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核黃素激酶調節有絲分裂的意外作用

An Unexpected Role for Riboflavin Kinase in Regulating Mitosis

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國立臺灣大學碩士學位論文
口試委員會審定書

核黃素激酶調節有絲分裂的意外作用
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本論文係林依潔君（R04B48001）在國立臺灣大學基因體與系統生物學學位學程完成之碩士學位論文，於民國 107 年 6 月 29 日承下列考試委員審查通過及口試及格，特此證明

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中文摘要

為了確保細胞內基因的穩定性，在有絲分裂期間調節染色體分離是非常重要的一個過程。有絲分裂主要是由細胞週期蛋白所調控，但合適的營養條件對於細胞週期的影響也是非常重要。有趣的是，我們發現產生能量的輔因子:黃素單核苷酸（FMN)在調節有絲分裂中具有重要的角色,黃素單核苷酸 是由核黃素激酶（Fmn1）催化核黃素（維生素 B_2）所形成。在酵母菌中，細胞週期結束時必須要透過 mitotic exit network（MEN） 的訊息調節，而 Lte1 是 MEN 的正調控因子。和組蛋白修飾有關的基因，*SET1*，也被報導和 mitotic exit 有關。有趣的是，我們發現 *FMN1* 和 *SET1* 還有 *LTE1* 皆有基因相互作用。我證實了有絲分裂的延遲造成此基因相互作用，而且，*FMN1* 的功能與 MEN 是平行的，因為 *FMN1* 會表現出與 *CLA4* 和 *CDC15* 有基因的相互作用，而此兩者都是參與 MEN 的調控。另外，*lte1Δ fmn1Δ* 的生長缺陷可以通過缺失 *BUB2* 來解救。而 *BUB2* 是 MEN 的負調控因子，也就是說，我發現，很重要地，*FMN1* 會與細胞週期的調控有關，而且是跟 mitotic exit network 有平行關係。更進一步地，我們發現 *FMN1* 參與在 anaphase promoting complex（APC）中，而且會有 metaphase-anaphase 時期轉換的缺陷。Ras2 會抑制 APC 的功能,而我們發現 *fmn1* 突變體的生長缺陷可通過缺失 *RAS2* 而得以挽救。我們也發現了 *fmn1* 突變體中表現 APC 缺陷的症狀: *fmn1* 突變體導致染色體分離錯誤和有絲分裂週期蛋白 Clb2 的異常積累。綜上所述，*FMN1* 缺陷會導致 metaphase 和 anaphase 轉換上出現錯誤。因此,*FMN1* 可能在調節有絲分裂中扮演重要的角色。

關鍵字：黃素單核苷酸，有絲分裂，染色體分離，MEN，Lte1，APC

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英文摘要

The regulation of chromosome separation during the mitotic phase is essential to ensure genome stability. Mitosis is controlled by complex regulation network, including proper nutritional and metabolic status to ensure smooth cell cycle transition. Interestingly, our genetic screening identified that an energy producing cofactor, flavin mononucleotide (FMN) has an important function in regulating mitosis in budding yeast. The Mitotic Exit Network (MEN) is a signaling pathway known to drive cells out of mitosis and to promote the faithful division of cells. We found that *FMN1*, encoding a Riboflavin kinase, confers a genetic interaction with *LTE1*, coding for a positive regulator of MEN. In addition, *FMN1* displayed negative interactions with *CLA4* and *CDC15*, both of which are involved in the regulation of MEN. Furthermore, the growth defect of *lte1Δ fmn1Δ* could be rescued by the deletion of *BUB2*, which is a negative regulator to the MEN activity. The growth defect of *fmn1Δ* could be rescued by the deletion of *RAS2*, which inhibits the function of anaphase promoting complex (APC), indicating that the Fmn1 may play a role in regulating APC. Furthermore, the *fmn1Δ* mutants exhibited anaphase entry delay, chromosome segregation error and abnormally accumulation of mitotic cyclin, Clb2. Taken together, ablation of *FMN1* leads to defects in metaphase-anaphase transition. Thus, *FMN1* may play a role in regulating mitosis.

Key word: riboflavin kinase, mitosis, mitotic exit, Lte1, MEN, APC, Clb2

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INTRODUTION

1 The function of riboflavin

Riboflavin, also called Vitamin B_2. The richest natural source is yeast. Riboflavin is involved in many processes in the body and is necessary for normal cell growth and function. Riboflavin deficiency in the body may cause various types of cancer, and for migraine headaches. Taking riboflavin helps for acne, muscle cramps, burning feet syndrome, carpal tunnel syndrome, and blood disorders such as congenital methemoglobinemia and red blood cell aplasia.

Riboflavin is one of eight B vitamins. B vitamins are a class of water-soluble vitamins that play important roles in cell metabolism. Though these vitamins share similar names, they are chemically distinct that often coexist in the same foods. All B vitamins help the body to convert carbohydrates into glucose, which is used to produce energy. These B vitamins also help the body metabolize fats and protein.

Riboflavin functions as a coenzyme, meaning that it is required for enzymes (usually proteins) to perform normal physiological actions. Riboflavin functions for the metabolism of carbohydrates, proteins, and lipids. It plays a crucial role in the production of hematopoietic stem cell, Nervi nervorum and protein synthesis. It acts as a coenzyme in the intermediate metabolism and participates in the reaction of folate synthase and vitamin B_6 production.

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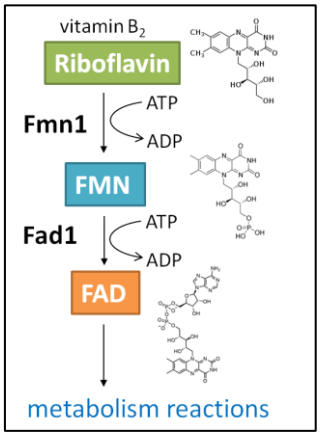
The using forms of riboflavin are riboflavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in eukaryotes. FMN and FAD bind to proteins to form the flavoproteins. This utilization of FMN and FAD are similar to the distribution across all kingdoms of life (Koch & Macheroux, 2016).

1.1 The function of Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)

FMN and FAD are important for many metabolic reactions. Riboflavin serves as a precursor, riboflavin kinase (Fmn1) phosphorylate riboflavin to form FMN. FAD synthetase (Fad1) catalyzes adenylylation of the FMN to form FAD. These riboflavin coenzymes play important roles in many metabolic reactions including amino acid carbohydrate and lipid, protein metabolism and in the conversion of folic acid and pyridoxine into their coenzyme forms.



FMN and FAD are the redox cofactor for the prosthetic group of many metabolic proteins. These proteins are called flavoproteins. Flavoproteins contain a

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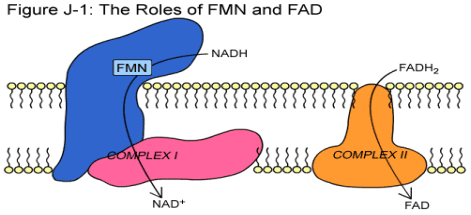
flavin moiety and it is essential for the protein function. Flavoproteins utilize the unique and versatile structure of flavin moieties to catalyze difficult redox reactions. Since flavins have, multiple redox states they can participate in processes that involve the transfer of one or two electrons, hydrogen atoms, or hydronium ions.

One well-known reaction is part of the citric acid cycle (also known as the TCA or Krebs cycle). In the electron transport chain, FMN is one of the components of complex I while FAD is involved in the activity of complex II (Succinate dehydrogenase). FAD acts as an electron carrier and takes part in both the Kreb's Cycle and oxidative phosphorylation. It accepts electrons and is transformed into $FADH_2$ that stored momentarily of the high-energy electrons from this oxidation. $FADH_2$ then transfers its electrons to complex II of the electron transport chain. For each pair of electrons from $FADH_2$ passed along the electron transport chain, 1.5 units of ATP molecules are formed.

Figure J-1: The Roles of FMN and FAD



The Complex I protein of the Electron Transport Chain is composed of an FMN group and a variety of other proteins. NADH, an electron carrier, binds to Complex I and transfers its electrons to FMN. $FADH_2$, another electron carrier, transfers its electrons to Complex II. Once $FADH_2$ transfers its electrons, it is converted back to FAD and used again as an electron carrier in metabolic processes.

http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/riboflavin/

The genome of *Saccharomyces cerevisiae* contains 68 genes (~1% of all yeast

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proteins) which encode for flavin-dependent proteins. Thirty-five flavoproteins require FAD (74%) and fifteen require FMN (26%). A large number of flavoproteins are present in cells suggest their important roles in cells. However, many yeast flavoenzymes are poorly characterized. Biochemical properties such as substrate specificity, kinetic parameters and reaction partners need to be determined to improve our understanding of human orthologs (Koch and Macheroux, 2016).

FMN functions as prosthetic group of various oxidoreductases including NADH dehydrogenase as well as cofactor in biological blue-light photoreceptors (Kerscher, 2000; Joseph-Horne, *et al.,* 2001; Christie, *et al.,* 1999). FMN is the form in which riboflavin is found in cells and tissues. It requires more energy to produce, but is more soluble than riboflavin. FMN is a stronger oxidizing agent than NAD and is particularly useful because it can take part in both one- and two-electron transfers (Casaus, *et al.,* 2002). During the catalytic cycle, a reversible interconversion of the oxidized (FMN), semiquinone ($FMNH^{\cdot}$) and reduced ($FMNH_2$) forms occurs in the various oxidoreductases (Jablonski & DeLuca, 1977).

FAD displays important roles in many metabolic processes. FAD is the more complex and abundant form of flavin. FAD is required to convert retinol (vitamin A) to retinoic acid via cytosolic retinal dehydrogenase and tryptophan to niacin (vitamin B_3) (Dalfó, 2007; Powers, 1999). FAD is also required for the production of pyridoxic acid

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from pyridoxal (vitamin B_6) by pyridoxine 5'-phosphate oxidase and the fatty acid oxidation by fatty acyl CoA dehydrogenase (Powers, 1999). Oxidation of pyruvate, α-ketoglutarate, and branched-chain amino acids requires FAD in the shared E3 portion of their respective dehydrogenase complexes (Islam, *et al*., 2009). Synthesis of an active form of folate (5-methyltetrahydrofolate) from 5, 10-methylenetetrahydrofolate by Methylenetetrahydrofolate reductase is $FADH_2$ dependent (Lubran, 1971). Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) by Glutathione reductase is also FAD dependent (Pai & Schulz, 1983).

FAD can exist in four different redox states, which are the flavin-N (5)-oxide, quinone, semiquinone, and hydroquinone (Iyanagi & Mason, 1973; Teufel, *et al.*, 2013). FAD is converted between these states by accepting or donating electrons. FAD, in its fully oxidized form, or quinone form, accepts two electrons and two protons to become $FADH_2$ (hydroquinone form). The semiquinone ($FADH\cdot$) can be formed by either reduction of FAD or oxidation of $FADH_2$ by accepting or donating one electron and one proton, respectively. FAD has a more positive reduction potential than NAD^+ and is a very strong oxidizing agent (Kumar & Chen, 2007). The cell utilizes this in many energetically difficult oxidation reactions such as dehydrogenation of a C-C bond to an alkene (Büch, 1995). FAD-dependent proteins function in a large variety of metabolic pathways including electron transport, DNA repair, and nucleotide biosynthesis,

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beta-oxidation of fatty acids, amino acid catabolism, as well as synthesis of other cofactors such as CoA, CoQ and heme groups (Preedy, 2012).

1.2 The *FMN1*, Fmn1 and FMN

The *FMN1* that encodes for yeast riboflavin kinase was found in 2000 (Santos, *et al*., 2000). The *FMN1* is an essential gene and the growth defect shown by the *fmn1Δ* null mutants could be rescued by supplementing the medium with externally high concentration of FMN suggests that a diffusion mechanism would be involved in the uptake of FMN by yeast cells (Santos, *et al*., 2000). Depleted *FMN1* causes abnormal mitochondrial morphology (Altmann & Westermann, 2005). Overexpression *FMN1* causes slow growth.

The riboflavin kinase (Fmn1) synthesis of FMN is essential for cell viability and that no redundant activities (Santos, *et al*., 2000). Riboflavin kinase from rat liver shows an activity increase with low concentrations of Zn^{2+} unlike other ATP-dependent enzymes that prefer Mg^{2+}, indicating its unusual properties (Merrill and McCormick, *et al.*, 1980). Fmn1 predominantly localizes to the microsomal fraction and also found in the mitochondrial inner membrane, exposing its COOH terminal domain to the matrix space (Santos, *et al*., 2000).

Riboflavin kinase of the *Schizosaccharomyces pombe* represents a novel family

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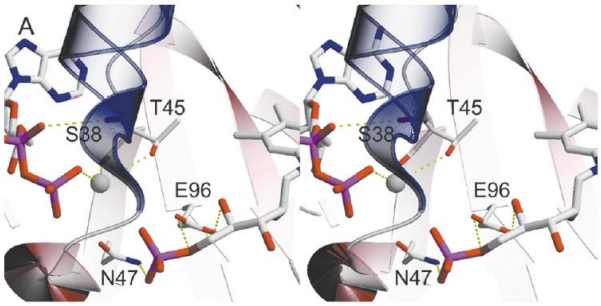
of phosphoryl transferring enzymes (Bauer, *et al.*, 2003). It is a monomer comprising a central β-barrel clasped on one side by two C-terminal helices that display an L-like shape. The opposite side of the β-barrel serves as a platform for substrate binding as demonstrated by complexes with ADP and FMN (Bauer, *et al.*, 2003).



(Bauer, et al., 2003)

FMN functions as prosthetic group of various oxidoreductases including NADH dehydrogenase as well as cofactor in biological blue-light photoreceptors (Kerscher, 2000; Joseph-Horne, *et al.*, 2001; Christie, *et al.*, 1999). FMN functions as a coenzyme, meaning that it is involved in many biological functions but not specific one. That is, deficiency of FMN makes a global effect in cells due to the defect of energy metabolism (Barile, *et al.*, 1997). However, the characterization of *FMN1*, FMN has not well reported until very recently. It is very valuable to investigate the multiple roles of *FMN1*, Fmn1 and FMN in cells

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2 Cell cycle regulation

2.1 The cell cycle

It is important for an organism to produce new daughter cells to maintain its structure and inherited material by cell divides into two cells. Cell-division cycle or cell cycle is a well regulating works in cells. The cells go through the cell cycle progression and pass all the necessary checkpoints that rely on specific control mechanisms to ensure it never returns to previous event. Many factors including cyclins, cyclin-dependent kinases (CDKs), ubiquitin ligases, inhibitors of cyclin-dependent kinases, and reversible phosphorylation to ensure that cell cycle events occur in correct order with least amount of errors (Nigg, 1995; Kaldis, *et al.*, 2013; D'Angiolella, *et al*., 2003; Fujimitsu, *et al*., 2016).

In each turn of cell cycle, cells take place series of events lead to duplicate its DNA (DNA replication) and divide to two daughter cells. According to the cell morphology, cell cycle can be briefly dividing into three periods: interphase, the mitosis, and cytokinesis (Cooper, 2000). Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase (Patt & Quastler, 1963). Although the various detailed stages of cell cycle are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of

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specialized biochemical processes that prepare the cell for initiation of cell division (Wieser & Pines, 2015).

Activation of each phase is dependent on the proper progression and completion of the previous one. The interphase is for cells to grow and prepare their DNA duplication. The interphase consists of three distinct phases: G1 phase, S (synthesis) phase, G2 phase (Israels & Israels, 2001). During G1 phase, the cells grow and accumulate nutrients needed for mitosis. During S phase, the cells duplicate its DNA. During G2 phase, the cells prepare for the cell division and chromosome condensation (Sorenson, *et al*., 1990). Cell's chromatin condenses into chromosomes during the onset of the mitotic phase. During the mitotic phase, cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. The final stage, cytokinesis, the chromosomes and cytoplasm separate into two new daughter cells (Hartwell, 1971). After cell division, each of the daughter cells begins the interphase of a new cycle.

In budding yeast, the progression through the cell cycle is controlled by a single cyclin-dependent kinase known as CDK1 (Cdc28) (Hartwell, 1973). This kinase is constitutively present through the cell cycle but associated with particular cyclins to differentially modulate its activity at each cell cycle stage. There are three G1 cyclins (Cln1–3) and six B-type cyclins (Clb1–6) that control S phase, G2, and mitosis (Hadwiger, *et al*., 1989; Fitch, *et al.*, 1992; Dahmann & Futcher, 1995). G1 cyclins are

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required for bud emergence, spindle pole body duplication, and the expression of the B-type cyclins. Clb5 and Clb6 are involved in DNA replication and spindle pole body duplication and Clb1–4 promote spindle formation and entry into mitosis (Cepeda-García, 2017).

2.2 The mitotic phase and mitotic checkpoints

To ensure genome stability, it is very important to well regulate the chromosome separation during the mitotic phase (Hartwell & Weinert, 1989). In addition, the nuclear division (karyokinesis) during mitotic phase is a relatively short period of the cell cycle, that's why mitotic phase is complex and highly regulated (Ou & Rattner, 2002). The sequence of mitotic events is essential to the process of chromosome segregation which is orchestrated by microtubules (a polarized protein polymer of tubulin) as well as hundreds of other proteins that function together (Walczak & Heald, 2008; Sobel, 1997). The events divided into several phases: metaphase, anaphase and telophase. To distinguish each phase, it is useful to monitor the morphology of the spindles. The metaphase spindle typically reaches 1.5–2 μm in lengths (Winey, *et al.*, 1995) which present the chromosome attachment and spindle assembly. The proper bipolar attachments of all chromosomes trigger the signal of cell to enter anaphase, the stage at which chromosome segregation, and then the spindle elongation occurs. The nuclear

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envelope of budding yeast does not break down during mitosis; that is to say, it undergoes a closed mitosis (Cavalier-Smith, 2010; Patterson, 1999).

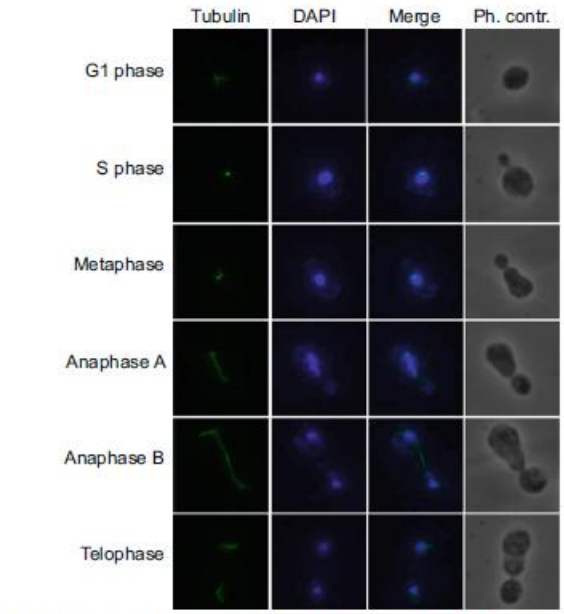

Fig. 2 Immunofluorescence images displaying the morphology of the spindle (tubulin) and the nucleus (DAPI) at different stages of the cell cycle. A merged image and a phase contrast image (Ph. contr.) image are also shown

(Marta and Fernando, 2017)

The process of the mitosis is to ensure that the mitotic spindle is correctly attached to the kinetochores and correctly positioned or oriented (McIntosh, *et al.*, 2002; Tanaka, 2002). If not, there are two mitotic checkpoints to inhibit mitotic progression in presence of errors. They are spindle assembly checkpoint (SAC) and spindle position checkpoint (SPOC or SPoC) (Rudner & Murray, 1996; Musacchio & Salmon, 2007; Yeh, *et al.*, 1995; Bloecher, *et al.*, 2000). The checkpoints and the regulation are essential to stop cell cycle progression in order to maintain genomic stability and cell viability.

The SAC monitors chromosome bi-orientation on the mitotic spindle and

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chromosomes attachment properly. It is a feedback-control system operates cells halts in mitosis and precludes passage into the final phases of cell division (Musacchio & Salmon, 2007). The function of SAC is preventing premature chromosome segregation and loss of sister chromatid cohesion to arrest the initiation of anaphase when unattached or incorrectly attached chromosomes is present (Shonn, *et al*., 2000). This is an important function for preserves the genome from alterations in chromosome copy number and their viability threats (Musacchio, 2015).

SAC consists of a group of proteins that monitor the attachment of spindle microtubules and kinetochores (for review see Musacchio & Salmon, 2007). Normally, the duplicated chromosomes properly attached to the mitotic spindle promoter cell to transit to anaphase. However, the SAC delays the metaphase-anaphase transition when duplicated chromosomes have not properly attached to the mitotic spindle (Nicklas, *et al*., 1995; Pinsky & Biggins, 2005). The highly conserved SAC consists of Mad1, Mad2, BubR1/Mad3, Bub1, Bub3, and Mps1 kinase (Foley & Kapoor, 2013; Jia *et al*., 2013). They associate with kinetochores, and target the anaphase-promoting complex (APC) to inhibit the anaphase progression. The APC is an E3 ubiquitin ligase that requires Cdc20 (Bloom, *et al.*, 2007) to recruit proteins for ubiquitination and subsequent degradation that promote the inactivation of Clb–Cdc28. The SAC proteins bind directly to Cdc20, preventing the function of APC, and then delays the metaphase-anaphase transition (Li,

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et al., 1997; Fang & Kirschner, 1998).

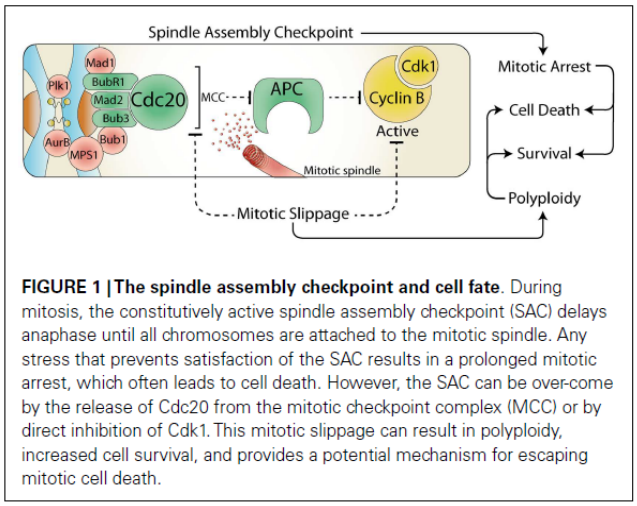
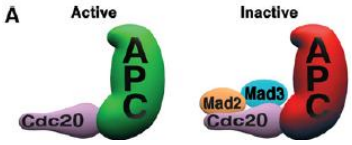


FIGURE 1 | The spindle assembly checkpoint and cell fate. During mitosis, the constitutively active spindle assembly checkpoint (SAC) delays anaphase until all chromosomes are attached to the mitotic spindle. Any stress that prevents satisfaction of the SAC results in a prolonged mitotic arrest, which often leads to cell death. However, the SAC can be over-come by the release of Cdc20 from the mitotic checkpoint complex (MCC) or by direct inhibition of Cdk1. This mitotic slippage can result in polyploidy, increased cell survival, and provides a potential mechanism for escaping mitotic cell death.

(Burgess, *et al.*, 2014)



(Schibler, *et al.*, 2016)

It is also important to silence the SAC when mistakes in chromosome attachment were resolved (Mao, *et al.*, 2003). However, the SAC silenced has received attention just in recent ten years (Vanoosthuyse and Hardwick, 2009). The Kinetochore-associated phosphatase protein 1 (PP1) is indicated to display an essential role in SAC silencing (Rosenberg, *et al.*, 2011). PP1 is likely to promote the removal of Mad1/Mad2 from kinetochores through the dephosphorylation of Bub1, and the dephosphorylation of Bub1 is likely a critical step in SAC silencing. In addition, the kinetochore-localized PP1 may antagonize checkpoint kinases Mps1 to promote anaphase onset and SAC silencing (Bokros and Wang, 2016).

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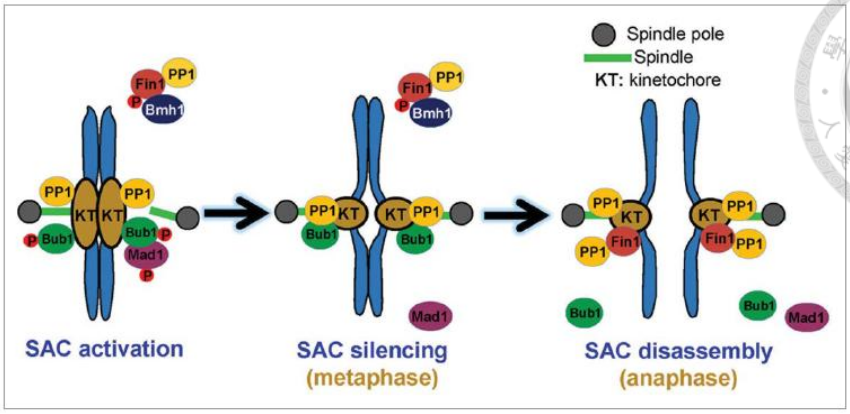

(Bokros and Wang, 2016)

For a daughter cell to receive a complete genomic complement, it is essential that the mitotic spindle be positioned accurately within the cell in the budding yeast (Falk, *et al.*, 2011). The spindle position checkpoint (SPOC) prevents cells from becoming anucleate/ binucleate by arresting cells in late anaphase until the spindle has realigned along the mother–bud axis. The SPOC is a feedback mechanism that delays cell-cycle progression in response to defects in spindle position (Muhua, *et al.*, 1998; Adames, *et al.*, 2001.). After the chromosomes attach properly to kinetochore, one copy of DNA will be separate into daughter cell. At this time, it is important to make sure that the spindle properly aligned along the mother–bud axis in budding yeast. Some case, the spindle becomes mispositioned in the mother cell compartment, if the (SPOC) are not activate, cells inappropriately exit from mitosis in the mother cell compartment and go on to produce one anucleate cell and one binucleate cell (Falk, *et al*., 2016).

The SPOC arrest cells from exit the mitosis until the spindle is properly aligned

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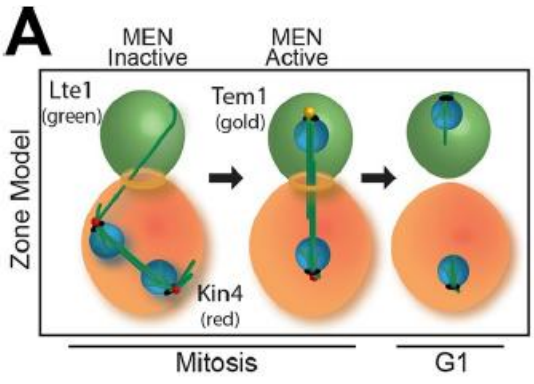
along the mother-bud axis (Yeh, *et al*., 1995; Lew & Burke, 2003). The mitotic exit is regulated by mitotic exit network (MEN), which promoters the activation of the phosphatase Cdc14 and causes cell exit from the mitosis (Visintin, *et al*., 1998). Key SPOC components are the kinase Kin4 and the Bub2–Bfa1 GAP complex that inhibit the key MEN component, Tem1. Kin4 primarily localizes to the mother cell and associates with spindle pole bodies (SPBs) located to inhibit MEN signaling. In contrast, the Kin4 does not associate with the SPBs in the bud. Thus, only when a MEN bearing SPB leaves the mother cell and the spindle is accurately positioned along the mother-bud axis can MEN signaling occur and cell division proceed (Falk, *et al.*, 2011).



(Falk, *et al.*, 2011)

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3 The mitotic exit

3.1 The mitotic exit

When the cell cycle progression goes to division, it is important to ensure the coordinated distribution of cellular material between the mother cell and the daughter cell (Jorgensen & Tyers, 2004; Goranov, *et al.*, 2009). On mitosis completion, mitotic structures disassemble, mitotic regulators are inactivated. This process, termed mitotic exit, starts with the inactivation of Cdk1, the master regulator of mitosis, and the inactivation of its substrates (Murray, *et al*., 1989; Hershko, *et al*., 1991; Holloway, *et al.*, 1993). Consequently, the mitotic spindle breaks down, cytokinesis occurs, chromosomes decondense, and interphase functions resume (Hotz, *et al.*, 2014). Mitotic Exit is an important transition point that signifies the end of mitosis and the onset of new G1 phase for a cell. For a normal eukaryotic cell, the mitotic exit is irreversible. Moreover, the cell needs to rely on specific control mechanisms to ensure it exits mitosis. The Mitotic Exit Network (MEN) is an extensively studied signaling pathway that promotes mitotic exit, facilitates the control of spindle orientation, and initiates cytokinesis in the budding yeast (Shou, *et al*., 1999; Visintin, *et al*., 1999). Another non-essential Cdc Fourteen Early Anaphase Release (FEAR) pathway also promotes this event (Stegmeier, *et al*., 2002). I will introduce FEAR on next section.

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The cyclin dependent kinase, Cdc28 must be inactive when cell exit from the mitosis. The Cdc28 was dephosphorylated and inactivated by Cdc14. Cdc14 is a conserved and essential protein phosphatase. Out of anaphase, Cdc14 is sequestered in the nucleolar chromatin bound to its inhibitor Net1, also called Cfi1. Net1 anchors Cdc14, and together with Sir2. They form the RENT (REgulator of Nucleolar silencing and Telophase) complex which inhibits transcription (Baro, *et al.*, 2017). The interaction is dissolved via the phosphorylation of Cfi/Net1 upon entry into anaphase. The release of Cdc14 from the nucleolus provides the signal coordinate the chromatin structure and chromosome segregation. The Cdc14 release liberates to dephosphorylate Cdc28 and promote exit from mitosis (Hwang, *et al.*, 2009).

3.2 The mitotic exit network

The Mitotic Exit Network (MEN) is the main signaling pathway that triggers Cdc28 inactivation and the onset of cytokinesis on segregation of the daughter nuclei (Murray, *et al*., 1989; Hershko, *et al*., 1991; Holloway, *et al.*, 1993). The MEN from budding yeast is closely related to the Septation Initiation Network (SIN) from the fission yeast *Schizosaccharomyces pombe* (Bardin & Amon, 2001) and the Hippo pathway from mammals (Hergovich & Hemmings, 2012). The MEN consists of GTPase Tem1, two serine/threonine kinases, Cdc15 and Dbf2- Mob1 (Baro, *et al.*,

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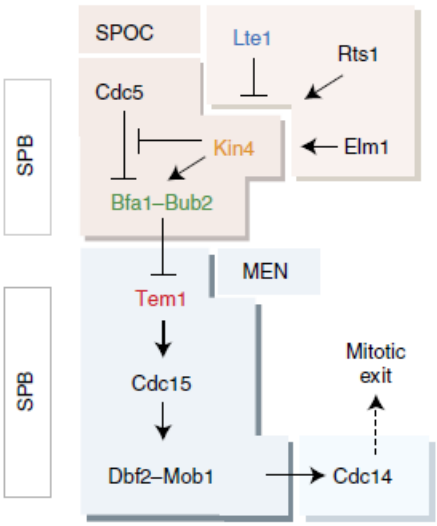
2017). The signaling pathway driven by the Tem1, which signals through the Cdc15. Cdc15, activates Dbf2, which works with the coactivator Mob1 to release the phosphatase Cdc14 from the nucleolus (Hotz, *et al*., 2014). Activation of the MEN is controlled through a complex relationship between all components and regulators of this signaling pathway (Bardin, *et al*., 2000; Pereira, *et al.*, 2000). Changes in the activity of these proteins and in their ability to interact with each other and to localize to different cell structures allow for a tight temporal and spatial regulation of the pathway (Jensen, *et al.*, 2004).



(Caydasi, *et al.*, 2012)

Regulation of the MEN is complex, with multiple, partially redundant pathways. The most studied regulators are Lte1 and the Bfa1-Bub2 complex, although Cdc5, Kin4, Kel1/2, Gic1 and -2, Ste20, Cla4, Cdc42, and Ras2 are also implicated (Geymonat, *et al.*, 2009). Lte1 (gene name means: low temperature essential) was seen as a positive mitotic regulator because *lte1* mutants undergo a telophase arrest at low temperature

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(Shirayama, *et al*., 1994). Lte1 shares homology with the guanosine nucleotide exchange domain of the Ras-guanosine nucleotide exchange factor (GEF) Cdc25 (Cherfils & Chardin, 1999; Quilliam, *et al*., 2002). Thus, it was proposed that Lte1 might be a GEF for Tem1. Cla4 kinase is essential for the activity of Lte1. Lte1 depends on Cla4 for its cell cycle-dependent phosphorylation that is essential for Lte1 function (Seshan & Amon, 2005). Bfa1 and Bub2 are negative regulators of the MEN that form a two-component GTPase-activating protein (GAP) for Tem1 *in vitro* (Geymonat, *et al*., 2002). The dissociation of Bfa1-Bub2 complex from Tem1 is dependent upon the Polo-like kinase Cdc5 to phosphorylate the Bfa1-Bub2 complex (Hu, *et al.*, 2001). The inactivation of MEN through Bfa1-Bub2 complex belongs to SPOC (Wang, *et al*., 2000; Li, 1999; Caydasi & Pereira, 2009). Thus, mitotic exit is prevented when there is spindle damage, spindle misorientation at G2/M phase or DNA damage (Hu and Elledge, 2002).

Lte1 has homology to GEF, and it has long been hypothesized that it could function as a GEF for Tem1 (Bardin, *et al*., 2000; Seshan and Amon, 2005). However, overexpression of the GEF domains of Lte1 could not suppress the low temperature sensitivity of *lte1* (Yoshida, *et al.*, 2003). In contrast, other (central) domain of Lte1 is enough to rescue the defect. Other observations indicated Lte1 cannot stimulate activity of Tem1 *in vitro* (Geymonat, *et al*., 2009). Rather than stimulating Tem1's nucleotide

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exchange, Lte1 was proposal to be a downstream effector protein of Ras , prevent the small amounts of Kin4 from associating with spindle pole bodies (SPB) in the bud (Bertazzi, *et al*., 2011; Falk, *et al*., 2011), or contribute the Bfa1 localization. Recently, it was shown that Lte1's GEF domain suppressed the lethality of a genetic mitotic exit defect strain by the allele of *lte1-ΔEcoRI* (Falk, *et al.*, 2016). But still, they could not demonstrate the Lte1's GEF activity *in vitro*. Thus, it is likely that Lte1 promotes exit form mitosis in multiple mechanisms (Falk, *et al.*, 2016).

Lte1 localized to the bud cortex is important for Lte1 activity (Bardin, *et al.*, 2000; Pereira, *et al.*, 2000). Additionally, the bud-localized activating signal is necessary for full MEN activation. The spatial information translate into chemical signal is coordinate by the Lte1 and Kin4. During metaphase, Tem1 and the Bfa1/Bub2 complex localize to both SPBs. During anaphase, they become concentrated at the daughter SPB (dSPB) that migrates into the bud (Fraschini, *et al.*, 1999; Pereira, *et al.*, 2000). At normal condition, SPB function as the sensor to translated the spatial information into chemical signal. The MEN signal will be activated when dSPB escapes the MEN inhibitor Kin4 in the mother cell and moves into the bud where the MEN activator Lte1 presented (Chan and Amon, 2010). In contrast, Bfa1-Bub2 and Kin4 remain on both SPBs when the spindle is misaligned in the mother cell and the SPOC is activated (Pereira, *et al.*, 2000, 2001; Molk, *et al.*, 2004; Pereira and Schiebel, 2005; Fraschini, *et al.*, 2006;

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Maekawa, *et al*., 2007). Thus, the switch from symmetrical to asymmetrical distributions of the MEN regulators precedes MEN activation.

3.3 The Cdc14 early anaphase release network

The Cdc Fourteen Early Anaphase Release (FEAR) network regulates the initiated inactivating of mitotic CDKs by triggering the Cdc14 releasing (Stegmeier, *et al*., 2002). The mitotic cyclin-CDK inactivation is important for exit from mitosis. The inactivation is initiated at the metaphase-anaphase transition which regulates by the FEAR in the budding yeast (Rahal & Amon, 2008). In addition, the FEAR is critical to ensure accurate anaphase chromosome segregation and the integration of this process with other anaphase events (Rock and Amon, 2009).

While FEAR pathway is not strictly essential for Cdc14 release, it helps coordinate the timing of Cdc14-driven anaphase events (Weiss, 2012). Proposed functions of FEAR include ensuring that the segregation of all chromosomes is initiated simultaneously, stabilization of anaphase spindles, localization of spindle proteins, positioning of the anaphase nucleus, resolution of the rDNA to allow its segregation, and priming of the MEN so that mitotic exit can occur promptly and efficiently (Rock and Amon 2009; Yellman and Roeder, 2015). That is, the FEAR, although not essential, has an important biological role in the metaphase-anaphase transition for

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timely controlling the cell cycle progression.

The FEAR network promotes Cdc14 release by multiple ways. At anaphase onset, the APC be activated by associate with Cdc20, and the FEAR occurs. The Cdc20-APC then degrades the Securin (Pds1), which is an anaphase inhibitor. The Securin inhibits the Seperase (Esp1), which cleavages the cohesion and let the chromosome segregate. Slk19, Kinetochore-associated protein, forms complex with Seperase. The Separase-Slk19 and the proteins Zds1 and Zds2 are thought to down-regulate PP2A-Cdc55 phosphatase activity (Yasutis, *et al.*, 2010; Wicky, *et al.*, 2011). This down-regulation of PP2A-Cdc55 phosphatase activity allows phosphorylation of Cfi1/Net1, which sequesters Cdc14 and localizes in the nucleolus (Visintin, *et al.*, 1999). Another pathway involves Spo12 (Tomson, *et al.*, 2009). Spo12 is a nucleolar phosphoprotein that binds to Fob1, which binds to Cfi1/Net1 and prevents Cdc14 release. The proteins of Spo12, Fob1 and Net1 regulate for the Cdc14's sequestration. CDK phosphorylation on the Spo12 would reduce Fob1's ability to inhibit Cdc14 release (Stegmeier, *et al.*, 2004). The Spo12 promotes the Cdc14 release by an indirect way. Cdc5 also is thought to be contributing for FEAR-dependent Cdc14 release. Cdc5 phosphorylates Net1 and results in Cdc14 disassociations (Shou, *et al.*, 2002; Yoshida and Toh-e, 2002). However, it has been argued that Cdc5, instead of directly phosphorylating Net1, primarily stimulating degradation of kinase Swe1, which enables

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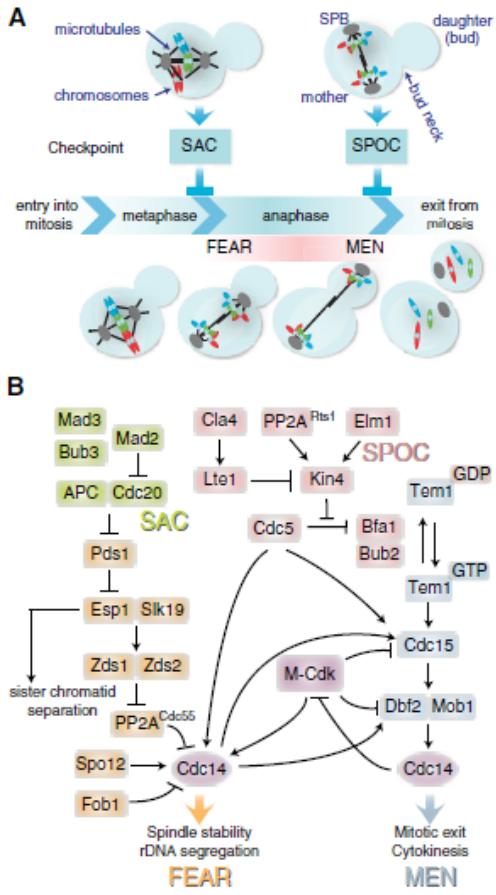
Clb2-Cdk1 to phosphorylate Net1 (Liang, *et al.*, 2009). Some evidence indicates that the direct phosphorylation of Cdc14 by Cdc5 may facilitate Cdc14 release (Visintin, *et al.*, 2003; Rahal and Amon, 2008). Also, the degradation of Cdc5 protein is essential for the return of Cdc14 to the nucleolus after exit mitosis (Visintin, *et al.*, 2008). Moreover, Cdc5 also controls the MEN by phosphorylating Bfa1-Bub2 complex which leads to the activation the MEN (Botchkarev and Haber, 2017).



(Caydasi and Pereira, 2012)

While the FEAR and the MEN both control the Cdc14 release and mitotic exit occurrence, they still have distinct contribution. The MEN drives export of Cdc14

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from nucleus to the cytoplasm (Mohl, *et al.*, 2009). Export from the nucleus would allow Cdc14 access to mitotic exit regulators in the cytoplasm. It has been suggested that Cdc14 release by the FEAR pathway might be limited to the nucleus (Lu and Cross, 2009; Yellman and Roeder, 2015), or alternatively that there could be some export to the cytoplasm (Rock and Amon, 2009).

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4 Histone protein modification and their function

Eukaryotic genomic DNA wraps around histones to form the nucleosome units of chromatin. Nucleosomes allow for regulated access of nuclear factors to DNA and provide a platform for recruitment of factors that do not interact directly with nucleic acids. Posttranslational modifications of histone tails mediate selective recruitment of factors with roles in gene transcription, DNA damage response, and other nuclear processes. Combinatorial and interdependent patterns of histone modifications create a multilayered system of chromatin domains and gene expression control (Suganuma and Workman, 2011).

Ubiquitination of histone H2B (H2Bub) are the most abundant ubiquitin conjugates in yeast corresponding to ~10% of H2B. Monoubiquitination of H2B is by far the most abundant form (Robzyk, *et al*., 2000). The H2Bub as a landmark is important for gene activation. In addition, H2Bub has histone crosstalk to promote histone H3 lysine 4 di- and trimethylation (H3K4me2 and H3K4me3) at actively transcribing genes.

4.1 H2B ubiquitylation and H3K4 methylation

Monoubiquitination of histone H2B has emerged as an important chromatin modification with roles not only in transcription but also in cell differentiation. The

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primary role for H2Bub is modifying nucleosome accessibility or recruiting the multicomponent histone methyltransferase. Additionally, H2Bub was indicated to be involving in many biological functions, such as transcription, RNA processing, DNA repair, cell differentiation, regulation of chromosome segregation by the kinetochore machinery (Soares & Buratowski, 2013).

Histone H2B is monoubituitinated (H2Bub) on lysine 123 in yeast or the corresponding lysine 120 in mammals (Osley, 2006). In *Saccharomyces cerevisiae*, Rad6 was identified as the only H2B ubiquitinconjugating enzyme (E2) and Bre1 as the only H2B ubiquitin ligase (E3) (Wood, *et al.*, 2003). H2Bub also regulates the activity of Dot1 and activates of the Set1 complex (COMPASS) who mediated H3 lysine 79 and H3 lysine 4 methylation (Nakanishi, *et al.*, 2009).

H2Bub regulates the methylation of histone H3 lysine 4 (H3K4me) by Set1 methyltransferase complex. This signal is linked to gene activation from yeast to humans. The requirement of H2Bub to regulate the H3K4 methylatation by Set1 complex is explained by at least three nonexclusive models. The crosstalk between H2Bub and H3K4 methylation (Soares and Buratowski, 2013) is explained for the followings. First, H2Bub may change the configuration of the nucleosome H3 tail to make it more accessible to the histone methyltransferase (HMT). A second simple model is that H2Bub may bind one of the HMT subunits, tethering the complex to

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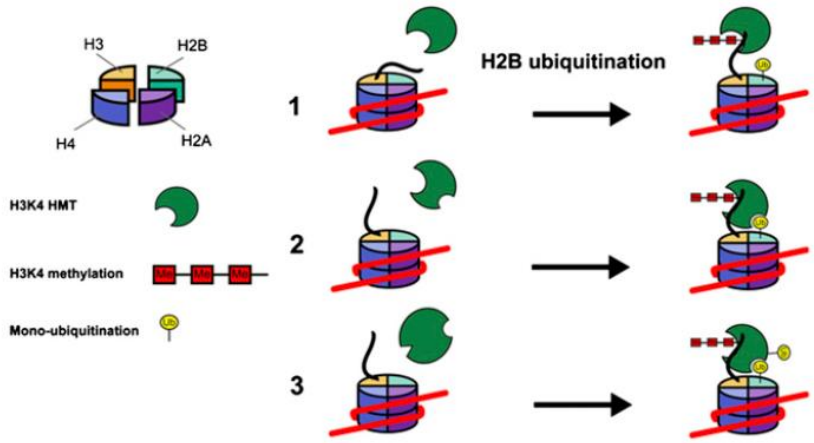
promote higher-level methylation by increasing the residence time. Alternatively, H2Bub binding to the HMT may trigger a conformation change that exposes or activates the catalytic site. Some experimental evidence exists for each of these possibilities, and all three mechanisms may contribute (Soares and Buratowski, 2013).



(Soares and Buratowski, 2013)

4.2 The Set1 complex and the regulation of H3K4 methylation

The H3K4 methylation (H3K4me) is important for gene transcription. Moreover, the different levels of H3K4me still have different functions. There are three levels of H3K4me: mono-, di-, and trimethylation; yet they have different functions and location (Soares and Buratowski, 2013). H3K4me1 is furthest from transcriptional start sites (TSSs) and is linked to nucleosome dynamics and chromatin remodeling at stress-responsive genes in yeast (Nadal-Ribelles, *et al.*, 2015). H3K4me2 peaks slightly further downstream from TSSs and recruits a histone deacetylase. And it is linked to

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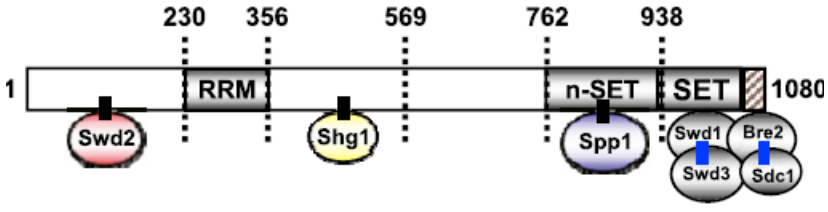
ongoing transcription as well as gene repression in yeast (Margaritis, *et al.*, 2012). H3K4me3 is highest near active promoters or around TSSs and recruits multiple factors that promote transcription, including TFIID, histone acetyltransferases, and chromatin remodelers (De Santa, *et al.*, 2010; Zhang, *et al*., 2016). In general, H3K4me3 is considered a universal hallmark of active transcription, as genome-wide studies from yeast to humans have shown a strong correlation between active transcriptions (Weiner, *et al.*, 2015).

All the status of H3K4 methylation is only performed by Set1 complex in *Saccharomyces cerevisiae*. The *SET1* encodes H3K4 methyltransferase (Set1) and the single H3K4 methyltransferase is found in a complex (ySet1C/COMPASS) with seven other subunits (Swd1, Swd3, Bre2, Sdc1, Spp1, Swd2, and Shg1) (Dehe and Geli, 2006). Set1 is homologous to human MLL1 (Roguev, *et al.*, 2001). Six of COMPASS subunits conserved in the metazoan SET1 complexes, with four also conserved in the metazoan MLL complexes.



Set1 domain and COMPASS (Kim *et al.*, 2013)

The Set1 methylated histone H3K4 is regulated by H2Bub. Set1, Swd1, Swd3,

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Bre2, and Sdc1 are the minimal Set1 complex subunits required for H2B ubiquitylation-dependent H3K4 methylation in the *in vitro* system. The same group also reveals that the n-SET Domain of Set1 is essential for H2B ubiquitylation-dependent H3K4 methylation (Kim, *et al.*, 2013). In contrast, the independent of the requirement for H2Bub regulation is linked by the Set1C/COMPASS subunit, Swd2. Previous reports (Vitaliano-Prunier, *et al.*, 2008) showed that Swd2 is required for H2B ubiquitylation-dependent H3K4 methylation *in vivo*. Histone H2B promotes ubiquitylation at Lys 68 and Lys 69 of Swd2, and mutation of the two sites reduced H3K4 trimethylation. Additionally, Swd2 ubiquitylation controls the recruitment of Spp1, which is specific necessary for H3K4 trimethylation.

Each subunits of Set1C/COMPASS differentially affects Set1 stability, complex integrity, global H3K4methylation, and distribution of H3K4 methylation along active genes (Dehe and Geli, 2006). The effects of them were analyzed by the loss of individual Set1C/COMPASS subunits. The complex requires Set1, Swd1, and Swd3 for integrity, and the Swd1-Swd3 heterodimer present is important for amount of Set1. Bre2 and Sdc1 also form a heteromeric subunit, which requires the SET domain of Set1 for interaction with the complex. Sdc1 strongly interacts with itself. Inactivation of either Bre2 or Sdc1 has very similar effects. The presence of Spp1 is important for the amount of Set1 and retains trimethylated H3K4, whereas, Shg1 slightly reduce levels of

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both di- and trimethylation (Dehé, *et al.*, 2006).

4.3 The relation between Set1/H3K4me and mitosis

H3K4 methylation by Set1 complex is important in regulating cell cycle beside of the function in regulating gene expression. H3K4me alters chromatin structure and serves to recruit or exclude binding of nonhistone proteins to chromatin. H3K4me is known to be associated with gene expression; however, H3K4me also contributes to cell cycle, such as DNA damage repair, timely DNA replication (Sollier, *et al.*, 2004), and mitosis (Schibler, *et al.*, 2016). Although the mechanism of Set1 complex regulating mitosis had not well studied, there still have some puzzle pieces

The Set1C complex has been involved in the regulation of chromosome segregation during mitosis (Zhang, *et al.*, 2005). Proper chromosome segregation requires that centromeres of sister chromatids be attached to microtubules. The attachments are mediated by kinetochores. Set1 is required for the methylation of conserved lysines in a kinetochore protein, Dam1 (Zhang, *et al.*,2005). Dam1 is phosphorylated by Ipl1 in response to inappropriate microtubule–kinetochore interactions (Keating, *et al.*, 2009). The Dam1 phosphorylation is inhibited by Set1 methylation of flanking amino acid. Set1 opposes the functions of Ipl1 in regulating chromosome segregation, and this suppression is not linked to methylation of H3.

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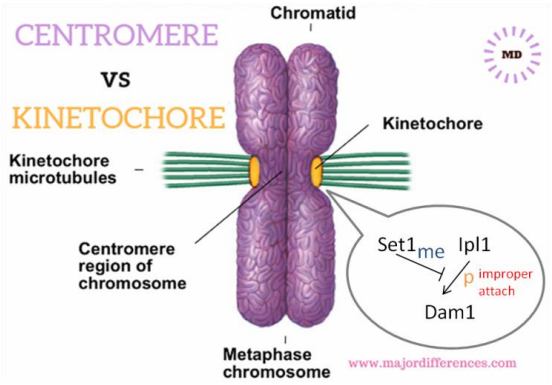

The Ipl1 phosphorylate Dam1 lead to triggering of the spindle assembly checkpoint (SAC) (Biggins and Murray, 2001), which inhibited the anaphase promoting processes. The fact that Set1 is opposite of the Ipl1 function suggest that loss of *SET1* may bypass the SAC. Indeed, deletion of *SET1* leads to resist Benomyl, which interferes mitotic spindle polymerizing and induces cell cycle arrest at metaphase (Schibler, *et al.*, 2016; Beilharz, *et al.*, 2017). The resistance was proposed that Set1/H3K4 stable the tubulin formation by delaying the G1/S or resolving the SAC. Another explanation is that the SAC component, Mad2, binds to H3K4me and leads to the relief from the SAC. The highly conserved HORMA domain in Mad2 is indicated to be a novel H3K4 methyl reader (Schibler, *et al.*, 2016). The interaction of Mad2 and H3K4 limits the capability of Mad2 to inhibit Cdc20 function, and then promote the relief of the SAC.

Set1/H3K4 also is indicated to regulate Cdc14 release which is a critical event during mitotic exit in the budding yeast (Hwang & Madhani, 2009). The Cdc14 is a phosphatase, which is requiring for proteins degradation during anaphase, leads to mitotic exit and restart the cell cycle (Hwang, 2009). The cross talk between

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chromosome and mitosis progression is important. The soluble proteins, Cdc14 is one of them, tightly chromatin-bound factors on chromosomes, is part of the regulatory complexity involves the requirement that a variety of communications. In addition, the Set1 is the factor that involved in the important control to ensure the timely and correct inheritance of sister chromatids followed by cytokinesis (Hwang & Madhani, 2009).

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5 The relationship between *SET1, FMN1* and mitosis

In summary, properly mitosis is important for genome stability. Previous studies indicate that the mitosis is critically regulated by chromatin factors (Zlotorynski, 2016; Aguilera, *et al*., 2000; Kadauke & Blobel, 2013; Chen, *et al.*, 2005; Black, *et al*., 2016). In addition, the mitosis progression is dependent on its nutrition condition. However, how the metabolism affects mitosis is not well defined. Our unpublished data have revealed a genetic interaction between *SET1* and *FMN1*. The *SET1* is important in regulating mitosis, thus, I aimed to study if the *FMN1* functions in mitosis, and the role of *FMN1* in regulating mitosis.

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RESULTS

1 *FMN1* genetically interacts with *SET1*

Our genetic screen recovered the *htb-K123R* mutation, which abolishes the H2B ubiquitylation, displays synthetic lethality with a point mutation resides in *FMN1* which encodes Riboflavin kinase, produces riboflavin monophosphate (FMN); FMN is a necessary cofactor for many enzymes (Figure 1A). To explore a potential link between H2Bub and *FMN1*, we created temperature sensitive alleles of *FMN1* by random mutagenesis. In total, we identified nine *fmn1* alleles and all of them showed synthetic sickness with *htb-K123R*, and they could be rescued when supplied with the addition of FMN in the culture medium. This observation may indicate that the mutation of *FMN1* may reduce the Fmn1 protein kinase activity and the production of FMN. In addition, the synthetic phenotype between *FMN1* and *htb-K123R* could be rescued with the addition of FMN in the culture medium. The result of this screening suggested that the FMN synthesis is likely the only function of Fmn1. And the interaction with *FMN1* suggests H2Bub may carry an unappreciated role in regulating cellular metabolism. Alternatively, FMN may have an unknown function that regulates a cellular process downstream of H2Bub pathway. Among those *FMN1* alleles, the *fmn1-105* mutation showed relatively minor temperature sensitivity but still displayed significant sickness than wild-type when combined with *htb-K123R*. The *fmn1-105* allele bears a point

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mutation at Phenylalanine 95, replaced by Serine. This site is near V91, which is homological to human flavin binding site. Its temperature sensitivity rises around 30~33 °C depending strain backgrounds. The *fmn1-105* allele was thus selected to further characterization of the genetic interaction between H2Bub and *FMN1*.

Monoubiquitination of histone H2B is catalyzed by Rad6-Bre1 (Wood, *et al.*, 2003). To investigate the requirement of FMN in *htb-K123R* cells is due to the H2B ubiquitination abolished, we confirmed the synthetic interaction and observed the viability of the *bre1⊿ fmn1-105* and *rad6⊿ fmn1-105* cells (Figure 1B). The *fmn1-105* mutant displayed strong genetic interaction with *BRE1* and *RAD6*, suggesting that the *FMN1* is important for cellular functions that regulated by H2B ubiquitination. H2Bub regulates Set1-mediated H3K4 methylation and Dot1-mediated H3K79 methylation. We found that *FMN1* does not have genetic interaction with *DOT1* while the *fmn1-105* mutant specifically displayed genetic interaction with *SET1*, suggesting that the *FMN1* is important for regulating some of the function of *SET1* (Figure 1B).

It has been proposed that *set1⊿* promotes microtubule stability as the mutation was deficient in spindle elongation (Reijo, *et al*., 1994). Benomyl is a microtubule destabilizing drug, which contains the active compound methyl benzimidazol-2-yl-carbamate (MBC). MBC is known to cause the depolymerization of microtubules *in vivo* (Jacobs et al. 1988) and *in vitro* (Kilmartin 1981), most likely by

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directly binding to tubulin (Neff et al. 1983). I treated cells with 30 μg/ml Benomyl and in consistent with published results, *set1Δ* mutant cells were highly resistant to high levels of Benomyl than wild-type (Figure 2). The *fmn1-105* cells also display Benomyl resistance; suggest that *FMN1* may play a role in mitosis by a microtubule stability dependent manner. Interestingly, the double mutants of *fmn1-105 set1Δ* resisted to Benomyl at the same level as *fmn1-105* but less than the *set1Δ* single mutant. The loss of *fmn1-105* activity lead to reduce the Benomyl resistance of *set1Δ*, suggesting that the function of *SET1* in mitosis is partially dependent on *FMN1*. To determine whether the Benomyl resistance complementary defect of *set1Δ* is specifically to *fmn1-105* cells, we examined a microtubule-irrelevant gene, *ACS2*, is coding for an acetyl-coA synthetase, the nuclear source of acetyl-coA for histone acetylation (Frenkel & Kitchens, 1977). The *ACS2* display genetic interaction with *SET1*, however, the *acs2-ts1* could not resist to Benomyl, in addition, the loss of Acs2 activity did not lead to reduce the Benomyl resistance of *set1Δ*. These observations indicate that the Benomyl resistance of *set1Δ* is specifically dependent on the function of Fmn1.

FMN is involved in many metabolic pathways. To further explore the role of *FMN1* in mitosis, we used synthetic genetic array (SGA) analysis (Tong, 2001) to screen for genes that may cause synthetic sickness/lethality with *fmn1-105*. We mated the single mutant library of *MATα* strains with, *MATa fmn1-105*. After selection, the

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double mutation strains were picked up if they were significantly slow growing. A list of candidate genes is found (Table 1), 74% are involved in metabolic process, 45% are involved in gene expression and 21% are involved in regulation of transcription by RNA polymerase II. These three biological processes are large scale to analyze, however, there were ~15% of candidates were related "mitotic cell cycle process". It supports that *FMN1* could be involved in mitosis. Remarkably, *LTE1* was one of the candidates, which was reported to cause synthetic lethality when combine with *SET1* deletion. To ensure the genetic interaction between *LTE1* and *SET1*, I deleted the *lte1* in *set1Δ* cells. The *set1Δ lte1Δ* cells indeed showed synthetic lethal, in consistent with the previous study (Hwang & Madhani, 2009). To further confirm the genetic interaction between *FMN1* and *LTE1*, I created the double deletion of *FMN1* and *LTE1* in three different genetic backgrounds, null deletion of *FMN1* covered by a *fmn1-105* or wild-type plasmid (URA marker) in BY4741 or W303 (Figure 3A. B) and W303 *RAD5+* with *fmn1-105* mutation residing on the genome (Figure 3C). Whichever background, the *fmn1-105 lte1Δ* exhibited strongly genetic interaction at 33℃. TheW303 *RAD5+* background exhibited a growth defect in double mutation but not *fmn1-105* only at 33℃. Moreover, it exhibited the phenotype only when growing in SC medium, but not in rich YPD medium. Taking into account the convenience of operation, I chose the *fmn1-105* mutated on the genome background for subsequent

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experiments.

To further confirm that the growth defect of *fmn1-105 lte1Δ* was due to the loss the Fmn1 activity, I supplied the cells with 0.05 mM and 0.5 mM of FMN in the media. I found the supplement of 0.5 mM FMN recuses the growth defect of *fmn1-105 lte1Δ* to the level of *lte1Δ* (Figure 3C). However, supplied cells with 0.05 mM FMN could not rescue the growth defect of *fmn1-105 lte1Δ* to the level of *lte1Δ*. These observations indicated that FMN producing activity is required the proper growth of *fmn1-105 lte1Δ* cells. Furthermore, I found that the 0.05mM of FMN also rendered the *lte1Δ* cells sicker than growing on medium without supplement. These observations indicated that cells require proper concentration of cellular FMN when loss of Lte1 activity. In addition, *LTE1* was deleted in *set1Δ fmn1-105* cells. I found that deletion of *LTE1* causes the lethality of *set1Δ fmn1-105* cells (Figure 4). Collectively, these data indicated that *SET1* and *FMN1* both display strong interactions with *LTE1*, indicating that a role for *FMN1* in mitosis.

2 The synthetic interaction of *FMN1* and *SET1* leads to defective cell cycle progression

To study the role of *FMN1* and *SET1* during cell cycle, I monitored the DNA content in mutant cells throughout a complete cell cycle progression. To clearly reveal the pace of cell cycle, cells were arrested in G1 with 2 mg/ml α factor and released into

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pheromone-free media at 15 degrees to slow down the cell cycle. After 160 min, 4 mg/ml α factor was added to prevent entry into the subsequent cell cycle. Cells were collected every 40 min and stained with SYBR GREEN for analysis DNA content by flow cytometry (Figure 5). The *fmn1-105, set1Δ* and *fmn1-105 set1Δ* cells were progressing from G1 into S phase slower (80min) than the wild-type (40min). The duration of G2/M was no significant difference between the *fmn1-105, set1Δ* and *fmn1-105 set1Δ* cells. However, a subset of the *fmn1-105 set1Δ* cells accumulated cells at G1 without migrating into cell cycle. This observation indicated that the genetic interaction between *FMN1* and *SET1* may lead to the G1-S transition defect. Indeed, we found the evidences of the defect in S phase transition of *fmn1-105 set1Δ* cells by another group in our lab (data not show). The current work only focuses on the role of *FMN1* in regulating the mitosis.

3 Loss of Fmn1 activity leads to mitosis delay

To investigate the role of *FMN1* in mitosis, I characterized the role of *FMN1* in mitotic defective cells by deleting *LTE1*. The accurate determination of cell growth and viability is pivotal to monitoring the cells bioprocess, so I first determined the growth rate of mutant cells. I cultured cells in flasks in constant temperature rooms, and on shakers drums to provide sufficient aeration. At intervals of every one hour, a sample would be sterilely removed and measured the cell density by spectrophotometer. OD_{600}

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values between 0.1 and 1 at the temperature from 15 -37 degree were used to calculate the growth rate. I found that the growth rate of *fmn1-105 lte1Δ* cells was slowest in all the temperature (Figure 6) but compare to *lte1 Δ* cells, the growth defect was strongest at 37°C.

Next, I analyzed the distribution of cells in the cell cycle to determine whether a loss of *FMN1* function altered cell cycle progression. The *fmn1-105*, *fmn1-105 lte1Δ, lte1Δ* and W303 (wild-type) cells were grown asynchronously at 37℃ for 8 hours and budding morphology was scored (Figure 7A, B). 74% of *fmn1-105 lte1Δ* cells were large budded compared to 52% in *lte1Δ*, 40% of *fmn1-105* cells and 36% in WT (wild-type) cells. These data suggested that the majority of *fmn1-105 lte1Δ* cells was arrested at mitosis. Moreover, supplied *fmn1-105 lte1Δ* cells with high concentration of FMN in the medium, the proportion of large budded cells was reduce to 40%, similar to the level of *lte1Δ* single mutant (52%) (Figure 7A, C). The increased proportion of mitotic cell in *fmn1-105 lte1Δ* could be rescued to as *lte1Δ* by the addition of FMN, suggesting that the effect in mitosis of *fmn1-10 5lte1Δ* is caused by the insufficient cellular concentration of FMN.

To further investigate if loss of *FMN1* function altered the specific progression of mitosis, large budded cells' nuclear DNA was stained by the addition 4,6-diamidino-2-phenylindole (DAPI) and inspected under the microscope. I found that

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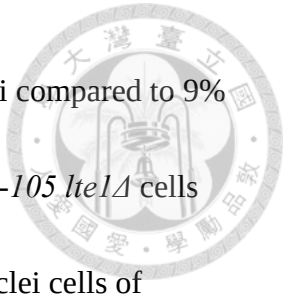

36% of *fmn1-105 lte1Δ* cells were large budded with undivided nuclei compared to 9% in *lte1Δ* cells (Figure 7D, E). This observation may suggest that *fmn1-105 lte1Δ* cells have delayed mitosis prior to anaphase. In addition, the undivided nuclei cells of *fmn1-105 lte1Δ* decreased when supplying high concentration of FMN in the medium, indicating that the proper FMN concentration is important for mitosis in *fmn1-105 lte1Δ* cells.

4 *FMN1* is involved in mitotic exit regulation

A previous study indicates that beside the function of mitotic exit network regulation (Geymonat, *et al.*, 2010), *lte1* mutants also displays altered bud morphology during mitotic arrest. Lte1 localizes specifically at the bud cortex, where it interacts with Ras2 and the polarity cap component, Kel1. Kel1 is required for bud site selection. Lte1 also can interact with and inhibit Bud1 to avoid untimely bud polarization. I therefore asked if *fmn1-105* activity affected the process of bud morphology. I examined the viability of *fmn1-105* cells with *BUD1* or *KEL1* deletion (Figure 8A), compare to single mutant, there was no growth defect in the double mutant cells, indicating that interfering Fmn1 activity did not influence the process of bud morphology.

The above observations narrowed down the impact of that the genetic interaction between *FMN1* and *LTE1* to the co-regulation mitotic exit events through the mitotic

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exit network. To determine if *fmn1-105* allele affected mitotic exit network (MEN) and the MEN activation. I examined the viability when mitotic exit network was ablated or the MEN were deactivated in *fmn1-105* cells. I deleted *CLA4*, which has an epistatic effect in Lte1 phosphorylation and found that *fmn1-105* mutants with *CLA4* deletion exhibited synthetic growth defects (Figure 8B), and the viability profile is similar to *fmn1-105 lte1Δ* cells. Next, to deactivate the MEN, I introduced a hypomorphic allele of *CDC15*, a MEN component, into cells harboring the *fmn1-105* allele. The double mutants exhibited enhanced temperature sensitivity than *fmn1-105* at 31~33℃, the temperature is lower than the *cdc15-2* cells deactivation temperature (Figure 8C). To further confirm that the MEN deactivation was due to loss the Fmn1 activity, I supply the cells with high concentration of FMN in the media. The supplement of FMN recused the growth defect of *cdc15-2 fmn1-105* to *cdc15-2* level, however, the high concentration of FMN also rendered the *cdc15-2* cells sicker than growing on medium without supplement. These observations indicated that the MEN activation requires proper concentration of cellular FMN.

The function of Bub2-Bfa1 antagonizes Lte1 to inhibit the MEN activity. Deletion of *BUB2* completely suppressed the proliferation defect of the *lte1Δ* mutant (Chan and Amon, 2010). This observation indicated that *BUB2* deletion in *lte1Δ* cells restored the MEN activity. I deleted *BUB2* in cells harboring the *set1Δ lteΔ*, and found the deletion

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of *BUB2* rescued the lethality of *set1Δ lteΔ*, consistent to the previous study (Chan and Amon, 2010; Hwang, *et al.*, 2009) (Figure 9A). To shed light on whether the MEN activity could be restrained in *fmn1-105 lte1Δ* cells, I then deleted *BUB2* in cells harboring the *fmn1-105 lteΔ*. The deletion of *BUB2* also rescued the growth defect of *fmn1-105 lteΔ* mutant, indicating that the sickness of the *fmn1-105 lte1Δ* was due to the restrained MEN activity (Figure 9B). Interestingly, the deletion had no effect on the viability of *fmn1-105 set1Δ*, suggesting that the interaction between *FMN1* and *SET1* may not lie at the process of mitosis (Figure 9C). In summary, *FMN1* and *SET1* may contribute independent roles to MEN which regulating mitotic exit and the genetic interaction between *FMN1* and *LTE1* resides at the regulation MEN activity.

5 Fmn1 activity is required for metaphase-anaphase transition

The mitotic exit regulation is an important function for anaphase progression (Queralt and Uhlmann, 2008). The anaphase progression is critically regulated by Cdc14 (Mohl, *et al.*, 2009). The Cdc14 phosphatase is essential for both CDK down regulation and reversal of the phosphorylation events mediated by CDK (Stegmeier and Amon, 2004). In addition to MEN, the FEAR (CdcFourteen Early Anaphase Release) network is prior to control the Cdc14 activity during early anaphase (Stegmeier, *et al.*, 2009). The previous result suggested that Fmn1 functions in parallel to MEN, to understand whether Fmn1 functions in the FEAR, I tested whether the deletion of Slk19,

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a component of FEAR would lead to cells growth defect in *fmn1-105* mutants. The interactions of *slk19Δ* and *fmn1-105* (Figure 10A) were relatively neutral, suggesting that *FMN1* may be epistatic to the FEAR pathway.

Another FEAR component, Spo12, contributes to FEAR by promoting dissociation of Cdc14 from the RENT (REgulator of Nucleolar silencing and Telophase) complex. I also found that the viability of *spo12Δ fmn1-105* double mutants were the same as the *fmn1-105* single mutant (Figure 10B). To ensure the previous reported that the *spo12Δ lte1Δ* double mutant exhibited lethality to cells, I deleted both of them and kept the cells alive by a centromeric URA3-based plasmid expressing *LTE1*. I treated cells with 5-FOA, leading the cells to abandon the plasmid. The result confirmed that the *spo12Δ lte1Δ* was inviable (Figure 10C). These observations indicate that the function of *FMN1* is epistatic to FEAR and in parallel to MEN.

At the metaphase-to-anaphase transition the anaphase promoting complex or cyclosome (APC/C) primes Pds1 for ubiquitin mediated degradation and promotes the FEAR (Rock and Amon, 2009). Ras2 signal is important for the metaphase-anaphase transition. A previous study indicated that APC/C defective mutants are suppressed by reduced Ras signaling activity, by a deletion of the *RAS2* gene (Irniger, *et al.*, 1999). If the Fmn1 is epistatic to regulates the APC/C, deletion of *RAS2* should rescue the viability of *fmn1-105*. Indeed, *fmn1-105 ras2Δ* cells exhibited significant improvement

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of growth at 33℃ relative to the *fmn1-105* single mutants (Figure 10D). The viability of *fmn1-105* cells could be partially suppressed by the perturbation of Ras2 function, suggesting that Ras2 may inhibit Fmn1 protein kinase activity.

The spindle assembly checkpoint (SAC) inhibits the APC/C functions through the mitotic checkpoint complex and arrests cell at metaphase when present not proper attachment of the spindle. *BUB3* is a non-essential gene whose product is involved in SAC (Hoyt, *et al*., 1991；Roberts, *et al*., 1994). Like other spindle checkpoint mutants, *bub3* loss-of-function mutants are sensitive to Benomyl and cannot delay cell division in response to spindle depolymerization. To further define the role of Fmn1 in metaphase-anaphase transition, I deleted the gene encoding SAC component in the *fmn1-105* strain. Deletion of *BUB3* enhanced the sensitivity of *fmn1-105* cells to Benomyl at 33 degrees (Figure 11A). The *bub3⊿* cells display more resistance to Benomyl at 33 degrees than 30 degrees may due to high temperature accelerate the break down rate of Benomyl (Mallat, *et al.*, 1977). This data indicated that *fmn1-105* mutant required intact SAC to grow when the proper chromosomes attachment is lost.

To determine whether the dependence of *fmn1-105* on the SAC defect in metaphase-anaphase transition, I treated SAC defective cells with Nocodazole. Nocodazole is a drug that induces microtubules disassembly; therefore, it destroys the mitotic spindle and activates the spindle assembly checkpoint (Künkel, 1980).

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Following Nocodazole treatment the entry into mitosis is blocked, so the cells are arrested in late G_2 with duplicated DNA and undivided sister chromatids, due to the persistence of the cohesion. However, SAC defective cells could not activate checkpoint as the cells bypassed metaphase-anaphase transition and formed new buds, accumulated as re-budded cells (Figure 11C). The budding pattern provided the information about defect of metaphase-anaphase transition in *bub3Δ*, *fmn1-105 bub3Δ* , *bub2Δ* and *fmn1-105bub2Δ* cells (Figure 11D). Bub2 is another SAC pathway-associated component. The Bub2 branches of the SAC pathway also limit cell cycle progression by preventing the activation of the APC/C (Bloom and Cross, 2007; Jessulat, *et al.*, 2015). I found all the cells bypassed the checkpoint and formed new buds, accumulated as re-budded cells. However, the re-budded cells in *fmn1-105 bub2Δ* were reduced, compared to *bub2Δ*. These data may suggest that loss of Fmn1 activity may require for metaphase-anaphase transition.

Failure of the checkpoint causes cells exited mitosis and underwent a new round of DNA replication (Fraschini, 2017). To ensure that loss of Fmn1 activity may suppress the checkpoint defect, I inspected the cell cyle profiles of WT and mutant cells using the FACS machine. The *bub3Δ* and *bub2Δ* cells accumulated with DNA content more than 2C indicates that the mitotic checkpoint was not activated and led to DNA re-replicated. However, the *fmn1-105* , *fmn1-105 bub3Δ* and *fmn1-105bub2Δ* cells are

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arrested with 2C DNA content means the mitotic checkpoint defect might be suppressed by *fmn1-105* (Figure 11B). Tight coordination of the cellular events associated with every phase of the cell cycle is essential for orderly progression through the cell cycle (Hartwell and Weinert, 1989). Taken together, these data indicated that *FMN1* mutants may require for metaphase-anaphase transition during SAC activation. Interestingly, I found *fmn1-105 bub3Δ* and *fmn1-105bub2Δ* mutants accumulated re-budded cells without DNA duplication. It suggested that loss of Fmn1 activity may lead to uncoordinated morphogenetic events with DNA replication.

Upon metaphase-anaphase transition, the APC/C also mediated chromosome segregation. To further explore the role of *FMN1* in metaphase-anaphase transition, cells were released into the restrictive temperature of 37 degreed, I monitored the chromosome segregation by fluorescence microscopic experiment (DAPI staining). Surprisingly, I found that the large budded cells displayed abnormal DNA signal in *fmn1-105* mutants (Figure 12A, B). Compared to wild-type cell where the fluorescent signal was even present in both daughter and mother-bud, or present in mother bud only, we observed abnormality in fluorescence signal in mutant strains compared to the wild-type. I found that abundant fluorescence signals were only present in daughter buds, predominantly accounted around 30% of total large budded cells in *fmn1-105 lte1Δ* mutants, and 17% of total large budded cells in *fmn1-105* mutants. The presence

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of abnormal DNA signal in *fmn1-105 lte1Δ* and *fmn1-105* cells suggested that chromosome had failed to separate or that sister chromatids had separated but had failed to be properly segregated to the opposite sites of the poles (Francisco, *et al.*, 1994; Kumar, 2018).

Chromosome mis-segregation leads to aneuploidy or polyploidy (Storchova, 2014). To examine more directly the way in which ploidy or chromosome number increases in mutant cells, I monitored the DNA content of wild-type, *fmn1-105, lte1Δ* and *fmn1-105 lte1Δ* cells. Wild-type and mutant haploid cells were grown exponentially at 28 were shifted to 37 degree and after two doublings, the DNA content of individual cells was measured by flow cytometry (Figure 12C). The distribution of DNA content for *fmn1-105 lte1Δ* cells was different from wild-type and signal mutants in the frequency of G1 peak. The *fmn1-105 lte1Δ* exhibited half of frequency of G1 peak compare to *lte1Δ*, at the level less than half of that in WT and *fmn1-105* cells. In addition, the *fmn1-105 lte1Δ* appeared with 0N peak which represent aploid cells with the chromosome loss (Chad and Botsteid, 1993) or indicates DNA fragmentation by apoptosis. The frequency of 0N is as high as 13.4% in *fmn1-105 lte1 Δ* cells. The presence of the high frequency of 0N would suggest an abnormal mitosis in which daughter cells received none of the duplicated chromosomes. However, there was no apparent 4N peak, which represented aneuploidy cells with gained chromosomes, so the

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possibility of cell lysis/apoptosis could not be excluded, and it needs more analysis.

A previous study also showed that both activation of the APC/C and chromosome segregation require for spindle stabilization and elongation (Severin, *et al*., 2001). To determine if the spindle elongation defect and the chromosome segregation error will lead to synthetic lethality, I tested if a deletion of the genes encoding for motor proteins needed for spindle elongation would affect the viability of *fmn1-105*. Cin8 is a microtubule-associated kinesin-5 protein and has been observed on the SPBs, kinetochores, spindles, and midzone (Tytell and Sorger, 2006; Gardner, *et al*., 2008). Cin8 has been shown to be required for spindle elongation (Saunders, *et al*., 1997), specifically for the rapid phase of mitotic spindle elongation (Straight, *et al*., 1998). The *fmn1-105 cin8Δ* cells exhibited strongly growth defect when compare to single mutants (Figure 13). This data supported the idea that the unrestrained spindle elongation (Chai, *et al*., 2010) leads to the inability of the *fmn1-105* cells to survive when chromosomes could not be segregated; thus the chromosome segregation error in *fmn1-105* could be due to spindle elongation restrained.

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DISSCUSION

Our initial goal was to identify the role of *FMN1* in regulating mitosis. We found the genetic interaction between *FMN1* and *LTE1*, and we believe that the genetic interaction due to the mitosis progression defect. Loss the riboflavin kinase activity leads to late for transition into anaphase that demonstrates mitosis progression defect. We put forward discover for the role of riboflavin kinase effect in mitosis, we observe that loss the riboflavin kinase activity lead to chromosome segregation error and subsequent late for mitosis for the first time.

Riboflavin kinase mutants performed mitosis deregulating

Loss of *FMN1* function leads to delayed mitotic progression, the *fmn1* mutants arrested at anaphase with undivided and divided nuclei. Cell division required the production of large amounts of energy and biomass, would explain why reduce the producing of energy or their co-enzyme may due to abnormal cell division progression such as Fmn1. In every cell cycle, the condition of nutrients for cell is an important signal to determine the progression transited. A previous study indicated that the duration of mitosis is increased when the rate of growth is slowed by poor carbon nutrients (Mendes, 2017). Emerging evidence reveals that metabolism is not only undergoing substantial changes during the cell cycle, but it is becoming equally clear that metabolism regulates cell cycle progression. The nutrients modulated the daughter

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cell size, because buds need to reach the threshold amount of growth to complete mitosis. It would make sense that the metabolic factor plays a significant role in cell cycle control. The important metabolic cofactor, FMN, also not surprisingly was regulating the cell cycle progression. FMN is a bound prosthetic group serves as an important role in glycolysis, which could explain why loss of riboflavin kinase activity causes only modest effects on mitosis in the cell cycle.

An alternative explain is that the cell cycle needs amount of protein degradation to lead to turn the cell cycle. Protein degradation uses up a lot of energy. Cell cycle proteins are largely degraded by two key E3 ubiquitin ligases (Koepp, 2014). One is response to DNA replication; another is response to anaphase progression. Thus, the two stage require more energy to transited the stages, in another words, an insufficient of energy producing mutants will exhibit predominate effects in the two stages. Indeed, we found that *fmn1* mutants display predominate defect in anaphase progression and DNA replication (Investigate by another group in our lab).

We believe that the deficiency of FMN affect the mitosis is credible. However, if the delay were a consequence of a reduction in energy needed for mitotic events, one would expect to see delays in both metaphase and anaphase. Next, I try to show that the why loss the riboflavin kinase activity will predominate affect the metaphase-anaphase transition.

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Loss the riboflavin kinase activity leads to chromosome segregation error

We discovery loss the riboflavin kinase activity leads to chromosome segregation error for the first time. Cells accumulate undivided nuclear with large budded when reducing the FMN producing activity. This effect can be rescue by supplement with additional FMN.

FMN were proposed as electron donors to oxygen, In addition, it was found that the production of reactive oxygen species (ROS) is enhanced in defective Complex I which has the prosthetic group of FMN (Raha and Robinson, 2000). The enhanced oxidative stress can affect the spindle assembly checkpoint (SAC) defect and chromosomal instability. Failure of the SAC may create a condition for chromosomal instability by increasing the rate of chromosome gain, loss or inducing tetraploidy. In addition, SAC delays metaphase-anaphase transition until all chromosomes are properly attached to the mitotic spindle. Consist to this study, we found that reduce the FMN may trigger the metaphase-anaphase transition defect and lead to chromosome dis-segregation and chromosome loss. It seems that FMN deficiency cells may have a condition for chromosomal instability. Indeed, the *fmn1* mutants display defect in chromosome segregation. Accurate chromosome segregation is required of Bub3 and Bub1 more than another SAC component. Cells containing *bub1* and *bub3* mutations were more Benomyl sensitive than *mad1*, *mad2*, or *mad3* mutants. The Benomyl

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sensitivity is elicited by the absence of kinetochore surveillance. The *fmn1* mutants enhance the Benomyl sensitivity of *bub3* cells. It may suggest that the chromosome segregation error may due to loss the kinetochore surveillance.

At anaphase onset, microtubules suddenly become more stable and it is importance for the ensuing chromosome segregation. We found that *fmn1* mutants were required for proper spindle elongation, means that microtubules are instable in *fmn1* mutants. That is the reason why the unstable microtubules in Fmn1 defective cells may lead to metaphase-anaphase transition defect. One flavoenzyme, Irc15 was found that loss its function resulted in delayed mitotic progression. Irc15 required FAD, and associated with microtubules and displayed an influence on the dynamics of microtubules. The *irc15* mutants resulted the failure to create enough tension to separate sister chromatids. Although the function of FAD and FMN are not the same, but it seems loss both of them lead to chromosome segregation error.

Defects in chromosome segregation are variable but classifiable. Mutations in the subunits of the same complexes often produce similar phenotypes. The type of chromosome segregation error in *fmn1* mutants is similar to *ipl1* mutants; their mitotic phenotype presented unequal chromosome segregation, which can be visualized by the sizes of the nuclear chromatin or the actual number of chromosomes in the daughter nuclei. Only detailed phenotypic analyses can reveal molecular mechanism in *fmn1*

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mutants. However, loss of *set1* suppresses chromosome segregation defects which are caused by the *ipl1* mutants (Zhang, 2005). Interestingly, the functions of *set1* and *Ipl1* in chromosome segregation is opposite, suggesting the role between Set1 and Fmn1 is more complex.

Another powerful interpretation is monitor the degradation of the chromosome segregation inhibitor, securin. The securin (Pds1) degraded at a slower rate depicted the metaphase-anaphase transition defect in mutants (Cohen-Fix, *et al.*, 1996). However, we are failure to tag the Pds1 (securin). Interestingly, *set1* mutants were found to display the slower degraded of Pds1 suggested that their metaphase-anaphase transition also had defect (Schibler, *et al.*, 2016). We also report that the *set1* mutants are resisted to depolymerizing drug microtubules more effectively than *fmn1* mutants. In addition, I fail to attend the *set1 cin8* double deletion mutants for three times suggested that Set1 regulate the metaphase-anaphase transition by a microtubules-dependent manner.

We still cannot rule out the possibility of chromosome dis-attached to kinetochore, spindle elongation or spindle position error lead to dis-segregated chromosome in *fmn1* mutants. Interestingly, MEN defective cells results in mis-segregation of chromosomes due to unrestrained spindle elongation with undivided SPB and unattached Kinetochores (Chai, 2010). The *cdc15-2* mutants presented premature spindle elongation after releasing from SAC. The *fmn1* mutants presented faster exit from the

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mitosis after releasing for SAC (data not show), which is similar to the phenotype with *cdc15-2* mutants, suggesting the potential of *fmn1* mutants to had premature spindle elongation. The chromosome segregation error may be extent the frequency if cells had premature spindle elongation. Both of Lte1 and Cdc15 are MEN component, due to chromosome segregation error in *cdc15-2* mutants, which may explain why the *fmn1lte1Δ* double mutants exhibited more chromosome segregation error than *fmn1* single mutants.

A previous study suggested that the H2B is essential for proper chromosome segregation (Maruyama, 2006). The improper chromosome segregation leads to unequal nuclear division phenotype in *htb1-223* mutants. It is similar to our *fmn1* mutants, suggesting the genetic interaction between *H2B* and *FMN1* are due to the chromosome segregation error. In addition, the H2B mutants were defective centromere/kinetochore functions. In the future, we also need to investigate the centromere/kinetochore functions in *fmn1* mutants.

We still need more explore for analyzing why loss the Fmn1 activity may lead to chromosome segregation error. Next, we try to define the role in *fmn1* mutants for the differences underlying these phenotypes for aberrantly formed kinetochore, kinetochores mis-attaching the chromatid pair to the spindle, non-dynamic microtubules, etc.

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METHODS AND MATERIALS

Yeast Strains and Growth Conditions.

Yeast strains used in this study are listed in Table S1. For gene disruptions, the indicated gene was replaced with the KanMX gene (deletion library from the Saccharomyces Genome Database) or disrupted through a PCR-based strategy (Janke C, et al., 2004). All yeast cells were grown in selective media (SC). All analyses were performed during the log phase of growth. Cells were arrested in G1 by the addition of a-factor to a final concentration of 2ug/mL (*BAR1* strain) for 2 h. Cells were released from G1 arrest by washing with 2 volume of H_2O twice before being suspended in fresh media. Cells were arrested in metaphase by the addition of Nocodazole to a final concentration of 5ug/mL for 2.5 h. Cells were released from metaphase arrest by washing with 2 volume of H_2O twice before being suspended in fresh media.

Spot Assays.

For spot assays, 10-fold serial dilutions of exponential yeast cultures were dropped onto SC plates or containing the indicated concentrations of Benomyl (Sigma-Aldrich) and were incubated at 25 ℃ for 2 days.

FACS.

For DNA content analysis, ~5×10^6 cells were collected at each time point, suspended in room temperature 70% (vol/vol) ethanol, and stored at −80 ℃ (Fixed cells can be

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stored for a very long time). Cells were washed twice with 50 mM Tris (pH 8.0), and RNA and proteins were removed by RNaseA (0.4 mg/mL) and proteaseK (1 mg/mL) treatment. Finally, cells were stained with SYBR GREEN I at 4 ℃ overnight or over weekend (Cells can be stored at 4 ℃ for a week). The cell size and DNA were examined on a FACSCanto II (BD). The result was used the Flowjo 7.6 for analysis the relative ratio of the phase of G1, S, G2/M and super G2.

Immunofluorescence imaging.

Cells were fixed with 3.7% formaldehyde fix solution (1/10 37% formaldehyde, 9/10 0.1M KPO_4, pH 6.4) for overnight at 4℃, washed with 0.1M KPO_4, pH6.4, and suspend in 1.2M sorbitol-citrate. Spin down and resuspend cells in digestion mix (1.2M sorbitol-citrate, Glusulase, zymolyase) for 20 minutes. Cells were again washed with 1.2M sorbitol-citrate buffer, and resuspended. Primary antibody used anti-tubulin (Sigma-Aldrich). Secondary antibodies were Alexa Fluor 488 (anti–rat). DNA was stained with 1 μg/ml DAPI solution (1.2M Sorbitol, 1% Triton, 0.1M KPO_4, pH7.5, DAPI). Fluorescence microscopy used Zeiss LSM880 with AiryScanon an inverted microscope with a 63× F1.4 objective. Cell images were captured and manipulated using Zen Black edition software.

Cells Collection.

~5×10^6 cells were collect at indicate time for 1 ml. Treat with 1/100 volume of 10%

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Sodium azide to stop the cell progression on ice.

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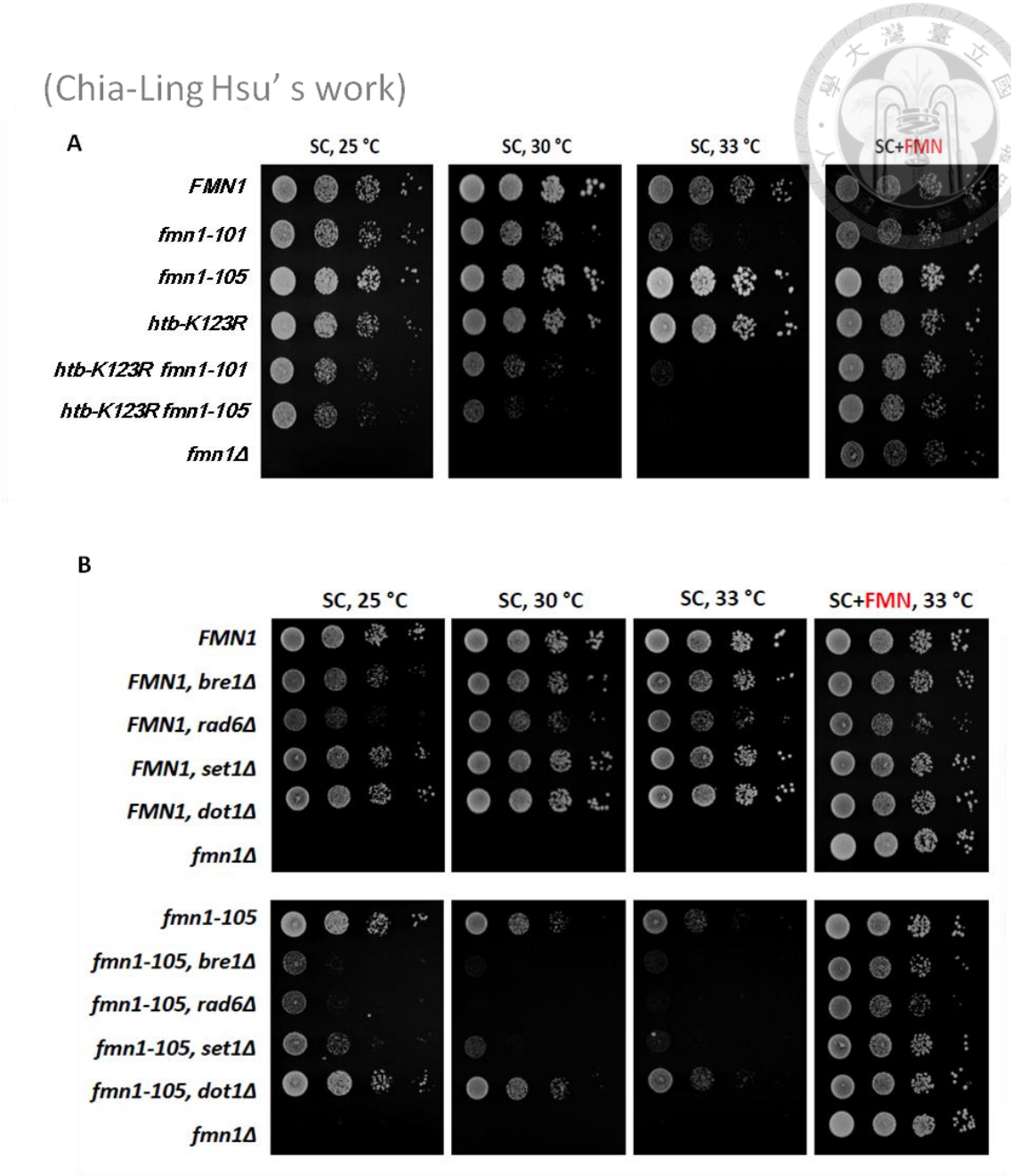

Figure 1. Mutation of *FMN1* displays genetic interaction with *SET1* and its upstream regulator H2Bub

(A and B) Yeast strains with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates, or SC containing 0.5mM FMN plates and grown at the indicated temperatures for 2 or3 (SC+FMN) days.

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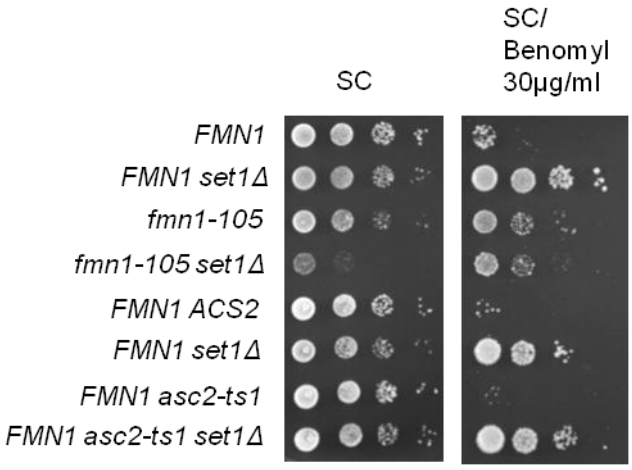

Figure 2. *fmn1-105* mutants exhibited slight resistance to Benomyl

Yeast strains with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates or SC containing Benomyl plates and grown at the indicated temperatures for 2 or 3 (SC+Benomyl) days.

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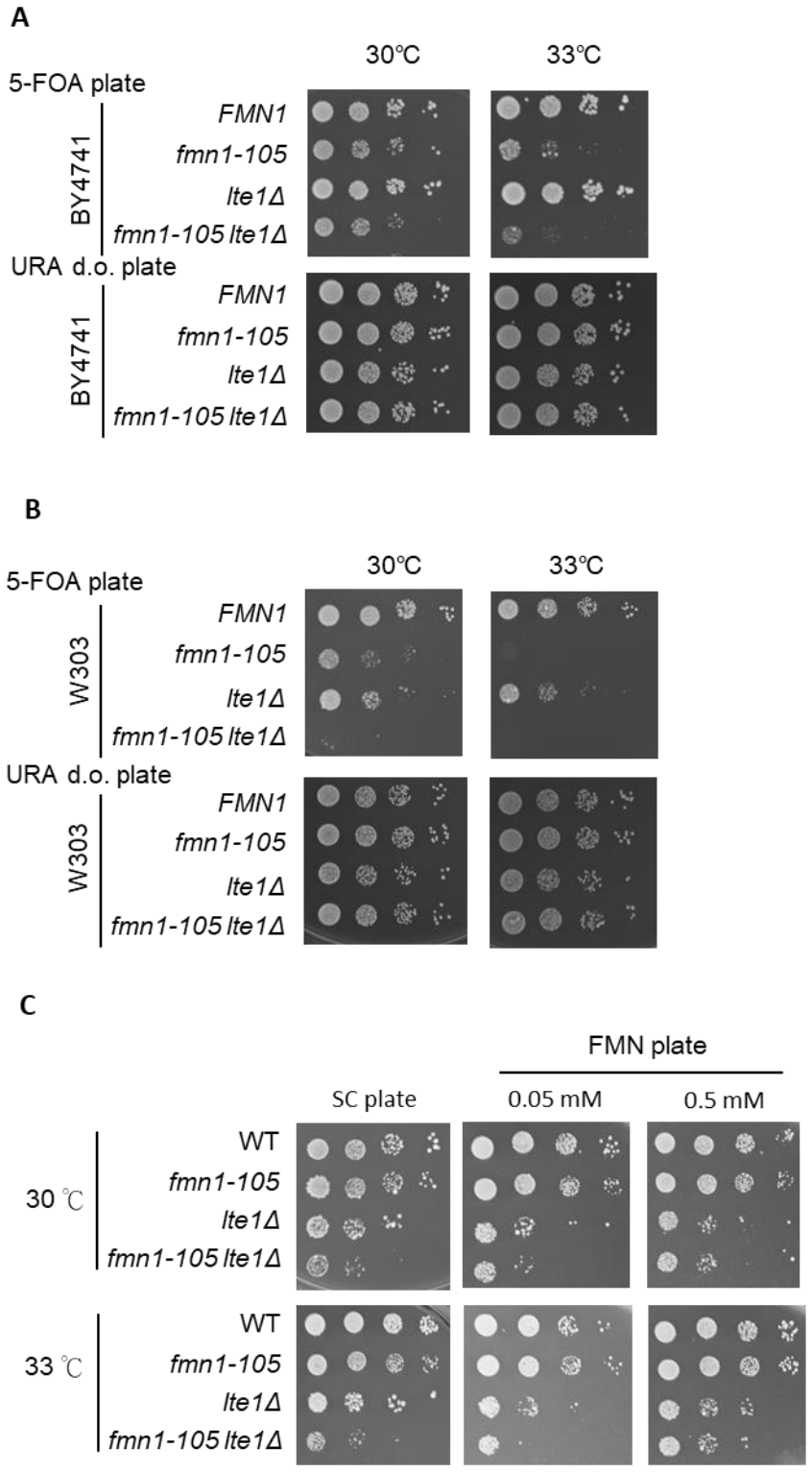

Figure 3. *FMN1* displays genetic interaction with *LTE1* in three different backgrounds

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(A)Yeast strains of BY4741 carried a URA3-based plasmid expressing wild-type *FMN1* to complement the loss of genome *FMN1*. After transformation with a HIS3-based plasmid expressing wild-type *FMN1* or *fmn1-105*, cells were serially diluted 10-fold, spotted onto SC containing 5-Fluoroorotic acid (5-FOA) plates and URA dropping out plate for 3 days.

(B)Yeast strains of W303 carried a URA3-based plasmid expressing wild-type *FMN1* to complement the loss of genome *FMN1*. After transformation with a HIS3-based plasmid expressing wild-type *FMN1* or *fmn1-105*, cells were serially diluted 10-fold, spotted onto SC containing 5-Fluoroorotic acid (5-FOA) plates and URA dropping out plate for 3 days.

(C) Yeast strains of W303 with wild-type or mutation *FMN1* with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates , or SC containing 0.05/ 0.5 mM FMN plates and grown at the indicated temperatures for 2 or 3 (SC+FMN) days.

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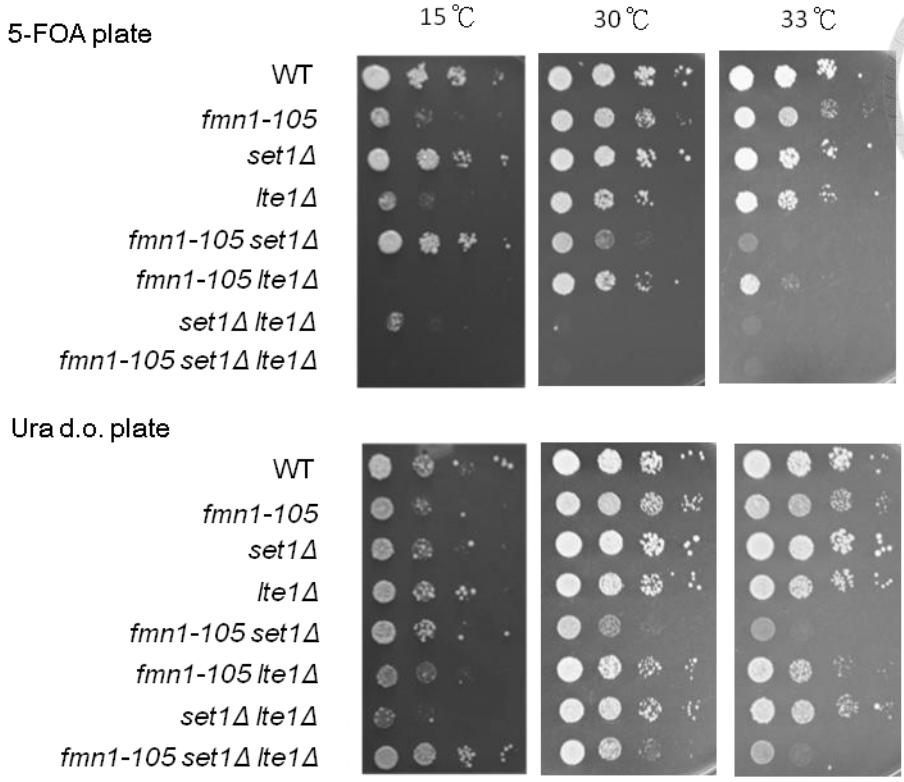

Figure 4. The viability of *fmn1-105 set1Δ* cells were dependent for Lte1

Yeast strains carried a URA3-based plasmid expressing wild-type LTE1 or not with the indicated genotypes were serially diluted 10-fold, spotted onto SC containing 5-Fluoroorotic acid (5-FOA) plates and URA dropping out plate and grown at the indicated temperatures for 3 days.

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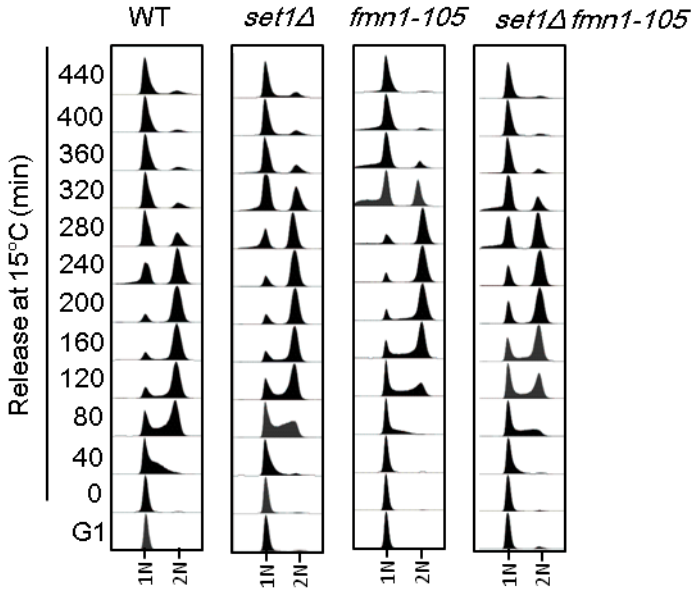

Figure 5. ***fmn1-105 lte1Δ*** **cells delay in S phase**

Cells were arrested with a-factor then released into SC media at 15℃ to induce the low temperature phenotype. Cells were sampled at 40 min intervals after release from α-factor at 15℃ for 440 minutes. Cell cycle progression as indicated time was monitored by flow cytometry.

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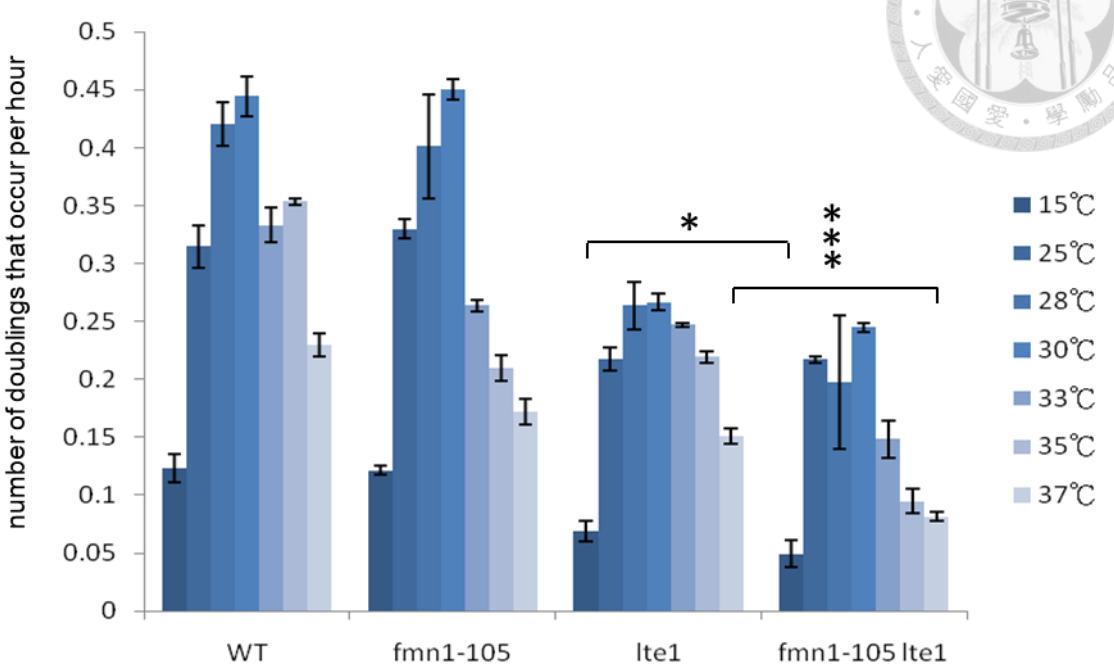

Figure 6. The growth rate of *fmn1-105 lte1Δ* cells is lower than wild-type and single mutants cells

The cells growth rates were show with indicated temperature. Cells were cultured in constant temperature rooms. At intervals hour, a sample would be sterilely removed and measured the cell density by spectrophotometer. An OD value between 0.1 and 1 is used to calculate the growth rate by Roth V. 2006 Doubling Time Computing. The growth rate means number of doublings that occur per unit of time. P values <0.1 were considered significant and are denoted by an asterisk in the figure. P values <0.01 were considered significant and are denoted by three asterisk in the figure.

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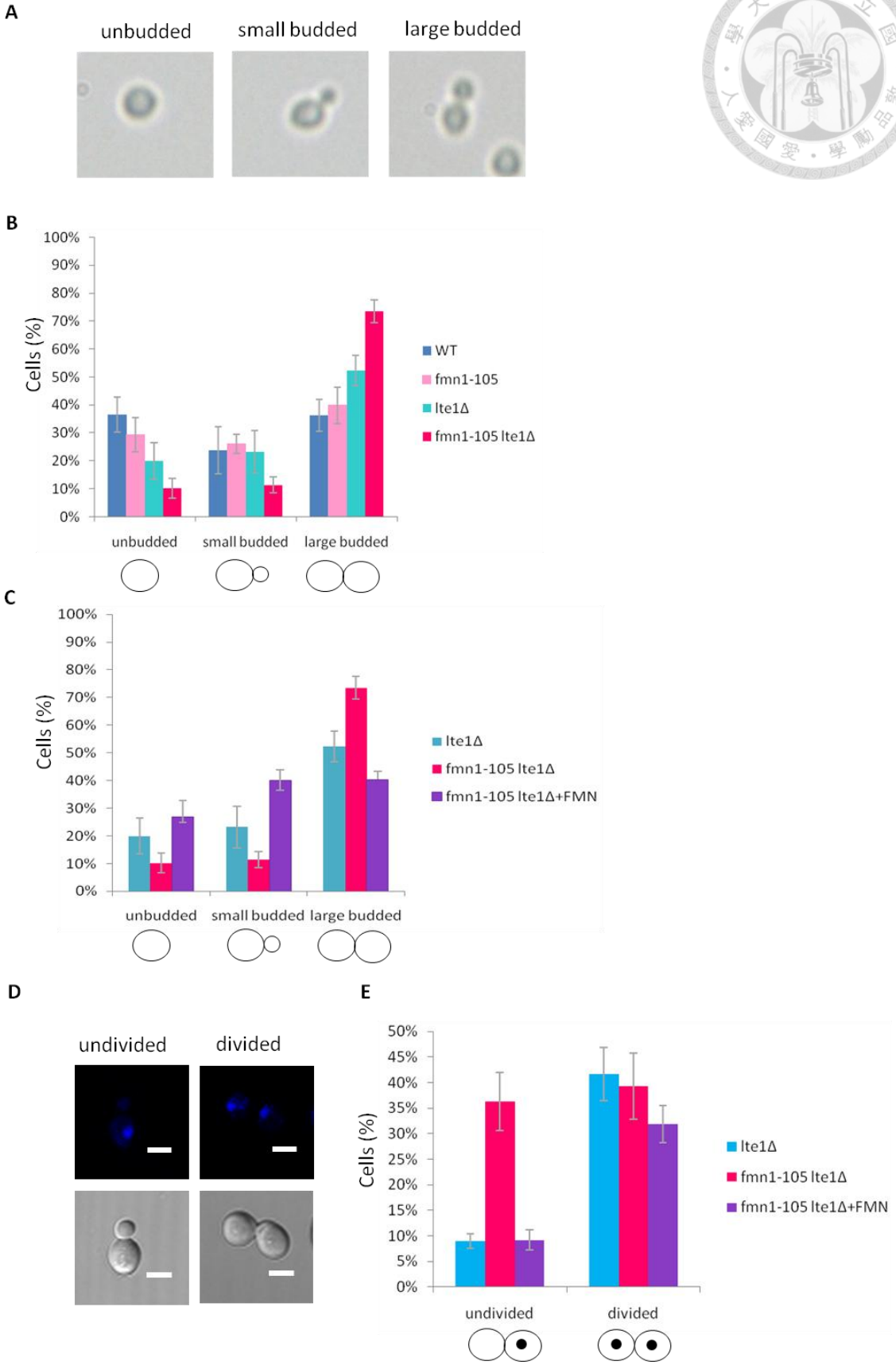

Figure 7. *fmn1-105 lte1Δ* cells are delayed in mitosis

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(A) The budding morphology analysis. Cells were distinguishing for small budded cells with the daughter cell less than one-half of mother cell.

(B) Budding morphology of asynchronous population of wild-type, *fmn1-105*, *lte1Δ* and *fmn1-105 lte1Δ* cells.

(C) Budding morphology of asynchronous population of, *lte1Δ*, *fmn1-105 lte1Δ* and *fmn1-105 lte1Δ* incubated in 0.5mM FMN media cells.

(D) The morphology of nuclear division imaging. bar= 5μm.

(E) Nuclear division in large budded cells was scored by DAPI staining in wild-type, *fmn1-105, lte1Δ* and *fmn1-105 lte1Δ* cells.

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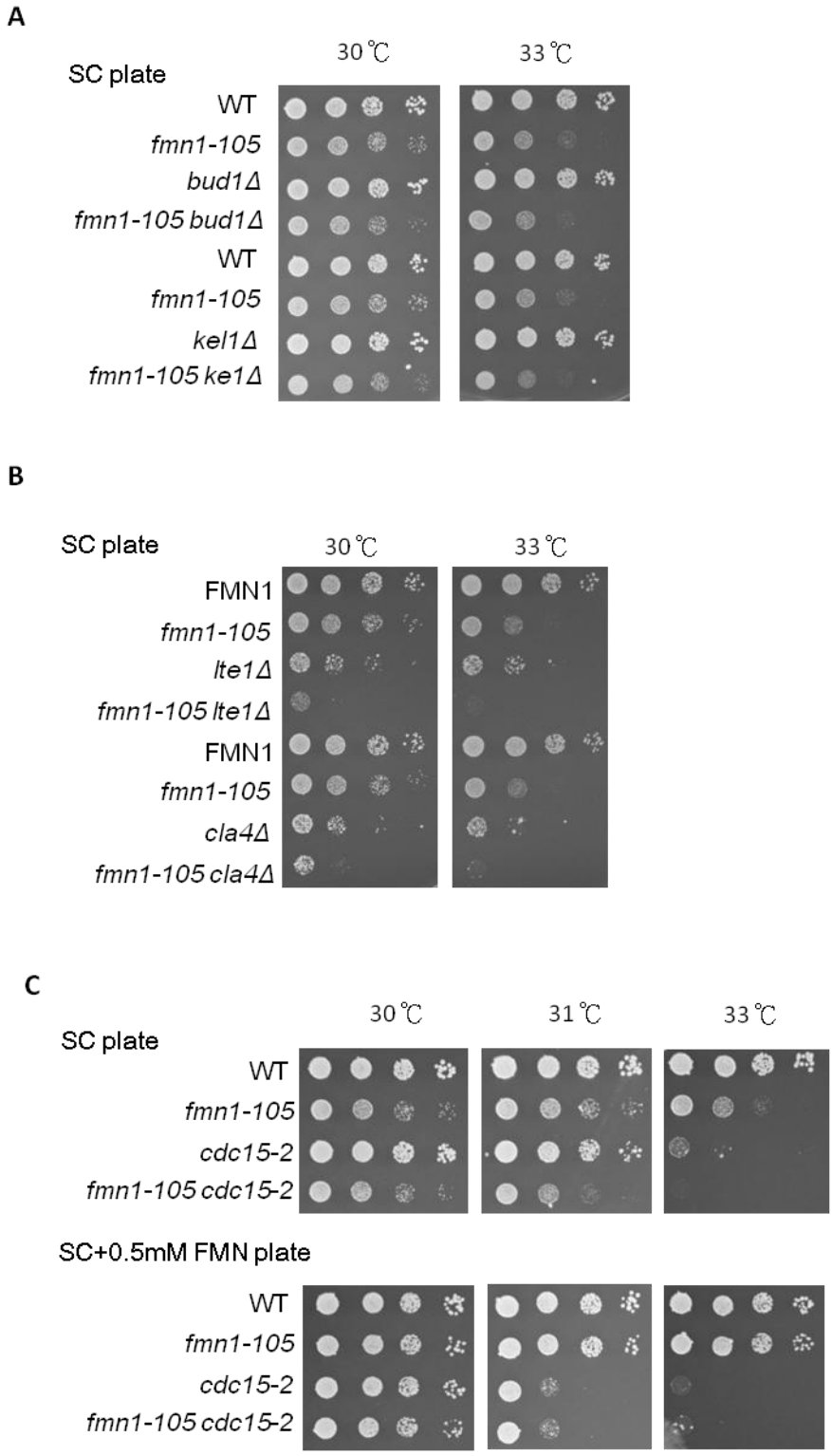

Figure 8. *FMN1* displays genetic interaction with mitotic exit network regulators

(A, B, C and C) Yeast strains with the indicated genotypes were serially diluted 10-fold,

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spotted onto SC plates, or SC containing 0.5mM FMN plates and grown at the indicated temperatures for 2 or3 (SC+FMN) days.

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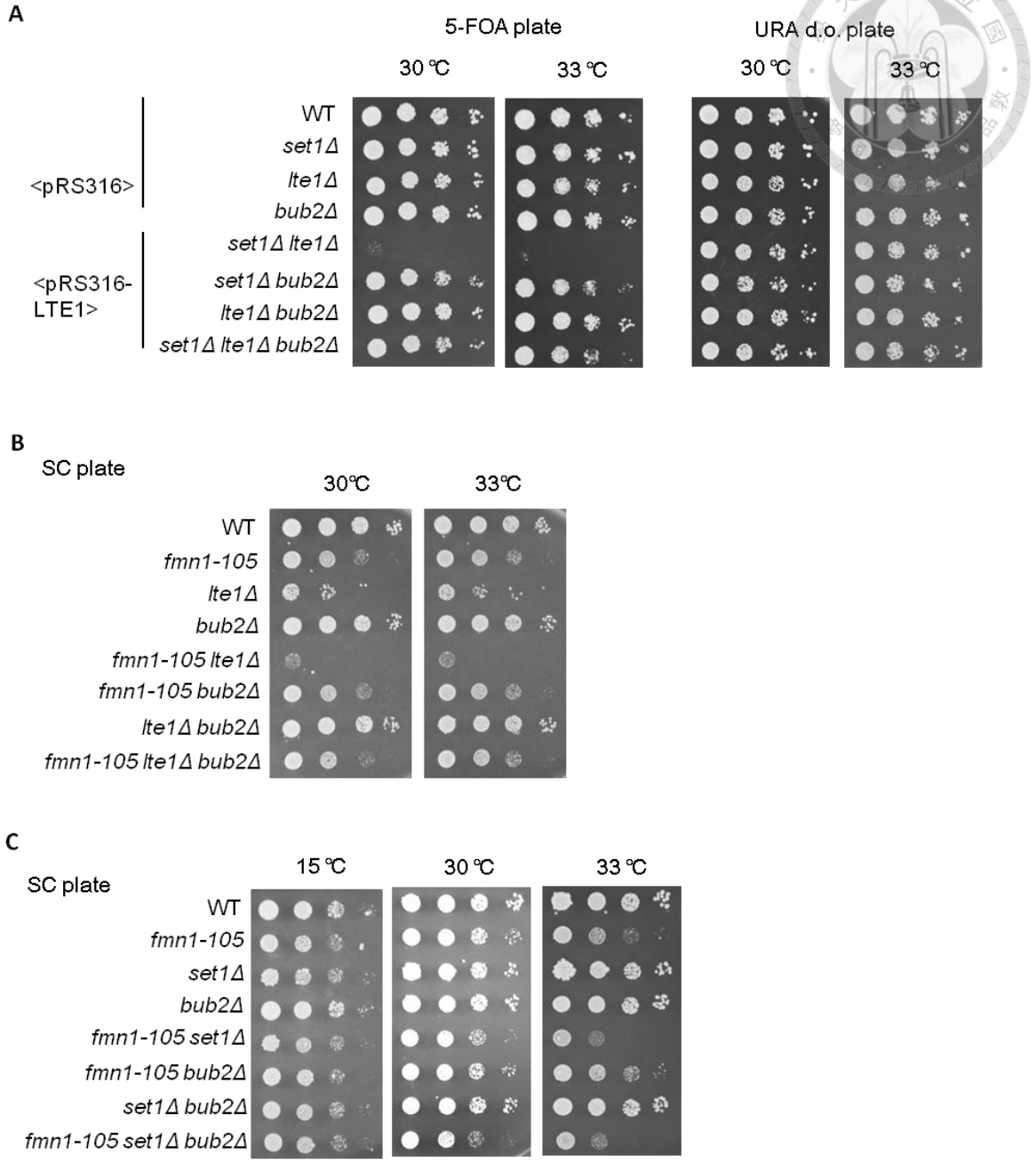

Figure 9. The growth defect of *fmn1-105 lte1Δ* is due to MEN restrain

(A and C) Yeast strains with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates and grown at the indicated temperatures for 2 days.

(B) Yeast strains carried a URA3-based plasmid expressing wild-type *LTE1* or not with the indicated genotypes were serially diluted 10-fold, spotted onto SC containing

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5-Fluoroorotic acid (5-FOA) plates and URA dropping out plate and grown at the indicated temperatures for 3 days.

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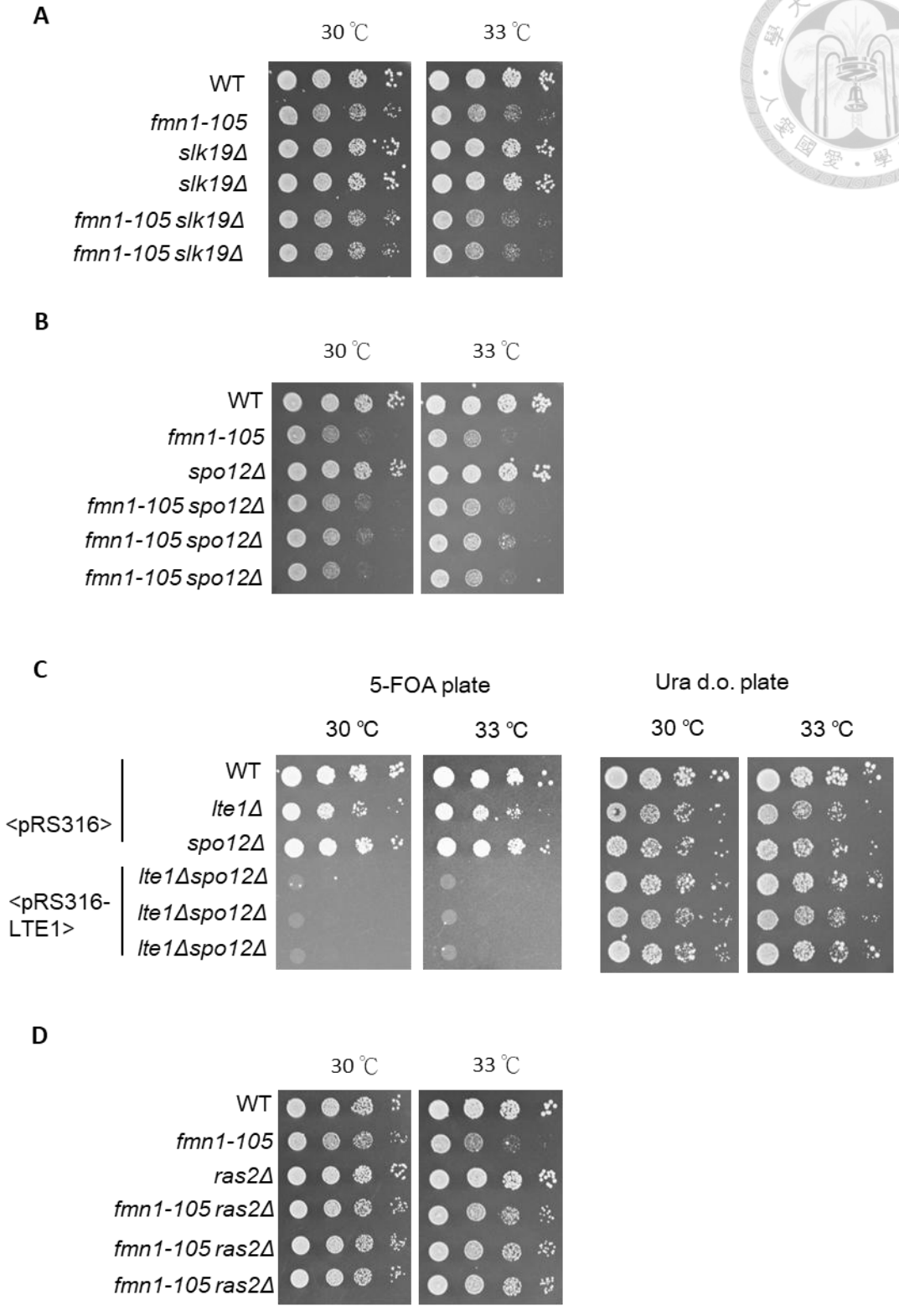

Figure 10. The genetic interaction between *FMN1*, FEAR components and APC regulator

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(A, B, and D) Yeast strains with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates and grown at the indicated temperatures for 2 days.

(C) Yeast strains carried a URA3-based plasmid expressing wild-type *LTE1* or not with the indicated genotypes were serially diluted 10-fold, spotted onto SC containing 5-Fluoroorotic acid (5-FOA) plates and URA dropping out plate and grown at the indicated temperatures for 3 days.

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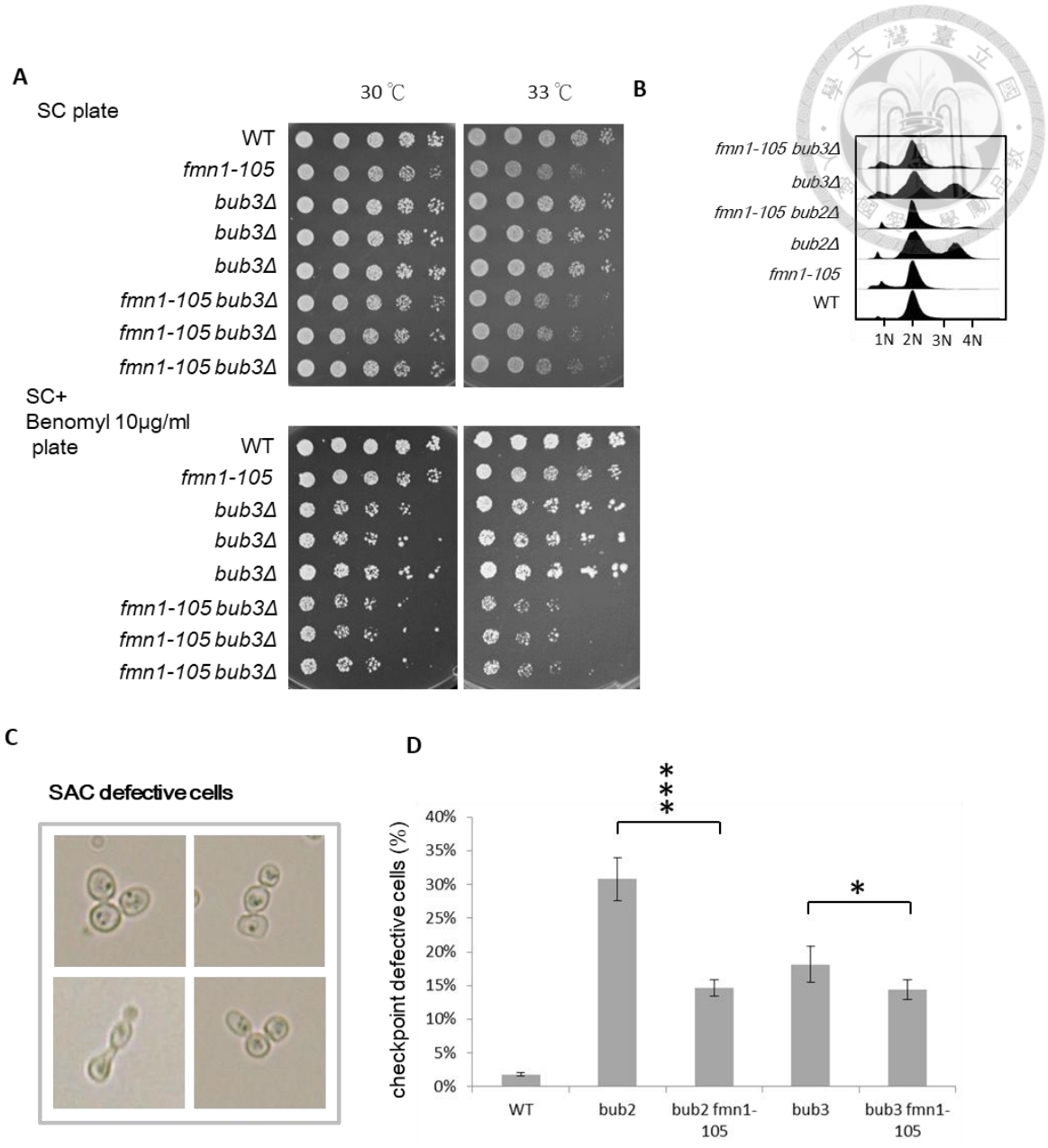

Figure 11. *fmn1-105* cells are later for metaphase-anaphase transition

(A) Yeast strains with the indicated genotypes were serially diluted 5-fold, spotted onto SC plates or SC containing Benomyl plates and grown at the indicated temperatures for 2 or 3 (SC+Benomyl) days.

(B) Cells were arrested with α-factor then released into YPD containing Nocodazole media for 3.5h. Cell cycle progression was monitored by flow cytometry.

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(C) The cells morphology with spindle assembly checkpoint defect was show in the panel.

(D) The frequency of spindle assembly checkpoint defective cells was show in the panel. P values <0.1 were considered significant and are denoted by an asterisk in the figure. P values <0.01 were considered significant and are denoted by three asterisk in the figure.

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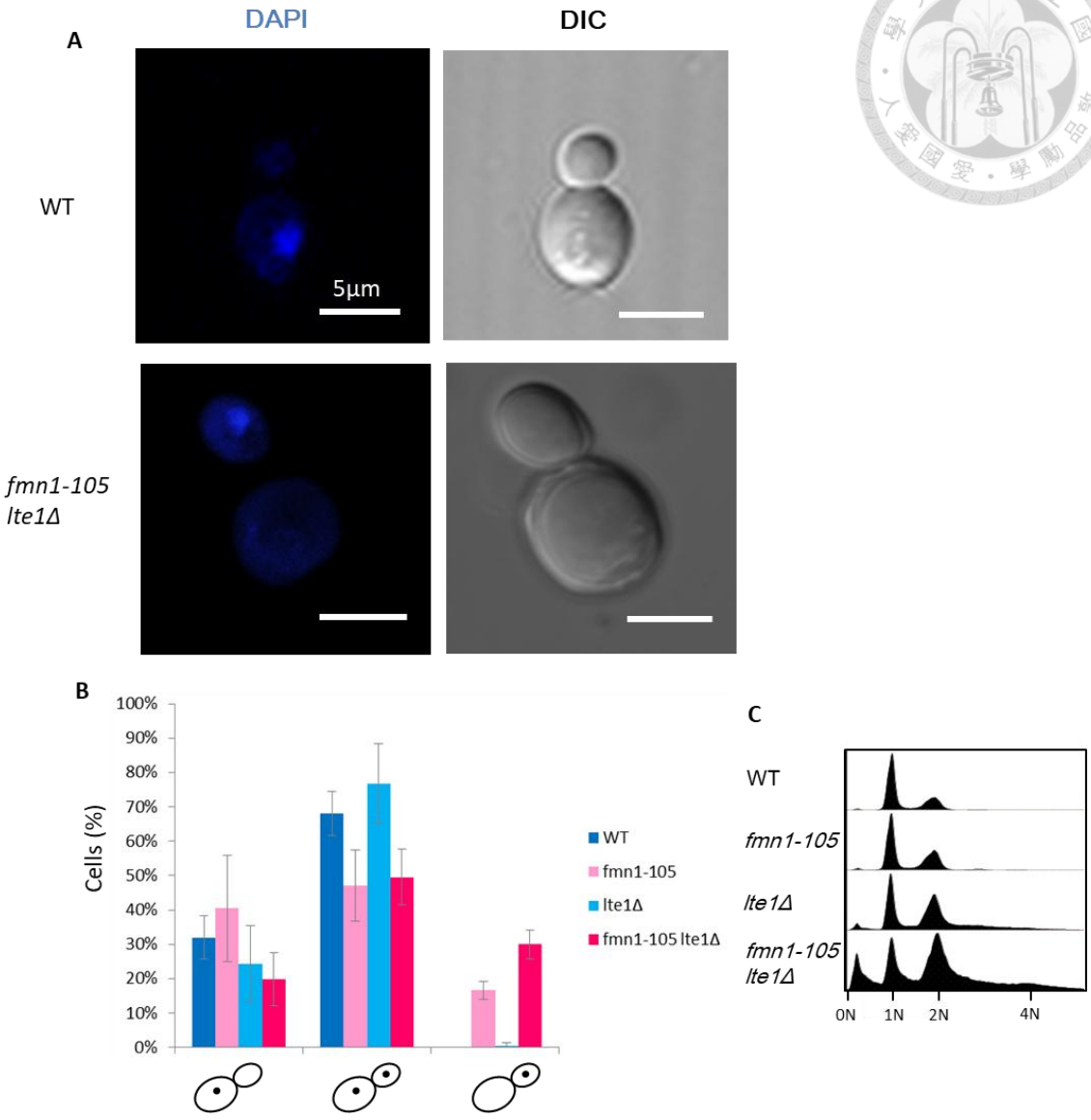

Figure 12. *fmn1-105* cells exhibit chromosome segregation error

(A) The morphology of nuclear division imaging. bar= 5μm. The daughter bud *of fmn1-105 lte1Δ* displays DAPI signal but no of mother bud.

(B) Nuclear division types in large budded cells was scored by DAPI staining in wild-type, *fmn1-105*, *lte1Δ* and *fmn1-105 lte1Δ* cells.

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(C) The cell cycle progression of asynchronous population of wild-type, *fmn1-105*, *lte1Δ* and *fmn1-105 lte1Δ* cells at 37℃ were monitored by flow cytometry.

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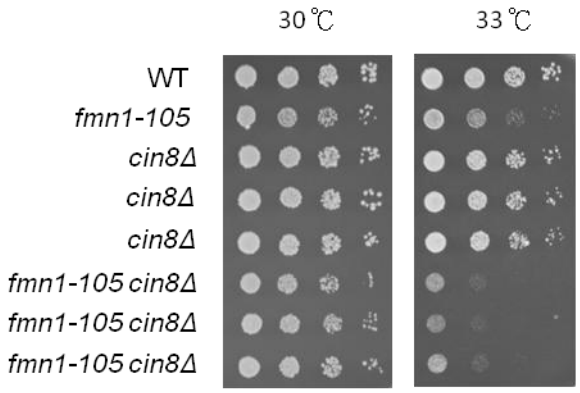

Figure 13. The spindle elongation defect affects the viability of *fmn1-105* cells

Yeast strains with the indicated genotypes were serially diluted 10-fold, spotted onto SC plates and grown at the indicated temperatures for 2 days.

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Table 1. Summary of the result of synthetic genetic array analysis of *fmn1-105*.

(Adopt from Tien-Hsien Chang's lab)

AMD2	PRB1	COG6	MNN10	RXT2
ARO1	RPN10	CTF18	MSH4	SAP30
BRE1	RPS18B	CTK1	NCS2	SDC1
BRE2	RPS21B	DAL81	NCS6	SDS3
BUB3	RTF1	EBS1	NEW1	SGF73
CLB2	SCS7	ELP4	PUS4	SHE4
DBF2	SEC22	ELP6	RIM21	SIN3
FLX1	SEM1	ERG3	RMD5	SWD1
IZH1	SOH1	FYV4	RMD7	SWM1
LGE1	SUR1	GIM3	RPA34	SWR1
LRP1	TOP1	GIS2	RPE1	TIM18
LTE1	UME6	HDA1	RPL16B	TOF2
MKT1	VHS1	HTZ1	RPL42A	TOM37
MSN5	ADR1	IKI3	RPS1B	UBA3
NPL4	AFG1	IVY1	RPS4A	VPS24
NUP188	BUD13	KAR3	RPS27B	VPS4
PEX5	CHL4	MET2	RPS29B	VPS53
PHO80	CIN8	MMS22	RPS4A	YME1
				ZDS1

Gene Ontology/ biological process	#	%
metabolic process	67	74%
gene expression	41	45%
regulation of transcription by RNA polymerase II	19	21%
mitotic cell cycle process	14	15%
chromatin silencing	11	12%
nuclear division	9	10%
nuclear chromosome segregation	8	9%
histone deacetylation	5	5%
histone H3-K4 methylation	3	3%

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Table 2. *Saccharomyces cerevisiae* strains used in this study

Strain name	**Relevant genotype**	**Source or reference**
ICL 1	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::NAT <pRS313-FMN1>*	This study
ICL 2	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::NAT set1:: KanMX <pRS313-FMN1>*	This study
ICL 9	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::NAT <pRS313-fmn1-105>*	This study
ICL 10	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::NAT set1:: KanMX <pRS313-fmn1-105>*	This study
ICL 19	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Mata acs2::HPH fmn1::10xp LEU2 <PHT112-1(WT)><pRS313-FMN1>*	This study
ICL 20	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Mata acs2::HPH fmn1::10xp LEU2 set1:: KanMX <PHT112-1(WT)><pRS313-FMN1>*	This study
ICL 21	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Mata acs2::HPH fmn1::10xp LEU2 <PHT215(acstTS1><pRS313-FMN1>*	This study
ICL 22	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Mata acs2::HPH fmn1::10xp LEU2 set1:: KanMX <PHT215(acstTS1><pRS313-FMN1>*	This study
ICL 29	*MATa RAD5^{+} leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100*	from Susan M. Gasser
ICL 38	*MATa RAD5^{+} leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105*	This study

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ICL 42	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1::KanMX*	This study
ICL 43	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1::KanMX fmn1::fmn1-105*	This study
ICL 46	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::Nat*	This study
ICL 48	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::loxp <pRS316-FMN1><pRS313-FMN1>*	This study
ICL 49	*MATa leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::loxp <pRS316-FMN1><pRS313-fmn1-105>*	This study
ICL 50	*MATa leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::loxp lte1:: KanMX <pRS316-FMN1><pRS316-FMN1>*	This study
ICL 51	*MATa leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::loxp lte1:: KanMX <pRS316-FMN1><pRS313-fmn1-105>*	This study
ICL 52	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::loxp <pRS316-FMN1><pRS313-FMN1>*	This study
ICL 53	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::loxp <pRS316-FMN1><pRS313-fmn1-105>*	This study
ICL 54	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::loxp lte1:: KanMX <pRS316-FMN1><pRS316-FMN1>*	This study
ICL 55	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fmn1::loxp lte1:: KanMX <pRS316-FMN1><pRS313-fmn1-105>*	This study

doi:10.6342/NTU201803272

ICL 60	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 cla4:: KanMX*	This study
ICL 61	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 cla4:: KanMX fmn1::fmn1-105*	This study
ICL 63	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 <pRS316>*	This study
ICL 64	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105 <pRS316>*	This study
ICL 65	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1:: KanMX <pRS316>*	This study
ICL 66	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105 lte1:: KanMX <pRS316>*	This study
ICL 67	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1:: KanMX <pRS316-LTE1>*	This study
ICL 68	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105 lte1:: KanMX <pRS316-LTE1>*	This study
ICL 73	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub1:: KanMX*	This study
ICL 74	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub1:: KanMX fmn1::fmn1-105*	This study
ICL 77	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 ras2:: KanMX*	This study
ICL 78	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 ras2:: KanMX fmn1::fmn1-105*	This study

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ICL 82	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1:: KanMX set1::Nat <pRS316-LTE1>*	This study
ICL 84	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::Nat fmn1::fmn1-105*	This study
ICL 85	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub2:: KanMX fmn1::fmn1-105*	This study
ICL 86	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub2:: KanMX*	This study
ICL 89	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 kel1:: KanMX*	This study
ICL 90	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 kel1:: KanMX fmn1::fmn1-105*	This study
ICL 100	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1:: KanMX set1::Nat fmn1:fmn1-105 <pRS316-LTE1>*	This study
ICL 104	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::Nat <pRS316>*	This study
ICL 141	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::Nat fmn1::fmn1-105 <pRS316>*	This study
ICL 143	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105-HPH*	This study
ICL 149	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1::Nat <pRS316>*	This study

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ICL 176	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 spo12:: KanMX*	This study
ICL 177	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 spo12:: KanMX fmn1::fmn1-105*	This study
ICL 178	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 spo12:: KanMX lte1::Nat <pRS316-LTE1>*	This study
ICL 181	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 spo12:: KanMX <pRS316>*	This study
ICL 200	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::HPH bud2:: KanMX*	This study
ICL 209	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::Nat bub2:: KanMX fmn1::fmn1-105*	This study
ICL 218	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::HPH lte1::Nat bub2:: KanMX <pRS316-LTE1>*	This study
ICL 219	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::HPH lte1::Nat <pRS316-LTE1>*	This study
ICL 221	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1::Nat bub2:: KanMX*	This study
ICL 222	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 lte1::Nat bub2:: KanMX <pRS316-LTE1>*	This study
ICL 229	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 set1::HPH bub2:: KanMX <pRS316-LTE1>*	This study
ICL 238	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105 bub3:: KanMX*	This study

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ICL 239	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 fmn1::fmn1-105 cin8:: KanMX*	This study
ICL 241	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 slk19:: KanMX fmn1::fmn1-105*	This study
ICL 280	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub3:: KanMX*	This study
ICL 281	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 cin8:: KanMX*	This study
ICL 283	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 slk19:: KanMX*	This study
ICL 291	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 bub2:: KanMX <pRS316>*	This study
ICL 304	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 cdc15::cdc15-2 LTE1::LTE2-ProA- KanMX*	This study
ICL 308	*MATa RAD5$^+$ leu2-3 leu2-112 ade2-1 his3-11 his-15 ura3-1 trp1-1 can1-100 cdc15::cdc15-2 fmn1::fmn1-105-HPH LTE1::LTE4-ProA- KanMX*	This study

doi:10.6342/NTU201803272

REFERENCE

Adames, N. R., Oberle, J. R., & Cooper, J. A. (2001). The surveillance mechanism of the spindle position checkpoint in yeast. *The Journal of Cell Biology*, *153*(1), 159-168.

Aguilera, A., Chávez, S., & Malagón, F. (2000). Mitotic recombination in yeast: elements controlling its incidence. *Yeast*, *16*(8), 731-754.

Altmann, K., & Westermann, B. (2005). Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae. *Molecular biology of the cell*, *16*(11), 5410-5417.

Bardin, A. J., & Amon, A. (2001). Men and sin: what's the difference?. *Nature Reviews Molecular Cell Biology*, *2*(11), 815-826.

Bardin, A. J., Visintin, R., & Amon, A. (2000). A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell*, *102*(1), 21-31.

Barile, M., Brizio, C., De Virgilio, C., Delfine, S., Quagliariello, E., & Passarella, S. (1997). Flavin adenine dinucleotide and flavin mononucleotide metabolism in rat liver: the occurrence of FAD pyrophosphatase and FMN phosphohydrolase in isolated mitochondria. *European journal of biochemistry*, *249*(3), 777-785.

Baro, B., Queralt, E., & Monje-Casas, F. (2017). Regulation of Mitotic Exit in Saccharomyces cerevisiae. *The Mitotic Exit Network: Methods and Protocols*, 3-17.

Bauer, S., Kemter, K., Bacher, A., Huber, R., Fischer, M., & Steinbacher, S. (2003). Crystal structure of Schizosaccharomyces pombe riboflavin kinase reveals a novel ATP and riboflavin-binding fold. *Journal of molecular biology*, *326*(5), 1463-1473.

Beilharz, T. H., Harrison, P. F., Miles, D. M., See, M. M., Le, U. M. M., Kalanon, M., ... & Holstege, F. (2017). Coordination of cell cycle progression and mitotic spindle assembly involves histone H3 lysine 4 methylation by Set1/COMPASS. *Genetics*, *205*(1), 185-199.

Bertazzi, D. T., Kurtulmus, B., & Pereira, G. (2011). The cortical protein Lte1 promotes mitotic exit by inhibiting the spindle position checkpoint kinase Kin4. *The Journal of cell biology*, *193*(6), 1033-1048.

Black, K. L., Petruk, S., Fenstermaker, T. K., Hodgson, J. W., Caplan, J. L., Brock, H. W., & Mazo, A. (2016). Chromatin proteins and RNA are associated with DNA during all phases of mitosis. *Cell discovery*, *2*, 16038.

Bloecher, A., Venturi, G. M., & Tatchell, K. (2000). Anaphase spindle position is monitored by the BUB2 checkpoint. *Nature Cell Biology*, *2*(8), 556.

Bloom, J., & Cross, F. R. (2007). Multiple levels of cyclin specificity in cell-cycle control. *Nature Reviews Molecular Cell Biology*, *8*(2), 149-160.

doi:10.6342/NTU201803272

Bokros, M., & Wang, Y. (2016). Spindle assembly checkpoint silencing and beyond. *Cell Cycle*, *15*(13), 1661.

Bonnet, J., Devys, D., & Tora, L. (2014). Histone H2B ubiquitination: signaling not scrapping. *Drug Discovery Today: Technologies*, *12*, e19-e27.

Botchkarev, V. V., & Haber, J. E. (2017). Functions and regulation of the Polo-like kinase Cdc5 in the absence and presence of DNA damage. *Current genetics*, 1-10.

Büch, K., Stransky, H., & Hager, A. (1995). FAD is a further essential cofactor of the NAD (P) H and O2-dependent zeaxanthin-epoxidase. *FEBS letters*, *376*(1-2), 45-48.

Burgess, A., Rasouli, M., & Rogers, S. (2014). Stressing mitosis to death. *Frontiers in oncology*, *4*, 140.

Casaus, J. L., Navarro, J. A., Hervás, M., Lostao, A., Miguel, A., Gómez-Moreno, C., ... & Medina, M. (2002). Anabaena sp. PCC 7119 Flavodoxin as Electron Carrier from Photosystem I to Ferredoxin-NADP+ Reductase ROLE OF TRP57 AND TYR94. *Journal of Biological Chemistry*, *277*(25), 22338-22344.

Cavalier-Smith, T. (2010). Origin of the cell nucleus, mitosis and sex: roles of intracellular coevolution. *Biology direct*, *5*(1), 7.

Caydasi, A. K., & Pereira, G. (2009). Spindle alignment regulates the dynamic association of checkpoint proteins with yeast spindle pole bodies. *Developmental cell*, *16*(1), 146-156.

Caydasi, A. K., & Pereira, G. (2012). SPOC alert—when chromosomes get the wrong direction. *Experimental cell research*, *318*(12), 1421-1427.

Caydasi, A. K., Lohel, M., Grünert, G., Dittrich, P., Pereira, G., & Ibrahim, B. (2012). A dynamical model of the spindle position checkpoint. *Molecular systems biology*, *8*(1), 582.

Cepeda-García, C. (2017). Determination of Cell Cycle Stage and Mitotic Exit Through the Quantification of the Protein Levels of Known Mitotic Regulators. In *The Mitotic Exit Network* (pp. 45-57). Humana Press, New York, NY.

Chai, C. C., Teh, E. M., & Yeong, F. M. (2010). Unrestrained spindle elongation during recovery from spindle checkpoint activation in cdc15-2 cells results in mis-segregation of chromosomes. *Molecular biology of the cell*, *21*(14), 2384-2398.

Chan, L. Y., & Amon, A. (2010). Spindle position is coordinated with cell-cycle progression through establishment of mitotic exit-activating and-inhibitory zones. *Molecular cell*, *39*(3), 444-454.

Chen, D., Dundr, M., Wang, C., Leung, A., Lamond, A., Misteli, T., & Huang, S. (2005). Condensed mitotic chromatin is accessible to transcription factors and chromatin structural proteins. *The Journal of cell biology*, *168*(1), 41-54.

Cherfils, J., & Chardin, P. (1999). GEFs: structural basis for their activation of small GTP-binding proteins. *Trends in biochemical sciences*, *24*(8), 306-311.

doi:10.6342/NTU201803272

Christie, J. M., Salomon, M., Nozue, K., Wada, M., & Briggs, W. R. (1999). LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide. *Proceedings of the National Academy of Sciences*, *96*(15), 8779-8783.

Cooper, G. M. (2000). The Cell: A Molecular Approach, 2nd edn. The Cell: A Molecular Approach. Sunderland, MA.

Dahmann, C., & Futcher, B. (1995). Specialization of B-type cyclins for mitosis or meiosis in S. cerevisiae. *Genetics*, *140*(3), 957-963.

Dalfó, D., Marqués, N., & Albalat, R. (2007). Analysis of the NADH- dependent retinaldehyde reductase activity of amphioxus retinol dehydrogenase enzymes enhances our understanding of the evolution of the retinol dehydrogenase family. *The FEBS journal*, *274*(14), 3739-3752.

D'Angiolella, V., Mari, C., Nocera, D., Rametti, L., & Grieco, D. (2003). The spindle checkpoint requires cyclin-dependent kinase activity. *Genes & development*, *17*(20), 2520-2525.

D'Angiolella, V., Santarpia, C., & Grieco, D. (2007). Oxidative stress overrides the spindle checkpoint. *Cell Cycle*, *6*(5), 576-579.

De Santa, F., Barozzi, I., Mietton, F., Ghisletti, S., Polletti, S., Tusi, B. K., ... & Natoli, G. (2010). A large fraction of extragenic RNA pol II transcription sites overlap enhancers. *PLoS biology*, *8*(5), e1000384.

Dehe, P. M., & Geli, V. (2006). The multiple faces of Set1. *Biochemistry and cell biology*, *84*(4), 536-548.

Dehé, P. M., Dichtl, B., Schaft, D., Roguev, A., Pamblanco, M., Lebrun, R., ... & Shevchenko, A. (2006). Protein interactions within the Set1 complex and their roles in the regulation of histone 3 lysine 4 methylation. *Journal of biological chemistry*, *281*(46), 35404-35412.

Falk, J. E., Campbell, I. W., Joyce, K., Whalen, J., Seshan, A., & Amon, A. (2016). LTE1 promotes exit from mitosis by multiple mechanisms. *Molecular biology of the cell*, *27*(25), 3991-4001.

Falk, J. E., Chan, L. Y., & Amon, A. (2011). Lte1 promotes mitotic exit by controlling the localization of the spindle position checkpoint kinase Kin4. *Proceedings of the National Academy of Sciences*, *108*(31), 12584-12590.

Falk, J. E., Tsuchiya, D., Verdaasdonk, J., Lacefield, S., Bloom, K., & Amon, A. (2016). Spatial signals link exit from mitosis to spindle position. *Elife*, *5*, e14036.

Fang, G., Yu, H., & Kirschner, M. W. (1998). The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes & development*, *12*(12), 1871-1883.

Fitch, I., Dahmann, C., Surana, U., Amon, A., Nasmyth, K., Goetsch, L., ... & Futcher,

doi:10.6342/NTU201803272

B. (1992). Characterization of four B-type cyclin genes of the budding yeast Saccharomyces cerevisiae. *Molecular biology of the cell*, *3*(7), 805-818.

Foley, E. A., & Kapoor, T. M. (2013). Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. *Nature reviews Molecular cell biology*, *14*(1), 25.

Francisco, L., Wang, W., & Chan, C. S. (1994). Type 1 protein phosphatase acts in opposition to IpL1 protein kinase in regulating yeast chromosome segregation. *Molecular and cellular biology*, *14*(7), 4731-4740.

Fraschini, R., Formenti, E., Lucchini, G., & Piatti, S. (1999). Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2. The Journal of cell biology, 145(5), 979-991.

Frenkel, E. P., & Kitchens, R. L. (1977). Purification and properties of acetyl coenzyme A synthetase from bakers' yeast. *Journal of Biological Chemistry*, *252*(2), 504-507.

Fujimitsu, K., Grimaldi, M., & Yamano, H. (2016). Cyclin-dependent kinase 1–dependent activation of APC/C ubiquitin ligase. *Science*, *352*(6289), 1121-1124.

Gardner, M. K., Bouck, D. C., Paliulis, L. V., Meehl, J. B., O'Toole, E. T., Haase, J., ... & Bloom, K. (2008). Chromosome congression by Kinesin-5 motor-mediated disassembly of longer kinetochore microtubules. *Cell*, *135*(5), 894-906.

Geymonat, M., Spanos, A., de Bettignies, G., & Sedgwick, S. G. (2009). Lte1 contributes to Bfa1 localization rather than stimulating nucleotide exchange by Tem1. *The Journal of cell biology*, *187*(4), 497-511.

Geymonat, M., Spanos, A., Jensen, S., & Sedgwick, S. G. (2010). Phosphorylation of Lte1 by Cdk prevents polarized growth during mitotic arrest in S. cerevisiae. *The Journal of cell biology*, *191*(6), 1097-1112.

Goranov, A. I., Cook, M., Ricicova, M., Ben-Ari, G., Gonzalez, C., Hansen, C., ... & Amon, A. (2009). The rate of cell growth is governed by cell cycle stage. *Genes & development*, *23*(12), 1408-1422.

Hadwiger, J. A., Wittenberg, C., Richardson, H. E., de Barros Lopes, M., & Reed, S. I. (1989). A family of cyclin homologs that control the G1 phase in yeast. *Proceedings of the National Academy of Sciences*, *86*(16), 6255-6259.

Hartwell, L. H. (1971). Genetic control of the cell division cycle in yeast: IV. Genes controlling bud emergence and cytokinesis. *Experimental cell research*, *69*(2), 265-276.

Hartwell, L. H., & Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science*, *246*(4930), 629-634.

Hartwell, L. H., Mortimer, R. K., Culotti, J., & Culotti, M. (1973). Genetic control of the cell division cycle in yeast: V. Genetic analysis of cdc mutants. *Genetics*, *74*(2), 267-286.

doi:10.6342/NTU201803272

Hergovich, A., & Hemmings, B. A. (2012, September). Hippo signalling in the G2/M cell cycle phase: lessons learned from the yeast MEN and SIN pathways. In *Seminars in cell & developmental biology* (Vol. 23, No. 7, pp. 794-802). Academic Press.

Hershko, A., Ganoth, D., Pehrson, J., Palazzo, R. E., & Cohen, L. H. (1991). Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts. *Journal of Biological Chemistry*, *266*(25), 16376-16379.

Holloway, S. L., Glotzer, M., King, R. W., & Murray, A. W. (1993). Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell*, *73*(7), 1393-1402.

Hotz, M., & Barral, Y. (2014). The Mitotic Exit Network: new turns on old pathways. *Trends in cell biology*, *24*(3), 145-152.

Hoyt, M. A., Totis, L., & Roberts, B. T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, *66*(3), 507-517.

Hu, F., & Elledge, S. J. (2002). Bub2 is a cell cycle regulated phospho-protein controlled by multiple checkpoints. *Cell Cycle*, *1*(5), 340-344.

Hu, F., Wang, Y., Liu, D., Li, Y., Qin, J., & Elledge, S. J. (2001). Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell*, *107*(5), 655-665.

Hwang, W. W., & Madhani, H. D. (2009). Nonredundant requirement for multiple histone modifications for the early anaphase release of the mitotic exit regulator Cdc14 from nucleolar chromatin. *PLoS genetics*, *5*(8), e1000588.

Islam, M. M., Nautiyal, M., Wynn, R. M., Mobley, J. A., Chuang, D. T., & Hutson, S. M. (2009). The branched chain amino acid (BCAA) metabolon: interation of glutamate dehydrogenase with the mitochondrial branched chain aminotransferase (BCATm). *Journal of Biological Chemistry*, jbc-M109.

Israels, E. D., & Israels, L. G. (2001). The cell cycle. *Stem cells*, *19*(1), 88-91.

Iyanagi, T., & Mason, H. S. (1973). Properties of hepatic reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase. *Biochemistry*, *12*(12), 2297-2308.

Jablonski, E., & DeLuca, M. (1977). Purification and properties of the NADH and NADPH specific FMN oxidoreductases from Beneckea harveyi. *Biochemistry*, *16*(13), 2932-2936.

Janke, C., Magiera, M. M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., ... & Knop, M. (2004). A versatile toolbox for PCR- based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast*, *21*(11), 947-962.

Jensen, S., Johnson, A. L., Johnston, L. H., & Segal, M. (2004). Temporal coupling of spindle disassembly and cytokinesis is disrupted by deletion of LTE1 in budding yeast. *Cell Cycle*, *3*(6), 815-820.

Jia, L., Kim, S., & Yu, H. (2013). Tracking spindle checkpoint signals from

doi:10.6342/NTU201803272

kinetochores to APC/C. *Trends in biochemical sciences*, *38*(6), 302-311.

Jorgensen, P., & Tyers, M. (2004). How cells coordinate growth and division. *Current Biology*, *14*(23), R1014-R1027.

Joseph-Horne, T. I. M., Hollomon, D. W., & Wood, P. M. (2001). Fungal respiration: a fusion of standard and alternative components. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1504*(2-3), 179-195.

Kadauke, S., & Blobel, G. A. (2013). Mitotic bookmarking by transcription factors. *Epigenetics & chromatin*, *6*(1), 6.

Kaldis, P., Tsakraklides, V., Ross, K. E., Winter, E., & Cheng, A. (2013). Activating phosphorylation of cyclin-dependent kinases in budding yeast.

Keating, P., Rachidi, N., Tanaka, T. U., & Stark, M. J. (2009). Ipl1-dependent phosphorylation of Dam1 is reduced by tension applied on kinetochores. *Journal of cell science*, *122*(23), 4375-4382.

Kerscher, S. J. (2000). Diversity and origin of alternative NADH: ubiquinone oxidoreductases. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1459*(2-3), 274-283.

Kim, J., Kim, J. A., McGinty, R. K., Nguyen, U. T., Muir, T. W., Allis, C. D., & Roeder, R. G. (2013). The n-SET domain of Set1 regulates H2B ubiquitylation-dependent H3K4 methylation. *Molecular cell*, *49*(6), 1121-1133.

Koch, K., & Macheroux, P. (2016, July). The protective/regulative role of the antioxidant enzyme Irc15 on the microtubule network of S. cerevisiae. In *8th OxiZymes Meeting 2016*.

Koepp, D. M. (2014). Cell cycle regulation by protein degradation. In *Cell Cycle Control* (pp. 61-73). Humana Press, New York, NY.

Krogan, N. J., Dover, J., Khorrami, S., Greenblatt, J. F., Schneider, J., Johnston, M., & Shilatifard, A. (2002). COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. *Journal of biological chemistry*, *277*(13), 10753-10755.

Kuczera, T., Bayram, Ö ., Sari, F., Braus, G. H., & Irniger, S. (2010). Dissection of mitotic functions of the yeast cyclin Clb2. *Cell cycle*, *9*(13), 2611-2619.

Kumar, R. (2018). Differential abundance and transcription of 14-3-3 proteins during vegetative growth and sexual reproduction in budding yeast. *Scientific reports*, *8*(1), 2145.

Kumar, S. A., & Chen, S. M. (2007). Electrochemically polymerized composites of conducting poly (p-ABSA) and flavins (FAD, FMN, RF) films and their use as electrochemical sensors: A new potent electroanalysis of NADH and NAD+. *Sensors and Actuators B: Chemical*, *123*(2), 964-977.

Künkel, W. (1980). Effects of the antimicrotubular cancerostatic drug nocodazole on the

doi:10.6342/NTU201803272

yeast Saccharomyces cerevisiae. *Journal of Basic Microbiology*, *20*(5), 315-324.
Lew, D. J., & Burke, D. J. (2003). The spindle assembly and spindle position checkpoints. *Annual review of genetics*, *37*(1), 251-282.
Li, R. (1999). Bifurcation of the mitotic checkpoint pathway in budding yeast. *Proceedings of the National Academy of Sciences*, *96*(9), 4989-4994.
Li, Y., Gorbea, C., Mahaffey, D., Rechsteiner, M., & Benezra, R. (1997). MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proceedings of the National Academy of Sciences*, *94*(23), 12431-12436.
Liang, F., Jin, F., Liu, H., & Wang, Y. (2009). The molecular function of the yeast polo-like kinase Cdc5 in Cdc14 release during early anaphase. *Molecular biology of the cell*, *20*(16), 3671-3679.
Lu, Y., & Cross, F. (2009). Mitotic exit in the absence of separase activity. *Molecular biology of the cell*, *20*(5), 1576-1591.
Lubran, M. M. (1971). The Biochemistry of Folic Acid and Vitamin B12. *Annals of Clinical & Laboratory Science*, *1*(3), 236-244.
Mao, Y., Abrieu, A., & Cleveland, D. W. (2003). Activating and silencing the mitotic checkpoint through CENP-E-dependent activation/inactivation of BubR1. *Cell*, *114*(1), 87-98.
Margaritis, T., Oreal, V., Brabers, N., Maestroni, L., Vitaliano-Prunier, A., Benschop, J. J., ... & Holstege, F. C. (2012). Two distinct repressive mechanisms for histone 3 lysine 4 methylation through promoting 3′-end antisense transcription. *PLoS genetics*, *8*(9), e1002952.
Marschall, L. G., Jeng, R. L., Mulholland, J., & Stearns, T. (1996). Analysis of Tub4p, a yeast gamma-tubulin-like protein: implications for microtubule-organizing center function. *The Journal of cell biology*, *134*(2), 443-454.
Maruyama, T., Nakamura, T., Hayashi, T., & Yanagida, M. (2006). Histone H2B mutations in inner region affect ubiquitination, centromere function, silencing and chromosome segregation. *The EMBO journal*, *25*(11), 2420-2431.
McIntosh, J. R., Grishchuk, E. L., & West, R. R. (2002). Chromosome-microtubule interactions during mitosis. *Annual review of cell and developmental biology*, *18*(1), 193-219.
Mendes Leitao, R. M. (2017). *The duration of mitosis and daughter cell size are modulated by nutrients in budding yeast*(Doctoral dissertation, UC Santa Cruz).
Merrill, A. H., & McCormick, D. B. (1980). Affinity chromatographic purification and properties of flavokinase (ATP: riboflavin 5'-phosphotransferase) from rat liver. *Journal of Biological Chemistry*, *255*(4), 1335-1338.
Mohl, D. A., Huddleston, M. J., Collingwood, T. S., Annan, R. S., & Deshaies, R. J. (2009). Dbf2–Mob1 drives relocalization of protein phosphatase Cdc14 to the

doi:10.6342/NTU201803272

cytoplasm during exit from mitosis. *The Journal of cell biology*, *184*(4), 527-539.

Muhua, L., Adames, N. R., Murphy, M. D., Shields, C. R., & Cooper, J. A. (1998). A cytokinesis checkpoint requiring the yeast homologue of an APC-binding protein. *Nature*, *393*(6684), 487.

Muñoz-Barrera, M., & Monje-Casas, F. (2017). Analysis of Cell Cycle Progression in Saccharomyces cerevisiae Using the Budding Index and Tubulin Staining. *The Mitotic Exit Network: Methods and Protocols*, 35-44.

Murray, A. W., Solomon, M. J., & Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature*, *339*(6222), 280.

Musacchio, A. (2015). The molecular biology of spindle assembly checkpoint signaling dynamics. *Current biology*, *25*(20), R1002-R1018.

Musacchio, A., & Salmon, E. D. (2007). The spindle-assembly checkpoint in space and time. *Nature reviews Molecular cell biology*, *8*(5), 379.

Nadal-Ribelles, M., Mas, G., Millán-Zambrano, G., Solé, C., Ammerer, G., Chávez, S., ... & de Nadal, E. (2015). H3K4 monomethylation dictates nucleosome dynamics and chromatin remodeling at stress-responsive genes. *Nucleic acids research*, *43*(10), 4937-4949.

Nakanishi, S., Lee, J. S., Gardner, K. E., Gardner, J. M., Takahashi, Y. H., Chandrasekharan, M. B., ... & Shilatifard, A. (2009). Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. *The Journal of cell biology*, *186*(3), 371-377.

Nelson, S. A., & Cooper, J. A. (2007). A novel pathway that coordinates mitotic exit with spindle position. *Molecular biology of the cell*, *18*(9), 3440-3450.

Nicklas, R. B., Ward, S. C., & Gorbsky, G. J. (1995). Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *The Journal of cell biology*, *130*(4), 929-939.

Nigg, E. A. (1995). Cyclin- dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays*, *17*(6), 471-480.

Osley, M. A. (2006). Regulation of histone H2A and H2B ubiquitylation. *Briefings in Functional Genomics*, *5*(3), 179-189.

Ou, Y. Y., & Rattner, J. B. (2002). Post- karyokinesis centrosome movement leaves a trail of unanswered questions. *Cell motility and the cytoskeleton*, *51*(3), 123-132.

Pai, E. F., & Schulz, G. E. (1983). The catalytic mechanism of glutathione reductase as derived from x-ray diffraction analyses of reaction intermediates. *Journal of Biological Chemistry*, *258*(3), 1752-1757.

Patt, H. M., & Quastler, H. (1963). Radiation effects on cell renewal and related systems. *Physiological reviews*, *43*(3), 357-396.

doi:10.6342/NTU201803272

Patterson, D. J. (1999). The diversity of eukaryotes. *the american naturalist*, *154*(S4), 96-124.

Pereira, G., Höfken, T., Grindlay, J., Manson, C., & Schiebel, E. (2000). The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Molecular cell*, *6*(1), 1-10.

Pinsky, B. A., & Biggins, S. (2005). The spindle checkpoint: tension versus attachment. *Trends in cell biology*, *15*(9), 486-493.

Powers, H. J. (1999). Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. *Proceedings of the Nutrition Society*, *58*(2), 435-440.

Preedy, V. R. (Ed.). (2012). *B vitamins and folate: chemistry, analysis, function and effects*. Royal Society of Chemistry.

Quilliam, L. A., Rebhun, J. F., & Castro, A. F. (2002). A growing family of guanine nucleotide exchange factors is responsible for activation of Ras-family GTPases.

Raha, S., & Robinson, B. H. (2000). Mitochondria, oxygen free radicals, disease and ageing. *Trends in biochemical sciences*, *25*(10), 502-508.

Rahal, R., & Amon, A. (2008). Mitotic CDKs control the metaphase–anaphase transition and trigger spindle elongation. *Genes & development*, *22*(11), 1534-1548.

Rahal, R., & Amon, A. (2008). The Polo-like kinase Cdc5 interacts with FEAR network components and Cdc14. *Cell Cycle*, *7*(20), 3262-3272.

Reijo, R. A., Cooper, E. M., Beagle, G. J., & Huffaker, T. C. (1994). Systematic mutational analysis of the yeast beta-tubulin gene. *Molecular biology of the cell*, *5*(1), 29-43.

Roberts, B. T., Farr, K. A., & Hoyt, M. A. (1994). The Saccharomyces cerevisiae checkpoint gene BUB1 encodes a novel protein kinase. *Molecular and Cellular Biology*, *14*(12), 8282-8291.

Robzyk, K., Recht, J., & Osley, M. A. (2000). Rad6-dependent ubiquitination of histone H2B in yeast. *Science*, *287*(5452), 501-504.

Rock, J. M., & Amon, A. (2009). The FEAR network. *Current Biology*, *19*(23), R1063-R1068.

Roguev, A., Schaft, D., Shevchenko, A., Pijnappel, W. P., Wilm, M., Aasland, R., & Stewart, A. F. (2001). The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. *The EMBO journal*, *20*(24), 7137-7148.

Rosenberg, J. S., Cross, F. R., & Funabiki, H. (2011). KNL1/Spc105 recruits PP1 to silence the spindle assembly checkpoint. *Current Biology*, *21*(11), 942-947.

Rudner, A. D., & Murray, A. W. (1996). The spindle assembly checkpoint. *Current opinion in cell biology*, *8*(6), 773-780.

doi:10.6342/NTU201803272

Santos, M. A., Jiménez, A., & Revuelta, J. (2000). Molecular characterization of FMN1, the structural gene for the monofunctional flavokinase of Saccharomyces cerevisiae. *Journal of Biological Chemistry*, *275*(37), 28618-28624.

Saunders, W., Lengyel, V., & Hoyt, M. A. (1997). Mitotic spindle function in Saccharomyces cerevisiae requires a balance between different types of kinesin-related motors. *Molecular biology of the cell*, *8*(6), 1025-1033.

Schibler, A., Koutelou, E., Tomida, J., Wilson-Pham, M., Wang, L., Lu, Y., ... & Shi, X. (2016). Histone H3K4 methylation regulates deactivation of the spindle assembly checkpoint through direct binding of Mad2. *Genes & development*, *30*(10), 1187-1197.

Seshan, A., & Amon, A. (2005). Ras and the Rho effector Cla4 collaborate to target and anchor Lte1 at the bud cortex. *Cell Cycle*, *4*(7), 940-946.

Shilatifard, A. (2012). The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annual review of biochemistry*, *81*, 65-95.

Shirayama, M., Matsui, Y., Tanaka, K., & Toh- E, A. (1994). Isolation of a CDC25 family gene, MSI2/LTE1, as a multicopy suppressor of ira1. *Yeast*, *10*(4), 451-461.

Shonn, M. A., McCarroll, R., & Murray, A. W. (2000). Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science*, *289*(5477), 300-303.

Shou, W., Azzam, R., Chen, S. L., Huddleston, M. J., Baskerville, C., Charbonneau, H., ... & Deshaies, R. J. (2002). Cdc5 influences phosphorylation of Net1 and disassembly of the RENT complex. *BMC molecular biology*, *3*(1), 3.

Shou, W., Seol, J. H., Shevchenko, A., Baskerville, C., Moazed, D., Chen, Z. S., ... & Deshaies, R. J. (1999). Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell*, *97*(2), 233-244.

Soares, L. M., & Buratowski, S. (2013). Histone crosstalk: H2Bub and H3K4 methylation. *Molecular cell*, *49*(6), 1019-1020.

Sobel, S. G. (1997). Mini review: mitosis and the spindle pole body in Saccharomyces cerevisiae. *Journal of Experimental Zoology*, *277*(2), 120-138.

Sobel, S. G., & Snyder, M. (1995). A highly divergent gamma-tubulin gene is essential for cell growth and proper microtubule organization in Saccharomyces cerevisiae. *The Journal of Cell Biology*, *131*(6), 1775-1788.

Sollier, J., Lin, W., Soustelle, C., Suhre, K., Nicolas, A., Géli, V., & Saint- André, C. D. L. R. (2004). Set1 is required for meiotic S- phase onset, double- strand break formation and middle gene expression. *The EMBO journal*, *23*(9), 1957-1967.

Sorenson, C. M., Barry, M. A., & Eastman, A. (1990). Analysis of events associated with cell cycle arrest at G2 phase and cell death induced by cisplatin. *JNCI: Journal*

doi:10.6342/NTU201803272

of the National Cancer Institute, *82*(9), 749-755.

Stearns, T., Hoyt, M. A., & Botstein, D. (1990). Yeast mutants sensitive to antimicrotubule drugs define three genes that affect microtubule function. *Genetics*, *124*(2), 251-262.

Stegmeier, F., Huang, J., Rahal, R., Zmolik, J., Moazed, D., & Amon, A. (2004). The replication fork block protein Fob1 functions as a negative regulator of the FEAR network. *Current biology*, *14*(6), 467-480.

Stegmeier, F., Visintin, R., & Amon, A. (2002). Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. *Cell*, *108*(2), 207-220.

Straight, A. F., Sedat, J. W., & Murray, A. W. (1998). Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. *The Journal of cell biology*, *143*(3), 687-694.

Suganuma, T., & Workman, J. L. (2011). Signals and combinatorial functions of histone modifications. *Annual review of biochemistry*, *80*, 473-499.

Tanaka, T. U. (2002). Bi-orienting chromosomes on the mitotic spindle. *Current opinion in cell biology*, *14*(3), 365-371.

Teufel, R., Miyanaga, A., Michaudel, Q., Stull, F., Louie, G., Noel, J. P., ... & Moore, B. S. (2013). Flavin-mediated dual oxidation controls an enzymatic Favorskii-type rearrangement. *Nature*, *503*(7477), 552.

Tomson, B. N., Rahal, R., Reiser, V., Monje-Casas, F., Mekhail, K., Moazed, D., & Amon, A. (2009). Regulation of Spo12 phosphorylation and its essential role in the FEAR network. *Current Biology*, *19*(6), 449-460.

Tong, A. H. Y., Evangelista, M., Parsons, A. B., Xu, H., Bader, G. D., Page, N., ... & Andrews, B. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science*, *294*(5550), 2364-2368.

Tytell, J. D., & Sorger, P. K. (2006). Analysis of kinesin motor function at budding yeast kinetochores. *J Cell Biol*, *172*(6), 861-874.

Vanoosthuyse, V., & Hardwick, K. G. (2009). Overcoming inhibition in the spindle checkpoint. *Genes & development*, *23*(24), 2799-2805.

Visintin, C., Tomson, B. N., Rahal, R., Paulson, J., Cohen, M., Taunton, J., ... & Visintin, R. (2008). APC/C-Cdh1-mediated degradation of the Polo kinase Cdc5 promotes the return of Cdc14 into the nucleolus. *Genes & development*, *22*(1), 79-90.

Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., & Amon, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Molecular cell*, *2*(6), 709-718.

Visintin, R., Hwang, E. S., & Amon, A. (1999). Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature*, *398*(6730),

doi:10.6342/NTU201803272

818-823.

Visintin, R., Stegmeier, F., & Amon, A. (2003). The role of the polo kinase Cdc5 in controlling Cdc14 localization. *Molecular biology of the cell*, *14*(11), 4486-4498.

Vitaliano-Prunier, A., Menant, A., Hobeika, M., Géli, V., Gwizdek, C., & Dargemont, C. (2008). Ubiquitylation of the COMPASS component Swd2 links H2B ubiquitylation to H3K4 trimethylation. *Nature cell biology*, *10*(11), 1365.

Walczak, C. E., & Heald, R. (2008). Mechanisms of mitotic spindle assembly and function. In *International review of cytology* (Vol. 265, pp. 111-158). Academic Press.

Wang, Y., Hu, F., & Elledge, S. J. (2000). The Bfa1/Bub2 GAP complex comprises a universal checkpoint required to prevent mitotic exit. *Current Biology*, *10*(21), 1379-1382.

Weiner, A., Hsieh, T. H. S., Appleboim, A., Chen, H. V., Rahat, A., Amit, I., ... & Friedman, N. (2015). High-resolution chromatin dynamics during a yeast stress response. *Molecular cell*, *58*(2), 371-386.

Weiss, E. L. (2012). Mitotic exit and separation of mother and daughter cells. *Genetics*, *192*(4), 1165-1202.

Wicky, S., Tjandra, H., Schieltz, D., Yates, J., & Kellogg, D. R. (2011). The Zds proteins control entry into mitosis and target protein phosphatase 2A to the Cdc25 phosphatase. *Molecular biology of the cell*, *22*(1), 20-32.

Wieser, S., & Pines, J. (2015). The biochemistry of mitosis. *Cold Spring Harbor perspectives in biology*, a015776.

Winey, M., Mamay, C. L., O'toole, E. T., Mastronarde, D. N., Giddings, T. H., McDonald, K. L., & McIntosh, J. R. (1995). Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. *The Journal of cell biology*, *129*(6), 1601-1615.

Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., ... & Johnston, M. (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Molecular cell*, *11*(1), 267-274.

Wyrick, J. J., & Parra, M. A. (2009). The role of histone H2A and H2B post-translational modifications in transcription: a genomic perspective. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1789*(1), 37-44.

Yasutis, K., Vignali, M., Ryder, M., Tameire, F., Dighe, S. A., Fields, S., & Kozminski, K. G. (2010). Zds2p regulates Swe1p-dependent polarized cell growth in Saccharomyces cerevisiae via a novel Cdc55p interaction domain. *Molecular biology of the cell*, *21*(24), 4373-4386.

Yeh, E., Skibbens, R. V., Cheng, J. W., Salmon, E. D., & Bloom, K. (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast, Saccharomyces

doi:10.6342/NTU201803272

cerevisiae. *The Journal of cell biology*, *130*(3), 687-700.

Yellman, C. M., & Roeder, G. S. (2015). Cdc14 early anaphase release, FEAR, is limited to the nucleus and dispensable for efficient mitotic exit. *PloS one*, *10*(6), e0128604.

Yoshida, S., & Toh-e, A. (2002). Budding yeast Cdc5 phosphorylates Net1 and assists Cdc14 release from the nucleolus. *Biochemical and biophysical research communications*, *294*(3), 687-691.

Yoshida, S., Ichihashi, R., & Toh-e, A. (2003). Ras recruits mitotic exit regulator Lte1 to the bud cortex in budding yeast. *The Journal of cell biology*, *161*(5), 889-897.

Zhang, K., Lin, W., Latham, J. A., Riefler, G. M., Schumacher, J. M., Chan, C., ... & Dent, S. Y. (2005). The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. *Cell*, *122*(5), 723-734.

Zhang, Z., Shi, L., Dawany, N., Kelsen, J., Petri, M. A., & Sullivan, K. E. (2016). H3K4 tri-methylation breadth at transcription start sites impacts the transcriptome of systemic lupus erythematosus. *Clinical epigenetics*, *8*(1), 14.

Zlotorynski, E. (2016). Chromosome biology: Mitotic chromatin condensin'. *Nature Reviews Molecular Cell Biology*, *17*(10), 610-610.

doi:10.6342/NTU201803272