cultured BY-2 cells. J Exp Bot. 2009; 60(1):197-212. doi:10.1093/jxb/ern280.

[11] Yokota E, Yukawa C, Muto S, Sonobe S, Shimmen T. Biochemical and Immunocytochemical Characterization of Two Types of Myosins in Cultured Tobacco. Plant Physiol. 1999; 121:525-534.

[12] Molchan TM, Valster AH, Hepler PK. Actomyosin promotes cell plate alignment and late lateral expansion in Tradescantia stamen hair cells. Planta. 2002; 214:683-693. doi:10.1007/s004250100672.

[13] Bond LM, Tumbarello D, Kendrick-jones J, Buss F. Small-molecule inhibitors of myosin proteins. Futur Med Chem. 2014; 5(1):41-52. doi:10.4155/fmc.12.185

[14] Fedorov O, Niesen FH, Knapp S. Kinase Inhibitors. Kuster B, ed. Methods Mol Biol. 2012; 795:109-118. doi:10.1007/978-1-61779-337-0.

[15] Wang F, Ulyanova NP, Daum JR, Patnaik D, Kateneva AV, Gorbsky GJ, Higgins JM. Haspin inhibitors reveal centromeric functions of Aurora B in chromosome segregation. J Cell Biol. 2012; 199(2):251-268. doi:10.1083/jcb.201205106.

Figure 1. Distribution of kinesin NACK1 throughout the cell cycle.

Live-cell imaging was performed on BY-2 cells expressing GFP-NACK1 after a 1-h treatment with DMSO (control) or 1 μM 5-ITu. Images for GFP-NACK1 and brightfield are a single focal plane acquired every 10 min. Numbers indicate time (hh:mm); the starting point of cytokinesis, when GFP-NACK1 was first observed on the cell plate, is shown in the 00:00 column. Scale bars = 10 μm.

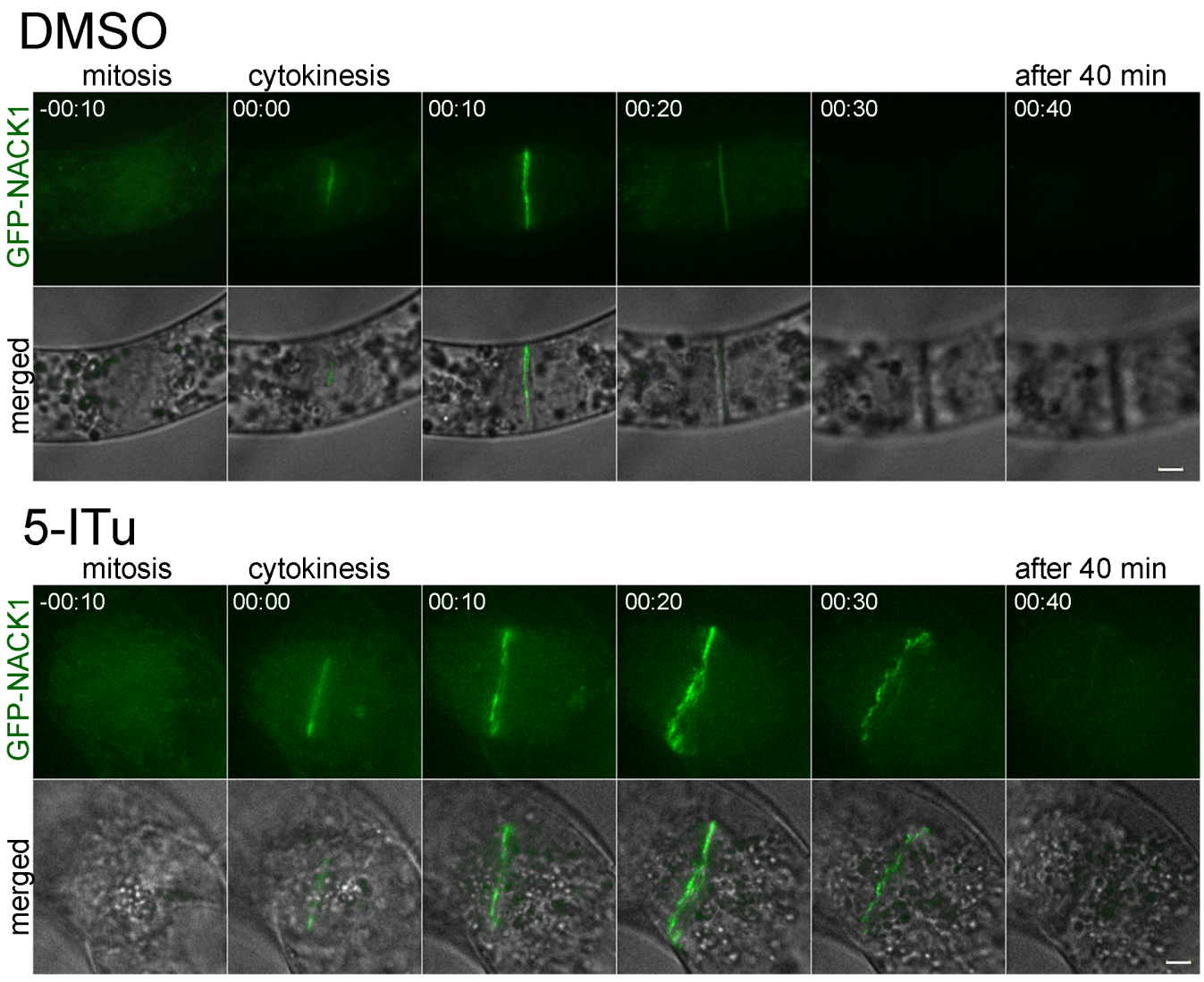
Figure 2. Cell plate expansion defect in BY-2 cells caused by ATP-competitive inhibitors 5-ITu and ML-7.

(A) Live-cell imaging of the BY-2 cells expressing cell plate marker GFP-KNOLLE after a 1-h treatment with DMSO (control), 1 μM 5-ITu or 200 μM ML-7. Numbers indicate time (hh:mm). First column (time frame 00:00) is first appearance of GFP-KNOLLE on the cell plate, and is considered a starting point of the cell plate expansion. Second column (time frame 60:00) shows same cell plate after 1 hr. Images for GFP-KNOLLE are the maximum projection of Z-planes. Scale bar = 10 μm. (B) Quantitative data for cytokinesis defect phenotype. BY-2 cells expressing GFP-KNOLLE were treated with DMSO (control; n = 19), 1 μM 5-ITu (n = 14) or 200 μM ML-7 (n = 20). Cytokinesis defect was categorized into two types: cell plate orientation defect (tilted cell plates, grey bars) and cell plate expansion defect (cell plates that did not complete expansion within 2-h, black bars).

Figure 3. Schematic representation of cytokinesis process in plant cells.

Cell plate expansion is guided by phragmoplast, which consists of MTs and AFs. Kinesin proteins are associated with MTs, facilitating vesicle transport

(unidentified kinesin) or promoting MT turnover (kinesin NACK1). Myosin proteins are present on the cell plate and cell borders, presumably using AFs as a bridge to guide cell plate towards cell borders.


DMSO
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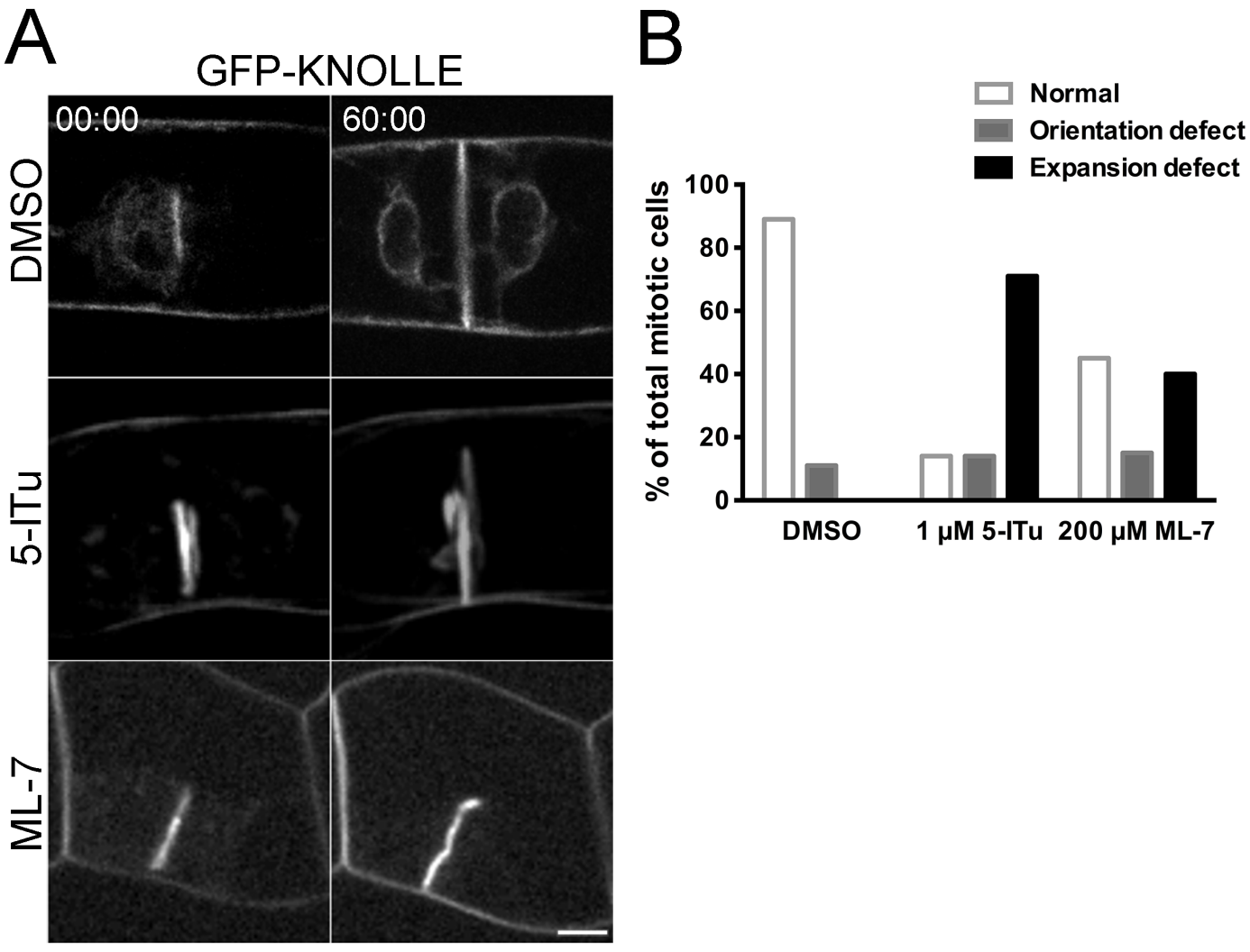
A
GFP-KNOLLE
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DMSO
5-ITu
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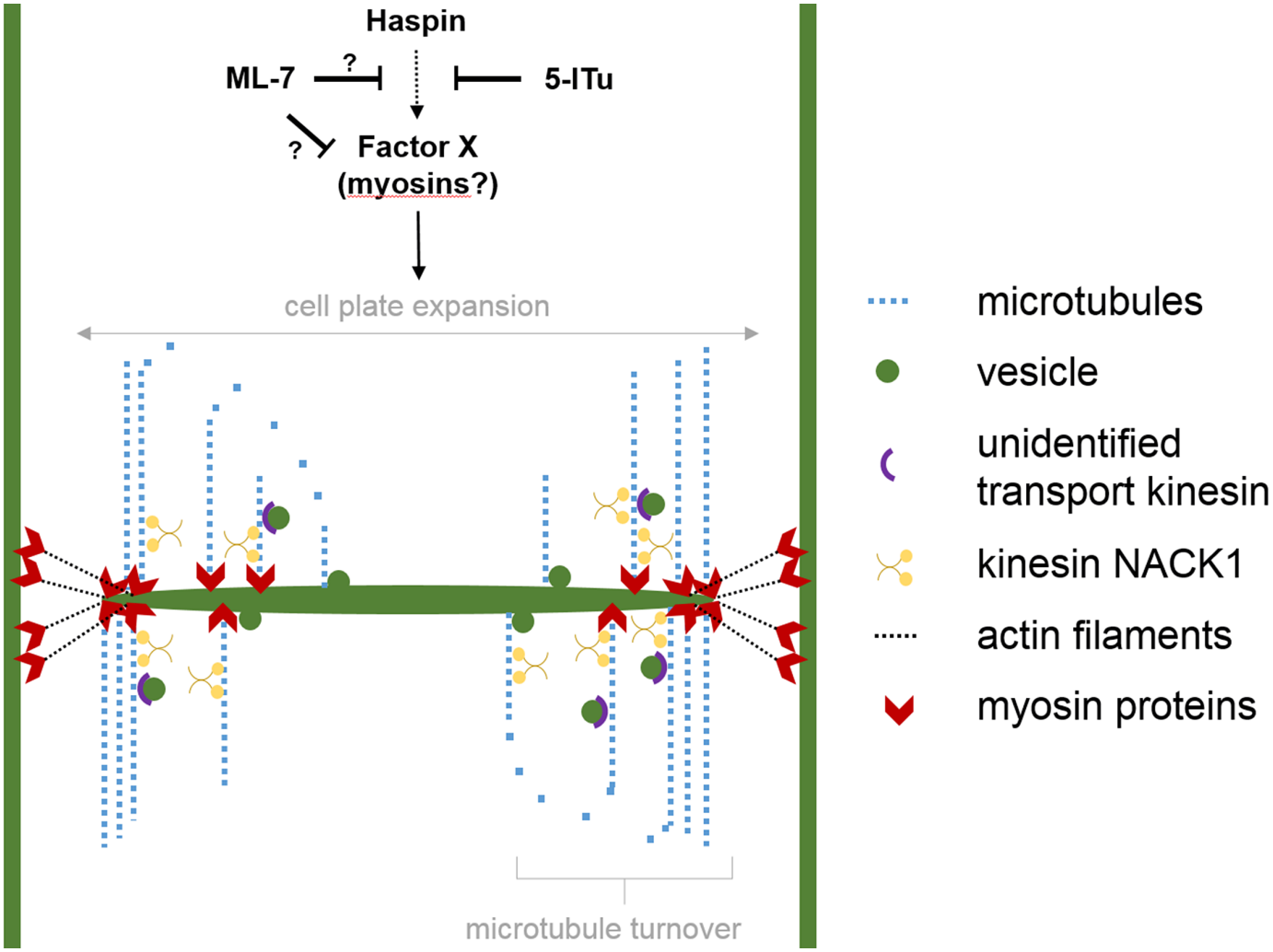
B
Normal
Orientation defect
Expansion defect
% of total mitotic cells
100
80
60
40
20
0
DMSO
1 µM 5-ITu
200 µM ML-7


Haspin
ML-7
?
5-ITu
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(myosins?)
cell plate expansion
microtubule turnover
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vesicle
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transport kinesin
kinesin NACK1
actin filaments
myosin proteins