Research article

**Multiple telophase arrest bypassed (tab) mutants alleviate the essential requirement for Cdc15 in exit from mitosis in S. cerevisiae**

Wenying Shou and Raymond J Deshaies*

Address: Division of Biology, 156-29 Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125, USA
E-mail: Wenying Shou - shouw@its.caltech.edu; Raymond J Deshaies* - deshaies@its.caltech.edu
*Corresponding author

**Abstract**

**Background:** The Mitotic Exit Network (MEN) proteins – including the protein kinase Cdc15 and the protein phosphatase Cdc14 – are essential for exit from mitosis in *Saccharomyces cerevisiae*. To identify downstream targets of the MEN, we sought telophase arrest bypassed (tab) mutations that bypassed the essential requirement for *CDC15*. Previous studies identified *net1*tab2-1 and *CDC14TAB6-1* as mutations in the RENT complex subunits Net1 and Cdc14, respectively, and revealed that the MEN acts by promoting release of Cdc14 from its nucleolar Net1 anchor during anaphase. However, the remaining tab mutants were not characterized.

**Results:** Fourteen out of fifteen tab mutants were mapped to three recessive (tab1-tab3) and three dominant (TAB5-TAB7) linkage groups. We show that *net1*tab2-1 enables growth of *tem1Δ, cdc15Δ, dbf2Δ dbf20Δ, and mob1Δ*, but not *cdc5Δ or cdc14Δ*, arguing that whereas the essential task of the first four genes is to promote exit from mitosis, *CDC5* possesses additional essential function(s). *net1*tab2-1 but not *CDC14TAB6-1* resulted in a high rate of chromosome loss, indicating that Net1 promotes accurate chromosome segregation in addition to its multiple known roles. Finally, *TAB1* was shown to be *MTR10*, a gene encoding nuclear transport receptor/adaptor. In some of the tab mutants including *mtr10tab1-1*, defective nuclear export of the ribosomal protein Rpl11b was observed. Furthermore, the transport-defective -31 allele of the karyopherin *SRP1*, but not the transport competent -49 allele, exhibited a tab phenotype.

**Conclusions:** Transport-defective mutations in two karyopherins can bypass *cdc15Δ*, suggesting that the function of the MEN is to promote mitotic exit by regulating nuclear transport.

**Background**

In *S. cerevisiae*, a key event accompanying exit from mitosis is the inactivation of Clb/Cdc28 protein kinase achieved through degradation of Clb and accumulation of the Clb/Cdc28 inhibitor Sic1 (reviewed in [1]). Anaphase-Promoting Complex/Cyclosome (APC/C) ubiquitin ligase and its substrate specific activator Hct1/Cdh1 are required for degradation of Clb2, the major mitotic cyclin in the budding yeast (reviewed in [2]). Whereas *hct1Δ* and *sic1Δ* cells appear relatively normal, *hct1Δ sic1Δ* cells are inviable, presumably due to their inability to extinguish Cdc28 activity in telophase [3].

The complexity in the regulation of mitotic exit is underscored by the existence of a set of genes essential for this process, referred to as the "mitotic exit network (MEN)".
They encode the GTP-binding protein Tem1 and its putative guanine nucleotide releasing factor Lte1, the dual specificity protein phosphatase Cdc14, protein kinases Cdc5, Cdc15, and Dbf2/Dbf20, the Dbf2-binding protein Mob1 (reviewed in [4]) and the spindle-pole-body component Nud1 [5]. Conditional-lethal temperature sensitive (ts) mutations in any of the MEN genes cause cells to arrest in late mitosis with elevated mitotic Cdk activity when shifted to the restrictive temperature. Inactivation of Cdc28 by overexpression of Sic1 suppresses multiple MEN mutants and hyperactivation of Cdc28 by overexpression of Clb2 exacerbates their phenotypes, consistent with the idea that a key function of the MEN proteins is to eradicate Cdc28 activity during the transition from anaphase/telophase to G1 [6–9].

Recently, important advances have been made in our understanding of how the MEN is switched on during anaphase. Tem1 is thought to be kept inactive throughout early mitosis by the two-component GTPase-activating protein complex Bub2/Bfa1 which co-localizes with Tem1 at the spindle pole body [10,11]. When the mitotic spindle penetrates the mother-daughter neck, the Tem1-bound spindle pole body is translocated into the daughter cell where a cortical pool of Lte1 is thought to activate Tem1 [12,13]. In addition to this spatial regulation, the GTPase activity of Bfa1 is thought to be inactivated through its phosphorylation by Cdc5 [14], but it remains unknown how this process is coordinated with the presumed juxtaposition of Lte1 and Tem1 in the daughter cell. Despite this rapid progress in understanding the signals that regulate the MEN, the direct downstream targets of the MEN have yet to be apprehended.

To address how the MEN components downstream of Cdc15 are organized and what their targets are, we sought tab (telophase arrest bypassed) mutants that alleviate the essential requirement for CDC15. In theory, tab mutants could act downstream of or parallel to CDC15, and could be either loss-of-function mutations in genes that inhibit exit from mitosis or gain-of-function mutations in genes that promote exit from mitosis. To our surprise, mutations in six linkage groups were uncovered, with three groups being recessive (tab1-tab3) and three groups being dominant (TAB5-7). We have shown in earlier studies that tab2-1 is a reduction-of-function mutation in NET1 (a.k.a. CFI1) [15–17] and that TAB6 is a gain-of-function mutation in CDC14[18]. Net1 sequesters Cdc14 in the nucleolus and inactivates it in preanaphase cells. During exit from mitosis, a TEM1/CDC15-dependent signal frees Cdc14 from the nucleolus and allows it to promote inactivation of Clb/Cdc28 [15,17,19]. The fact that both Net1 and Cdc14 are directly involved in exit from mitosis validates the effectiveness of the tab screen and heightens the potential value of the uncharacterized tab mutants. Here, we present a detailed description of the tab mutant screen, and describe a third TAB gene, TAB1/MTR10.

Results

Cdc15 promotes activation of Clb proteolysis by a post-translational mechanism

Transcription has been postulated to play an important role in triggering exit from mitosis [20]. For example, Dbf2, a protein kinase that promotes exit from mitosis, is associated with the CCR4 transcription regulatory complex [21]. In addition, the Swi5 transcription factor activates SIC1 expression during late anaphase, and both sic1Δ and swi5Δ are synthetically lethal with dbf2A[22,7]. To address if production of new proteins is essential for exit from mitosis, we examined the effects of the protein synthesis inhibitor cycloheximide on Clb2 degradation, a key aspect of this process. Mutant cdc15-2 cells were uniformly arrested in late anaphase/telophase by incubation at the restrictive temperature 37°C. The cultures were then supplemented with 0, 10, or 100 µg/ml cycloheximide before being returned to 25°C to reverse the cdc15-2 arrest. In the absence of cycloheximide, Sic1 accumulated and Clb2 disappeared as cells exited mitosis (Figure 1, lanes 1–4). In the presence of cycloheximide, Sic1 accumulation was abolished, but Clb2 degradation still occurred with apparently normal kinetics (Figure 1, lanes 5–8 and 9–12). Although this result does not exclude the likely possibility that synthesis of new proteins (e.g. Sic1) normally facilitates the exit from mitosis, it reveals that a key aspect of mitotic exit – inactivation of Cdc28 protein kinase via degradation of the mitotic cyclin Clb2 – can proceed beyond the Cdc15-dependent step without synthesis of new proteins.

A screen for telophase arrest bypassed (tab) mutants

To understand how the MEN mobilizes post-translational mechanisms to trigger mitotic exit, we conducted a genetic screen for ‘telophase arrest bypassed’ (tab) mutants that bypassed the essential requirement for CDC15 (Figure 2). Because the essential requirement for CLNs 1, 2, and 3 in entry into S phase can be bypassed by deletion of a gene (SIC1) that inhibits entry into S phase [23,24], we reasoned by analogy that our screen might reveal key negative regulators of the anaphase/telophase -> G1 transition.

Ninety independent cultures of cdc15A [pMET3-cdc15-2, URA3] were first grown in inducing medium (- methionine) at permissive temperature (25°C) to allow occurrence of spontaneous mutations. The cultures were subsequently plated on repressing medium (+ methionine) at a temperature semi-restrictive for the cdc15-2 allele (30°C) to enrich for mutations that supported the growth of CDC15- deficient cells. To identify mutations that allowed complete bypass of cdc15A, representative colonies from each plate were tested for their ability to
grow on 5-FOA-containing medium (5-FOA selectively blocks the growth of plasmid-bearing URA3+ cells). The parental strain failed to grow due to its inability to proliferate without the [MET3-cdc15-2, URA3] plasmid. In contrast, twenty five mutants survived on 5-FOA. Furthermore, all of them had lost the plasmid as determined by PCR analysis (data not shown). Fifteen of these mutants were amenable to genetic manipulation and harbored single mutations. Fourteen mutants were assigned to three recessive and three semi-dominant/dominant linkage groups (Table 2). We referred to these mutants as tab mutants for their telophase-arrest-bypass (Tab+) phenotype. In addition, we serendipitously found that srp1-31 but not srp1-49 could bypass cdc15Δ (Table 2; data not shown).

Earlier studies identified tab2 and TAB6 as mutations in NET1 and CDC14, respectively [15,18]. Net1 detains Cdc14 in the nucleolus and inactivates it until a Tem1-dependent signal sets Cdc14 free to promote exit from mitosis [15–17]. The fact that both Net1 and Cdc14 are directly involved in exit from mitosis prompted us to further analyze tab mutants.

does bypass of cdc15Δ require Sic1 accumulation and Clb2 degradation?

Either Sic1 accumulation or Clb2 degradation is proposed to be sufficient to sustain mitotic exit [3]. Although cdc15-2 mutants are defective in both processes, ectopic activation of either one should restore exit from mitosis and growth. Thus, to gain insight into their molecular targets, we asked if bypass of cdc15Δ by tab mutations required SIC1 or HCT1. Whereas all tab mutants tested were able to bypass cdc15Δ in the absence of SIC1, we tested if the same bypass efficiency could be achieved by tab3-1, a

Figure 1
Post-translational control of Clb2 proteolysis by CDC15. Exponentially growing cdc15-2 (RJD619) cells were arrested in late anaphase by shifting the culture to 37°C for three hours. The culture was split in three and either mock-treated (lanes 1–4) or supplemented with the protein synthesis inhibitor cycloheximide (CHX) at 10 µg/ml (lanes 5–8) or100 µ g/ml (lanes 9–12). After five minutes (time = 0), the cultures were released from cell cycle arrest by downshift to 25°C. At indicated time points, samples were withdrawn, and the levels of Clb2, Sic1 and Cdc28 proteins were assayed by SDS-PAGE and immunoblotting.

Figure 2
Scheme for isolating telophase arrest bypassed (tab) mutants. See text for details.
Table 2: Characterization of tab mutants

| Name  | # alleles | recessive/dominant | identity | growth at 37°C | Nop1 pattern |
|-------|-----------|---------------------|----------|---------------|--------------|
| tab/1 | 5         | recessive           | MTR10°   | inviable      | -            |
| tab2  | 2         | recessive           | NET1     | slow          | -            |
| tab3  | 1         | recessive           | slow     | normal        | +            |
| TAB5  | 2         | semi-dominant       | normal   | +             |              |
| TAB6  | 1         | dominant            | CDC14    | slow          | +            |
| TAB7  | 3         | semi-dominant (-1)  |         | normal        | +            |
| srpl-31 |        | recessive           |          |               |              |

Notes: 
- We have not determined if srpl-31 is allelic to tab3, TAB5, or TAB7. 
- All alleles were tested for recessiveness/dominance of Tab except for TAB7. 

Table 3: Does bypass require SIC1 or HCT1?

| Strain   | Sic1 Δ | Hct1 Δ | +/− |
|----------|--------|--------|-----|
| tab-1    |        |        | +   |
| tab-2    | +/-    |        | +   |
| tab-3    | -      |        | +   |
| TAB-5    | -      |        | +   |
| TAB-6    | +/-    |        | +   |
| TAB-7    | +/-    |        | +   |

Notes: 
- We have not determined if srpl-31 is allelic to tab3, TAB5, or TAB7. 
- All alleles were tested for recessiveness/dominance of Tab except for TAB7.

**Table 3: Does bypass require SIC1 or HCT1?**

| Strain | Sic1 Δ | Hct1 Δ | +/− |
|--------|--------|--------|-----|
| tab-1  |        |        | +   |
| tab-2  | +/-    |        | +   |
| tab-3  | -      |        | +   |
| TAB-5  | -      |        | +   |
| TAB-6  | +/-    |        | +   |
| TAB-7  | +/-    |        | +   |

Notes: 
- We have not determined if srpl-31 is allelic to tab3, TAB5, or TAB7. 
- All alleles were tested for recessiveness/dominance of Tab except for TAB7.
tants relates more to the strength of the particular mutation, rather than the mechanism of bypass. In support of this hypothesis, we recently observed that the \( SIC1 \)-independent bypass mutant 15D2 is allelic to the \( SIC1 \)-dependent \( tab1-1 \) mutant (R. Azzam, unpublished data).

\( tab2-1 \) bypasses multiple deletion mutants of MEN

All MEN genes are required for exit from mitosis, but it has not yet been tested whether this is the sole essential function of all MEN genes, or if some MEN genes play critical roles in other processes. This question is amplified by the observations that Cdc5 participates in both chromosome segregation and cytokinesis ([25,26]; Park et al., submitted), Mob1 binds an essential protein kinase involved in spindle pole body duplication [27], and Tem1, Cdc15, Dbf2, and Mob1 have all been implicated in cytokinesis ([28–31]; reviewed in [1]). The \( tab \) mutants provide a unique opportunity to address this issue. Accordingly, we tested if \( net1tab2-1 \) could bypass deletion mutants of other MEN genes. \( net1tab2-1 \) bypassed \( cdc15\Delta, tem1\Delta, dbf2\Delta dbf20\Delta, mob1\Delta, \) but not \( cdc14\Delta \) (Figure 4A; [15]). In addition, it bypassed \( cdc5\Delta \) with negligible (1000–10,000 fold lower) efficiency (Fig. 4A), suggesting that although the sole essential function of \( CDC15, TEM1, DBF2, \) and \( MOB1 \) is to drive cells out of mitosis, \( CDC5 \) has other critical cellular functions.

Some but not all \( tab \) mutants exhibit additional phenotypes including growth defects, perturbations in the nucleolus, and elevated rates of chromosome loss

To further characterize the \( tab \) mutants, we tested whether they display other phenotypes in an otherwise wild type background. \( tab \) mutants grown to exponential phase at 25°C and shifted to 37°C for 3 or 6 hours did not arrest at a particular stage of the cell cycle (data not shown). When \( tab \) mutants were plated on YPD plates and grown at 37°C, \( TAB5 \) and \( TAB7 \) grew normally (Table 2), suggesting that bypass of \( CDC15, TEM1, DBF2, \) and \( MOB1 \) is to drive cells out of mitosis, \( CDC5 \) has other critical cellular functions.

**Figure 4**

\( net1tab2-1 \) efficiently bypasses multiple deletion mutants of MEN and extends the permissive temperature range for \( cdc15\Delta, cdc14\Delta, \) and \( cdc5\Delta. \) (A) The bypass specificity of \( net1tab2-1 \) (\( net1-1 \)). (B) Cells with specified combinations of \( NET1 \) alleles (indicated at the top) and \( CDC \) alleles (indicated on the side) were spotted onto YPD plates in 5-fold serial dilutions. Plates were incubated at 24°C (left panel) or 32.5°C (center and right panels) for 2–4 days before being photographed. \( net1tab2-1 \) was observed to suppress the ts phenotype of \( dbf2-1 \) at 35.5°C (not shown).
that have multiple functions. Indeed, NET1 additionally modulates nucleolar structure and transcription [16,18].

The release of Cdc14 from nucleolar Net1 promotes mitotic exit [15,17,18], net1^{tab2-1} cells display a panel of nucleolar defects including reduction of nucleolar silencing, mislocalization of nucleolar antigens, alteration of rDNA morphology, and reduction of rRNA synthesis [16,18]. To test whether perturbation of nucleolar structure is consistently associated with bypass of cde15Δ, we immunolocalized the nucleolar antigen Nop1 in all tab mutants. Nop1 localization was also perturbed in tab1-1 and srp1-31, but was normal in tab3, TAB5, TAB6, and TAB7 cells (Table 2; [18]), suggesting that a gross change in nucleolar structure is not required for bypass.

Because the putative Tem1 antagonists and mitotic checkpoint proteins Bub2 and Bfa1 negatively regulate exit from mitosis and cytokinesis in response to both DNA damage and spindle misorientation ([32]; reviewed in [33]), we asked whether bypass of TEM1-dependent regulation can cause chromosome instability. All tab mutants are in the W303 background in which the ade2-1 mutation makes colonies red after adenine in the growth medium has been depleted. A URA3-marked tester mini-chromosome carrying the SUP11 suppressor transfer RNA gene [34]) was introduced into net1^{tab2-1} and CDC14^{TAB6-1}, the two tab mutants with well-characterized bypass mechanisms. Cells that retain the mini-chromosome should form white colonies due to suppression of ade2-1 by SUP11, whereas loss of this chromosome should result in a red sector or sectors. The extent of chromosome loss (expressed as % colonies that were red or had red sectors) was low in wild-type and CDC14^{TAB6-1} cells (< 5%), and high (94%) in net1^{tab2-1} cells (Figure 5). In comparison, in a strain lacking the anaphase-inhibitor Pds1, ~18% of the colonies suffered chromosome loss events (Figure 5). Since net1^{tab2-1} but not CDC14^{TAB6-1} also perturbs nucleolar functions [18], CDC14^{TAB6-1} is a more specific and representative bypass mutant. Thus, TEM1 regulation can be short-circuited without a major effect on chromosome loss. Consistent with this notion, cells lacking Bub2 (an inhibitor of Tem1) showed a similarly moderate extent of chromosome loss (5%) (Figure 5). We propose that additional functions of Net1 ensure faithful transmission of chromosomes.

**Molecular cloning of TAB genes and implication of nuclear transport in the regulation of mitotic exit**

We attempted to clone by complementation genes corresponding to the recessive tab mutations by exploiting the fact that all of the recessive tab mutants are ts for growth (see below). By this approach, TAB1 was revealed to be MTR10, a gene previously implicated in nuclear transport [35–37]. We were unable to isolate plasmids that complemented tab3 in all libraries tested.

Both MTR10 (TAB1) and SRP1 belong to the nuclear transport receptor/adaptor family (reviewed by [38]). Mutant srp1-31 and mtr10^{tab1-1} cells accumulate the ribosomal protein Rpb11b in the nucleus [39]. Thus, to evaluate a potential link between nuclear transport and the tab phenotype, we evaluated the localization of Rpb11b-GFP [39].
in all tab mutants. Mutants expressing Rpl11b-GFP were grown to early-log phase at 25°C, shifted to 37°C to induce the ts mutant phenotype (which also reduces synthesis of ribosomal proteins including Rpl11b), and shifted back to 25°C to induce new ribosomal protein synthesis. If the rate of mutant phenotype reversal is slower than restoration of ribosome synthesis, then Rpl11b should accumulate in the nucleus. Whereas Rpl11b-GFP was distributed relatively evenly in wild-type cells, it accumulated in the nucleus in a significant fraction of srp1-31 and net1tab2-1 cells at 25°C and after 25°C -> 37°C shift (Figure 6; [39]). In addition, although ntr1tab1-1 cells were relatively normal at 25°C, 30% of them accumulated nuclear Rpl11b after 25°C -> 37°C -> 25°C shift (Figure 6). Thus, multiple tab mutants accumulate Rpl11b in the nucleus, implying that nucleocytoplasmic trafficking is involved in the regulation of mitotic exit. Other tab mutants (such as tab3, TAB5, CDC14TAB6 and TAB7) showed no defect in this assay, suggesting that bypass of cdc15Δ can be achieved without perturbing nucleocytoplasmic transport.

**Discussion**

**MEN: A signalling network that can post-translationally trigger exit from mitosis**

Both transcriptional and post-translational controls have been implicated in the operation of the Mitotic Exit Network and the regulation of the anaphase/telophase -> G1 transition [40,7,19,41]. To address whether the portion of the MEN that acts downstream of CDC15 can trigger a key aspect of the anaphase/telophase -> G1 transition (i.e. inactivation of Clb/Cdc28 via proteolysis of Clb2) in the absence of new gene expression, we evaluated the turnover of Clb2 in cells released from a cdc15-2 arrest in the presence of cycloheximide. Interestingly, Clb2 was rapidly and efficiently degraded upon reversal of the cdc15-2 block regardless of whether cycloheximide was present (Figure 1) although both cytokinesis and cell separation require protein synthesis [42]. This observation suggests that the exit from mitosis in budding yeast may be controlled by mechanisms fundamentally similar to those employed by animal cells, which proceed through mitosis with highly condensed, transcriptionally silent chromosomes. Interestingly, all of the MEN genes have putative homologues in the *C. elegans* genome [http://genome-www.stanford.edu/Saccharomyces/worm/].

**Isolation of tab mutants**

A deliberate search for bypass mutants was a key aspect of the screen reported here, since numerous genetic interactions have been observed among components of the MEN (consult [9] for a summary). Formally speaking, it is not possible to order gene functions based on suppressive interactions involving reduction-of-function (e.g. ts) alleles. For example, the net1-1 allele suppressed ts mutations in both its downstream target CDC14 and its upstream regulators CDC5, CDC15, and DBF2 (Figure 4B). Since we sought mutants that bypassed cdc15Δ, the tab genes are predicted to function either downstream of or parallel to CDC15, but not upstream of CDC15.

The tab screen yielded both dominant and recessive mutants which mapped to six linkage groups. The pattern of alleles (Table 2) does not satisfy a Poisson distribution, suggesting that the screen has not reached saturation. The recessive tab mutants are likely to represent reduction-of-function mutations in genes that inhibit exit from mitosis, and the dominant TAB mutants are likely to result from gain-of-function mutations in genes that promote exit from mitosis. This prediction is supported by our prior characterization of the tab2-1 and TAB6-1 alleles of NET1 and CDC14, respectively (see next section). The fact that we recovered recessive, semi-dominant, and dominant mutants in additional tab genes suggests that there are likely to be multiple dosage-sensitive positive and negative regulatory controls that act downstream of or parallel to CDC15.

**tab mutants can be classified into two groups:** those that bypass cdc15Δ only in the presence of SIC1 (e.g. tab3-1), and those that can bypass in the absence of SIC1 (e.g. net1tab2-1 and CDC14TAB6-1). It remains unclear whether mutants distribute into these classes based on bypass...
mechanism or allelic potency, but we favor the latter possibility, because some tab1 alleles required Sic1 to bypass cdc15Δ, whereas others did not (R. Azzam, unpublished data). Importantly, tab mutants from both classes appeared to bypass mitotic arrest in Tem1-depleted cells by enabling degradation of Clb2 and accumulation of Sic1 – two hallmark-marks of mitotic exit (Figure 3; [15,18]).

**A genetic pathway for anaphase -> G1 transition**

Biochemical and cell biological experiments indicate that Net1 acts as part of a complex named RENT that tethers Cdc14 to the nucleolus and inhibits Cdc14 phosphatase activity, and that CDC15 and TEM1 are required for the release of Cdc14 from Net1 at the end of mitosis [15–17,43]. Consistent with this notion, both net1tab2-1 (which encodes a mutant version of Net1 with presumably reduced affinity for Cdc14) and CDC14TAB6-1 (which encodes a mutant version of Cdc14 with reduced affinity for Tab2/Net1) bypass cdc15Δ [15,18]. Both net1tab2-1 and CDC14TAB6-1 impinge directly on mitotic exit, suggesting that other TAB genes may encode physiological regulators and effectors of the Mitotic Exit Network.

The net1tab2-1 mutation efficiently bypassed tem1Δ, cdc15Δ, dbf2adb20AΔ and mob1Δ, consistent with the notion that the sole essential function of all these genes is to inhibit Net1. net1tab2-1 failed to bypass cdc14Δ, because Cdc14 is the downstream target of Net1 [15,17]. Cdc5 also regulates Net1 (Shou et al., manuscript in preparation), but net1tab2-1 bypassed cdc5Δ with very low efficiency, presumably because CDC5 has other essential functions [44,25,26,45].

The most parsimonious model that unifies all of these observations is as follows: Tem1 functions upstream of Cdc15, which in turn activates the Dbf2-Mob1 protein kinase complex [46–49]. Together with Cdc5, these proteins dislodge Cdc14 from nucleolar Net1. Cdc14 subsequently dephosphorylates (and thereby activates) proteins involved in Sic1 expression (Swi5) and C1b degradation (Hct1/Cdh1) [19,41,50], thereby eliminating mitotic Cdk activity and promoting exit from mitosis. The mechanisms by which Cdc5 and Dbf2/Mob1 promote the disassembly of RENT remain unknown, raising the possibility that other Tab proteins serve as intermediaries in this process. One attractive hypothesis based on our identification of MTR10 as a tab gene is that MEN proteins regulate the nucleocytoplasmic distribution of a key regulator of the release process.

**Chromosome instability in tab mutants**

The Bub2/Bfa1 GTPase-activating protein (GAP) complex inhibits the MEN and cytokinesis in response to DNA damage and spindle misorientation ([32]; reviewed in [33]). bub2Δ and CDC14TAB6-1 cells show similar chromosome instability as wild-type cells. In contrast, net1tab2-1 cells have a dramatic chromosome instability phenotype, with some colonies showing multiple red sectors indicative of multiple chromosome-loss events, and many colonies completely red on the surface (Figure 5A). Net1 is unlikely to directly regulate transmission of the tester chromosome, because it is only localized to rDNA in the nucleolus [15–17]. Thus, the difference between net1tab2-1 and CDC14TAB6-1 presumably results from molecular defects unique to the former mutant, including defective nucleolar structure and function, or perhaps defective nuclear transport ([18]; Figure 6). Further study is required to reveal how the multifunctional Net1 protein helps to ensure chromosome transmission fidelity.

**Multiple tab mutations influence nucleocytoplasmic transport**

Nuclear transport-defective mutations in MTR10 and SRP1 (mtr10tab1-1 and srp1-31, respectively) bypassed cdc15Δ. Mtr10 assembles with and functions as a nuclear import receptor for the mRNA-binding protein Npl3 [36,37]. Interestingly, srp1-31 also inhibits nuclear import of multiple proteins including Npl3 [51,52]. Remarkably, reduction-of-function alleles of the karyopherin KAP104 were also recovered in a screen for mutations that suppressed cdc15-2ts (A. Toh-e, personal communication). KAP104 is required for proper localization of mRNA binding proteins Nab2 and Hrp1 to the nucleus [53].

The only phenotype known to be shared by srp1, mtr10, and kap104 mutants is accumulation of the 60S ribosomal protein Rpl11b in the nucleus [39]. SRP1, MTR10, and KAP104 may also collaborate to maintain the proper nucleocytoplasmic partitioning of α factor (or factors) that regulates mitotic exit. An alternative possibility is that they sequester an inactive stockpile of α factor that, upon mutation or signalling by the MEN, is released from these transportins and enables exit from mitosis [54,55]. Finally, it is possible that the transportin mutants enable bypass through an indirect perturbation of nucleolar architecture (Table 2). Given that some of the MEN proteins reside in the cytoplasm whereas their target – the RENT complex – resides in the nucleolus, it is tempting to speculate that nuclear transport is a target of MEN action. Our failure to observe nuclear accumulation of Rpl11b in tab3 and TAB5-TAB7 mutants suggests that these tab genes bypassed cdc15Δ by a distinct mechanism. Regardless of the exact mechanisms of bypass, it is clear from these analyses that the release of Cdc14 from Net1 is more intricately connected to cell regulation than was previously imagined.

**Conclusions**

Three main conclusions on how exit from mitosis is regulated in the budding yeast can be derived. First, the sole es-
sential function of the Tem1, Cdc15, Dbf2/Dbf20, and Mob1 (but not Cdc5) proteins are to promote the release of Cdc14 from the nucleolus, as cells lacking these proteins can grow upon simultaneous reduction of NET1 activity. Second, high chromosome loss rate was observed in net1ab-2 but not CDC14TAB-6, suggesting that although CDC15-independent mitotic exit does not dramatically affect chromosome transmission, additional functions of Net1 do. Third, both mtr10ab-1 and srp1-31 affect nuclear transport, suggesting that nuclear transport regulates mitotic exit.

Materials and Methods

Strains and plasmids
All yeast strains used in this study (listed in Table 1) are isogenic to W303. Standard methods were employed for the culturing and manipulation of yeast [56]. All plasmid constructions were based on the pRS vector series [57]. To replace CDC15 with TRP1, polymerase chain reaction (PCR) products containing TRP1 flanked by 200-base pair (bp) homology to the 5’ and 3’ untranslated regions of the targeted gene were used to transform diploid strain RJD381. Correct integrants were verified by PCR using primers that amplified a DNA fragment that spanned the recombination junction. Deletions of CDC5 and TEM1 were carried out similarly. To construct [pMET3-CDC15, URA3] (pWS100) and [pMET3-cdc15-2, URA3] (pWS109), the corresponding genes (from start codon to 300 bps downstream of the stop codon) were amplified by PCR from genomic DNA of wild type (RJD381) or cdc15-2 (RJD619) cells, and cloned into the SpeI and SacI sites of RDB620 (a derivative of pRS316 containing the MET3 promoter inserted between the HindIII and EcoRI sites).

Isolation of tab mutants
The scheme is outlined in Figure 2. Ninety independent cultures of cdc15A [pMET3-cdc15-2, URA3] (WY221) were grown at 25°C in synthetic minimal medium + 2% glucose (SD) in the absence of methionine. For each culture, ~8 x 10^6 cells were plated on SD+ methionine (2 mM) at 30°C (to simultaneously repress expression and partially inactivate the Cdc14 from the nucleolus, as cells lacking these proteins can grow upon simultaneous reduction of NET1 activity. Second, high chromosome loss rate was observed in net1ab-2 but not CDC14TAB-6, suggesting that although CDC15-independent mitotic exit does not dramatically affect chromosome transmission, additional functions of Net1 do. Third, both mtr10ab-1 and srp1-31 affect nuclear transport, suggesting that nuclear transport regulates mitotic exit.

Materials and Methods

Strains and plasmids
All yeast strains used in this study (listed in Table 1) are isogenic to W303. Standard methods were employed for the culturing and manipulation of yeast [56]. All plasmid constructions were based on the pRS vector series [57]. To replace CDC15 with TRP1, polymerase chain reaction (PCR) products containing TRP1 flanked by 200-base pair (bp) homology to the 5’ and 3’ untranslated regions of the targeted gene were used to transform diploid strain RJD381. Correct integrants were verified by PCR using primers that amplified a DNA fragment that spanned the recombination junction. Deletions of CDC5 and TEM1 were carried out similarly. To construct [pMET3-CDC15, URA3] (pWS100) and [pMET3-cdc15-2, URA3] (pWS109), the corresponding genes (from start codon to 300 bps downstream of the stop codon) were amplified by PCR from genomic DNA of wild type (RJD381) or cdc15-2 (RJD619) cells, and cloned into the SpeI and SacI sites of RDB620 (a derivative of pRS316 containing the MET3 promoter inserted between the HindIII and EcoRI sites).

Isolation of tab mutants
The scheme is outlined in Figure 2. Ninety independent cultures of cdc15A [pMET3-cdc15-2, URA3] (WY221) were grown at 25°C in synthetic minimal medium + 2% glucose (SD) in the absence of methionine. For each culture, ~8 x 10^6 cells were plated on SD+ methionine (2 mM) at 30°C (to simultaneously repress expression and partially inactivate the cdc15-2 allele). One to two colonies were picked from each plate and tested for viability on SD+5-FOA medium. When more than one colony was picked from each plate and tested for viability on SD+ 5-FOA medium, care was taken to pick colonies of different size or morphology. Out of ~90 colonies tested, 25 survived on 5-FOA medium (Tab+). PCR analysis confirmed that all twenty five colonies had lost the [pMET3-cdc15-2, URA3] plasmid, indicating that they were true bypassers of cdc15A.

Mutant characterization
To determine if Tab+ phenotypes were due to single mutations, cdc15A tab strains were crossed to cdc15A [pMET3-cdc15-2, URA3] and the resulting diploids were sporulated and dissected. Approximately 50% of the viable spores should bypass cdc15A (as indicated by 5-FOA resistance) if the Tab+ phenotype was due to a single mutation. Out of twenty five mutants, fifteen satisfied this criterion, and the other ten were discarded because they either failed to sporulate, or yielded few viable spores after dissection, or did not segregate as single mutations. The same crosses were also used to determine if Tab+ was recessive or dominant: for every

Table 1: S. cerevisiae strains

| Strain | Genotype |
|--------|----------|
| RJD381 | MATa/MATα |
| RJD619 | cdc15-2 pep4::TRP1 MATα |
| WY74   | TAB7-2 MATα |
| WY9    | cdc15-2::TRP1 TAB5-1 [pMET3-CDC15, URA3] [pRS315, LEU2] MATα |
| WY10   | TAB5-1 [pRS315, LEU2] MATα |
| WY11   | cdc15-2::TRP1 tab2-1 [pMET3-CDC15, URA3] MATα |
| WY14   | tab3-1 MATα |
| WY17   | cdc15-2::TRP1 TAB6-1 [pMET3-CDC15, URA3] [pRS315, LEU2] MATα |
| WY18   | TAB6-1MATα |
| WY21   | cdc15-2::TRP1 tab1-1 [pMET3-CDC15, URA3] [pRS315, LEU2] MATα |
| WY34   | cdc15-2::TRP1 TAB7-1 [pMET3-CDC15, URA3] [pRS315, LEU2] MATα |
| WY38   | tab1-1 MATα |
| WY39   | tab2-1 [net1-1] MATα |
| WY41   | cdc15-2::TRP1 tab3-1 [pMET3-CDC15, URA3] MATα |
| WY46   | tem1::GAL-UPL-TEM1/TPR1 bar1::his5+ MATα |
| WY97   | tab3-1 tem1::GAL-UPL-TEM1/TPR1 bar1::his5+ MATα |
| WY214  | cdc5-2::TRP1 [pMET3-CDC5, URA3] MATα |
| WY217  | cdc15-2::TRP1 [pMET3-CDC15, URA3] MATα |
| WY218  | tem1::TRP1 [pGAL1-TEM1, URA3] MATα |
| WY221  | cdc15-2::TRP1 [pMET3-cdc15-2, URA3] MATα |
| WY224  | cdc14::his5+ [HA3-CDC14, URA3] MATα |
| WY280  | dbf2-1::LEU2 dbf20::TRP1 [DBF2, URA3] MATα |
| WY288  | mob1::HIS3 [MOB1, URA3] MATα |

*All strains are in the W303 background (ade2-1 can1-100 his3-11-15 leu2-3-112 trp1-1 ura3-1), which was provided by B. Fuller. [] indicates CEN/ARS plasmid. WY280 and WY288 were derived after backcrossing the original strains into W303 three times.
and tab spores were verified by crossing to cdc15Δ [pMET3::CDC15, URA3], and demonstrating that half of the resulting cdc15Δ segregants were 5-FOA resistant. All cdc15Δ tab mutants were also crossed to strains harboring either [pMET3::CDC15, URA3] [pRS313, HIS3] or [pMET3::CDC15, URA3] [pRS315, LEU2] to introduce these plasmids. The resulting strains grew better than the original cdc15Δ tab strains, and harbored selectable markers that facilitated subsequent crosses.

To test whether net1tab-1 could bypass tem1Δ, dbf2Δ dbf2Δ mob1Δ, cdc14Δ, and cdc5Δ, we crossed net1tab-1 (WY39) to WY218, WY280, WY288, WY243, and WY214, respectively. The diploids were sporulated, and if ~50% of the MEN-deleted spores were resistant to 5-FOA, then net1-1 could bypass the MEN null mutation.

To test temperature sensitivity (ts) of tab mutants, cells were spotted or streaked onto YPD plates, and incubated at 37°C. Their growth was scored after one to two days.

Nop1 immunolocalization was performed as described earlier [15].

The chromosome loss assay was carried out using strain [34] backcrossed into the W303 background by F. Uhlmann. When cells were pre-grown in YPD liquid (instead of SD-URA solid) media before being tested on YPD plates, the percentage of colonies that were red or had red sectors remained similar for wild-type, CDC14TAB-6-1, and bub2Δ strains, but was ~25% for net1tab-1. This number was lower than the 94% value observed in Figure 5, but still significantly higher than the wild-type value (~5%).

**Linkage analysis**

To assign tab mutants to linkage groups, cdc15Δ tab [pMET3::CDC15, URA3] strains were crossed against each other, and the diploids were sporulated and dissected. If two tab mutations belonged to the same linkage group, then all spores were expected to survive on 5-FOA. Otherwise, approximately one quarter of the spores would fail to bypass cdc15Δ. The alleles of TAB2, TAB3, TAB5, TAB6, and TAB7 (see Table 2) were assigned this way. The recessive tab mutants exhibited a ts growth phenotype that cosegregated with the Tab+ phenotype. Complementation tests were used to assign two of the ts mutations as alleles of tab 1. After TAB1 was shown to be MTR10, other tab1 alleles were ascertained by their linkage to the MTR10 locus.

**Molecular cloning of TAB1 and TAB2**

To clone TAB1 and TAB2, tab1-1 and tab2-1 mutant strains were transformed with plasmid libraries harboring yeast genomic DNA fragments, and incubated at 25°C for one day before being shifted to 37°C [59]. Libraries constructed by the laboratories of P. Heiter (ATCC#77164) and R. Young [60] rescued tab1-1, and a 2µ library (ATCC#37323, [61]) rescued tab2-1. Transformants (enough to cover multiple genome equivalents) were screened, and plasmids retrieved from colonies that grew at 37°C were re-transformed into the original mutant strain to verify their complementation activity. Candidate plasmids were sequenced, and the genomic regions containing the complementing activity were identified. TAB1 resided on Chromosome XV, 631,500–637,950, and TAB2, on Chromosome X, 291,900–299,466. To confirm that these genomic fragments indeed carried the TAB genes, complementing fragments were cloned into the integrating vector [pRS305, LEU2], and linearized to target integration into the tab locus in a cdc15Δ::TRP1 tab [pMET3::CDC15, URA3] strain. The transformants lost their Tab+ phenotype, and when crossed to cdc15Δ::TRP1 [pMET3::CDC15, URA3], the diploid yielded no spores that could bypass cdc15Δ (n = 40–50). Since both genomic fragments contained multiple open reading frames (ORFs), each ORF was amplified by PCR from yeast genomic DNA and cloned into the integrating plasmid pRS305. The resulting plasmids were transformed into cdc15Δ::TRP1 tab [pMET3::CDC15, URA3] to identify the ORF that reversed the Tab+ phenotype. This analysis revealed TAB1 to be MTR10, and TAB2 to be YJL076W (NET1).

**Gene replacement**

For gene replacement, the Schizosaccharomyces. pombe his5+ PCR amplification/ transformation method was used [62]. Correct integrants were verified by PCR using primers that amplified a DNA fragment that spanned the recombination junction. To assay if SIC1 or HCT1 was required for the Tab+ phenotype of tab mutants, SIC1 or HCT1 was replaced by his5+ in cdc15Δ::TRP1 tab [pMET3::CDC15, URA3] strains. Each transformant was assayed for the occurrence of correct integration and its ability to survive on 5-FOA.

**Cell cycle synchronization**

Cells were grown in 1% yeast extract/2% peptone (YP) with 2% glucose (YPD), 2% galactose (YPG), or 2% raffinose (YPY). For the experiment described in Figure 1, cdc15-2 (RJD 619) cells were grown to exponential phase in YPD at 30°C, and shifted to 37°C for three hours so that >95% cells were arrested as large-budded cells. Cycloheximide (CHX) was supplemented to a final concentration of 0, 10, or 100 µg/ml to inhibit translation, and α factor (10 µg/ml) was added to trap cells in the subsequent G1 phase. After five minutes, the cultures were released to 25°C at time 0. Samples were taken at various time points after release. The experiment in Figure 3 was carried out as previously described [15].
Cell extract preparation and protein detection

Detection of proteins from crude yeast extracts was as described [15]. The following primary antibodies were used in immunoblotting: anti-Sic1 (1:8000), anti-Cdc2 (1:2500), anti-Cdc28 (1:3000), and 12CA5 (directed against the haemagglutinin (HA) epitope; 1:1000, with 0.5 M NaCl). The first three antibodies were raised in rabbits and affinity purified, and the last one was from mouse.

Acknowledgements

Special thanks go to Ramzi Azzam for making the putative “tab4” mutation to the tab1 linkage group (Table 2) and Kathleen Sakamoto for performing the Nop1 immunofluorescence experiment (Table 2). We thank R. Azzam, K. Sakamoto, M. Budd, R. Feldman, B. Hay, P. Sternberg, and R. Verma for discussions; H. Mountain, P. Lipshitz, A. Varshavsky, B. Futcher, and P. Silber for pMET3, S. pombe his5+, UPL, HAA3, and RPL1 1B-GFP plasmids, respectively; F. Uhlmann and H. Rao for the SUP1/ URA3 sectoring strain; M. Nomura, L. Johnston, and F. Luca for spl, dbf2, dbd20, [pRS316-DBF2], and mob1 [pRS316-MOB1] strains, respectively; C. Thompson for yeast genomic library, and D. Kellogg, R. Feldman, and J. Aris for anti-Cdc2, anti-Sic1, and anti-Nop1, respectively. W.S. was a Howard Hughes Medical Institute predoctoral fellow.

References

1. McCollem D, Gould KM: Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. Trends in Cell Biology 2001, 11:89-95.
2. Peters JM: SCF and APC: the Yin and Yang of cell cycle regulation. Current Opinion in Cell Biology 1998, 10:759-768.
3. Schwab M, Lutum AS, Seufert W: Yeast Hct1 is a regulator of cell cycle exit, independent of controlling mitotic exit. Nature Cell Biology 1999, 1:E47-E53.
4. Morgan DO: Regulation of the APC and the exit from mitosis. Nature Cell Biology 1999, 1:E47-E53.
5. Gruneberg U, Campbell K, Simpson C, Grindlay J, Schiebel E: Nud1p links astral microtubule organization and the control of exit from mitosis. Embo Journal 2000, 19:6475-6488.
6. Donovan JD, Toyn JH, Johnston LH: P40(Sdb2), a putative Cdk inhibitor, has a role in the M/G1 (1) transition in Saccharomyces cerevisiae. Genes & Development 1994, 8:1640-1653.
7. Toyn JH, Johnston AL, Donovan JD, Toome WM, Johnston LH: The Swi5 transcription factor of Saccharomyces cerevisiae has a role in exit from mitosis through induction of the cdk-inhibitor Sic1 in telophase. Genetics 1997, 145:85-96.
8. Charles JF, Jaspersen SL, Tinker-Kulberg RL, Hwang L, Sidon A, Morgan DO: The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in S. cerevisiae. Current Biology 1998, 8:497-507.
9. Jaspersen SL, Charles JF, Tinker-Kulberg RL, Morgan DO: A late mitotic regulatory network controlling cyclin destruction in Saccharomyces cerevisiae. Molecular Biology of the Cell 1998, 9:2803-2817.
10. Fraschini R, Formenti E, Lucchini F, Piatti S: Bifurcation of the mitotic checkpoint pathway in budding yeast. Proceedings of the National Academy of Sciences of the United States of America 1999, 96:4989-4994.
11. Bardin AJ, Visintin R, Amon A: A mechanism for coupling exit from mitosis to partitioning of the nucleus. Cell 2000, 102:21-31.
12. Pereira G, Holfken T, Grindlay J, Manson C, Schiebel E: The Bud2p spindle checkpoint links nuclear migration with mitotic exit. Molecular Cell 2000, 6:1-10.
13. Hu FH, Wang YC, Liu D, Li YM, Qin J, Elledge SJ: Regulation of the Bud2p/Bif1l GAP complex by Cdc5 and cell cycle checkpoints. Cell 2001, 107:653-665.
14. Shou WY, Seol JH, Shvchenko A, Baskerville C, Moazed D, Chen ZHWS, Jang J, Shvchenko A, Charbonneau H, Deshaies RJ: Exit from mitosis is triggered by Tem1-dependent release of the protease Cdc14 from nuclear RENT complex. Cell 1999, 97:233-244.
15. Straight AF, Shou WY, Dowd GJ, Turk C, Deshaies RJ, Johnson AD, Moazed D: Net1, a Sir2-associated nuclear protein required for rDNA silencing and nuclear integrity. Cell 1999, 97:245-256.
16. Visintin R, Hwang ES, Amon A: Cdf1 prevents premature exit from mitosis by anchoring Cdc14 phosphate in the nucleus. Nature 1999, 398:818-823.
17. Shou WY, Sakamoto KM, Keener J, Morimotow K, Traverso EE, Azzam R, Hoppe G, Feldman RM, DeMondea J, Moazed D, Charbonneau H, Nomura M, Deshaies RJ: Net1 stimulates RNA polymerase I transcription and regulates nuclear structure independently of controlling mitotic exit. Molecular Cell 2001, 8:45-55.
18. Visintin R, Craig K, Hwang ES, Prinz S, Tyers M, Amon A: The phosphatase Cdc14 triggers mitotic exit by reversal of CDK-dependent phosphorylations of Sic1. Cell 1999, 97:279-288.
19. Aernle BL, Johnson AL, Toyn JH, Johnston LH: SWI5 controls a novel wave of cyclin synthesis in late mitosis. Molecular Biology of the Cell 1998, 9:945-956.
20. Liu HY, Toyn JH, Chiang YC, Draper MP, Johnston LH, Denis CL: DBF2, a cell cycle-regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. Embo Journal 1997, 16:5289-5298.
21. Liu HY, Toyn JH, Chiang YC, Draper MP, Johnston LH, Denis CL: DBF2, a cell cycle-regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. Embo Journal 1997, 16:5289-5298.
22. Schneider BL, Yang QH, Futcher AB: Linkage of replication to start by the Cdk inhibitor Sic1. Science 1999, 282:560-562.
23. Tyers M: The cyclin-dependent kinase inhibitor p40(SIC1) imposes the requirement for Cln G1 cyclin function at start. Proceedings of the National Academy of Sciences of the United States of America 1999, 96:7772-7776.
24. Alexander G, Uhlmann F, Mechtler K, Poupaut MA, Nasmyth K: Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. Cell 2001, 104:459-472.
25. Schneider BL, Yang QH, Futcher AB: Linkage of replication to start by the Cdk inhibitor Sic1. Science 1999, 282:560-562.
26. Song SG, Lee KS: A novel function of Saccharomyces cerevisiae Cdc5 in cytokinesis. Journal of Cell Biology 2001, 152:451-469.
27. Luca FC, Winey M: MOB1, an essential yeast gene required for completion of mitosis and maintenance of ploidy. Molecular Biology of the Cell 1998, 9:29-46.
28. Frenz LM, Lee SE, Fesquet D, Johnston LH: The budding yeast Dbf2 protein kinase localises to the centromere and moves to the bud neck in late mitosis. Journal of Cell Science 2000, 113:339-3408.
29. Lippincott J, Shannon KB, Shou WY, Deshaies J, Li R: The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. Journal of Cell Science 2001, 114:1379-1386.
30. Menssen R, Neutzner A, Seufert W: Asymmetric spindle pole localization of yeast Cdc15 kinase links mitotic exit and cytokinesis. Current Biology 2001, 11:345-350.
31. Luca FC, Mody M, Kurischko C, Roof DM, Giddings TH, Winey M: Saccharomyces cerevisiae Mob1p is required for cytokinesis and mitotic exit. Molecular and Cellular Biology 2001, 21:6972-6983.
32. Wang YC, Hsu FH, Elledge SJ: The Bfa1p/Bub2 GAP complex comprises a universal checkpoint required to prevent mitotic exit. Current Biology 2000, 10:1379-1382.
33. Schuyler SC, Peilman D: Search, capture and signal: games microtubules and centrosomes play. Journal of Cell Science 2001, 114:247-255.
34. Spencer F, Gerring SL, Connelly C, Hieter P: Mitotic Chromosome Transmission Fidelity Mutants in Saccharomyces-Cerevisiae. Genetics 1990, 124:237-249.
35. Kadowaki T, Chen YP, Hackett M, Jacobs E, Kumagi C, Liang S, Schieder R, Singleton D, Wistow J, Tartakoff AM: Isolation and Characterization of Saccharomyces-Cerevisiae Messenger-Rna Transport-Defective (Mtr) Muts. Journal of Cell Biology 1994, 126:649-659.
36. Pemberton LF, Rosenblum JS, Blobel G: A distinct and parallel pathway for the nuclear import of an mRNA-binding protein. Journal of Cell Biology 1997, 139:1645-1653.
37. Senger B, Simos G, Bischoff FR, Podtelejnikov A, Mann M, Hurt E: Mtr1Op functions as a nuclear import receptor for the mRNA-binding protein Npl3p. Embo Journal 1998, 17:2196-2207.
38. Nakielny S, Dreyfuss G: Transport of proteins and RNAs in and out of the nucleus. Cell 1999, 99:677-80
39. Stage-Zaher U, Zepeda-U, Silver PA: Factors affecting nuclear export of the 60S ribosomal subunit in vivo. Molecular Biology of the Cell 2000, 11:3777-3789
40. Johnston LH, Eberly SL, Chapman JW, Araki H, Sugino A: The Product of the Saccharomyces-Cerevisiae Cell-Cycle Gene Dbf2 Has Homology with Protein-Kinases and Is Periodically Expressed in the Cell-Cycle. Molecular and Cellular Biology 1990, 10:1358-1366
41. Zachariae W, Schwab M, Nasmyth K, Seufert W: Control of cyclin ubiquitination by Cdk-regulated binding of Hcti to the anaphase promoting complex. Science 1998, 282:1721-1724
42. Burke DJ, Church D: Protein-Synthesis Requirements for Nuclear Division, Cytokinesis, and Cell-Separation in Saccharomyces-Cerevisiae. Molecular and Cellular Biology 1991, 11:3691-3698
43. Charbonneau H: Characterization of the Net1 cell cycle-dependent regulator of the Cdc14 phosphatase from budding yeast. Journal of Biological Chemistry 2001, 276:21924-21931
44. Shirayama M, Zachariae W, Ciosk R, Nasmyth K: The polo-like kinase Cdc5p and the WD-repeat protein Cdc30p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae. Embo Journal 1998, 17:1336-1349
45. Sanchez Y, Bachan J, Wang H, Hu FH, Liu D, Tetzlaff M, Elledge SJ: Control of the DNA damage checkpoint by Chkl and Rad53 protein kinases through distinct mechanisms. Science 1999, 286:1166-1171
46. Shirayama M, Matsui Y, Tohe A: The Yeast Tem1 Gene, Which Encodes a GTP-Binding Protein, Is Involved in Termination of M-Phase. Molecular and Cellular Biology 1994, 14:7476-7482
47. Lee SE, Frenz LM, Wells NJ, Johnson AL, Johnston LH: Order of function of the budding-yeast mitotic exit-network proteins Tem1, Cdc5, Mobi, Dbf2, and Cdc5. Current Biology 2001, 11:784-788
48. Malik AS, Jang J, Deshaies RJ: Protein kinase Cdc15 activates the Dbf2-Mobi kinase complex. Proceedings of the National Academy of Sciences of the United States of America 2001, 98:7325-7330
49. Visintin R, Amon A: Regulation of the mitotic exit protein kinases Cdc15 and Dbf2. Molecular and Cellular Biology 2001, 12:2961-2974
50. Jaspersen SL, Charles JF, Morgan DO: Inhibitory phosphorylation of the APC regulator Hcti is controlled by the kinase Cdc28 and the phosphatase Cdc14. Current Biology 1999, 9:227-236
51. Proick DJ, Schlenstedt G, Pellman D, Korshunov D, Fink GR: The Yeast Nuclear Import Receptor Is Required for Mitosis. Molecular Biology of the Cell 1995, 6:1994
52. Liu Y, Guo W, Tartakoff PY, Tartakoff AM: A Crmlp-independent nuclear export path for the mRNA-associated protein, Npl3p/Mtr1p. Proceedings of the National Academy of Sciences of the United States of America 1999, 96:6739-6744
53. Arlatchon JD, Blobel G, Rout MP: Kap104p: A karyopherin involved in the nuclear transport of messenger RNA binding proteins. Science 1996, 274:624-627
54. Nachury MV, Maresja T, Salmon WG, Waterman-Storer CM, Heald R, Wei K: Importin beta is a mitotic target of the small GTPase ran in spindle assembly. Cell 2001, 104:95-106
55. Wiese C, Wilde A, Moore MS, Adam SA, Merdes A, Zheng YX: Role of importin-beta in coupling ran to downstream targets in microtubule assembly. Science 2001, 291:653-656
56. Sherman F: Getting Started with Yeast. Methods in Enzymology 1991, 194:3-21
57. Sikorski RS, Hieter P: A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces-Cerevisiae. Genetics 1989, 122:19-27
58. Sikorski RS, Boeke JD: In vitro Mutagenesis and Plasmid Shuffling – from Cloned Gene to Mutant Yeast. Methods in Enzymology 1991, 194:302-318
59. Rose MD, Broach JR: Cloning Genes by Complementation in Yeast. Methods in Enzymology 1991, 194:195-230
60. Thompson CM, Koleske AJ, Chao DM, Young RA: A Multisubunit Complex-Associated with the Rna Polymerase-ii Ctd and TATA-Binding Protein in Yeast. Cell 1993, 73:1361-1375
61. Nasmyth KA, Reed SI: Isolation of Genes by Complementation in Yeast Molecular Cloning of a Cell-Cycle Gene. Proc Natl Acad Sci USA 1980, 77:219-2123
62. Wach A, Brachat A, AlbertsSegu C, Rebischung C, Philippsen P: Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in Saccharomyces cerevisiae. Yeast 1997, 13:1065-1075