Slk19-dependent mid-anaphase pause in kinesin-5-mutated cells

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Summary

We examined spindle elongation in anaphase in Saccharomyces cerevisiae cells mutated for the kinesin-5 motor proteins Cin8 and Kip1. Cells were deleted for KIP1 and/or expressed one of two motor-domain Cin8 mutants (Cin8-F467A or Cin8-R196K), which differ in their ability to bind microtubules in vitro, with Cin8-F467A having the weakest ability). We found that, in kinesin-5-mutated cells, predominantly in kip1Δcin8-F467A cells, anaphase spindle elongation was frequently interrupted after the fast phase, resulting in a mid-anaphase pause. Expression of kinesin-5 mutants also caused an asymmetric midzone location and enlarged midzone size, suggesting that proper organization of the midzone is required for continuous spindle elongation. We also examined the effects of components of the FEAR pathway, which is involved in the early-anaphase activation of Cdc14 regulatory phosphatase, on anaphase spindle elongation in kip1Δcin8-F467A cells. Deletion of SLK19, but not SPO12, eliminated the mid-anaphase pause, caused premature anaphase onset and defects in DNA division during anaphase, and reduced viability in these cells. Finally, overriding of the pre-anaphase checkpoint by overexpression of Cdc20 also eliminated the mid-anaphase pause and caused DNA deformation during anaphase in kip1Δcin8-F467A cells. We propose that transient activation of the pre-anaphase checkpoint in kinesin-5-mutated cells induces a Slk19-dependent mid-anaphase pause, which might be important for proper DNA segregation.

Supplementary material available online at http://jcs.biologists.org/cgi/content/full/121/15/2529/DC1

Key words: Anaphase B, Kinesin-5, Midzone, Slk19, Spo12

Introduction

Anaphase-B spindle elongation is the final phase of bipolar spindle morphogenesis, during which the spindle elongates dramatically, as much as two to five times its pre-anaphase length, thus spatially separating sister chromatids. This process is achieved by the dynamics of spindle microtubules (MTs) (reviewed in Howard and Hyman, 2003; Kline-Smith and Walczak, 2004), by protein complexes that stabilize the spindle (Higuchi and Uhlmann, 2005; Pereira and Schiebel, 2003; Schuyler et al., 2003; Severin et al., 2001; Zeng et al., 1999), and by the activity of molecular motor proteins from the dynein and kinesin superfamilies (reviewed in Barton and Goldstein, 1996; Bloom and Endow, 1995).

Among the kinesin-related motor proteins, kinesin-5 family members have been shown to play major roles in mitotic spindle morphogenesis. Kinesin-5 homologues are found throughout the eukaryotes (Blangy et al., 1995; Goshima and Vale, 2005; Hagan and Yanagida, 1992; Heck et al., 1993; Hoyt et al., 1992; Le Guelllec et al., 1991; Roof et al., 1992). They are conserved in the amino acid sequence of the motor (force-producing) domain and function as homotetramers, with two catalytic motor domains located on opposite sides of the active motor complex (Gordon and Roof, 1999; Kashina et al., 1996). This special architecture enables the kinesin-5 motors to crosslink and slide anti-parallel MTs (Gheber et al., 1999; Kapitein et al., 2005) originating from opposite spindle poles and thereby perform their essential functions in mitotic spindle dynamics (Barton and Goldstein, 1996; Kashina et al., 1997). In addition to their well-established roles in spindle assembly (Hoyt et al., 1992), kinesin-5 motor proteins were also suggested to have a role in anaphase-B spindle elongation in the budding yeast Saccharomyces cerevisiae (Saunders et al., 1995; Straight et al., 1998) as well as in other organisms (Sharp et al., 1999; Touitou et al., 2001). However, the mechanisms by which kinesin-5 motor proteins perform their anaphase-spindle-elongation functions are not well understood.

In S. cerevisiae cells, anaphase-B spindle elongation proceeds in two phases. During the first fast phase, the spindle elongates from 1-2 μm to 4-5 μm, whereas during the second slow phase, the spindle elongates up to 7-10 μm (Kahana et al., 1995; Straight et al., 1998). The mechanisms that control the transition between the fast and slow anaphase-B phases have not yet been elucidated. However, recent studies have indicated that this transition coincides with early-anaphase Cdc14 release from the nucleolus to the nucleus (Stegmeier et al., 2002), a process that is governed by the FEAR (Cdc fourteen early anaphase release) pathway. The FEAR pathway consists of separate (Esp1), the polo-like Cdc5 kinase, the kinetochore protein Slk19, Spo12 and its homologue Bns1, and Fob1. Cdc14 phosphatase is sequestered in the nucleolus during most of the cell cycle via its interaction with Cfi1 (also known as Net1), which is stabilized by Fob1 (reviewed in D’Amours and Amon, 2004). Spo12 activation during early anaphase antagonizes the Fob1 functions, thus promoting the release of Cdc14 from the nucleolus. Esp1 and Slk19 were suggested to activate Cdc5, which
phosphorylates Cdc14, thus promoting Cdc14 release from the nucleolus. Although the main role of early Cdc14 release has been suggested to mediate activation of the mitotic-exit network (MEN) (D’Amours and Amon, 2004), recent studies have indicated that the FEAR pathway participates in nuclear positioning (Ross and Cohen-Fix, 2004) and in stabilization of the mitotic spindle during anaphase (Higuchi and Uhlmann, 2005; Khmelinskii et al., 2007; Pereira and Schiebel, 2003).

The proper organization of the anaphase spindle midzone, the region of overlap between anti-parallel spindle MTs, becomes crucial as the spindle elongates during anaphase B. Following anaphase onset, this region provides the structural stability for the elongating spindle (Schuyler et al., 2003), and serves as the attachment site for kinetochore proteins and molecular complexes that control spindle MT dynamics (Buvelot et al., 2003; Higuchi and Uhlmann, 2005; Pereira and Schiebel, 2003; Schuyler et al., 2003; Thomas and Kaplan, 2007; Widlund et al., 2006). Thus far, two major midzone-localizing factors, Ase1 and Slk19, have been shown to contribute to anaphase spindle stability (Schuyler et al., 2003; Zeng et al., 1999), through their role in midzone organization and focusing (Khmelinskii et al., 2007). Although kinesin-5 motors have also been shown to localize to the spindle midzone (Tytell and Sorger, 2006), their function in anaphase midzone organization has not yet been demonstrated.

In the present study we examined anaphase B progression in S. cerevisiae cells carrying mutations in kinesin-5 motor proteins. Specifically, we analyzed anaphase B in cells carrying one of two Cin8 motor-domain mutations, Cin8-F467A (Cin8-FA) and Cin8-R196K (Cin8-3), which differ in their ability to bind MTs in vitro, with this ability being impaired more severely by the Cin8-FA mutant (Gheber et al., 1999). By analyzing these two Cin8 mutants, either alone or in the genetic background of KIP1 chromosomal deletion, we assessed the effect of kinesin-5 proteins on anaphase spindle morphology and spindle-elongation kinetics. In addition, we examined the regulatory factors that contribute to correct DNA segregation during anaphase in kinesin-5-mutated cells.

**Results**

Mutations in kinesin-5 motor proteins induce a mid-anaphase pause

To explore the effect of Cin8 mutants on anaphase-B spindle elongation, we examined real-time anaphase-B kinetics in cells expressing the kinetochore-localized Nufl-GFP fusion protein (Kahana et al., 1995). It had been demonstrated that, in S. cerevisiae cells, the anaphase-A movement of the kinetochores towards the spindle pole bodies (SPBs) occurs within 1 minute following anaphase-B onset (Pearson et al., 2001). Because, during most of anaphase B, kinetochores colocalize with the SPBs, monitoring the distance between the two Nufl-GFP signals allows spindle length to be measured during anaphase spindle elongation (Kahana et al., 1995) (Fig. 1A,B). In agreement with previous reports (Kahana et al., 1995; Straight et al., 1998), we found that wild-type (WT) and kip1Δ cells exhibited bi-phasic anaphase-B kinetics (Fig. 1 and Table 1). In WT cells, the average rate of the initial fast phase was 0.85 μm/minute, whereas the average spindle length after the fast phase was 4.86 μm (Table 1). The rate of the second, slow phase was 0.25 μm/minute and the average final spindle length was 7.35 μm (Table 1). Examination of kip1Δ cin8 cells revealed that anaphase-B spindle elongation during both the fast and slow phases was significantly slower in kip1Δ cin8-FA cells compared with kip1Δ cells (Table 1). These results indicate that, in the absence of Kip1, Cin8 binding is important for spindle elongation during the fast and slow phases.

In contrast to WT and kip1Δ cells (Fig. 1Ca,b), in a subset of cells that express the Cin8 motor-domain mutant either alone or in combination with kip1Δ, anaphase spindle elongation was interrupted after the fast phase. This interruption created a mid-anaphase pause in which, following the fast phase, the spindle length remained roughly constant (Fig. 1Cc-e). The percentage of cells exhibiting such a delay and the duration of the delay varied between cells with different genotypes (see below). In about 50%
of the cells exhibiting the mid-anaphase delay, slow-phase spindle elongation was observed up to 7–9 μm (Fig. 1Cc,d), whereas the remaining 50% of the cells appeared to be arrested after the fast phase with spindles of intermediate length (Fig. 1Ce). Because of the decay of the GFP signal, we cannot rule out the possibility that the slow phase would have also been eventually observed in cells that appeared to be arrested in mid-anaphase. Because both types of anaphase kinetics – the mid-anaphase pause and the apparent mid-anaphase arrest – indicate that anaphase B is interrupted after the fast phase, we refer to both as the mid-anaphase pause. To ensure that the differences in anaphase progression result from impaired activity of Cin8 mutants, we examined expression levels of these mutants and found no differences in expression between the WT and mutant Cin8 variants (data not shown).

The severity of the pause phenotype varied among cells depending on the combination of kinesin-5 mutants. First, the percentage of cells with the mid-anaphase-pause phenotype was higher in cells expressing the cin8-FA mutation than in cells expressing the cin8-3 mutation, either expressed alone or in combination with kip1Δ (Table 1). Second, the duration of the pause was longer in double-mutant kip1Δ cin8-FA or kip1Δ cin8-3 cells (average pause duration = 19.3±5.0 minutes, ± s.d., n=7), compared with single-mutant cin8-FA or cin8-3 cells (average pause duration = 5.2±2.5 minutes, ± s.d., n=5). This indicates that the combination of CIN8 motor-domain mutations with KIP1 chromosomal deletion enhanced the mid-anaphase-pause phenotype compared with expression of CIN8 mutants alone.

Kinesin-5 mutations affect anaphase spindle symmetry and midzone organization
To address the possibility that the mid-anaphase pause is related to structural defects of the anaphase spindles caused by kinesin-5 mutations, we examined anaphase spindle morphology by high-resolution fluorescence microscopy (see Materials and Methods) in cells expressing tubulin-GFP. The majority of intermediate (<5 μm) and long (>5 μm) anaphase spindles in WT and kip1Δ cells had a normal symmetric shape with a clearly visible midzone located in the middle of the spindles (Fig. 2A,B). We also noticed that, in kip1Δ cells, the midzone appeared larger than in WT cells, but quantization of this effect did not reach statistical significance. In contrast to in WT and kip1Δ cells, in cells expressing the cin8-FA mutation, either alone or in combination with kip1Δ, the majority of intermediate anaphase spindles appeared asymmetric (Fig. 2C,D), with one side being considerably brighter and/or there being a substantial shift of the midzone region towards one pole (Fig. 2C,D, arrows). The percentage of asymmetric spindles was similar in cin8-FA and kip1Δ cin8-FA cells (Fig. 2E, left), indicating that Cin8 has a more important role than Kip1 in centering the midzone. Finally, we observed that about 40% of kip1Δ cin8-FA cells had no distinguishable midzone (Fig. 2E, right). This result indicates that both Cin8 and Kip1 are required for midzone organization and focusing, and that the differential severity of spindle abnormalities is in good correlation with the varying

Table 1. Characterization of anaphase-B spindle elongation in cells expressing kinesin-5 mutants and Ase1-GFP

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fast phase</th>
<th>Slow phase</th>
<th>Pause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate μm/minute</td>
<td>Spindle length μm</td>
<td>Rate μm/minute</td>
</tr>
<tr>
<td>WT</td>
<td>0.85±0.03</td>
<td>4.86±0.24</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td>kip1Δ</td>
<td>0.74±0.04*</td>
<td>4.85±0.26</td>
<td>0.20±0.01*</td>
</tr>
<tr>
<td>cin8-FA</td>
<td>0.84±0.06</td>
<td>4.78±0.18</td>
<td>0.19±0.01*</td>
</tr>
<tr>
<td>cin8-3</td>
<td>0.77±0.05</td>
<td>6.40±0.44**</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>kip1Δ cin8-FA</td>
<td>0.50±0.04***</td>
<td>3.89±0.23***</td>
<td>0.17±0.01**</td>
</tr>
<tr>
<td>kip1Δ cin8-3</td>
<td>0.78±0.06</td>
<td>5.16±0.17</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Ase1-GFP</td>
<td>0.73±0.05</td>
<td>5.8±0.24</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>kip1Δ Ase1-GFP</td>
<td>0.62±0.04**</td>
<td>4.80±0.19</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>kip1Δ Ase1-GFP (pASe1)</td>
<td>0.62±0.10</td>
<td>4.5±1.09</td>
<td>0.24±0.05</td>
</tr>
</tbody>
</table>

*Significance compared with WT cells.
**Significance compared with kip1Δ cells.
***Significance compared with WT cells.

*a Rates of spindle elongation determined for those cases in which there were at least four time points on the slope or the plot of SPB distance vs time.
*b Numbers in parentheses indicate the number of averaged experiments.
*c Determined for those cases in which there were at least four time points on the slope or the plot of SPB distance vs time.
*d Extra copy of ASE1 is expressed from a centromeric plasmid.

*p<0.05; **p<0.01; ***p<0.001.
severity, from no altered phenotype to severely affected, of the mid-
anaphase-pause phenotype in WT, kip1Δ, cin8-FΔ and kip1Δ cin8-
FA cells, respectively.

To further assess the effect of kinesin-5 mutants on midzone
focusing, we examined the localization of two non-motor midzone
proteins, Ase1 and Slk19 (Schuyler et al., 2003; Zeng et al., 1999),
by GFP imaging. We observed a significant difference between
kip1Δ and kip1Δ cin8-FA cells in the midzone localization of Ase1
and Slk19. In kip1Δ cin8-FA cells, the localization of Ase1 and Slk19
was more diffuse and asymmetric (Fig. 2F), and occupied larger
portions of the spindle (Fig. 2G). On average, there was an increase
of ~15% in the portion of the spindles occupied by either Ase1 or
Slk19 in kip1Δ cin8-FA cells, compared with kip1Δ cells (Fig. 2G).
These results further support the idea that combined mutations in
the two S. cerevisiae kinesin-5 homologues interfere with midzone
organization and focusing, supporting the correlation between
incorrect midzone organization and the occurrence of the mid-
anaphase pause in kip1Δ cin8-FA cells. Interestingly, we found that
chromosomal tagging of Ase1 with GFP in kip1Δ cells introduced
a 7.4±0.2 minute (average ± s.e.m., n=8) mid-anaphase pause (Table
1). This pause was not observed in WT Ase1-GFP cells and was
eliminated by expression of an additional WT Ase1 copy from a
centromeric plasmid in kip1Δ ASE1-GFP cells (Table 1). Thus, the
mid-anaphase pause in kip1Δ ASE1-GFP cells is likely to be an
outcome of the elimination of Kip1 function combined with
compromised or altered Ase1 activity caused by GFP tagging.
Because Ase1 is a major midzone-stabilizing and -organizing protein
(Khmelinskii et al., 2007; Schuyler et al., 2003), these results further
support the notion that the mid-anaphase pause in kinesin-5-
mutated cells results from defects in midzone organization.

SLK19 and SPO12 deletions produce opposite effects
on anaphase spindle elongation in kinesin-5-mutated cells

The occurrence of the mid-anaphase pause (Fig. 1B,Cc-e) coincides
with the timing of early-anaphase Cdc14 release, which is triggered
by the FEAR pathway (Stegmeier et al., 2002). Examination of
Cdc14 release by GFP imaging revealed that there is no significant
difference between the timing of Cdc14 release in kip1Δ and kip1Δ
cin8-FA cells. In both cell types, Cdc14 was released within 0-3
minutes after the completion of the fast phase (supplementary
material Fig. S1). We next investigated whether deletion of the
function of the SLK19 or SPO12 gene products, components of the
FEAR pathway, influences the mid-anaphase-pause phenotype and
the spindle-elongation rates in kip1Δ cin8-FA cells. We first
examined the effect of SLK19 or SPO12 deletions on
anaphase-B progression in WT cells. In spo12Δ cells, the rate of the fast phase was slower and the final spindle
length was shorter as compared with that of WT cells (Table
2). By contrast, in slk19Δ cells, the rates of both anaphase-B phases were faster and the spindle length
after the fast phase was longer compared with WT cells (Table
2).

We found that SPO12 deletion in kip1Δ cin8-FA cells introduced various abnormalities in anaphase
spindle elongation, such as short spindle length, slow
and irregular spindle elongation or spindle disassembly, and re-assembly during the elongation
process (supplementary material Fig. S2D-H). Despite
these defects, we observed translocation to the bud of
one of the spindle poles during various stages of
spindle elongation. In addition, all kip1Δ cin8-FA
spo12Δ cells with 4-5 μm spindles had divided nuclei

Fig. 2. (A–D) Anaphase spindle morphologies in WT (A), kip1Δ
(B) cin8-FA (C) and kip1Δ cin8-FA (D) cells. Cells expressing
tubulin-GFP were examined by high-resolution fluorescence
microscopy (see Materials and Methods). Representative 2D
images are shown. Spindle length (μm) is indicated at the bottom
of each spindle image. Normal, symmetric and indistinguishable
midzone (ind. midzone) morphologies are indicated at the top.
Arrows indicate the location of the midzone region.
(E) Percentage of spindles with defects. Left, percentage of
intermediate (3-5 μm) asymmetric spindles; right, percentage of
anaphase spindles with an indistinguishable midzone region. For
each genotype, 80-120 anaphase (>3 μm) spindles were
analyzed, half of which were of intermediate length. (F,G) Spindle
localization of Ase1 and Slk19 proteins. (G) Images of
unsynchronized cells expressing either Ase1-GFP (left) or Slk19-
GFP (right) were acquired during anaphase. Representative
images (2D projection) of spindles are shown. SPBs are labelled
with Nuf2-GFP. Spindle length (μm) is indicated on the bottom
of each image. (F) Length of Ase1-GFP signal (left) and Slk19-GFP
signal (right), expressed as a percentage of spindle length in
intermediate (3-5 μm) and long (>5 μm) spindles. Average and
s.d. of 25-35 measurements are shown. Genotypes are as
indicated: kip1Δ, white; kip1Δ cin8-FA, grey.
that were evenly distributed between mother and daughter cells (Fig. 3Ae). These results indicate that, although continuous anaphase-B progression is disrupted in \textit{kip1}Δ \textit{cin8-FA} \textit{spo12}Δ cells, successful DNA partitioning between mother and daughter cells takes place.

Examination of anaphase-B kinetics in \textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ cells revealed that deletion of the \textit{SLK19} gene completely eliminated the occurrence of the mid-anaphase pause observed in \textit{kip1}Δ \textit{cin8-FA} cells (Table 2 and supplementary material Fig. S2C). This result was confirmed in three independent \textit{slk19}Δ transformants. Also, in \textit{kip1}Δ \textit{cin8-FA} cells, \textit{SLK19} deletion increased the rates of the fast and the slow anaphase-B phases as well as the average spindle length after the fast phase (Table 2). Examination of spindle and nuclear morphology, revealed that, following release from S-phase arrest, about 60% of \textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ cells with intermediate (4-6 μm) spindles had undivided, deformed or unevenly distributed DNA morphology (Fig. 3Ad). Uneven distribution of DNA was also observed in about 25% of \textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ cells with long (>6 μm) spindles (Fig. 3Ad, top panels). In contrast to \textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ cells, the majority of \textit{kip1}Δ \textit{cin8-FA} and \textit{slk19}Δ cells with intermediate spindles, had normal DNA morphology, with the two divided nuclei evenly distributed between mother and daughter cells (Fig. 3Ab,c). A deformed undivided nucleus was observed in only 10% of \textit{kip1}Δ \textit{cin8-FA} cells and in 20% of \textit{slk19}Δ cells with intermediate spindles and no deformed nuclear morphology was observed in \textit{kip1}Δ cells (Fig. 3Aa). We also found that deletion of \textit{SLK19} reduced the viability of \textit{kip1}Δ \textit{cin8-FA} cells at elevated temperatures. \textit{kip1}Δ \textit{cin8-FA} cells are viable at temperatures lower than 35°C (Gheber et al., 1999), but addition of \textit{SLK19} deletion to these cells eliminated cell viability at 33°C (Fig. 3B). Thus, in \textit{kip1}Δ \textit{cin8-FA} cells, the intact function of Slk19 is important for maintaining cell viability at elevated temperatures.

![Effect of SLK19 or SPO12 chromosomal deletion on spindle and nuclear morphology](image1)

**Table 2. Characterization of anaphase-B spindle elongation in \textit{slk19}Δ and \textit{spo12}Δ cells**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rate of spindle elongation (μm/minute)</th>
<th>Spindle length (μm)</th>
<th>Rate of spindle elongation (μm/minute)</th>
<th>Final spindle length (μm)</th>
<th>% occurrence of spindle disassembly</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.85±0.03</td>
<td>4.86±0.24</td>
<td>0.25±0.02</td>
<td>7.35±0.48</td>
<td>0</td>
</tr>
<tr>
<td>\textit{slk19}Δ</td>
<td>0.92±0.02***</td>
<td>5.31±0.30</td>
<td>0.31±0.03</td>
<td>7.49±0.41</td>
<td>0</td>
</tr>
<tr>
<td>\textit{spo12}Δ</td>
<td>0.67±0.07***</td>
<td>4.53±0.40</td>
<td>0.28±0.04</td>
<td>6.80±0.22</td>
<td>0</td>
</tr>
<tr>
<td>\textit{kip1}Δ \textit{cin8-FA}</td>
<td>0.50±0.04</td>
<td>3.89±0.23</td>
<td>0.17±0.01</td>
<td>7.41±1.15</td>
<td>76</td>
</tr>
<tr>
<td>\textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ</td>
<td>0.69±0.03***</td>
<td>5.70±0.25***</td>
<td>0.30±0.02***</td>
<td>7.69±0.17</td>
<td>0</td>
</tr>
</tbody>
</table>

\*Rates of spindle elongation determined for those cases in which there were at least four time points on the slope or the plot of SPB distance vs time.

\*\*Significance compared with \textit{kip1}Δ \textit{cin8-FA} cells.

\*\*\*Significance compared with wild-type (WT) cells.

\*\*\*\*Significance compared with \textit{kip1}Δ \textit{cin8-FA} \textit{slk19}Δ cells.

\*P<0.05; **P<0.01; ***P<0.001.
Pre-anaphase checkpoint is required for the mid-anaphase pause and correct DNA segregation in *kip1Δ cin8-FA* cells

We next examined whether DNA deformation during anaphase and altered spindle-elongation kinetics in *kip1Δ cin8-FA slk19Δ* cells might be related to the timing of anaphase onset in these cells. For this purpose, we examined spindle elongation and cleavage of the Scc1 subunit of the cohesion complex (Ciosk et al., 1998) in a population of cells following release from S-phase arrest (Fig. 4).

We found that, based on the increase in the percentage of cells with elongated (>2.5 μm) spindles and the appearance of the Scc1 cleavage product, anaphase onset in WT and *kip1Δ* cells occurs within 60 minutes following release from S-phase arrest. In *kip1Δ cin8-FA* cells, anaphase entry was delayed by about 15 minutes, compared with WT and *kip1Δ* cells, indicating that the pre-anaphase checkpoint (Hoyt et al., 1991; Li and Murray, 1991; Roberts et al., 1994; Winey et al., 1991) is transiently activated in these cells. We also found that, in *kip1Δ cin8-FA slk19Δ* cells, anaphase entry occurred 15-30 minutes earlier compared with *kip1Δ cin8-FA* cells. These results indicate that deletion of *SLK19* overrides the transiently activated pre-anaphase checkpoint in *kip1Δ cin8-FA* cells.

To examine whether the premature anaphase onset induced by deletion of *SLK19* is related to the elimination of the mid-anaphase pause in *kip1Δ cin8-FA* cells, we followed the kinetics of anaphase spindle elongation in cells moderately overexpressing Cdc20 (*CDC20-OX*). Cdc20 is an activator of anaphase-promoting complex/cyclosome (APC/C), which is required for metaphase-to-anaphase transition, and whose overexpression was shown to override the pre-anaphase checkpoint (Pan and Chen, 2004; Schott and Hoyt, 1998; Zachariae et al., 1996). Cdc20 overexpression was achieved by introducing a plasmid in which *CDC20* was placed downstream of the MET25 promoter (*PMET*; a,b) or vector only (c,d) were grown to log phase in medium containing methionine. To induce Cdc20 overexpression, cells were placed into methionine-lacking medium (– methionine; b,d). The distance between SPBs, monitored by Nuf2-GFP fluorescence, was measured as a function of time. ‘R’ represents the rate of spindle elongation. The different anaphase-B phases are indicated by the following symbols: fast phase, diamond; pause, square; slow phase, triangle; prior to spindle elongation, post-anaphase spindle elongation or spindle collapse, circle. (B) Representative anaphase DNA morphologies in *kip1Δ cin8-FA* cells overexpressing Cdc20. Normal and deformed DNA morphologies are shown. Arrows point to lagging DNA in cells with two DNA masses separated by more than 5 μm. Scale bar: 5 μm. (C) Percentage of cells with all DNA deformations (left) and percentage of cells with two deformed DNA masses separated by more than 5 μm (right) in anaphase cells. In each genotype, indicated at the bottom, 200-220 cells were analyzed.

**Fig. 4.** Scc1 cleavage as a landmark of anaphase onset. Cells expressing Scc1-HA were arrested in S-phase of the cell cycle by hydroxyurea then processed for tubulin immunostaining and western blot analysis (anti-HA) following release. The Scc1 band (asterisk) and cleavage-product band (arrowhead) are shown. Genotypes are indicated on the top of each panel. The time after release from S-phase arrest are indicated at the top of each lane. The percentage of cells with elongated anaphase spindles (>2.5 μm) are indicated at the bottom of each lane (% ana). Results are representative of three independent experiments. In each experiment, tubulin morphology of 150-200 anaphase cells was examined.

**Fig. 5.** (A) Effect of the overexpression of Cdc20 on spindle elongation kinetics in *kip1Δ cin8-FA* cells. Cells either carrying plasmid containing *CDC20* under the control of the MET25 promoter (*PMET–CDC20*, a,b) or vector only (c,d) were grown to log phase in medium containing methionine. To induce Cdc20 overexpression, cells were placed into methionine-lacking medium (~ methionine; b,d). The distance between SPBs, monitored by Nuf2-GFP fluorescence, was measured as a function of time. ‘R’ represents the rate of spindle elongation. The different anaphase-B phases are indicated by the following symbols: fast phase, diamond; pause, square; slow phase, triangle; prior to spindle elongation, post-anaphase spindle elongation or spindle collapse, circle. (B) Representative anaphase DNA morphologies in *kip1Δ cin8-FA* cells overexpressing Cdc20. Normal and deformed DNA morphologies are shown. Arrows point to lagging DNA in cells with two DNA masses separated by more than 5 μm. Scale bar: 5 μm. (C) Percentage of cells with all DNA deformations (left) and percentage of cells with two deformed DNA masses separated by more than 5 μm (right) in anaphase cells. In each genotype, indicated at the bottom, 200-220 cells were analyzed.
1998). Anaphase kinetics were followed in cells at log phase that were subjected to CDC20 overexpression for 4 hours. Control experiments were performed with cells carrying the vector only (+methionine) and in cells carrying the PMET–CDC20 plasmid that were grown in medium containing methionine (Fig. 5 and Table 3). We found that, in kip1Δ cin8-FA cells containing the vector or the PMET–CDC20 plasmid that were grown in medium containing methionine, the rate of the slow phase was faster and the duration of the pause was shorter (10.7±5.1 minutes, ± s.d., n=19) compared with cells without the plasmids (Tables 2 and 3). In addition, in 20% of cells carrying the plasmids, spindle elongation proceeded in one continuous phase, with a rate similar to that of the slow phase (~0.2 μm/minute, data not shown). We also found that when Cdc20 was overexpressed, the rate of spindle elongation was significantly slower, compared with control experiments with vector only or with the plasmid in the presence of methionine (Table 3). Most importantly, we found that, whereas mid-anaphase pause was observed in 60-90% of cells that carried the vector only (+methionine) or that carried the PMET–CDC20 plasmids in the presence of methionine (Table 3), moderate overexpression of Cdc20 completely eliminated the pause in kip1Δ cin8-FA cells (Fig. 5 and Table 3). These results strongly indicate that the functional metaphase-to-anaphase transition checkpoint is required for the occurrence of the mid-anaphase pause in kip1Δ cin8-FA cells.

To further assess the connection between elimination of the mid-anaphase pause and DNA deformation during anaphase, we examined DNA morphology during anaphase by DAPI staining in cells overexpressing Cdc20, following release from S-phase arrest (Fig. 5B,C). In control experiments in which Cdc20 was not overexpressed, more than 70% of anaphase DNA masses, either one elongated mass or two separated masses, were symmetric (Fig. 5B, top, and C). By contrast, in kip1Δ cin8-FA cells that either overexpressed Cdc20 or carried a deletion of Slk19, more than 45% of anaphase DNA masses were deformed, had a non-symmetrical shape or had two DNA masses with lagging DNA in between (Fig. 5B, two bottom rows, and C). This result strongly indicates that premature anaphase onset causes DNA deformation during anaphase in kinesin-5-mutated cells. In addition, the percentage of kip1Δ cin8-FA slk19Δ cells with two separated DNA masses that had either non-symmetric staining or had lagging DNA (Fig. 5B, bottom, arrows) was twice as much as found in kip1Δ cin8-FA CDC20-OX cells (Fig. 5C, right). Because the rate of spindle elongation and final spindle length were considerably higher in kip1Δ cin8-FA slk19Δ cells compared with kip1Δ cin8-FA CDC20-OX cells (Tables 2 and 3), our results suggest that fast spindle elongation induced by Slk19 deletion exacerbates DNA-segregation defects in anaphase in kinesin-5-mutated cells.

**Discussion**

The functions of kinesin-5 motor proteins in metaphase spindle assembly and maintenance have been well characterized in S. cerevisiae cells (Gordon and Roof, 1999; Kashina et al., 1996) as well as in higher eukaryotes (Barton and Goldstein, 1996; Kashina et al., 1997). By contrast, the functions of these motor proteins during anaphase spindle elongation have not as yet been sufficiently characterized. The present work reveals that, during anaphase spindle elongation in S. cerevisiae cells, these motor proteins are required for the focusing and central positioning of the midzone, and for uninterrupted and continuous spindle elongation.

**Kinesin-5 function is important for anaphase midzone organization and positioning**

We have demonstrated that a combination of mutations in the Cin8 motor domain with deletion of Kip1 function increases the size of the midzone region in which the plus-ends of the interpolar MTs (iMTs) overlap (Fig. 2F,G). One of the factors that determines the size of the midzone is the dynamics (polymerization and depolymerization) of the plus-ends of iMTs. The enlarged midzone in kinesin-5-mutated cells suggests that the plus-ends of iMTs in these cells are stabilized either by altered rates of polymerization and/or depolymerization of the iMTs, or altered frequency of catastrophe and/or rescue of the iMTs. Numerous kinesin-related proteins have been shown to affect MT dynamics (reviewed in Howard and Hyman, 2007). Therefore, it is possible that, in kinesin-5-mutated cells, altered iMT plus-end dynamics is directly caused by the kinesin-5 mutations. This suggests that, by crosslinking activity, kinesin-5 motors also destabilize MTs. Another possibility is that MT stabilization in kinesin-5-mutated cells is an indirect effect, caused by incorrect midzone organization and the subsequent mislocalization of proteins that affect spindle elongation and MT dynamics, such as Slk19 (Khmelinskii et al., 2007; Zeng et al., 1999), Stu2 (Severin et al., 2001), Ndc10 (Bouck and Bloom, 2005; Widlund et al., 2006) or Ipl1-Sli15 (Buvelot et al., 2003; Pereira and Schiebel, 2003; Thomas and Kaplan, 2007). It has recently been reported that mutations in non-motor midzone-stabilizing proteins, such as Ase1 and Slk19, interfere with midzone...
organization and focusing (Khmelinskii et al., 2007). However, it has also been reported that single deletions of either Kip1 or Cin8 have no effect on midzone organization (Khmelinskii et al., 2007; Straight et al., 1998). Here, we demonstrate that a combination of Cin8 motor-domain mutation with deletion of KIP1 introduces pronounced aberrations in spindle midzone organization. Thus, our results indicate that kinesin-5 motor activity is among the major factors that organize the midzone of anaphase spindles.

We have also shown here that expression of the Cin8-FA mutation that is severely defective for the MT-binding function (Gheber et al., 1999) causes asymmetric positioning of the midzone (Fig. 2A-E). This result indicates that the MT-crosslinking function of Cin8 is an important factor for maintaining the central positioning of the midzone and the symmetric spindle shape. Consistent with a previous report indicating that Cin8 plays a more important role in spindle elongation compared with Kip1 (Saunders et al., 1995), we found that the function of Kip1 has a minor contribution to centering of the midzone, because KIP1 deletion had no effect on the asymmetry of anaphase spindles in cin8-FA cells (Fig. 2E). A possible mechanism by which Cin8 can facilitate midzone centering is illustrated in Fig. 6. In WT cells, MT crosslinking by Cin8 organizes the midzone MTs in a tight bundle. The dynamics of iMT plus-ends is restricted by this midzone bundle to a small portion of their length (Fig. 6A). In cin8-FA cells, in which the MT crosslinking activity is significantly reduced, MT bundles are considerably less tight, as indeed was previously demonstrated by us in vitro (Gheber et al., 1999). As a result, iMT plus-end dynamics is not restricted and iMTs are exchangeable throughout a larger portion of their length. In the absence of tight MT crosslinking of Cin8, iMT dynamics at the two halves of the spindle was independent, which might result in asymmetric midzone positioning (Fig. 6B). In this regard, the role of Cin8 can be viewed as a coupler between the iMT plus-end dynamics of the two halves of the spindle. Positioning of the spindle in general and location of the midzone in particular have been shown to determine the positioning of the cleavage furrow during telophase and cytokinesis in higher-eukaryote cells (D’Avino et al., 2005). Because kinesin-5 proteins are highly conserved among the eukaryotes, we propose that the MT-crosslinking function of these motor proteins might have a role in controlling anaphase midzone centering and accurate cytokinesis in higher-eukaryote cells.

Differential functions of Slk19 and Spo12 during spindle elongation

Although both Slk19 and Spo12 are required for Cdc14 release in early anaphase (Stegmeier et al., 2002), we demonstrated that they influence the kinetics of anaphase spindle elongation differently (Table 2 and supplementary material Fig. S2). In kip1Δ cin8-FA cells, SPO12 deletion reduced the spindle-elongation rate and induced spindle instability. By contrast, deletion of SLK19 increased spindle-elongation rates and spindle length in WT and kip1Δ cin8-FA cells. The differential effects of Spo12 and Slk19 on anaphase-B spindle elongation might be explained by their different anaphase functions. Spo12 is localized to the nucleolus, where its main function is the release of Cdc14 from the nucleolus to the nucleus in early anaphase (Stegmeier et al., 2002). One of the functions of Cdc14 at this stage is to stabilize the anaphase spindles (Higuchi and Uhlmann, 2005; Khmelinskii et al., 2007). Therefore, the spindle-elongation defects in kip1Δ cin8-FA spo12Δ cells are likely to reflect a decrease in anaphase spindle stability caused by inhibition of the early release of Cdc14 in Spo12-deficient cells.

The mechanism(s) by which Slk19 reduces the spindle-elongation rate in WT and kip1Δ cin8-FA cells is not clear. It is not likely that these mechanisms are related to the function of Slk19 in the FEAR pathway, because the timing of Cdc14 release is similar in kip1Δ and kip1Δ cin8-FA cells (supplementary material Fig. S1) and deletion of Spo12 has an effect opposite to that of deletion of Slk19. Also, these mechanisms are probably not directly related to the role of Slk19 in midzone focusing (Khmelinskii et al., 2007), because lack of midzone organization and focusing reduced spindle-elongation rates and imposed discontinuous anaphase-B progression (Khmelinskii et al., 2007) (and this study). One possibility is that Slk19 slows down spindle elongation through its activation of Cdc5 kinase (Visintin et al., 2003), which in turn phosphorylates unknown downstream targets. The activation of Cdc5 by Slk19 or FEAR-pathway-independent Slk19 functions (Sullivan et al., 2001; Zeng et al., 1999; Zhang et al., 2006) might explain the unique role of Slk19 in anaphase-B progression in cells impaired in kinesin-5 motor-protein functions. Another possibility is that Slk19, either directly or indirectly, influences iMT plus-end dynamics. Indeed, it has been shown that deletion of SLK19 increases the dynamics of pre-anaphase spindles (Zhang et al., 2006). Increased spindle and/or MT dynamics might extend into anaphase and explain the increase of spindle-elongation rates in slk19Δ and kip1Δ cin8-FA slk19Δ cells.
The functional pre-anaphase checkpoint is required for the mid-anaphase pause

Several lines of evidence presented here indicate that the mid-anaphase pause is not merely the slowing of anaphase by reduced kinesin-5 activity but rather is triggered by a specific mechanism that is dependent on the functional pre-anaphase spindle checkpoint. First, the well-established role of kinesin-5 proteins in spindle assembly (Blangy et al., 1995; Goshima and Vale, 2005; Hagan and Yanagida, 1992; Heck et al., 1993; Hoyt et al., 1992; Le Guellec et al., 1991; Roof et al., 1992) suggests that the pre-anaphase spindle checkpoint is transiently activated in cells in which kinesin-5 proteins are partially active. This notion is supported by our result that anaphase onset is delayed in kip1Δ cin8-F4A cells (Fig. 4). Second, the variable severity of the mid-anaphase phenotype among the different combinations of kinesin-5 mutations correlates with the variability in MT-binding activity of these mutants (Table 1). Our previous in vitro work demonstrated that the MT-binding activity of Cin8-F4A is substantially decreased compared with the ability of Cin8-3 (Gheber et al., 1999). Based on this difference, we expect that MT-crosslinking function provided by kinesin-5 will be lower in cells that express Cin8-F4A compared with cells that express Cin8-3. Similarly, we expect that this activity will be lower in the double mutants that contain both KIP1 deletion and cin8 motor-domain mutation, compared with the single mutants. Because we have also previously shown that Cin8 MT-binding activity is crucial for spindle assembly (Gheber et al., 1999), we expect the pre-anaphase checkpoint to be activated to various degrees in the different cells examined here by us, with a rank similar to the defect in MT-crosslinking activity. The fact that we have observed the same rank in the severity of the mid-anaphase pause (Table 1) indicates that the mid-anaphase pause and the pre-anaphase spindle checkpoint are closely related in kinesin-5-mutated cells. Finally, we have directly demonstrated that moderate overexpression of Cdc20, which is known to override the pre-anaphase checkpoint (Pan and Chen, 2004; Schott and Hoyt, 1998; Zachariae et al., 1996), eliminated the mid-anaphase pause in kip1Δ cin8-F4A cells (Fig. 5).

A possible connection between the pre-anaphase spindle checkpoint, which acts to inhibit Cdc20 activity, and the mid-anaphase pause could be the set of proteins that are bound to the kinetochores prior to anaphase but relocate to the spindle midzone following anaphase onset. One example of a complex that exhibits this localization pattern is the chromosomal passenger complex, composed of the conserved Aurora B kinase (Ipl1), the inner centromere-like protein (INCENP, Sli15) and survivin (Bir1) (reviewed in Vader et al., 2005). This complex was shown to perform essential mitotic functions in the pre-anaphase spindle–assembly checkpoint (Pinsky et al., 2006; Pinsky et al., 2003), in performing essential mitotic functions in the pre-anaphase spindle–assembly checkpoint (reviewed in Vader et al., 2006). This complex was shown to be required for correct DNA segregation in these cells – i.e. deformed DNA morphology, and longer and possibly more dynamic spindles. It is therefore likely that pre-anaphase Slk19 function is important for strengthening sister-chromatid cohesion (Zhang et al., 2006) and preventing premature sister-chromatid separation in the presence of a spindle-assembly defect and the pre-anaphase spindle-checkpoint activation in kinesin-5-mutated cells. Indeed, we have shown that, in kip1Δ cin8-F4A slk19Δ cells, anaphase onset occurs earlier compared with kip1Δ cin8-F4A cells, indicating that anaphase delay is bypassed and premature DNA segregation takes place. The DNA deformation during anaphase that we observed in kip1Δ cin8-F4A slk19Δ and kip1Δ cin8-F4A CDC20-OX cells (Fig. 3A and Fig. 5B) is likely to be a result of this premature anaphase onset. In kip1Δ cin8-F4A slk19Δ cells, these DNA-segregation defects are further augmented (Fig. 5C, right) by the dramatic increase in the spindle-elongation rate in these cells (Table 2). This is likely to cause the decreased viability of kip1Δ cin8-F4A slk19Δ cells (Fig. 3B).

It has been reported that slk19Δ is synthetically lethal with cin8Δ, but an explanation for this lethality has not been proposed (Zeng et al., 1999). The data presented here indicates that, in kinesin-5 mutated cells, the function of Slk19 is required to maintain the pre-anaphase checkpoint and to restrict the spindle-elongation rate in order to allow for correct DNA segregation in these cells.

Materials and Methods

Yeast strains, media and genomic manipulations

The S. cerevisiae strains (supplementary material Table S1) used in this work are derivatives of the S288C strain. Rich (YPD) and minimal (SD) media were described previously Cin8 motor-domain mutations (cin8-3 and cin8-F4A) were described previously (Gheber et al., 1999; Hoyt et al., 1992). Nuf2-GFP chromosomal integration was performed as previously described (Kahana et al., 1995). All anaphase-B examination experiments were performed at room temperature (24°C). For examination of cell viability, cells were grown overnight in liquid medium, diluted to the same optical density in each sample, plated in serial dilutions and grown at various temperatures for 3 days. For arrest in S-phase, 0.1 M hydroxyurea (Sigma Chemical Company) was added to log-phase cells in liquid minimal medium lacking tryptophan, pH 5.2. To release cells from hydroxyurea arrest, they were washed and resuspended in fresh medium. Cdc20 overexpression was achieved by introducing a plasmid that contained the CDC20 coding sequence under the control of the MET25 promoter then growing cells in medium lacking methionine for 4 hours. To combine Cdc20 overexpression with S-phase arrest, cells were grown to log phase, arrested with hydroxyurea for 2 hours then hydroxyurea-containing medium was changed to medium lacking methionine and arrest was maintained for an additional 2 hours. For release from S-phase arrest under Cdc20 overexpression conditions, cells were washed and placed into fresh medium lacking methionine. DNA morphology was observed ~50 minutes following release from hydroxyurea arrest. A PCR-generated strategy was used to replace slk19 and spo12 open reading frames (ORFs) with a KanMX module and to tag Ase1, Slk19 and Cdc14 with GFP, and Sce1 with 3xHA (Baudin et al., 1993; Wach et al., 1994). Genomic DNA prepared from MATa haploid complete set deletion and GFP strain collections (Invitrogen, California) was used as a template for amplification of spo12::KanMX and slk19::KanMX gene disruption cassettes, and Ase1, Slk19 and Kaplan, 2007) or the mid-anaphase pause. Overriding of the pre-anaphase checkpoint by overexpression of Cdc20 might induce premature relocation of these proteins to the spindle, thus eliminating the mid-anaphase pause.

The elimination of the mid-anaphase pause by deletion of SLK19 can also be explained by the connection between the pre-anaphase spindle checkpoint and the pause. Prior to the anaphase onset, Slk19 is localized to the kinetochore (Sullivan et al., 2001; Zeng et al., 1999), where it was shown to associate with the sister-chromatid cohesion-complex subunit Sec1 (Zhang et al., 2006). It has also been shown that metaphase-arrested slk19Δ cells have more dynamic and longer spindles, lose their centromeric DNA plasticity, and have a deformed nucleus that translocates through the mother-bud neck (Zhang et al., 2006). These phenotypes resemble the ones we reported here during anaphase in kip1Δ cin8-F4A slk19Δ cells – i.e. deformed DNA morphology, and longer and possibly more dynamic spindles. It is therefore likely that pre-anaphase Slk19 function is important for strengthening sister-chromatid cohesion (Zhang et al., 2006) and preventing premature sister-chromatid separation in the presence of a spindle-assembly defect and the pre-anaphase spindle-checkpoint activation in kinesin-5-mutated cells.
Cdc14 tagging with GFP-SphIS5 and PCR products were introduced into the target yeast strain by a standard lithium-acetate transformation protocol (Gietz et al., 1995; Gietz and Woods, 2002). The specific primers used for amplification of the deletion cassettes, tagging, confirmation of the correct integration location and for confirmation of the absence of the WT SLK19 and SPO12 genes are listed in supplementary material Table S2. For elimination of the H3IS gene, partial ORF sequence of the S. cerevisiae HIS3 gene (16-370 bp) was amplified with primers that introduced XhoI and NotI sites at the 5’ side and PCR product was cloned in pRS315. For integration, the resulting plasmid (pV10) was cut with NdeI and yeast cells were transformed using a standard protocol. Transformants positive for LEU2 and negative for HIS3 were selected.

**Microscopy**

Immunostaining was performed using a standard procedure (Gheber et al., 1999). To follow real-time anaphase-B kinetics, we used the fluorescence 3D time-lapse microscopy assay. This assay is based on real-time imaging of kinetochore kinetics in dividing yeast cells. The kinetochores are labelled by fusion of the Nuf2 kinetochore motor protein essential for bipolar spindle formation in vivo. Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor protein (Kahana, J. A., 2005). The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. Nature 435, 114-118.

**Statistical analysis**

Values in all tables represent average ± s.e.m. The significance of the differences between the average values was determined using Student’s t-test. *P＜0.05; **P＜0.01; ***P＜0.001.

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**References**


Mid-anaphase pause in kinesin-5-mutated cells


Cdc14 release during anaphase B in kinesin-5 mutated cells. (A and B) Representative images (2D projection) of Cdc14-GFP localization in Nuf2-GFP labeled cells. Images represent six \textit{kip1Δ} and fourteen \textit{kip1Δ cin8-FA} cells filmed for each strain. (A) \textit{kip1Δ CDC14-GFP} strain. (B) \textit{kip1Δ cin8FA CDC14-GFP} strain. Time interval between frames – 1 min; bar – 2 μm; frame labeling: (*) - end of the fast phase; (**) - Cdc14-GFP release from the nucleolus; (***), (****) - end of the slow phase; (****) - end of the mid-anaphase pause. (C) Anaphase B progression of the cells presented in A and B. Distance between spindle pole bodies (SPB), monitored by Nuf2-GFP fluorescence, was measured as a function of time. “R” represents the rate of spindle elongation. The different anaphase B phases are indicated by the following symbols: (●) – fast phase, (■) – pause, (▲) – slow phase, (●) post anaphase spindle elongation or spindle collapse. The genotypes are indicated at the bottom of each plot.
Supplementary Figure S2

Effect of SLK19 or SPO12 chromosomal deletion on anaphase B spindle elongation. Cells were arrested in S-phase of the cell-cycle for 2 h and released from arrest. The distance between spindle pole bodies (SPB), monitored by Nuf2-GFP fluorescence, was measured as a function of time. Time of initiation of spindle elongation was assigned as 0 min.

Representative plots are shown. “R” represents the rate of spindle elongation. The different anaphase B phases are indicated by different symbols: (♦) – fast phase, (■) – pause, (▲) – slow phase, (●) – post spindle elongation. The various genotypes are indicated at the bottom of each plot: (A) – slk19Δ; (B) – spo12Δ; (C) – kip1Δ cin8-FA slk19Δ; (D-H) – kip1Δ cin8-FA spo12Δ.
**Supplementary table TS1. S. cerevisiae strains (S288C) and plasmids used in this study**

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<td>a, lys2, ura3, leu2, his3, cyh2', ADE, nuf2::NUF2-GFP-URA3, ASE1-GFP::SpHIS5</td>
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<td>LGY1510</td>
<td>a, lys2, ura3, leu2, his3, ADE, nuf2::NUF2-GFP-URA3, cin8-F467A, kip1::KanMX, ASE1-GFP::SpHIS5</td>
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<td>LGY1806</td>
<td>a, lys2, ura3, leu2, his3, ADE, nuf2::NUF2-GFP-URA3, kip1::KanMX, ASE1-GFP::SpHIS5, ASE1-CEN-LEU2 (pB243)</td>
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<tr>
<td>LGY1808</td>
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<tr>
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<tr>
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<td>a, lys2, his3, ura3, leu2, ADE, cyh2', cin8-F467A, kip1::KanMX, nuf2::NUF2-GFP-URA3, Slk19-GFP::SpHIS5</td>
</tr>
<tr>
<td>Strain</td>
<td>Genotype</td>
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<tr>
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<td>---------------------------------------------------------------------------</td>
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<tr>
<td>LGY1968</td>
<td>ade2, his3, leu2, lys2, ura3, cyh2', can1, kip1::HIS3, cin8-F467A, nuf2::URA-NUF2-GFP (pES36)</td>
</tr>
<tr>
<td>LGY1971</td>
<td>ade2, his3, leu2, lys2, ura3, cyh2', can1, kip1::HIS3, cin8-F467A, nuf2::URA-NUF2-GFP (p415-MET25)</td>
</tr>
<tr>
<td>LGY14800</td>
<td>his3, leu2, lys2, ura3, cyh2', NUF2::URA-NUF2-GFP, leu2::LEU2-TUB1-GFP</td>
</tr>
<tr>
<td>LGY1125</td>
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</tr>
<tr>
<td>LGY1147</td>
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<td>LGY1260</td>
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</tr>
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**Supplementary table TS2.** A list of the oligonucleotides used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Purpose</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Slk19_del_f</td>
<td>Forward primer for <em>slk19::KanMX</em> amplification</td>
<td>5'-GTATGGCCTTGATTTGTGTG-3'</td>
</tr>
<tr>
<td>Slk19_del_r</td>
<td>Reverse primer for <em>slk19::KanMX</em> amplification</td>
<td>5'-GGCCAACCAAGAGACATATG-3'</td>
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<tr>
<td>Spo12_del_f</td>
<td>Forward primer for <em>spo12::KanMX</em> amplification</td>
<td>5'-GGGTTACAGAAAAAAAGGC-3'</td>
</tr>
<tr>
<td>Spo12_del_r</td>
<td>Reverse primer for <em>spo12::KanMX</em> amplification</td>
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</tr>
<tr>
<td>KanMX_del_con_f</td>
<td>Forward primer for correct KanMX insertion verification</td>
<td>5'-GGTATTGATAAATCTCGATATG-3'</td>
</tr>
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<td>KanMX_del_con_r</td>
<td>Reverse primer for correct KanMX insertion verification</td>
<td>5'-CCATGTTGGAATTAATCGG-3'</td>
</tr>
<tr>
<td>Slk19_conf_r</td>
<td>Reverse primer for confirmation of <em>SLK19</em> substitution</td>
<td>5'-CGCCCTTATATGATTATACT-3'</td>
</tr>
<tr>
<td>Spo12_conf_r</td>
<td>Reverse primer for confirmation of <em>SPO12</em> substitution</td>
<td>5'-TCATCGATTTTCTACATCTCT-3'</td>
</tr>
<tr>
<td>ASE1GFPF</td>
<td>Forward primer for yeast genomic Ase1-GFP amplification</td>
<td>5'-ACTGGCACCACGGGTAAAGG-3'</td>
</tr>
<tr>
<td>ASE1GFPR</td>
<td>Reverse primer for yeast genomic Ase1-GFP amplification</td>
<td>5'-TCCAGAGTCAGCGTAATGG-3'</td>
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<td>CDC14GFPF</td>
<td>Forward primer for yeast genomic Cdc14-GFP amplification</td>
<td>5'-ACAACACAGATGAGCAATTC-3'</td>
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<td>CDC14GFPR</td>
<td>Reverse primer for yeast genomic Cdc14-GFP amplification</td>
<td>5'-CTATTGCGGTATACATATGAC-3'</td>
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<td>SLK19GFPF</td>
<td>Forward primer for yeast genomic Slk19-GFP amplification</td>
<td>5'-GGCTGAGCACATCAAGATG-3'</td>
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<td>SLK19-delr</td>
<td>Reverse primer for yeast genomic Slk19-GFP amplification</td>
<td>5'-GTATGGCCTTGATTTGTGTG-3'</td>
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<tr>
<td>SCC1-3HA-F</td>
<td>Forward primer for yeast genomic Scc1-3HA tagging</td>
<td>5'-GACGCCAACACCTGCACTATTG AAGGGTATCAATGCTaacatatttacagt-3'</td>
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<td>SCC1-3HA-F</td>
<td>Reverse primer for yeast genomic Scc1-3HA tagging</td>
<td>5'-ATTGGTCCACCAAGAATCC CTCCGCTAAGTACATTCCGTTtacaattgac gctgt-3'</td>
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<tr>
<td>HIS-DEL_F</td>
<td>Forward primer for partial HIS3 ORF amplification</td>
<td>5'-CTCACGAGGCCTAGCTAAGG CTATTAC-3'</td>
</tr>
<tr>
<td>HIS-DEL_R</td>
<td>Reverse primer for partial HIS3 ORF amplification</td>
<td>5'-CTCACGAGGCCTAGCTAAGG CTATTAC-3'</td>
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