

Research progress on the regulation mechanism of the mitotic spindle localization in budding yeast cells

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Abstract. Eukaryotic cells distribute genetic material equally to two daughter cells through mitosis to maintain the stability of genetic traits between parents and offspring. The key to the precise distribution of genetic material is spindle localization. Many studies have shown that spindle localization is a very complex life process regulated by many related proteins such as tubulin and dynein. It is not only essential for normal cell activities such as cell division, differentiation, and embryonic development but also related to the occurrence of diseases such as cancer. Over the years, although the regulatory mechanism of spindle localization in different organisms has been reported successively, the research on its regulatory mechanism in budding yeast is more in-depth. Based on the research status of mitotic spindle regulation mechanism of budding yeast cells at home and abroad, this paper summarizes the research progress of spindle localization regulation mechanism and related protein function in budding yeast in recent years.

Keywords: num1; budding yeast; spindle localization; mitosis.

1. Introduction

The mitosis of eukaryotic cells maintains the stability of the genome by transferring a copy of genetic material to daughter cells with the nucleus and spindle. In this process, spindle localization plays an essential role in the equal distribution of genetic material to daughter cells. If the distribution of chromosomes in daughter cells in mitosis is wrong, aneuploidy will appear, leading to tumors. In this process, the precise distribution of genetic material is inseparable from the correct positioning of the spindle. Therefore, clarifying the regulatory mechanism of spindle localization in mitosis is essential for the continuation and healthy development of human life.

In recent years, research on the regulation mechanism of mitotic spindle localization has been carried out in different organisms. As a single-cell eukaryote, Budding yeast has been favored by most researchers because of its short culture cycle, simple genetic operation, and perfect expression control system. Therefore, the regulation mechanism of spindle localization is more precise in budding yeast.

Yeast cell cycle regulation is similar to the standard cell cycle, and many of the genes involved in cell cycle regulation are essentially the same as those of higher organisms. The yeast cell cycle has distinctive features: the duration is short, about 90 min; the cell division process is closed, and the nuclear membrane does not depolymerize; and the spindle, which is directly related to nuclear division, is located in the nucleus, not in the cytoplasm. *Saccharomyces cerevisiae* divides in a budding manner (hence the name budding yeast) and divides unequally, i.e., the two daughter cells are unequal in size so that a rough estimate of the cell period can be made on the size ratio of the budding bodies.

In budding yeast, nuclear migration protein num1 plays an essential role in spindle localization. The initial research shows that cytoplasmic dynein binds to the "+" end of stellar microtubules far away from the spindle in budding yeast. At the same time, dynein is anchored to the cell cortex by using the motility of stellar microtubules. The dynein anchored to the cortex applies tension to a stellar microtubule through its anchoring protein num1 to move the spindle connected with microtubule "-" through the bud neck^[1]. Before that, myosin V interacted with the positive end of stellar microtubules through kar9-bim1 / EB1 and pulled the positive end along the actin cable, bringing the spindle close to the bud neck^[2,3]. The spindle is correctly located through the above two

processes so that the genetic material can be accurately distributed to the daughter cells. These studies probably reveal the basic process of spindle localization, but spindle localization is a very complex and accurate life activity in which many signal molecules will participate, including some non-essential proteins involved in regulation. Therefore, if you want to explore further the regulation mechanism of spindle localization,^[4] it is undoubtedly an excellent choice to start with the known interacting proteins of proteins involved in spindle localization. In addition, it is not clear how the functions of these essential proteins are regulated^[5]. However, we can explore their biochemical regulation by analyzing the phosphorylation and ubiquitination of essential proteins, which will also help to reveal the regulatory mechanism of spindle localization at a deeper level. This paper will review the regulatory mechanism of spindle localization based on the function of num1 and its related proteins.

2. Summary of basic characteristics of *Saccharomyces cerevisiae*

As a unicellular eukaryote, *Saccharomyces cerevisiae* has two life forms: haploid and diploid. The reproduction mode of yeast haploid is relatively simple, which is generally budding reproduction. A copy of genetic material is transmitted to daughter cells by budding, but it will die when the environmental survival pressure is high; Diploid cells mainly reproduce by mitosis, but they can reproduce by meiosis and produce haploid spores when the environmental conditions are bad. Haploids can be mated and fused to form diploid cells and continue to reproduce in mitosis.

Saccharomyces cerevisiae, as a model organism known as "Escherichia coli" in eukaryotes, is the preferred organism to study the life activities of eukaryotes. In addition to the above-mentioned stable growth and reproduction in the state of haploid and diploid, it can also easily convert between haploid and diploid under experimental conditions. Haploid cells can be any of the two mating types, type A and type B, respectively α Type. The two cells with different mating types are diploid cells (a)/ α . It can further proliferate through mitosis. It is undoubtedly very beneficial to the study of its gene function. In haploid *Saccharomyces cerevisiae*, the deletion mutant of a specific gene can be obtained by only one gene replacement by using the method of homologous recombination; For the gene with lethal deletion, the gene can be replaced in the diploid strain, and then the haploid strain with gene deletion can be obtained through spore screening. In addition, the regulation process of the *Saccharomyces cerevisiae* cell cycle is similar to the standard cell cycle, and many genes involved in the regulation of the cell cycle are the same as those of higher organisms. However, the yeast cell cycle has its obvious characteristics: the duration is short, about 90 min; The cell division process is closed, and the nuclear membrane does not depolymerize; The spindle directly related to nuclear division is located in the nucleus rather than in the cytoplasm; Unequal division, we can roughly estimate the period of the cell by the size of the daughter cell volume. In addition, *Saccharomyces cerevisiae* has the most basic characteristics of all eukaryotic cell life activities and has the advantages of easy culture and operation of microorganisms needed in experiments, rapid growth, etc. Therefore, single-cell eukaryotic budding yeast is a good model for studying mitotic spindle localization.

3. Protein molecules regulating spindle localization in budding yeast

In determining the direction of the mother-daughter cell axis and spindle localization in mitosis, in addition to various extracellular regulatory factors such as various physical conditions and environments, many necessary intracellular protein molecules also play a role. Together with mitotic cyclins, they are directly or indirectly involved in the directional process of cell mitosis. In recent years, the regulation mechanism of spindle localization in budding yeast has been studied clearly, and many proteins such as num1, kar9, and EBI have been found. These proteins orderly pull the spindle through the bud neck-through continuous and coordinated work to realize the balanced distribution of genetic material. Understanding the specific functions of these proteins will help to reveal the regulatory mechanism of spindle localization better.

3.1. Dynein binding tubulin controls spindle localization

Dynein is a regulatory protein found in the initial study of spindle localization in budding yeast. As a large protein complex in the cytoplasm, it has ATPase activity and can drive material transport in cells. The study is shown that the spindle is involved in the division and inactivation of the nuclear membrane, which is involved in the function of the spindle.

Dynein participates in the regulation of spindle localization in *Saccharomyces cerevisiae*. In *Saccharomyces cerevisiae* mitosis, one end of dynein and its complex is bound to the positive end of tubulin, and the other end is anchored to the cell cortex through the motility of microtubules. The dynein bound to the positive end of microtubules slides along the cell membrane to its opposing end, resulting in tension on cytoplasmic microtubules. Finally, microtubules move forward, driven by dynein. Thus, the spindle and nucleus connected to the negative end of microtubules are pulled through the bud neck^[6]. In the experiment, ADAMES et al. [7] found that microtubules keep the positive end forward and slide forward along the plasma membrane for up to half a minute. If the dynein and its complex are lost, *asus*, Overview of essential characteristics of *Saccharomyces cerevisiae*, After living, the spindle can not pass through the bud neck but stay near the bud neck. These phenomena indicate that dynein is a necessary regulatory molecule for spindle localization.

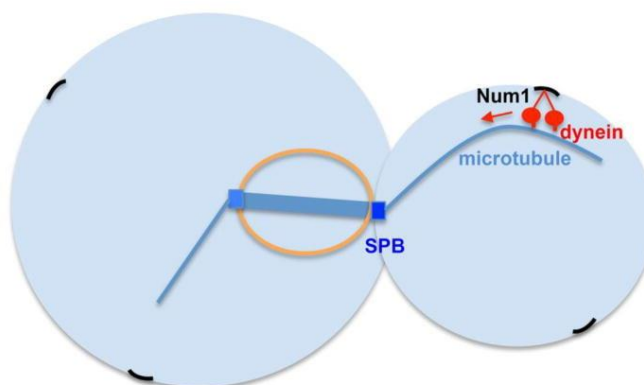


Fig.1 Dynein mediates the spindle through the bud neck^[3]

A cartoon depicts *Saccharomyces cerevisiae* dynein walking on an astral microtubule towards its minus end at the spindle pole body (SPB). It causes the microtubule to slide along the cortex. The anaphase spindle is positioned across the bud neck by dynein's activity.

In addition, dynein also plays an essential role in spindle checkpoint inactivation. After the cell enters mitosis, when it is found that there is no corresponding microtubule attachment at the centromere, Bub1, as the upstream component of spindle physical examination, can be located at the centromere and activated by its high phosphorylation to recruit Mad1 and Mad2 at the centromere. Mad1 stimulates the conformation change of MAD2, transforms from passive confirmation to active conformation, and helps bind Cdc20. Thus, bub1-bub3-mad2-cdc20 mitotic checkpoint complex is formed to inhibit Cdc20 and not activate ubiquitination ligase anaphase-promoting complex/cyclosome (APC / C) and inhibit its ubiquitination. To avoid the degradation of downstream factor securin, the activation of subsequent factor separase, and the cleavage of cohesion, prevent the premature separation of sister chromosomes^[8].^[9] Once the kinetochores on all chromosomes are respectively connected with the corresponding microtubules, Mad1, Mad2, and Bub1 move along the microtubules to the two poles of the spindle under the action of dynein and then separate APC from its inhibitory protein^[10], and the spindle physical examination point is closed. On the one hand, dynein controls spindle localization through attachment plasma membrane and microtubules; On the other hand, the spindle physical examination checkpoint is inactivated by acting on the spindle core checkpoint protein. Therefore, dynein is an essential protein molecule to ensure the smooth mitosis of budding yeast cells.

3.2. Effect of num1 on spindle positioning

Kormanecj et al. First isolated a new periodic gene num1 in *Saccharomyces cerevisiae*,^[11] which encodes a 313kda protein, which is composed of a predicted N-terminal helix domain, a predicted EF-hand, a central region containing 64 unknown functional amino acids in the 30th repeat and a C-terminal pleckstrin homologous (PH) domain. In cells lacking num1, mother cells often have two non-isolated nuclei. We know that similar phenotypes occur when cells are depleted of energy or treated with organic reagents that inhibit spindle syntheses, such as colchicine and benzoyl. Therefore, num1 is considered to affect nuclear migration by affecting the role of the spindle.

3.2.1 Two domains of num1

In budding yeast, the cortical attachment of dynein requires nuclear migration protein num1. It is found that num1 has two domains: the PA domain near the N-terminal and PH domain near the C-terminal. Among them, the PH domain at the C-terminal of num1 protein mediates the binding of num1 to the cell membrane by binding to inositol phosphate PI (4,5) P2 on the cell membrane, and PI (4,5) P2 is necessary for the PH domain to guide the cortical localization of num1, but not enough to mediate the cortical assembly of num1 plaques^[12]; Therefore, the only role of PH domain is to target num1 to the cell cortex. However, Tang et al.^[12] found that this targeting mechanism can be replaced entirely by another num1 targeting mechanism guided by the membrane targeting sequence of yeast ras2 protein CAAX motif. CAAX motif can replace the PH domain to target num1 to the cortex, but this targeting does not depend on the PI (4,5) P2 level of the cell cortex. However, CAAX targeted num1 and wild-type num1 show different localization to a great extent. This different localization does not affect the dynein pathway, and it still plays a role in the dynein pathway. In addition, the CAAX motif can also save the growth defect of kar9 deletion and form a normal colony similar to the wild type.

The^[1] PA domain located at the N-terminal of num1 protein mediates the formation of multi-molecular complexes on the cell membrane. In spindle localization, dimer num1 molecules are first recruited to the cell cortex. Further, dimer molecules are recognized and combined through their N-terminal PA domain and assembled into fixed plaques in the cell cortex. Then, the dynein connected to the positive end of microtubules interacts with num1 plaques through the PA domain and is anchored to the cell cortex.^[1] In addition, the PA domain also mediates the connection between num1 and mitochondria by binding to cardiolipin on mitochondria.

3.2.2 Num1 relieves PAC1 mediated inhibition and activation of the dynein movement mechanism

In addition to attaching to the cell cortex,^[13] dynein also needs to target the positive end of microtubules and then pull the spindle through the bud neck. This process requires Bik1 and dynein-related factor PAC1. PAC1 is connected with the positive terminal binding protein Bik1 and then anchored to the positive end of microtubules and prevents the negative end movement of dynein. Once dynein binds to num1cc, dynein may rotate randomly to make it directly contact the positive end through mtbd, then release PAC1, and then move to the negative end to pull the spindle slowly through the bud neck. Studies have shown that the ph-dyn1-num1cc complex may be oligomerized in the cell cortex, and the interaction between the three does not interfere.^[13] Lammerslg et al. Observed the following phenomena by inducing the overexpression of num1cc: the number of positive terminal lesions of dyn1 - (dynein heavy chain) and jnm1-3mcherry (P50 subunit of dynactin) decreased by about 5 times; Only 29.1% of ph-dyn1-3m cherry lesions contained pac1-3yfp fluorescence; In addition, fluorescence intensity measurements also showed a significant reduction in the number of PAC1 molecules associated with cortical pH dynein patches. In contrast, there was no significant change in the frequency or intensity of CO localized dynactin, and Western blot showed that the decrease in localization was not due to the decrease of PAC1 protein expression or stability. These suggest that num1cc activates dynein movement by consuming microtubule positive dynein dynactin by mediating the reduction of dynein PAC1 interaction.

3.2.3 Two traction mechanisms of spindle localization mediated by num1

Studies have shown that the PH domain at the C-terminal of num1 protein targets num1 to the whole-cell cortex by binding to inositol phosphate PI (4,5) P2 on the cell membrane. It is found that num1 targeting the cell cortex has different populations, and the functions of these different populations are also different. In wild-type cells, num1 is distributed along the bud cortex, and the dynein is connected with the positive end of microtubules. It is anchored to the cortex by microtubule dynamics. After num1, motility is activated and moves towards the negative end of microtubules, which makes microtubules slide along the bud cortex and pull the spindle through the bud neck. It is the first discovery of the num1 mediated spindle traction mechanism. Later ^[14], Omer et al. found that the cortical distribution of num1 significantly changed the dynein-dependent spindle traction mechanism. By deleting endoplasmic reticulum protein scs2 / 22, they found that num1 was only obviously located at the bud end but did not lead to the error of spindle positioning. At this time, the dynein mainly produced tension through the MT capture contraction mechanism to make the spindle pass through the bud neck. In this mechanism, anchored dynein is spatially limited to interact with the ends of stellar microtubules. Dynactinma acts as a cortical connector to maintain the connection between MT ends and the cortex. The attached MT causes the spindle to move towards the bud tip through shortening and contraction and further aligns the spindle along the mother bud axis. However, this tension, which depolymerizes MT into bud tip dynamin, is attenuated when Num1 is typically distributed along the bud cortex. Therefore, the two stretching mechanisms of NUM1-mediated spindle location can jointly regulate spindle movement into the bud neck, but in WT and Num1 GFP CAAX SCS2/22DKAR9D cells where Num1 is distributed along the bud cortex, the tension generated by dynamin through MT sliding mechanism is dominant.

3.2.4 The small cluster of num1 mediates the mechanism of dynein anchoring and spindle localization

Yeast dynein needs to be anchored to the clustered num1 to slide along the bud cortex, and the cortical aggregation of num1 is controlled by its assembly factor mdm36. Omer et al. Found that overexpression of mdm36 led to the overall enhancement of the num1 cluster but revealed the apoptosis of the dim num1 cluster anchored by cortical dynein. ^[15] Direct imaging showed that the bud localization fuzzy num1 cluster containing only six num1 molecules mediated dynein-dependent spindle traction through the transverse MT sliding mechanism. Moreover, the percentage of cells in cortical dyn1-3gfp lesions in mdm36 Δ deleted cells was not significantly different from that in WT. However, mdm36 Δ kar9 Δ The aggregation of num1 in the cortex was affected, with kar9 Δ Compared with a single mutant, its offspring formed viable colonies, did not show obvious growth defects, and the function of the dynein pathway was in mdm36 Δ There are no defects in cells, but this effect undoubtedly destroys the interaction between mitochondria and num1. Therefore, mutations affecting num1 aggregation will interfere with mitochondrial aggregation but will not interfere with the spindle traction function of num1 based on dynein, that is, with a small amount of num1 points, asus. The analysis here is good. There should be a comparative study. It is suggested that the previous analysis should also be written according to this template.,The small and dim plaques of the son are sufficient to realize the functions of cortical dynein anchoring and spindle positioning. It indicates that num1 aggregation plays a more important role in mitochondrial anchoring than dynein anchoring.

4. The function of Kar9 in spindle localization

The mitotic spindle localization of *Saccharomyces cerevisiae* is two continuous processes. One process is that the stellar microtubules mediated by dynein slide along the bud cortex and pull the spindle through the bud neck; Before that, myosin V interacted with the positive end of stellar microtubules through kar9-bim1 / EB1 and pulled the positive end along actin cable to make the spindle close to the bud neck.^[16-17] It was found that three sites in the C-terminal disordered domain of ^[18] kar9 mediated the close interaction between kar9 and the C-terminal dimer domain of bim1

(EB1 homology). Site1 and site2 contain sxip motifs, which mediate the recruitment, nuclear movement, and mitosis of kar9 to the end of microtubules, but only site2 plays a role in spindle localization in the metaphase; However, the site3 is the binding site of EBI. Liakopoulos^[19] et al. found that kar9 protein connects microtubules to the plasma membrane through myo2 (myosin V) and mediates the movement of microtubules along with the microfilament skeleton the plasma membrane to the budding end. Myo2 (myosin V) is connected to the plasma membrane through the interaction between kar9 and the positive end of astral microtubules. Bim1, located at the positive end of microtubules, grows to the bud cortex by increasing the length and dynamics of microtubules. At this time, bim1 interacts with cortical protein kar9. Then myosin V moves along the positive end of the actin cable, and the spindle is close to the bud neck.^[20] Cib4 / CDC28 enzyme can phosphorylate kar9 protein and prevent it from locating at one end of the mother cell of the spindle. In this way, the polar distribution of kar9 protein on the spindle ensures that one end of the spindle is pulled close to the bud end, which ensures the correct positioning of the spindle. Therefore, kar9 mediated spindle approaching the bud neck is the first step of spindle positioning. After the spindle approaches the bud neck, num1 and dynein then perform the function of the spindle passing through the bud neck. These two continuous and coordinated processes control the correct positioning of the spindle in each dividing cell.

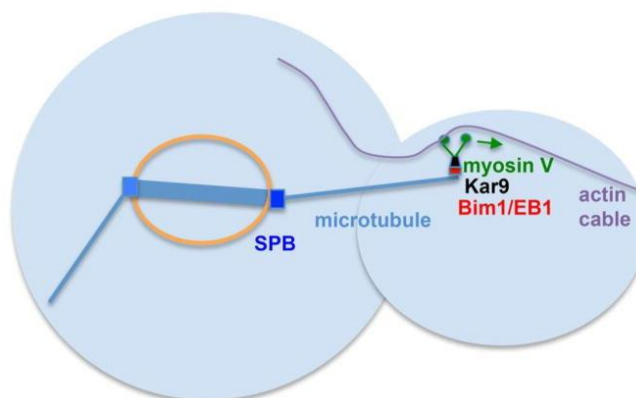


Fig.2 Kar9 mediates the spindle toward the bud neck^[3]

A picture depicts the movement of an anaphase spindle toward the bud neck in *S. cerevisiae*. Bim1/EB1 interacts with Kar9 at the microtubule plus end, which connects with the myosin V motor, and myosin V walks along the actin cable formed by the bud cortex, causing the spindle/nucleus to move toward the bud neck.

5. EBI controls the correct orientation of spindles in cells

EBI protein is a typical microtubule positive end binding protein composed of conserved N-terminal and C-terminal domains. It mediates the localization of a series of + T1P proteins at the positive end of growing microtubules and maintains a dynamic balance at the microtubule end.^[21] During mitosis, EB1 is considered to be a kinetochore protein. However, it is only located at the posterior follow-up point of microtubule growth, indicating that the kinetochore localization of EB1 depends on the growth of kinetochore microtubules and the N-terminal domain of EB1 protein mediates the direct binding of protein to microtubules. In addition, EBI also ensures that the microtubule cathode can effectively find its binding sites by increasing the dynamics of microtubules. EBI mainly controls the correct orientation of spindles in cells during mitosis.^[22] In normal mitosis, the spindle is usually located at the geometric center of the cell, while in cells without EBI, the spindle location often deviates from this center, resulting in the wrong location.^[23] Dema Alessandro et al. Found that in cells without EB1 activity, astral microtubules could not participate in cortical dynein mediated spindle migration. Therefore, EBI is also very important for the correct positioning of the cell spindle. In addition,^[24] the C-terminal of EBI can also bind to APC, a tumor suppressor protein.

The deletion of EBI and APC will lead to the wrong separation of metaphase sister chromosomes, which can not be detected by spindle physical examination, which will lead to aneuploidy or tumor.^[25] Moreover, in most rectal cancers, the interaction between EBI and APC is destroyed. Cells that lose their interaction can cause chromosomal instability, one of the carcinogenesis sources. Therefore, the regulation of EBI on spindle localization and chromosome segregation is closely related to tumors and other diseases, which is worthy of in-depth study.

6. Prospect

With the deepening of research, the mechanism of spindle localization regulation in *Saccharomyces cerevisiae* and the functions of related proteins involved in spindle localization regulation are gradually clear, but there are still many related problems worth exploring. On the one hand, the above research results only know more about some essential proteins for spindle localization regulation, such as num1 and dynein. Without these proteins, the spindle will not be located correctly. As we all know, spindle localization is a precise and complex process. In addition to these proteins, some proteins are still involved in the spindle. The regulation of spindle localization but its loss will not affect the correct localization of the spindle. Therefore, we want to elaborate on the spindle localization regulation mechanism further^[4] to explore its function of spindle regulation from the interaction proteins of known proteins is helpful to more comprehensively elaborate on the spindle localization regulation mechanism.^[26] Snf1 aids in correct spindle alignment in budding yeast mitosis, and snf1 acts parallel with Dyn1 and in synergy with Kar9 to promote spindle orientation. Therefore, studying the functions of these interacting proteins can provide a deeper insight into the mechanism of spindle localization. On the other hand, we only know the main functions of each protein, but how these functions are regulated is still unclear. Therefore, exploring the biochemical regulation of these functional proteins by analyzing the phosphorylation and ubiquitination of related proteins^[5]. It also helps reveal the regulatory mechanism of spindle localization on a deeper level. Spindle localization is closely related to tumors and other diseases. If the spindle localization is wrong, there will be an abnormal distribution of genetic material, which can lead to aneuploidy and even tumors. On the contrary, if the specific regulation mechanism of spindle localization can be clarified, the spindle can be mislocated by inhibiting the relevant targets of spindle localization. To curb the proliferation of cancer cells, the treatment of tumors will be improved to a great extent.

References

- [1] Tang X, Germain B S, Lee W L. A novel patch assembly domain in Num1 mediates dynein anchoring at the cortex during spindle positioning [J]. *Cell Biol*, 2012, 196(6): 743-56.
- [2] Fan Y, Yao N, Lu Y, Liu H. Role of EB1 protein in regulating microtubule dynamics, spindle localization, and chromosome stability [J]. *Journal of Shaanxi Normal University (Natural Science Edition)*, 2007, (S2): 95-98.
- [3] Xiang X. Nuclear movement in fungi. *Semin Cell Dev Biol*, 2018, 82: 3-16.
- [4] Tang X, Xiao Y, Hai L. The interaction proteins of Num1 were identified by in vitro binding assay and mass spectrometry [J]. *Journal of South-Central University for Nationalities (Natural Science Edition)*, 2018, 37(04): 27-30.
- [5] Xiao Y. Phosphorylation analysis of Num1 and identification of interacting proteins [D]. *South-central University for Nationalities*, 2018.
- [6] Yang M, Li B, Liu C, Tomchick D R, Machius M, Rizo J, Yu H, Luo X. Insights into mad2 regulation in the spindle checkpoint revealed by the crystal structure of the symmetric mad2 dimer. *PLoS Biol*, 2008, 6(3): e50.
- [7] Adames N R, Cooper J A. Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae* [J]. *Cell Biol*, 2000, 149(4): 863-874.

- [8] Kuhkian A, Han J S, Cleveland D W. Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding [J]. *Dev Cell*, 2009, 16(1): 105 — 117.
- [9] Pinsky B A, Kung C, Shokat K M, et al. The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores [J]. *Cel Biol*, 2006, 8;78-83
- [10] Howell B J, McEwen B F, Canman J C, Hoffman D B, Farrar E M, Rieder C L, Salmon E D. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J Cell Biol*, 2001, 155(7): 1159-72.
- [11] Ján Kormanec et al. Nuclear migration in *Saccharomyces cerevisiae* is controlled by the highly repetitive 313 kDa NUM1 protein [J]. *MGG Molecular & General Genetics*, 1991, 230(1-2): 277-787.
- [12] Tang X, Punch J J, Lee W L. A CAAX motif can compensate for the PH domain of Num1 for cortical dynein attachment. *Cell Cycle*, 2009, 8(19): 3182-90.
- [13] Lammers L G, Markus S M. The dynein cortical anchor Num1 activates dynein motility by relieving Pac1/LIS1-mediated inhibition. *J Cell Biol*, 2015, 211(2): 309-22.
- [14] Omer S, Greenberg S R, Lee W L. Cortical dynein pulling mechanism is regulated by differentially targeted attachment molecule Num1. *Elife*, 2018, 7: e36745.
- [15] DeZwaan TM, Ellingson E, Pellman D, Roof DM. Kinesin-related KIP3 of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration. *J Cell Biol*. 1997; 138(5): 1023–40.
- [16] Cottingham FR, Hoyt MA. Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically acting microtubule motor proteins. *J Cell Biol*. 1997; 138(5): 1041–53.
- [17] Omer S, Brock K, Beckford J, Lee W L. Overexpression of Mdm36 reveals Num1 foci that mediate dynein-dependent microtubule sliding in budding yeast. *J Cell Sci*, 2020, 133(20): jcs246363.
- [18] Manatschal C, Farcas A M, Degen M S, Bayer M, Kumar A, Landgraf C, Volkmer R, Barral Y, Steinmetz M O. Molecular basis of Kar9-Bim1 complex function during mating and spindle positioning. *Mol Biol Cell*, 2016, 27(23): 3729–45.
- [19] Liakopoulos D, Kusch J, Grava S, et al. Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment [J]. *Cell*, 2003, 112(4): 561-574.
- [20] Zhao L, Yang C, Zhuang F. Factors affecting the direction of mitosis in animal cells the role of stress fibers in spindle localization [J]. *Chinese Journal of Biochemistry and Molecular Biology*, 2010, 26(11): 981-987.
- [21] Wang Z. Functional Studies of microtubule terminal regulatory proteins CSPP1 and EB1 [D]. University of Science and Technology of China, 2014.
- [22] Dema A, van Haren J, Wittmann T. Optogenetic EB1 inactivation shortens metaphase spindles by disrupting cortical force-producing interactions with astral microtubules. *Curr Biol*, 2022, 32(5): 1197-1205.
- [23] Draviam V M, Shapiro I, Aldridge B, Sorger P K. Misorientation and reduced stretching of aligned sister kinetochores promote chromosome missegregation in EB1-or APC-depleted cells. *EMBO J*, 2006, 25(12): 2814-27.
- [24] Fodde R, Kuipers J, Rosenberg C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability [J]. *Nature Cell Biology*, 2006, 1(4): 149.
- [25] Tripodi F, Fraschini R, Zocchi M, Reghellin V, Coccetti P. Snf1/AMPK is involved in the mitotic spindle alignment in *Saccharomyces cerevisiae*. *Sci Rep*. 2018 Apr 11; 8(1): 5853.