Genetic characterization of the abscission pathway and of its regulation by
the NoCut checkpoint in the budding yeast Saccharomyces cerevisiae

A dissertation submitted to
ETH Zürich

for the degree of
Dr. sc. ETH Zurich

presented by

Harald M. Rauter

Magister rer. nat.
University of Vienna, Austria

born, November 3rd 1978
Klagenfurt, Austria

Accepted on the recommendation of

Prof. Yves Barral, examiner
Prof. Stephen J. Doxsey, co-examiner
Prof. Markus Aebi, co-examiner

2009
Acknowledgements:

I would like to thank Yves for the great opportunity and the challenge to perform my PhD training in your lab, for the support during this difficult and demanding time. I have profited massively for myself through your guidance and your critical mind - thank you very much!!

I would like to thank the entire Barral group for the support and the critical discussions during lab meetings and coffee breaks - I wish you guys all the best for your careers and in your private lives. I am tremendously grateful to the former members of the Barral group for introducing me to the work with yeast and for creating a wonderful atmosphere in lab.

A special thanks to my thesis committee - Steve Doxsey, Markus Aebi and Andreas Mayer - for taking the effort supporting me and my project during this time of my PhD.

I am thankful to the entire D-floor, groups of Patrick and Daniel, whose suggestions for good experiments often helped to see again light at the end of the tunnel.

Many, many thanks go to the IBC, that creates a very stimulating environment and to the staff, that supported me tremendously throughout my PhD, especially Toni, to whom I owe a lot, Roland and Nico for their patience with their most annoying client; the media and D-Biol shop people and Sonja for keeping things running!

A warm thank you to those, who are not listed here and have supported me throughout this period of my PhD.
Si non è vero, è molto ben trovato

(Giordano Bruno 1548 - 1600)
TABLE OF CONTENTS

Acknowledgements 1
Abstract 6
Zusammenfassung 7
Chapter I - Introduction 8
  General Principles In Cytokinesis 10
  Selection Of The Division Site 11
    Regulation Of The Formation Of The Acto-myosin Contractile Ring 13
      in S.cerevisiae 13
      in S.pombe 14
      in Metazoan 15
  In Yeast, Septum Formation And CAR Contraction Depend On Each Other 17
  Roles Of Rab And Rho Related GTPases In Cytokinesis 19
  Mitotic Exit Network/Septation Initiation Network 21
  Septins And Their Role In Cytokinesis 25
    Organization Of Septin Filaments 25
    Localization And Function Of Septins In Model Organisms 27
    Septin Dysfunction Is A Cause Of Many Diseases 28

Abscission 29
Checkpoints During The Cell Cycle 34
  The NoCut Checkpoint 35

Aim Of This Work 39

Chapter II - Results Section I - SL Screens Identify Myo1, Hof1 And Cyk3 Pathway Genes 43
  Synthetic Lethality Screens 43
  Rational For The Use Of SGA To Unravel 44
  Novel Genes Involved In Cytokinesis 44
  Generation And Analysis Of Bait Strains For The SGA 46
  Conducting SGA With The Bait Strain Of Interest 46
  Why To Do A Complementary ase1Δ Synthetic Lethal Screen 47
  Genes In The Myo1-pathway 49
  Genes In The Hof1-pathway 51
  Genes In The Cyk3-pathway 53
  Genes Present In All Six Screens 56
  Genes Present In The "sl With ase1Δ"-Pathway 58
Discussion

The Myo1-Pathway 59
The Hof1-Pathway 59
The Cyk3-Pathway 60
The Fourth Cytokinetic Pathway? 61
The "sl with aselΔ"-Pathway? 63

Chapter II - Results Section II - The NoCut Checkpoint Targets the GTPase Rho2 To Inhibit the Completion of Abscission

NoCut Interacts With Abscission 70
Tus1 And Cyk4 Are Displaced From The Bud Neck In The NoCut Response 70
Cytokinetic Roles Of The Cyk3-Pathway Components 73
Tus1 And Cyk4 Are Interdependent For Localization And Depend On Myosin II For Localization 76
Genetics And Localization Studies 77
Implicate The GTPase Rho2 In Abscission 77

Chapter II - Results Section III - Novel Cyk6 supports cytokinesis in S.cerevisiae

Swi5 Is Needed For Cell Integrity 83
The Swi5 Target Cyk6 Is Needed For Cell Integrity 84
Cyk6 Accumulates At The Bud Neck In Late Mitosis 85
Cyk6 Supports Acto-myosin Ring Contraction 87
Cyk6 Is Required For Plasma Membrane Resolution 89
Discussion 92

Chapter III - Conclusions and Perspectives

General Conclusions 96
Differential And Specific Activation Of Rho2 Is Required To Promote Abscission In S.cerevisiae 96
Outlook On Rho2 Function In Abscission 98
A Comprehensive Model Fort The Inhibition of abscission in response To Spindle and Chromosome Segregation Defects 101
What "really" triggers NoCut? 102
How Is The NoCut Signal Conveyed To The Bud Neck 104
NoCut Prevents Activating Factors Of Abscission From Localizing To The Bud Neck 106

Chapter IV - Materials and Methods

Strains, Plasmids And Growth Conditions 109
Synthetic Lethal Screens 110
Abstract

Abscission defines the terminal step of cytokinesis where one continuous plasma membrane is resolved into two independent membranes giving rise to a mother and a daughter cell. Although the mechanistic principles of abscission are only beginning to be understood, recent studies established that abscission is highly coordinated with chromosome segregation in yeast as well as in mammals. This co-ordination is ensured by a novel checkpoint called NoCut. The NoCut checkpoint reversibly inhibits abscission in cells with spindle mid-zone defects and/or defects in chromosome segregation. This inhibition is of particular importance as it prevents to “cut” chromosomes and thereby prevents gross chromosomal rearrangements (GCRs) in surviving daughters. However it is unknown how NoCut interferes with abscission itself.

In this work we performed comprehensive genetic analysis of the three major cytokinetic pathways Myo1, Hof1 and the less characterized Cyk3 pathway in *Saccharomyces cerevisiae*. We provide data showing that the Cyk3 pathway represents the abscission pathway. We show that high levels of Rho2 at the bud neck are required to promote abscission in *S. cerevisiae*. We further show that the active NoCut checkpoint displaces Tus1, a Rho2 GEF, from the bud neck, thereby inhibiting abscission. Tus1 re-localizes to the bud neck in cases where NoCut is artificially switched off, and promotes abscission. Thus, NoCut interferes with abscission by regulating Rho2 activity at the bud neck via its GEF Tus1.

Additionally we characterize four previously uncharacterized open reading frames and show their contribution to successful and complete budding yeast cytokinesis.

In conclusion, this work provides new insights in how the abscission machinery regulates membrane fission and provides new insights in the mechanism of how abscission is inhibited by the NoCut checkpoint.
Zusammenfassung:


Darüberhinaus charakterisieren wir vier neue, bislang unbeschriebene Leseraster und zeigen ihren Beitrag zu erfolgreicher Zytokinese.

Zusammenfassend beschreiben wir in dieser Arbeit eine erweiterte Sicht, wie Zellabtrennung in der Bäckerhefe reguliert wird, und beschreiben einen möglichen Mechanismus, wie der NoCut Prüfmechanismus die Zellabtrennung reguliert.
Chapter I: Introduction

Anaphase  Cytokinesis  Abscission
Front image: HeLa cells undergoing cytokinesis

Image taken from Zhao et al, Mol Biol Cell 2006
Spindle in red, Chromatin in blue and CPC in yellow
General principles in cytokinesis

Cytokinesis defines the cell division process itself that takes place at the end of mitosis. Through cytokinesis a binucleated cell irreversible and physically separated into two independent daughter cells, each containing a single nucleus. Ultimately, this process is completed by the resolution of the plasma membrane into two independent plasma membranes.

During the last 10 years, cytokinesis has become a widely studied field involving many model organisms ranging from yeasts *S.cerevisiae*, *S.pombe* to worms *C.elegans* and flies *D.melanogaster*. Ultimately, Technical advances like RNAi and automated high throughput microscopy have made it possible to study cytokinesis efficiently also in mammalian cells. These advances are of particular importance as a long - standing hypothesis on the mechanism of tumorigenesis states that the failure of cell division results in the formation of genetically unstable tetraploid cells. This tetraploidization is considered one of the major and common sources for various types of cancers. Ultimately, These ideas put cytokinesis into the spot-light in the quest for a cure for cancer.

Although different organisms from different kingdoms have evolved different strategies to accomplish cytokinesis some of the players and molecular cues involved are remarkably conserved throughout.

In the following introductory section, I will summarize the state of the art of our knowledge on how the cleavage plane is determined, how the contractile ring is formed, how furrow ingression is regulated and finally how abscission, the plasma membrane fission event, is regulated.
Selection of the division site

It seems intuitive to think that cytokinesis is tightly co-ordinated with chromosome segregation. Indeed, organisms from yeast to mammals spatially organize their cytokinetic apparatus relative to the chromosome segregation machinery but have evolved different mechanisms to do so.

Budding yeast *S. cerevisiae* has a rigid cell body due to a cell wall and positions the mitotic spindle relative to the predetermined cleavage plane and thus determine the orientation of the mitotic spindle perpendicular to the division site which ultimately allows chromosome segregation into mother and daughter cell. A Kar9 (REF Kusch/Barral 2002) and a Dynein (REF Grava/Barral 2006) dependent pathway ensure eventually in metaphase and anaphase respectively the correct positioning of the mitotic spindle perpendicular of the cleavage plane.

In yeast *S. pombe* and plants it is the position of the nucleus that determines the future position of the cleavage plane prior to mitosis. Mid1, an anillin homologue, is an early landmark that localizes to the cell cortex depending on the position of the nucleus (REF...
Plants position the nucleus in the cell by controlled interaction with the cell cortex. Early in mitosis, before prophase, a so-called pre-prophase band (PPB) is formed which consists to a great extend of microtubules, but also of F-actin filaments, which circumscribes the nucleus and thereby marks the future division site. This recruitment to the future division site depends on the microtubule dependent kinesins Pok1 and Pok2. This pre-prophase band then disassembles upon nuclear envelope breakdown and leaves the protein TANGLED back, which serves as a spatial landmark for the recruitment of the phragmoplast (REF Walker/Smith 2001). Subsequent to Cdk inactivation (REF Jürgens 2005) the phragmoplast facilitates construction of new cell wall material.

In higher eukaryotes e.g. *C.elegans, D.melanogaster* and mammals the position of the cleavage plane is determined by multiple factors. Early reports stated that the position of the mitotic spindle is key for the determination of the cleavage plane, specifically signals from the central spindle (REF Dechant/Glotzer 2003). The central mitotic spindle is the site where the anti-parallel, non-kinetochore microtubules are bundled, which enables the spindle to exhibit a pushing force during anaphase. It appears to be the critical signaling structure (REF Glotzer 2004) for cleavage furrow positioning. Additional supportive evidence comes from the fact that specific cells of *D.melanogaster* successfully accomplish cytokinesis although they lack centrioles and therefore lack astral microtubules (REF Basto/Raff 2006). On the other hand early embryonic cells from *C.elegans* manage to ingress a furrow at the right position devoid a functional central spindle suggesting an alternative mechanism to establish a cleave furrow (REF Jantsch-Plunger/Glotzer 2000), which requires centrosomes and astral microtubules for proper positioning of the cleavage furrow. However recent data from the *C.elegans* embryo are able to combine these two opposing observations. Elegant experiments using laser ablation and thereby dissecting the requirements of spindle midzone and astral microtubules suggest a model that correct positioning of the cleavage furrow depends on two consecutive signals first from the centrosome dependent microtubule asters and secondly by the signals specified by the spindle midzone (REF Bringmann/Hyman 2005), both of which are sufficient for cleavage, which is accordance with the observations outlined above. However robust positioning of the cleavage furrow requires both signals.
Regulation of the formation of the acto-myosin contractile ring structure

The acto-myosin based ring structure has been shown to play an important role in cytokinesis in fungi and animal cells, but not in plants. It is generally accepted that myosin II motors slide along actin filaments, which causes constriction, pulls the plasma membrane inwards and this spatial configuration ultimately allows abscission, which will be discussed extensively later.

Although the acto-myosin based ring structure has been described throughout all kingdoms except the plant kingdom (REF Barr/Gruneberg 2007) it is dispensable in many cases. Some sea urchins species accomplish cytokinesis without a contractile acto-myosin structure. Myosin encoding genes are dispensible in many strain backgrounds in yeast S.cerevisiae.

In the following paragraphs I will briefly review our state of the art understanding of how the actomyosin structure is established and controlled throughout the different model organisms.

in S.cerevisiae

The site where cytokinesis takes place in S.cerevisiae is called the mother bud-neck. It defines an already narrow constriction (≈1.1 - 1.2μm) between the mother and the daughter cell. This is exactly why it has been assumed for a very long time that S.cerevisiae accomplishes cytokinesis without the help of a contractile acto-myosin ring structure. However, S.cerevisiae cytokinesis does also relies on such a pathway for efficient cytokinesis although it is dispensable in many strain backgrounds (REF Bi/Pringle 1998). I will refer to this pathway as the Myo1-dependent pathway from now on according to the gene, which encodes the type II myosin, MYO1 (REF Bi, Pringle 1998). Myo1 is recruited to the bud-neck very early in the cell cycle, around the G1/S transition, as the bud starts to emerge (REF Vallen/Bi 2004), but isn’t yet activated. The recruitment to the bud-neck happens in a septin dependent manner. Along with Myo1 the formin Bnr1 is recruited to the bud-neck as well. Hof1, which is the homologue in S.pombe Cdc15 is recruited shortly after in a septin dependent but Myo1 independent fashion (REF Lippincott/Li 1998). Genetically Hof1 and Myo1 act in parallel pathways and Hof1 is thought to facilitate Myo1 contraction by (a) bending the plasma membrane (REF
Tsujita/Takenawa 2006, Chitu/Stanley 2007) and (b) remodeling the actin cytoskeleton (REF Aspenström/Richnau 2006). I refer to this genetically distinct pathway as the Hof1 pathway. Iqg1, an IQGAP homologue (REF Ko/Pringle 2007, Shannon/Li 2000), Bni1, the second yeast formin (REF Dong/Bretscher 2003, Buttery/Pellman 2007), Rho1 (REF Tolliday/Li 2002), and actin arrive at the bud-neck in late anaphase (REF Bi/Pringle 1998). Polo/Cdc5-dependent phosphorylation of Tus1 and Rom2, the GEFs of Rho1 supports the formation of the contractile ring (REF Yoshida/Pellman 2006). Contraction is triggered as the cells exit mitosis (REF Jensen/Segal 2004) and the mitotic spindle is disassembled (REF Buvelot/Biggins 2003).

How Myo1-dependent contraction is achieved, is not known at the molecular level. It has been shown that the contraction of the ring is interdependent with the formation of the cell wall (REF Schmidt/Cabib 2002). A recent report implicates the essential protein Inn1 in the process of contraction. Inn1 is not required for the formation of the contractile ring but is required to stabilize the contracting ring and possibly also to induce curvature of the plasma membrane adjacent to the contractile ring, which facilitates ingression of the membrane. The C2 domain of Inn1 seems to act both in the Myo1-dependent as well as in the Hof1-dependent pathway, which would explain the essential function of the gene in cytokinesis (REF Sanchez-Diaz/Labib 2008).

In S. pombe

The site of cytokinesis in fission yeast is the medial cortex. Many components required for assembly and contraction of the contractile acto-myosin ring, are conserved between the two yeast species S. pombe and S. cerevisiae. However, their timing and succession of recruitment and their function partially differs (REF Vavylonis/Pollard 2008, Wu/Pollard 2008). The most striking difference to S. cerevisiae is that septins, essential in yeast, are completely dispensable for cytokinesis in S. pombe. The priming molecule for the assembly of the contractile acto-myosin structure in fission yeast is the anillin-homologue Mid1. Mid1 establishes a broad band of nodes in the cortex. The duplicated type II myosins Myo2p and Myp2p, the myosin ligh chain Cdc4 and regulatory light chain Rlc1, as well as Rng2, the IQGAP homologue; Cdc15 and the formin Cdc12 are recruited subsequently to a band around the site of cytokinesis. This broad band of nodes coalesce into a compact ring structure once the formin Cdc12 and profilin Cdc3...
stimulate actin polymerization (REF Wu/Pollard 2005). It is worthwhile mentioning that there is strong evidence, that the Arp2/3 complex is not involved in the maturation of the contractile ring, yet Arp2/3 complex is required for cytokinesis.

The activated ring rests in check until the Septation Initiation Network (SIN) allows cytokinesis to start (REF Krapp/Simanis 2004).

How Myo2/Myp2-dependent contraction is achieved, is also not known at the molecular level in S. pombe. At least recent studies strongly suggest that a well defined stoichiometric equilibrium of all acting proteins is required to successfully contract the ring (REF Wu/Pollard 2006). Active myosin consists as a hexamer, which comprises two myosin heavy chains, where each one is again associated with an essential myosin light chain (ELC) and a regulatory light chain (RLC). This complex protein structure suggests a tight regulation of high complexity. However, S. pombe can accomplish fully functional contraction with the non catalytic tail domain alone devoid the catalytic head domain (REF Lord/Pollard 2005). These results shed new light on the anticipated mechanism on how active myosin binds actin and how the ATPase activity of myosin II drives cytokinesis and shows that the motor domain is dispensable for myosin II function in contractile ring contraction.

In Metazoan

The least understood cytokinetic system, both mechanistically and regulatory, is the metazoan system. As discussed earlier, the positioning of the cleavage furrow depends on consecutive signals from the astral microtubules first and secondly from the spindle midzone (REF Bringmann/Hyman 2005).

However, both theories agree on the fact that activated RhoA at the correct region at the cell cortex is required for the organization of the contractile ring in animal cells (REF Piekny/Glotzer 2005, Yuce/Glotzer 2005). RhoA is a typical GTPase, meaning that Guanine Exchange Factors (GEF) and GTPase Activating Proteins (GAP) regulate its activity. Small GTPases are discussed in detail below. During the cytokinesis of animal cells, Ect2 is the most prominent RhoA GEF. MgcRacGAP (REF Pienky/Glotzer 2005) is the most prominent GAP for RhoA. Both are essential for proper RhoA function in cytokinesis. Ect2 phosphorylation during metaphase is key for its function to activate
RhoA during cytokinesis (REF Tatsumoto/Miki 1999). However this seems not to be the sole function of Ect2, as genetic data suggest additional, undefined functions of Ect2 (REF Echard/O’Farrell 2004).

MgcRacGAP in turn forms together with the kinesin Mklp1 the so-called "centralspindlin" complex (REF Mishima/Glotzer 2002, Minoshima/Kitamura 2003), which is suggested to link RhoA regulation at the cell cortex with the central spindle microtubules (REF Guse/Glotzer 2005, Minoshima/Kitamura 2003). Polo-like serine/threonine kinase Plk1 phosphorylates MgcRacGAP (REF Burkard/Jallepalli 2007). Only upon this phosphorylation Ect2 translocates to the central spindle to become part of a ternary complex with MgcRacGAP and Mklp1 (REF Brennan/Straight 2007, Santamaria/Wortmann 2007, Petronczki/Peters 2007) and thus restrict RhoA activation to the cortex (REF Somers/Saint 2003). Recent studies reported that furrow positioning is a very dynamic process that involves feedback loops from the cortex to the microtubules and back (REF Hu/Mitchison 2008). The authors suggest that RhoA is transported to the cortex by kinesins, where it promotes acto-myosin assembly. This specialized cortex feeds back to the microtubules in the vicinity by a yet to be defined mechanism that promotes microtubule stabilization/bundling. In line with this, other groups (REF Miller/Bement 2009) suggest such a dynamic regulation as well for RhoA activation. The model proposes that Ect2 and MgcRacGAP act simultaneously and RhoA cycles constantly between the GTP and the GDP bound state. MgcRacGAP would bind to RhoA-GTP but as the affinity of activated RhoA to its substrates is higher than to the GAP, activated RhoA would signal downstream and only eventually RhoA will be inactivated by its GAP. In experiments, inactive GAP domains interfere with this flux balance and as a consequence the contractile ring structure becomes less refined. Aurora B phosphorylation of MgcRacGAP has been reported (REF Guse/Glotzer 2004) but seems to play a marginal role in this event as the consensus Aurora B phosphorylation (REF Minoshima/May 2003) site is not conserved throughout all species. It should be stated that the role of Aurora B in cytokinesis should not be neglected. Depletions of Aurora B result in hyperstabilized central spindles (REF Buvelot/Biggins 2003) and in defects in cleavage furrow ingression, which suggests a key role of Aurora B and its associated Chromosomal Passenger Complex in promoting several steps during cytokinesis (REF Kaitna/Glotzer 2000, Gruneberg/Barr 2006). However a couple of open questions remain on how the furrow is positioned and furrow ingression is
initiated. Recent studies have implicated phosphatidylinositol lipid PI(4,5)P2 in cytokinesis (REF Field/Cantley 2005, Janetopoulos/Devreotes 2005). PI(4,5)P2 lipids are enriched in the cleavage furrow, but one can only speculate about their direct involvement in furrow ingression. Yet an indirect mechanism might be, that PI(4,5)P2 lipids would facilitate furrow ingression by tethering many factors like Ect2 or actin binding protein Anillin, that harbor a PH domain to the ingressing furrow. Concerning furrow ingression it is certain that it involves actin, driven by formin mDia and possibly Arp2/3 complexes and myosin II. A tempting hypothesis proposes that comparably to fission yeast, nodes of actin-myosin and associated proteins (REF Eckley/Earnshaw 1997, Werner/Glotzer 2007) may coalesce and form an activated acto-myosin contractile ring. Recent advances in C.elegans embryo cytokinesis research suggest that the MgcRacGAP homolog Cyk-4 in the centralspindlin complex inactivates Rac and its effectors, which in turn allows RhoA activation and this differential activation of RhoA is vital to drive furrow ingression (REF Canman/Oegema 2008).

**In yeast, septum formation and CAR Contraction depend on each other**

In fungi, cell division has to be coordinated with the synthesis of new cell wall material (REF Schmidt/Cabib 2002). The cell wall has the function to keep the rigid cell-wall structure intact. As proteins required for cytokinesis and abscession, cell wall assembly enzymes and cell wall components (e.g. glucans in yeast) are delivered via vesicle-mediated transport. In both fission and budding yeast, cell wall synthesis pathways is coordinated with acto-myosin ring contraction (REF Schmidt/Cabib 2002). As the myosin II based acto-myosin ring contracts after exit of mitosis, the chitin synthase Chs2-dependent pathway synthesizes a chitin rich cell wall called the primary septum. Chs2 targeting to the site of septation depends on post Golgi-vesicles as well as on the exocyst. Additionally activation of the Chs2 pathway relies on cyclin degradation, like for activation of the Myo1 pathway (REF Verplank/Li 2005). The Myo1- and Chs2-dependent pathways are so tightly linked to each other that depletions of the two genes have no additive effects (see Fig.9). Once the ring has successfully contracted and the primary septum is deposited, the Chs3 and Fks1/2-dependent secondary septum formation machinery deposits a thick secondary septum rich in β-1,3-glucan, β-1,6-glucan, α-1,3-glucan, α-galactomannan (REF Humbel/Osumi 2001) and partially chitin. Chs3 localizes
to the bud-neck in a septin dependent manner (REF Schmidt/Cabib 2003) and is delivered to the bud-neck in a Ypt31/32-dependent manner (REF Ortiz/Novick 2006). In contrast to chs2Δ mutations in the chs3Δ deletion greatly worsens the phenotype of myo1Δ and chs2Δ deleted cells, suggesting that the Chs3 dependent pathway represents an essential additional pathway for successful cytokinesis in yeast (REF Schmidt/Cabib 2002). Co-ordination of Myo1/Chs2-dependent contraction of the acto-myosin ring and primary septum formation with subsequent Chs3-dependent secondary septa formation leads to the formation of a trilaminar structure as depicted in Fig.9. Ultimately the endochitinase Cts1 digests the primary septum, such as to separate the daughter cells. Cts1 is specifically expressed only once the cells have successfully undergone mitotic exit. This activation cascade is highly regulated and only activated in the daughter cell by the transcription factor Ace2 (REF O’Conallain/Butler 1999). The model proposes that this localization of Ace2 is controlled on three levels: Transcription, localization and total protein turnover (REF O’Conallain/Butler 1999).

**Fig.9:** Schematic view of septation in wild-type (A-D) and in chs2Δ or myo1Δ mutants (A,E-G). The neck region between the mother and daughter cell is represented, with the cell walls as the grey area and the plasma membrane as the brown line. The red spots indicate the location of Chs2p. Chitin is shown in green. In (B), the membrane invaginates and chitin is laid down in the invagination. Continuation of this process leads to generation of the primary septum disk and to pinching off of the membrane (C). Next, secondary septa (yellow) are built up from both the mother and daughter cell sides. A trilaminar septum results (D). In chs2Δ and myo1Δ mutants, invagination of the plasma membrane in a small area and growth of a primary septum do not take place. Instead, inward growth of cell wall material over a large portion of the plasma membrane pushes the membrane toward the center of the channel (E,F), finally closing the latter and generating a thick and uniform septum (G). In our hypothesis, this represents growth of secondary septa, which would occur at 90° to the normal direction. Thus, in the cell cycle, stage E would start between the normal times for (C) and (D). The green spots in (E-G) designate chitin formed by the action of chitin synthase III that is required for the remedial septa but not for the normal secondary septa. In (G), a lacuna resulting from the uneven fusion of the advancing secondary septum is also shown. Image taken from Schmidt et al, J Cell Sci. 2002 Jan 15;115(Pt 2): 293 - 302

**Roles of Rab and Rho related GTPases in cytokinesis**

Small GTP binding proteins are global key regulators of numerous cell biological processes. This involves prominent events like cytoskeletal organization and rearrangements throughout the entire cell cycle, cell polarization and migration, vesicle
trafficking and cytokinesis. It doesn’t come as a surprise that small GTP-binding proteins are conserved from yeast to mammals and have therefore been studies extensively.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome size (Mb)</th>
<th>Genes</th>
<th>Small GTPase Genes (%)</th>
<th>GAP (%)</th>
<th>The ratio of small GTPases to GAPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. sativa</td>
<td>420</td>
<td>32,000-30,000</td>
<td>111 (0.22-0.35)</td>
<td>85 (0.17-0.27)</td>
<td>1.31 : 1</td>
</tr>
<tr>
<td>A. thaliana</td>
<td>125</td>
<td>25,498</td>
<td>93 (0.30)</td>
<td>65 (0.25)</td>
<td>1.43 : 1</td>
</tr>
<tr>
<td>H. sapiens</td>
<td>2851</td>
<td>20,000-25,000</td>
<td>174 (0.70-0.87)</td>
<td>173 (0.69-0.87)</td>
<td>1.01 : 1</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>120</td>
<td>13,600</td>
<td>90 (0.66)</td>
<td>64 (0.47)</td>
<td>1.41 : 1</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>12</td>
<td>6,034</td>
<td>30 (0.50)</td>
<td>35 (0.58)</td>
<td>0.86 : 1</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>-</td>
<td>498</td>
<td>422</td>
<td>1.18 : 1</td>
</tr>
</tbody>
</table>

**Fig.2:** Overview over the predicted small GTPases in eukaryotes and their ratio to predicted GAPs. Image taken from Jiang et al., Physiol Genomics 24:235-251, 2006

Rab and Rho related GTPases represent a subgroup of the small GTPases family. The majority of the GTPases are "classically activated" GTPases meaning that their activity depends on associated proteins. Guanine Exchange Factors (GEF) activate small GTPases, GTPase Activating Enzyme and the Guanine Dissociation Inhibitor (GDI) recycle small GTPases and prepare them for a next round of activation (REF Heasman/Ridley 2008). A subgroup of the total small GTPases belongs to the "atypical" class of small GTPases, whose activity is not controlled by GTP - GDP cycling but by transcriptional control, protein modification and total protein turnover of the constitutively active form of the protein. Prominent members of this class are RhoH and Rnd1 - 3.

Of particular interest to this work is the fact that numerous reports throughout the last decade convincingly implied Rab and Rho related GTPases in cytokinesis (REF Simon/Prekeris 2008, Pohl/Jentsch 2008).

As discussed above, Rab and Rho related GTPases are essential for the establishment and formation of the acto-myosin contractile structure from yeast to mammals. In *S. cerevisiae*, Cdc5 phosphorylates Tus1 and Rom2, both prominent GEFs for Rho1,
which in turn promotes actin organization at the bud-neck and therefore contractile ring formation (REF Yoshida/Pellman 2006). Fission yeast Pxl1 has been shown to act as a GAP for Rho1 (REF Pinar/Perez 2008) and upon its deletion cells are not able to properly form a contractile ring and exhibit a delay in contraction. Interplay between Ect2 and MgcRacGAP is required to promote furrow positioning and possibly also furrow ingression in metazoan (REF Hu/Mitchison 2008). Interestingly parallel RhoA activation and inactivation of Rac and its effectors by MgcRacGAP is essential to ingress the cleavage furrow (REF Canman/Oegema 2008). This well described example highlights the required differential activation of GTPases and the enormous array of signal that can be generated through GTPases.

In budding yeast and fission yeast the entry into cytokinesis depends on the Mitotic Exit Network (REF Bardin/Amon 2003) and the Septation Initiation Network (REF Krapp/Simanis 2007) respectively. These networks ensure that cytokinesis only happens once chromosome segregation is successfully accomplished. In both networks a small GTPase is the most upstream factor and is thus essential for maintaining chromatin integrity at the end of anaphase. The MEN and the SIN will be discussed later in detail.

Vesicle mediated transport to the cleavage furrow has been shown to be essential for cytokinesis in numerous reports (REF Giansanti/Gatti 2007, Vjestica/Oliferenko 2008 Kasahara/Yamaguchi 2007). Rab proteins, the largest branch of the Ras GTPase, superfamily, orchestrate this intracellular traffic. Being classically activated GTPases Rabs use the guanine nucleotide-dependent switch mechanism to regulate each of the four major steps in membrane traffic: (1) vesicle budding from the donor membrane, (2) vesicle delivery to the target membrane, (3) vesicle tethering to the target membrane and (4) fusion of the vesicle with the target compartment (REF Grosshans/Novick 2006).

It is important to note that there are two kind of budding events. A vesicle can bud into the cytoplasm, which is accomplished by a fission event from outside. A vesicle can also but of away from the cytoplasm, which requires a fission event from inside. This event is in striking similarity with abscission that also requires a fission event from inside. Therefore it is tempting to speculate that there are conserved machineries between budding events and the final act of cytokinesis - abscission (REF Gromley/Doxsey 2005, Kasahara/Yamaguchi 2007, Carlton/Martin-Serrano 2007, Pohl/Jentsch 2008).

Abscission is defined as the process where one continuous plasma membrane is resolved into two independent plasma membranes giving rise to two independent
daughter cells. As the main goal of this work is to characterize the abscission pathway in budding yeast it will be discussed extensively later.

In budding yeast there are around 30 small GTPases known. Cdc42, Rho1, Rho2, Rho4 localize to the bud-neck and have at least partially been described to have a role in cytokinesis. Apart from the Rho proteins several GEFs - Cdc24, Tus1, Rom1 and Rom2 as well as several GAPs - Rgd2, Rga1, Rga2, Lrg1, Bem1 and Pxl1 localize to the bud neck. Fission yeast requires Cdc12 for acto-myosin ring formation(REF Pelham/Chang 2002).

**Mitotic Exit Network/Septation Initiation Network**

For both yeast model organisms *S.cerevisiae* and *S.pombe* cytokinesis can only occur once the cells have successfully exited mitosis. Both the Mitotic Exit Network(MEN) of budding yeast as well as the Septation Initiation Network(SIN) of fission yeast are signal cascade network that convey signals to the cytokinetic machinery. Both MEN and SIN are triggered upon the positioning of one spindle pole body in the bud during anaphase. This event is timely coordinated with chromosome segregation as the mitotic spindle elongates. On a molecular level, the essential output of mitotic exit is the inactivation of cyclin-dependent kinases, which earlier promoted entry into and progression through mitosis(REF Jin/Wang 2008). Molecularly, the network releases the essential phosphatase Cdc14 from the nucleolus, whose function it is to revert Cdk phosphorylations(REF Visintin/Amon 1998). As mentioned in the previous chapter, the Mitotic Exit Network resembles a classical small GTPases signaling cascade. The small GTPase Tem1 localizes asymmetrically to the spindle pole body, which faces the bud. It acts most upstream of the network and the GAP Bub2/Bfa1 suppresses its activity. As the mitotic spindle elongates during anaphase Tem1 gets in close proximity to its GEF Lte1, which localizes exclusively to the cell cortex of the bud. In turn, Lte1 activates Tem1 and Tem1 signals to downstream effectors. Whether Lte1 is indeed the decisive GEF is still debated, as the deletion of the lte1 gene is not lethal, which is not consistent with the predicted outcome of the inability to activate mitotic exit. On top of this, it has never been shown that Lte1 really functions as a GEF for Tem1 in *vitro*. However, despite these inconsistencies in the current model this checkpoint ensures that
activation of contractile ring contraction only happens once the spindle has properly aligned and elongated into the bud.

Activated Tem1 propagates a signal to the protein kinase Cdc15(REF Lee/Johnston 2001). Cdc15 signals to the Dbf2/Mob1 complex(REF Mah, Deshaies 2001), which upon its activation translocates to the bud-neck. Although the molecular details are still not clear, it is for certain, that Cdc14 is freed from its nucleolar inhibitor complex Cfi1/Net1 and therefore released into the cytoplasm. The polo-like kinase Cdc5 plays a very complex role in promotion of mitotic exit. As depicted in Fig.3.a, polo plays a dual role in negatively regulating Bfa1 by phosphorylation and thereby promoting Tem1 activation(REF Geymonat/Sedgwick 2003) and activating the Dbf2/Mob1 complex.

Another question, which is not solved and that remains is how the MEN interacts with the cytokinetic machinery. Recent reports suggest that MEN components Tem1, Cdc15, Dbf2/Dbf20 are required to re-polymerize the actin cytoskeleton on both sides of the bud neck, which corresponds to contractile ring activation. Additionally, phosphorylation of Hof1 is dependent on the activity of these MEN components. Defects during this
phosphorylation step lead to increased stability of the ring as Hof1 cannot be targeted for Grr1 dependent degradation (REF Corbett/Price 2006, Blondel/Peter 2005). This model suggests that the MEN controls acto-myosin ring contraction in a three fold fashion: (1) biochemical reversion of Cdk phosphorylations (see Fig. 4) (2) remodeling of the actin cytoskeleton at the bud neck and (3) timely activation of contraction via the Hof1 pathway. Supporting evidence comes from observations that MEN component Tem1 is required for the dynamics of the acto-myosin ring during contraction (REF Lippincott/Li 2001).

Fig. 4: Side by side illustration of the outputs of the Mitotic Exit Network of budding yeast (a) and the Septation Initiation Network in fission yeast (b). Image taken from Bardin et al, MEN and SIN - what's the difference, Nat Rev Mol Cell Biol. 2001 Nov;(11):815 - 26

At exit of mitosis fission yeast relies for on the Septation Initiation Network (SIN). The network structure of the SIN is organized very similarly to the MEN (REF Krapp/Simanis 2007). Most components involved in the SIN share similarities to components in the budding yeast MEN. It has to be noted though, that in contrast to the MEN, the SIN does not trigger CDK inactivation but its sole role is to regulate the formation of the division septum after mitotic exit. Comparably to budding yeast MEN, cells mutant for members of the SIN can form a contractile acto-myosin rings structure but are not able to contract it. The top of the cascade functions the small GTPase Spg1[S.c. Tem1](REF Schmidt/Simanis 1997), which is tethered to the Spindle Pole Body by the scaffold Cdc11/Sid4(REF Krapp/Simanis 2007, Tomlin/Gould 2002). Cdc16/Byr4[S.c. Bub2/Bfa1] GAP complex(REF Furge/Albright 1998) keeps Spg1[S.c. Tem1] in check throughout the
entire cell cycle. Only during anaphase Spg1 becomes activated and is able to recruit the downstream kinase Cdc7[S.c. Cdc15] (REF Sohrmann/Simanis 1998) to the proximal spindle pole. As in budding yeast, it is not exactly known, which GEF activates the small GTPase Spg1. During Anaphase B the GAP complex Cdc16/Byr4[S.c. Bub2/Bfa1] re-associates with the Spindle Pole Body and inactivates Spg1. Cdc7 signals, after dissociation from the Spindle Pole Body, to Sid1 with its co-factor Cdc14(REF Guertin/McCollum 2001). Sid1/Cdc14 signals to the the Sid2/Mob1[S.c. Dbf2/Mob1] complex(REF Hou/McCollom 2004). Sid2/Mob1 translocates from the spindle pole body to the contractile acto-myosin ring and triggers the formation of the septum (REF Jin/McCollum 2006). Like in the MEN, also the fission yeast homologue of the polo like kinase Plo1[S.c. Cdc5] regulates the SIN(REF Ohkura/Glover 1995), as plo1 shut-off alleles exhibit, amongst other phenotypes, a classical septation initiation phenotype and overexpression of Plo1 positively regulates the SIN(REF Tanaka/Hagan 2001). Although its role is not very well described, epistasis analysis suggests that the core SIN proteins described above act downstream of Plo1(REF Tanaka/Hagan 2001).

Higher eukaryotes lack real MEN/SIN components. However at a closer look many of the MEN/SIN members do also exist in higher eukaryotes. Most prominently, the polo kinase Plk1 has been implicated by many reports to be involved in cytokinesis(REF Bement 2007) in mammalian cells. Also Mob1 and Sid2 in fission yeast or Mob1 and Dbf2 in budding yeast find functional homologues in mammalian cells. Sid2 is a member of the NDR-family of protein kinases, which function as tumor suppressors and act at the centrosome(REF Hergovich/Hemmings 2006). MobKL2A is the human homolog of Mob1 and together with NDR-familily kinase both proteins have been implicated in cell division and proliferation(REF Citterio/Barcaccia 2006, Hammarton/Mottram 2007). Most recently, the homologue of Nud1 in budding yeast/Cdc11 in fission yeast, called centriolin has been clearly shown to be required for proper completion of abscission(REF Gromley/Doxsey 2003, Gromley/Doxsey 2005). Although the pathways seem not to be conserved, some of the acting members are. One possible explanation might be that due to the fact that both fission and budding yeast position their mitotic spindle according to a predetermined cleavage plane in contrast to mammalian cells where the cleavage plane is positioned according to the mitotic spindle, the exit strategies might have evolved differently as well.
**Septins and their role in cytokinesis**

Septins are conserved GTPases that can bind and hydrolyze GTP. They are found virtually in all eukaryotes except in protozoa and in most plants (REF Kinoshita 2003). They fulfill a variety of functions throughout the entire cell cycle. They serve as spatial landmarks, support cytokinesis and membrane remodeling events by recruiting effectors, establishment and maintenance of cell polarity (REF Barral/Snyder 2000, Takizawa/Vale 2000), and most recent findings described septins to function as a lateral diffusion barriers (REF Dobbelare/Barral 2004, Shcheprova/Barral 2008). How can septins act in so many processes and so dynamically throughout the entire cell cycle? At least a partial answer to this question lies in their striking ability to dynamically form and change higher-order structures like filaments, collars, gauzes and rings (REF Kinoshita/Mitchison 2002, Weirich/Barral 2008).

**Organization of septin filaments**

Recent advances in the septin research gave first structural insights into the organization of septin filaments of *S. cerevisiae, C. elegans* and mammals (REF McMurray/Thorner 2007, John/Barral 2007, Sirajuddin/Wittighofer 2007) and revealed that septins organize in a non-polar fashion (see Fig.5).

![Fig.5: Side by side illustration of the structural organization of the septin filament in mammals (top left), C. elegans (top right) and S. cerevisiae (bottom). All septin filaments form in a non-polar manner; Image taken from Weirich et al, Nat Rev Mol Cell Biol. 2008 Jun; 9(6):478 - 89](image)
To form these higher order structure septins depend on the GTPase domain and only to a minor extend on the coiled-coil domain (REF Sirajuddin/Wittighofer 2007). It is noteworthy that these structures additionally revealed a differential state of nucleotide loading. Whereas both Sept2 and Sept7 are GDP-bound, Sept7 is GTP-bound. This translates into two different G-dimer interfaces in the filament namely the Sept2-Sept6 GDP-GTP interface as well as the Sept7-Sept7 GTP-GTP interface (see Fig. 5). It might well be that this concept of differential nucleotide binding and hydrolysis is essential for filament formation and septin dynamics during the cell cycle.

The structure also gave valuable insights into how septins interact with membranes. Many experiments in vivo and in vitro predicted a polybasic tract to be the interface for interaction (REF Zhang/Trimble 1999, Casamayor/Snyder 2003) and the structure of the mammalian Sept2-Sept6-Sept7 filament supports that idea, although its not the sole interaction interface. The N-terminus proved crucial for septin-septin interaction and disruption leads to loss of filament formation in vivo (REF Sirajuddin/Wittighofer 2007).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Group 1A</th>
<th>Group 1B</th>
<th>Group 2A</th>
<th>Group 2B</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Cdc10</td>
<td>-</td>
<td>Cdc3</td>
<td>-</td>
<td>Cdc11</td>
<td>Shs1</td>
<td>Spr28</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>Spr2</td>
<td>-</td>
<td>Spr1</td>
<td>-</td>
<td>Spr3</td>
<td>Spr5</td>
<td>Spr7</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Cdc10</td>
<td>-</td>
<td>Cdc3</td>
<td>-</td>
<td>Cdc11</td>
<td>Sept7</td>
<td>Spr28</td>
</tr>
<tr>
<td>Eremoctochium gossypii</td>
<td>Hyp1</td>
<td>-</td>
<td>Hyp1</td>
<td>-</td>
<td>Hyp4</td>
<td>Hyp6</td>
<td>Hyp7</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>AspD</td>
<td>-</td>
<td>AspB</td>
<td>-</td>
<td>AspA</td>
<td>AspC</td>
<td>AspE</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td>Hyp3</td>
<td>-</td>
<td>Hyp1</td>
<td>-</td>
<td>Hyp4</td>
<td>Hyp2</td>
<td>Hyp5</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>-</td>
<td>UNC-61</td>
<td>-</td>
<td>UNC-59</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>-</td>
<td>SEP2</td>
<td>SEP5</td>
<td>-</td>
<td>Pnut</td>
<td>SEP1</td>
<td>SEP4</td>
</tr>
<tr>
<td>Xenopus laevis</td>
<td>Hyp1</td>
<td>-</td>
<td>-</td>
<td>SEP2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mammals</td>
<td>SEPT3</td>
<td>SEPT9</td>
<td>SEPT12</td>
<td>SEPT6</td>
<td>SEPT1</td>
<td>SEPT2</td>
<td>SEPT4</td>
</tr>
<tr>
<td></td>
<td>SEPT8</td>
<td>SEPT10</td>
<td>SEPT11</td>
<td>SEPT14</td>
<td>SEPT5</td>
<td>SEPT7</td>
<td>SEPT13</td>
</tr>
</tbody>
</table>

Fig.6: Overview over the diversity of all discovered septins in various model organisms; Image taken from Weirich et al, Nat Rev Mol Cell Biol. 2008 Jun; 9(6):478 - 89
Localization and function of septins in model organisms

Septins have been best studied in budding yeast \textit{S.cerevisiae}, where they also have been originally identified (REF Hartwell 1971). Budding yeast encodes five mitotic septins, which are named according to their essential function in the cell cycle Cdc3, Cdc10, Cdc11, Cdc12 and the only non-essential septin Shs1. Yeast encodes also two meiosis specific septins Spr3 and Spr28. The septins localize to the site of cytokinesis at the bud-neck very early in the cell cycle and are crucial already for the selection of the future bud site (REF Ford/Pringle 1991). During metaphase septins are required for proper positioning of the mitotic spindle (REF Kusch/Barral 2002, Grava/Barral 2006). At the end of mitosis septins are required for cytokinesis (REF Longtime/Bi 2000). Septins serve as a scaffold at the bud-neck, which subsequently recruits all proteins required for cytokinesis. Up to this point, there is not a single protein known, which localizes to the bud-neck in a septin independent manner. The cue of recruitment will be discussed later when I will discuss individually the various cytokinetic pathways in yeast and the recruitment of known and previously unknown cytokinetic proteins. This epistasis analysis will also be subject of this work and therefore discussed in the result section.

Fission yeast \textit{S.pombe} encodes four septins Spn1, Spn2, Spn3 and Spn4. They also form a septin ring at the cleavage furrow but in contrast to budding yeast their recruitment happens after the assembly of the acto-myosin ring (REF Berlin/Chang 2003). This is consistent with the fact, that fission yeast septins are non-essential.

In \textit{C.elegans} septins UNC-59 and UNC-61 localize to the leading edge of the ingressing furrow and after contraction to the midbody and the and have been shown to function in asymmetric furrow ingression (REF Meddox/Oegema 2007). Deletion of \textit{UNC}-59 and \textit{UNC}-61 has only moderate effects and as the gene names already tell us, worms depleted for septins show unc-coordinated locomotion and thus there is no direct evidence that septins are required for abscission.

In mammalians 12 septins have been identified with even more splice-isoforms (REF Kinoshita 2003). Septins are found to localize to the cytoplasm and as in all other organisms associated with membranes. During cytokinesis, septins localize with the ingressing furrow and in contrast to \textit{C.elegans} septins are required for abscission as depletion of septins leads to bi-nucleated cells (REF Nagata/Inagaki 2003).
Septin dysfunction is cause of many diseases

Septin function has also been implicated in human pathologies. Abnormal septin structures are found during development of Alzheimer's disease (REF Kinoshita/Kimura 1998). Septin function is also perturbed during Parkinson's disease (REF Ihara/Kinoshita 2007). It has been shown that septins form aggregates with one of the major components of the Parkinson plague - \( \beta \)-synuclein.

During oncogenesis there are implications that during development of leukaemia, Sept5, Septin6 (REF Ono/Hayashi 2002) and Sept9 are frequently found in fusion with MLL. MLL is a transcription factor that is overexpressed in leukaemia (REF Taki/Hayashi 1999). Although the function of these fusion-complexes is not known, septin dysfunction is considered one of the causes of leukaemia. Additionally, there is more and more evidence showing up that septin dysfunction is a cause for many types of cancers as reviewed in Hall/Russell 2005.

Abscission

Cytokinesis terminates with the cleavage of the intercellular membrane bridge in a poorly understood process called abscission. It has been considered for a very long time that abscission is a stochastic membrane rupture-and-repair event, which is facilitated as furrow ingression closes the gap. This event brings the plasma membrane into a spatial configuration, which ultimately allows the membrane fission event that leads to two independent daughter cells.

Meanwhile, numerous mutants have been identified that are specifically deficient in resolution of the plasma membrane while being fully proficient for furrow ingression. These results suggest that abscission is highly regulated and requires distinct cellular processes, which will be reviewed here. Experimental evidence of how abscission mechanistically works and of how abscission is regulated comes mainly from higher eukaryotic systems e.g. *C. elegans*, *D. melanogaster* and mammalian systems. In the following introductory section, I will summarize our knowledge on how post-furrowing abscission is accomplished.
Abscission is a membrane remodeling event therefore the lipid composition of the plasma membrane is greatly remodeled during furrow ingression and before abscission (REF Ng/Brugess 2005, Wachtler/Balasubramanian 2003). It has been shown that signaling lipids cluster at the intercellular bridge where abscission is about to take place. These specialized lipids are cholesterol (REF Kasahara/Yamaguchi 2007), phosphatidylethanolamines and signaling lipids like PtdIns(4,5)P2 (REF Janetopoulos/Devreotes 2006, Logan/Mandato 2006).

Cholesterol acts as a tether for members of the non-receptor-type tyrosine kinases at the midbody (REF Kasahara/Yamaguchi 2006). Interestingly Src-mediated tyrosine phosphorylation is required during early stages of mitosis even before a functional cleavage furrow has been formed. Activated Src is translocated to the midbody through Rab11 vesicles (REF Kasahara/Yamaguchi 2006). RNAi mediated depletion of Src yields HeLa cells incapable to abscise the plasma membrane (REF Kasahara/Yamaguchi 2007). Their role during the abscission event is not clear up to this point.

PtdIns(4,5)P2 are major signaling lipids (REF Santarius/Anderson 2006, Hawkins/Stephens 2006) and are required for cytokinesis in various organisms (REF Ng/Burgess 2005, Zhang/Kuno 2000, Field/Cantley 2005). PtdIns(4,5)P2 localize uniformly on the plasma membrane throughout the cell cycle, re-localizes and concentrate at the intercellular bridge during cytokinesis (REF Emoto/Umeda 2005). PtdIns(4,5)P2 fulfill multiple roles during cell division and are essential for bridge stability and the completion of abscission (REF Kouranti/Echard 2006). Conversely, it seems to be important that PtdIns(3,4,5)P3 are absent from the cleavage furrow (REF Janetopoulos/Devreotes 2005). Taking these results together it strongly suggests that differential polarization of signaling lipid domains during cell division is required to promote cytokinesis.

How this signaling lipid reorganization of the plasma membrane is accomplished is not clear. Experimental evidences suggest that involvement of both secretory and recycling endocytic pathways is crucial to differentially polarize signaling lipids in late phases of cytokinesis. On a molecular level, Rab35 recycling vesicles control the PtdIns(4,5)P2 enrichment/levels at the midbody by targeting the PtdIns4P5-kinase to the intercellular bridge (REF Kouranti/Echard in preparation). The generation of PtdIns(4,5)P2 allows the translocation of essential downstream factors to the intercellular bridge required for proper abscission (REF Emoto/Umeda 2000).
The GTPases Rab11 and Arf6 regulate the targeting of recycling endosomes to the cleavage furrow during cytokinesis (Ref Prekeris/Gould 2008, Prekeris/Scheller 2000). One cargo of endosomal vesicles has been recently described (Ref Simon/Prekeris 2008). Fip3 is a Rab11 family interacting protein, which is transported to the midbody by Rab11/Arf6 containing vesicles. There, Fip3 is tethered to the midbody ring by directly binding Cyk4, i.e. the centralspindlin complex. Fip3 replaces the RhoA GEF Ect2 from the centralspindlin complex and this step is required for abscission to proceed (Ref Simon/Prekeris 2008). How the replacement of Ect2 by Fip3 promotes abscission, and which molecular function Fip3 has, is not yet known.

The exocyst, which contributes to the docking of exoctic vesicles to the plasma membrane, plays an essential role in abscission. The exocyst is tethered to the midbody ring by centriolin, whose localization in turn depends on the centralspindlin complex (Ref Gromley/Doxsey 2005). These findings highlight the importance of recycling and secretory vesicle pathways in the directed targeting of components required for abscission to the midbody.
Cep55 is yet another protein, which, apart from its function as microtubule bundling protein, associates with the centralspindlin complex, directly binds Mklp1/MgcRacGAP and facilitates membrane fusion. It controls the localization of endobrevin to the midbody, a v-SNARE required for cell abscission (REF Zhao/Fang 2006, Fabbro/Khanna 2005).

It is common to all these proteins, that they all localize to the midbody ring structure, whose integrity is key for successful re-localization of all these factors and therefore for successful abscission. However, none of the proteins mentioned above impair integrity of the midbody ring structure itself or disrupt proper localization of the centralspindlin complex indicating that they function downstream of the centralspindlin complex.

BRUCE is a recently identified high molecular weight protein, which does not only localize to the midbody ring structure but is also required for its stability. As anticipated for such proteins it is essential for abscission (REF Pohl/Jentsch 2008). The discovery of BRUCE is particularly exciting, as BRUCE appears to serve as a scaffolding molecule that fulfills multiple functions during abscission. BRUCE localization to the midbody ring is dependent on Mklp1. In turn, cells depleted for BRUCE show significantly reduced Mklp1 positively stained midbodies indicating that BRUCE and Mklp1 are at least in part mutually dependent. BRUCE co-localizes with Rab8 and Rab11 vesicles as well as the exocyst component Sec8. At the midbody it is required for proper recruitment of Rab8/11 vesicles. Some components of the midbody ring is ubiquitinated during cytokinesis and abscission by BRUCE and deubiquitinated by UBPY (REF Pohl/Jentsch 2008). The question that still needs to be addressed is whether ubiquitination-dependent degradation of midbody ring components like Aurora B, Plk1 and Mklp1 is required for successful abscission and/or non-proteolytic ubiquitin signaling is required to accomplish abscission.

Apart from these proteins described above that lead to an abscission phenotype upon their deletion there is recent data emerging that might address how abscission mechanistically works.

The ESCRT pathways help to sort membrane proteins into vesicles that bud into the lumen to create multivesicular bodies (MVB) (REF Hurley/Emr 2006, Gill/Williams 2007). These vesicles are mostly targeted to the lysosome and for the degradation of its cargo. There are three Endosomal Sorting Complex Required for Transport machineries
ESCRT I, II and III respectively (REF Chu/Emr 2006). Tsg101 and Alix are subunits of the ESCRT machinery. Most strikingly, these vesicles bud of away from, rather than into the cytoplasm, which implies striking similarities between the fission event from inside the neck of a budding vesicle or a budding virus and membrane fission during abscission (see Fig. 8) (REF Morita/Sundquist 2007, Carlton/Martin-Serrano 2007). Especially ESCRT III is specifically thought to support direct membrane fission events for the release of cargo-filled vesicles, as it is anticipated during abscission as well.

Therefore it has been tested, whether ESCRT might function in abscission as well (REF Morita/Sundquist 2007, Carlton/Martin-Serrano 2007). Indeed, as this model predicts, Tsg101 and Alix localize to the midbody in a Cep55 dependent manner. Cells depleted of Tsg101 or Alix still recruit Cep55 and the centralspindlin complex to the midbody indistinguishable of wt cells (REF Morita/Sundquist 2007). However, these cells were unable to finish abscission and became multinucleated (REF Morita/Sundquist 2007, Carlton/Martin-Serrano 2007). These data indicate that ESCRT-mediated membrane
fission at the midbody might drive abscission, although the direct evidence is still lacking.

Remarkably, it should be mentioned that the abscission players presented here localize to the centrosome during interphase and re-localize to the midbody ring during cytokinesis. This is true for centriolin, Fip3, Cep55, Tsg101 and Alix. Whether the centrosome fulfills an active signaling function in the process of abscission or rather serves as a simple storage has still to be elucidated.

**Checkpoints during the cell cycle**

In order to successfully perform a complete cell cycle, cells from yeast to animal cells have evolved checkpoints, whose purpose it is to verify the completion and control the quality of the previous cell cycle step before entering the next cell cycle stage. These checkpoints enable the cell to correct mistakes in case they happen and make sure throughout the entire cell cycle that the daughter cell represents an identical copy of the mother; that is to say, that no mistakes are passed on to the next generation. As mentioned, checkpoints exist throughout the entire cell cycle and thus, I will just briefly review the cell cycle dependent checkpoints in yeast and will focus in detail on the cytokinetic checkpoint called NoCut in budding yeast and its functional homologue in animal cells.

Checkpoints exist for the successful DNA replication (REF Herrick/Bensimon 2008). The Septin Checkpoint ensures that segregation of the duplicated chromosomes cannot occur before the bud has emerged(REF Yeh/Lew 2000). The fidelity of chromosome segregation depends upon efficient kinetochore-microtubule attachment and proper bi-orientation of attachment. The checkpoint controlling these events is the Spindle Assembly Checkpoint - SAC. SAC acts in metaphase of mitosis and ensures that paired sister chromatids are prevented from segregating into mother and daughter compartment until full bipolar attachment to the mitotic spindle has been accomplished (REF Dobles/Sorger 2000). Premature onset of anaphase while not all chromosomes are properly attached to the mitotic spindle is particularly fatal, as loss of chromosomes and gross chromosomal rearrangements (GCR) are thought to be one of the major causes of cancer(REF Chen/Kolodner 1999, Myung/Kolodner 2004). Consistent with
these findings, malfunctioning of the SAC has been implicated in the development of cancer. SAC employs the conserved proteins Mad1, Mad2, Mad3/BubR1, Bub1, Bub3 and Mps1. Essentially, the SAC prevents anaphase to happen by keeping the APC/c activator Cdc20 in the BubR1/Bub3/Cdc20 complex in check (REF Nilsson/Pines 2008). Thereby the SAC prevents APC/c dependent degradation of Securin from Separase, which in turn cannot cleave cohesin and thus the cells are not able to progress into anaphase (REF Hwang/Murray 1998). Conversely, after successful attachment of all kinetochores and thereby all chromosomes to the mitotic spindle, the SAC allows Cdc20 to activate the APC/c and the APC/c sends securin for 26s proteasome degradation (REF Szaniecka/Hardwick 2008). This event frees Separase, and cohesin is cleaved, leading to anaphase initiation (REF Nakajima/Hirota 2007, Uhlmann 2004).

As discussed above the mitotic exit network in budding and the septation initiation network in fission yeast represent also checkpoints that prevents cells from initiating cytokinesis before the entire chromatin mass has been successfully segregated into the two prospective daughter cells (see above for extensive presentation of MEN and SIN). As discussed before, abscission represents a discrete step of cytokinesis, where one continuous plasma membrane is resolved into two independent membranes (REF Gruneberg/Barr 2008). Recent findings suggest that also the timing of abscission is controlled by checkpoints in budding yeast and mammals (REF Norden/Barral 2006, Steigemann/Gerlich 2009).

The NoCut checkpoint

Under certain circumstances chromatin bridges or lagging chromosomes can occur, which are not sensed by any checkpoints described so far. Indeed early observations from fission yeast described mutations in chromosome segregation that led to a "cut" phenotype (REF Hirano/Yanagida 1986) in fission yeast meaning that chromosome integrity was disrupted by the cytokinetic machinery.

After successful mitotic exit the acto-myosin ring contracts and ultimately allows abscission. Recent findings suggest a molecular checkpoint, called NoCut, which regulates the timing of abscission (REF Norden/Barral 2006). NoCut is a signal cascade that delays abscission in case of spindle midzone defects or defects in chromosome segregation to avoid chromosome breakage by the cytokinetic machinery. How might
this work? The mitotic spindle midzone is defined as a zone of the anaphase spindle that consists of bundled, overlapping microtubules and is equidistant to each pole (REF Khmelinskii/Schiebel 2007). Ase1 bundles anti-parallel microtubules specifically at the spindle midzone. Additionally to Ase1, many other proteins localize to the spindle midzone after anaphase onset, including the chromosomal passenger complex (REF Ruchaud/Earnshaw 2007), Ndc10 (REF Bouck/Bloom 2005) and Cbf3 (REF Bouck/Bloom 2005). All of them contribute to the stability of the spindle midzone during anaphase and the timely disassembly of the mitotic spindle at the time of mitotic exit (REF Buvelot/Biggins 2003).

Integrity of the mitotic spindle midzone is essential to generate a pushing force during chromosome segregation in Anaphase B. Disruption of the integrity of the mitotic spindle midzone also leads to a delay in the resolution of the plasma membrane during abscission (REF Norden/Barral 2006). This delay is dependent on the Aurora kinase Ipl1, which sends the anillin related proteins Boi1 and Boi2 to the bud neck, where they inhibit cytokinesis. How Boi1 and Boi2 are able to inhibit cytokinesis is not known, however it is clear that contraction of the acto-myosin ring remains unaffected, indicating that the process inhibited is abscission. Accordingly, combination of ndc10-1 and deletions in the genes encoding Boi1 and Boi2 restores wild type timing of abscission. The same effect was obtained when the ndc10-1 allele was combined with a temperature sensitive allele ipl1-321. Taking these data together, NoCut represents a reversible, inhibitory checkpoint that delays abscission in response to spindle midzone defects in an Ipl1, Boi1/Boi2 dependent manner (REF Norden/Barral 2006). In a physiological context NoCut prevents chromosome breakage by the cytokinetic machinery. Most likely spindle integrity is not the molecular event sensed by NoCut and unpublished data by Mendoza et. al. suggest that what is sensed is the proximity of chromatin to the midzone (REF Mendoza/Barral 2009 in press Nature Cell Biology - see appendix).
During early anaphase, surrounding chromatin activates Ipl1 at the central spindle, where Ipl1 localizes. Active Ipl1 causes Boi1 and Boi2 to translocate to the cortex, where they inhibit abscission. (B) Upon segregation of the chromosomes away from the central spindle, Ipl1 is no longer kept active by chromatin. Boi1 and Boi2 leave the bud neck. Abscission can take place. (C) In cells with midzone defects, Ipl1 stays close to chromatin even after chromosome segregation. Boi1 and Boi2 are not removed from the bud neck. Abscission remains inhibited.

In support of this hypothesis, recent research in animal cells suggests that the NoCut pathway is conserved in higher eukaryotes (REF Steigemann/Gerlich 2009). This is particularly exciting, as the topological requirements and the molecular cues that lead to successful cytokinesis are significantly different between yeast and human cells, as outlined earlier. Also in animal cells, cytokinesis can be severely delayed due to chromatin bridges (REF Cimini/Degrassi 2003), which occurs at a regular frequency of about 1% (REF Gisselsson/Mandahl 2000). As mentioned earlier, animal cells do not really have a functional equivalent of the Mitotic exit network. It is particularly important to monitor the timing of completion of chromosome segregation with the timing of abscission, although experimental support for this prediction has so far been lacking completely. Interestingly these chromatin bridges delay abscission in an Aurora B dependent manner (REF Steigemenn/Gerlich 2009), which represents an additional function of Aurora B dependent pathways during cytokinesis as Aurora B is required to stimulate furrow ingression earlier on (REF Eggert/Field 2006, Ruchaud/Earnshaw 2007) in mammalian cells. Aurora B, as part of the chromosomal passenger complex, localizes to the midbody in late anaphase/telophase. Aurora B activity depends on phosphorylation of T232 residue (REF Yasui/Inagaki 2004). Dephosphorylation and thus Aurora B inactivation triggers midbody microtubule disassembly and provides a trigger...
for abscission (REF Steigemann/Gerlich 2009). Premature inactivation of Aurora B using Hesperadin (REF Hauf/Peters 2003) leads to accelerated abscission and conversely chromatin bridges keep Aurora B activity high in post-telophase stages (REF Steigemann/Gerlich 2009) and thus delay abscission. What are the molecular cues that lead to this inhibition of abscission? Aurora B does so by phosphorylating its downstream effector Mklp1 on S911. Active Mklp1 can be visualized by using phosphospecific antibodies (REF Neef/Barr 2006) and activated Mklp1 anchors the ingressed furrow at a stable intercellular canal (REF Steigemann/Gerlich 2009) where Mlkp1 has been reported to localize (REF Guse/Glotzer 2005, Neef/Barr 2006). Conversely, if Mklp1 phosphorylation by Aurora B is inhibited by the drug ZM1, Mklp1 levels is gradually lost from the midbody with simultaneous increase of furrow regression events.

Although both checkpoints regulate the timing of abscission it is noteworthy that the budding yeast NoCut checkpoint is required to prevent chromosome breakage by the cytokinetic machinery (REF Norden/Barral 2006) whereas the mammalian counterpart is required to prevent tetraploidization (REF Steigemann/Gerlich 2009). This is due to the fact that in *S. cerevisiae* the cytokinetic machinery is coupled to the cell wall machinery, which prevents a furrow regression event.

Although these studies emphasize the importance of the NoCut checkpoint, it is not clear up to this point how NoCut interferes with abscission. In fact it is not even known, how the cell carries out abscission and what are the molecules and molecular processes involved.
Aim of this work

Since the identification of a budding yeast contractile acto-myosin ring structure that "remains in the mother-bud neck until the end of anaphase, when a ring of F-actin forms and associates with it; then contracts to a point and disappears" (REF Bi/Pringle 1998) to our current understanding of cytokinesis and its regulation it has been a long path and significant progress has been made over the last decade. However virtually all efforts have been put into elucidating the mechanisms of acto-myosin ring contraction. The fact that abscission is an independent post-furrowing phenomenon with its own mechanistical hallmarks and regulatory cues that ultimately lead to plasma membrane cleavage has not yet been fully appreciated.

Therefore the overall aim of this work is to extend our knowledge on cytokinesis, and specifically about the molecular classes that are required to accomplish abscission. When I started this project in April 2005, there was not a single published report on how abscission might be carried out and even the term "abscission" was one of unofficial nature. However the work on the NoCut checkpoint in our lab had already progressed substantially (REF Norden/Barral 2006). It was already very clear, that abscission is actively regulated but mechanistical details lacked completely. Genetic evidences prompted us to explore the previously poorly characterized Cyk3 protein in more detail (REF Norden/Barral 2006, Korinek/Chant 2000) and ask whether or not genes involved in the Cyk3-pathway act indeed in abscission in S.cerevisiae. To address this, we made use of the power of yeast as a model. We employed genetic screens using the systematic gene deletion library of S.cerevisiae to identify genes that act in cytokinetic pathways Myo1, Hof1 and Cyk3.

Remarkably, in both yeast and mammalian cells NoCut activation does not interfere with furrow ingress, but solely with abscission, offering an opportunity to clearly separate these two processes. This observation already indicated that the yeast cytokinesis protein Cyk3 must be involved in abscission. Indeed, probably due to the narrow morphology of the mother-bud neck of budding yeast cells, yeast cells do not fully depend on actomyosin contraction for cytokinesis. Instead, genetic studies established that actomyosin ring contraction, powered by the myosin II molecule Myo1 (REF Bi/Pringle 1998), septum deposition, which is guided by the PCH protein Hof1 (REF Lippincott/Li 1998), and a third, poorly understood process dependent on the protein
Cyk3 (REF Korinek/Chant 2000) can at least partially compensate for each other and complete cytokinesis even when any one of these three processes is defective. As a consequence, cells carrying either one of the myo1Δ, hof1Δ and cyk3Δ deletions as a single mutation succeed to complete cytokinesis most of the time and are viable, while any combination of two of these mutations impairs cytokinesis and is lethal. Suggesting that the Cyk3 pathway contributes more specifically to abscission, conditions that leads to NoCut activation, such as the lack of the spindle stabilizing protein Ase1, are synthetic lethal with the myo1Δ and with hof1Δ mutations but not with cyk3Δ (REF Norden/Barral 2003). These data are consistent with NoCut activation leading to the inhibition of the Cyk3 pathway, specifically.

However, how NoCut interferes with the abscission machinery is unknown. At least in budding yeast, the proteins Boi1 and Boi2 ensure the communication between the spindle midzone, where the chromosome passenger kinase Aurora B/Ipl1 monitors chromosome segregation (REF Mendoza/Barral 2009 submitted), and the bud neck cortex, where abscission takes place. These two proteins shuttle between the nucleus and the cell cortex during the cell cycle, and their export from the nucleus requires Ipl1 activity. How Boi proteins reversibly inhibit abscission is not known.

Therefore, our first goal was to identify comprehensively genes that act in the Cyk3 pathway. The genetic redundancy of known cytokinetic pathways allowed us to draw a comprehensive genetic overview of the yeast cytokinetic pathways. After identifying the non-essential genes that act in the three cytokinetic pathways Myo1, Hof1 and Cyk3, we characterized selected genes mapped in the Cyk3 pathway further, using fluorescent reporter assays.

Secondly, to test candidates for their involvement in ring contraction and/or abscission we used high-resolution microscopy and probed for the kinetics of acto-myosin ring contraction and for membrane resolution over time. These assays were constructed to group the genetically identified candidates into functional classes.

Thirdly, this narrowed list allowed us to ask specific questions on the functional nature of our candidate genes. It was only then that educated guesses lead us to conclude that high Rho2 levels are required to promote abscission and that NoCut negatively regulates abscission by preventing Rho2-promoting factors of abscission to localize to the bud-neck.
Results I

SL screens identify Myo1, Hof1 and Cyk3 pathway genes
Front image: Final pinning of the hof1Δ synthetic lethal screen

finished 15/06/06; marked with white boxes
are postive genetic interactors with hof1Δ
**Synthetic lethality screens:**

The complete sequencing of the model organism *S.cerevisiae* provided novel tools to study molecular pathways in semi-forward genetic approaches. It revealed that 5100 of 6200 genes in the entire genome are non-essential upon their deletion in the haploid, and under laboratory conditions (REF Winzeler/Davis 1999). These findings highlight the enormous capacity of yeast cells to tolerate deletions and functionally buffer entire pathways in case a gene function is lost. This redundancy is also reflected by the fact that phenotypes are "buffered" by other genes of the same or parallel pathways, leaving still 30% of the yeast ORFs phenotypically uncharacterized (REF Hartman/Hartwell 2001). Cytokinesis is such a heavily buffered system, that most genes involved in cytokinesis are not essential in most yeast strain backgrounds. The concept of synthetic genetics proved to be a highly efficient method to investigate these "buffered phenotypes". Additionally synthetic genetics allows drawing of genetic interaction maps and pathways (REF Tong/Boone 2001, Hartman/Hartwell 2001). Two genes show a "synthetic interaction" if the combination of two gene deletions, neither of them being lethal by itself, causes cell death or slowed growth (REF Novick/Botstein 1989). This phenomenon proved useful to unravel "hidden" phenotypes associated with specific gene deletions. Synthetic Genetic Array (SGA) is a high throughput method to explore functional genetic relationships between genes and pathways (REF Tong/Boone 2001). In short, a bait strain carrying a mutation in the gene of interest is mated to the entire non-essential gene deletion mutant array of the opposite mating type. After successful mating, selection and sporulation the meiotic progenies carrying the double mutations are selected in a series of elegant selection steps. Ultimately, the growth of the haploid double mutant strain carrying both bait and prey mutation is visually inspected for colony size and growth rate (REF Tong/Boone 2001).
Rational for the use of SGA to unravel novel genes involved in cytokinesis

As an approach to learn more about novel genes involved in cytokinesis and abscission and furthermore to understand how the NoCut pathway interferes with cytokinesis, we first sought to identify all the genes involved in the individual cytokinetic pathways Myo1, Hof1 and Cyk3. Due to the redundancy between the Myo1-, Hof1- and Cyk3- dependent pathways of cytokinesis in yeast (REF Korinek/Chant 2000), we rationalized that genes acting specifically in the Myo1-, Hof1- and Cyk3-pathway individually, are most likely to be non-essential. Therefore, we sought to identify all the non-essential genes acting in the Cyk3-pathway, i.e. the disruption of which show the same genetic interaction pattern as the cyk3Δ mutation by itself. In other words we searched for all gene disruptions that are synthetic lethal with myo1Δ and hof1Δ mutations but not with cyk3Δ mutation (see Fig.12).
Respectively, we sought to identify all the non-essential genes acting in the Hof1-pathway, the disruption of which show the same genetic interaction pattern as the hof1Δ mutations, i.e., all the gene disruptions that are synthetic lethal with the myo1Δ and cyk3Δ mutations but not with hof1Δ. Using the same rational, our screens then identified as well all the genes of the Myo1-pathway.

**Fig.12:** Schematic representation of the pathway concept during yeast cytokinesis. Genes involved in the Myo1-pathway are synthetic lethal with the bait strain hof1Δ and cyk3Δ but not with myo1Δ. Genes involved in the Hof1-pathway are synthetic lethal with the bait strain myo1Δ and cyk3Δ but not with hof1Δ. Genes in the Cyk3-pathway are synthetic lethal with the bait strain myo1Δ and hof1Δ but not with cyk3Δ.

Genes synthetic lethal with all three bait strains myo1Δ, hof1Δ and cyk3Δ either represent a fourth cytokinetic pathway or are factors that are commonly required upstream of the Myo1-, Hof1-, and Cyk3-pathway.
Generation and analysis of the Bait strains for the SGA

Although many systematic synthetic lethal screens have been carried out in the past, none have been reported yet with either of myo1Δ, hof1Δ, cyk3Δ nor ase1Δ. One probable reason for this is, that in some frequently used strain backgrounds, such as W303, frequently the myo1Δ mutation is lethal, while in many others, such as S288C and A364a, they are viable. By comparison, the hof1Δ, cyk3Δ and ase1Δ mutations are viable in most backgrounds. Accordingly, the collection of strains carrying all the disruption of non-essential genes, which was generated in S288c, contains the strains where the genes HOF1, CYK3 and ASE1 are individually disrupted. However, it does not contain the myo1Δ strain(REF Winzeler/Davis 1999). Furthermore, careful analysis of the hof1Δ strain by PCR in the initial phase of this thesis indicated that it carried the wild type HOF1 gene, and that the KanMX6 insertion was probably integrated at another locus. Thus, we sought to create the myo1Δ::NAT, hof1Δ::NAT, cyk3::NAT and ase1::NAT mutations in the S288c background de novo with the goal to screen the entire genome for disruptions that are synthetic lethal with these mutations in a genetical isogenic background. All four mutations were verified by PCR and tetrad analysis, and were found to be viable, although both the myo1Δ and hof1Δ strains showed a slow growth phenotype when grown at 30°C and even exacerbated at 37°C. Furthermore, the myo1Δ and hof1Δ mutations were synthetic lethal with each other and both synthetic lethal with the cyk3Δ mutation from the strain collection. When crossed with a random strain of the disruption collection, haploid single (myo1Δ, hof1Δ, cyk3Δ and ase1Δ) and double mutant spores were recovered upon meiosis with reasonable frequencies (25% frequency for myo1Δ, hof1Δ, cyk3Δ and ase1Δ strains and 25% for the random double mutants). Thus, these strains behaved as expected and were suitable to carry out synthetic lethal screens.

Conducting SGA with the bait strains of interest

Thus, using a semi-robotized approach the bait strains myo1ΔNAT, hof1ΔNAT, cyk3ΔNAT and ase1ΔNAT were crossed individually with the entire gene deletion collection and synthetic lethal interactions were screened as reported(REF Tong/Boone 2001). The same screen was carried out twice with each strain, with the exception of the cyk3Δ screen, which was carried out only once. Instead, the second screen was carried
out with the \textit{ase1Δ} strain, which like \textit{cyk3Δ} is synthetic lethal with both \textit{myo1Δ} and \textit{hof1Δ} but not with \textit{cyk3Δ} (REF Norden/Barral 2006). The synthetic lethalities observed between \textit{ase1Δ} and both \textit{myo1Δ} and \textit{hof1Δ} are due to activation of the NoCut pathway in response to the \textit{ase1Δ} deletion and thus due to spindle midzone instability (see introductory section Checkpoints - NoCut for detail).

**Why to do a complementary \textit{ase1Δ} synthetic lethal screen**

Yeast cells lacking the microtubule bundling, central spindle component Ase1 form highly unstable mitotic spindles that break prematurely during anaphase (REF Schuyler/Pellman 2003). The \textit{ase1Δ} mutant cells are viable and activate NoCut, which in turn delays abscission to prevent chromosome breakage by the cytokinetic machinery. The Ase1 protein becomes essential in the absence of one of the two cytokinetic genes \textit{MYO1} and \textit{HOF1} (REF Norden/Barral 2006) as \textit{myo1Δ ase1Δ} doubles as well as \textit{hof1Δ ase1Δ} double cells form inviable progenies. However, \textit{ase1Δ cyk3Δ} double cells are fully viable indicating that Ase1 function is upstream required for proper activity of the Cyk3 pathway and that Ase1 or the spindle midzone help to promote abscission.

These data taken together suggest that \textit{ase1Δ} cells activate NoCut to inhibit the Cyk3 pathway and thereby inhibit abscission. In this model, this inhibition is the reason why \textit{ase1Δ myo1Δ} and \textit{ase1Δ hof1Δ} cells are inviable as these genetic setups mimic a \textit{myo1Δ cyk3Δ} or \textit{hof1Δ cyk3Δ} double mutant respectively. Additionally, it was shown that \textit{ase1Δ} is synthetic lethal with \textit{boi1Δ boi2Δ}, indicating that NoCut-pathway members become essential in an \textit{ase1Δ} mutant background (REF Norden/Barral 2006). The physiological reason for this genetic interaction is most likely that the co-ordination between abscission and chromosome segregation is lost due to the inactivation of NoCut. This has been shown to lead to chromosome breakage by the cytokinetic machinery and inviable progenies (REF Norden/Barral 2006). In this view we rationalized that (a) novel members of the NoCut pathway must become essential in the absence of the Ase1 protein, and that, (b) as Ase1 and Cyk3 act in the same pathway these screens can function to validate eachother.

Therefore we performed a synthetic lethality screen against a bait strain carrying a deletion in the \textit{ASE1} gene.
However, the results obtained in the aselΔ synthetic lethal screen cannot be interpreted that straightforward only. The aselΔ mutation has been shown to be synthetically lethal with the dynein pathway (REF Tong/Boone 2004) out of other reasons than NoCut activation. Cells lacking a functional mitotic spindle midzone cannot exhibit a pushing force during Anaphase B and depend completely on fully functional pulling force from the cell cortex, which is taken over by the dynein pathway. Cells lacking both forces are not able to undergo proper chromosome segregation, which explains physiologically the genetic interaction between those two genes.

Kar9 and the Kar9-dependent pathway as well as the dynein dependent pathway are redundant pathways ensuring proper alignment of the mitotic spindle (REF Liakopoulos/Barral 2003, Grava/Barral 2006). The kar9Δ dyn1Δ double mutant cells are inviable because they are not able to position the mitotic spindle properly and therefore fail to segregate the chromosomes faithfully into mother and daughter cell. All the hits obtained in the aselΔ synthetic lethal screen were subtracted by the common hits with a screen against the bait strain kar9Δ to define the subset of candidate genes that are actually members of the dynein pathway and thereby interact with aselΔ for reasons of spindle positioning problems rather than NoCut activation.

The candidate genes in the three cytokinetic pathways and the "aselΔ sl"-pathway

From these screens we derived the genes that belong to the Myo1-pathway (45 genes) as being those that were identified in both hof1Δ screens, and in each of the cyk3Δ and the aselΔ screens, but in none of the myo1Δ screens. Similarly, the genes required for the Hof1-pathway (42 genes) were defined as those identified in both myo1Δ screens and in each of the cyk3Δ and aselΔ screens, but in none of the hof1Δ screens. Finally, the genes defining the Cyk3-pathways (102 genes) are those identified in all myo1Δ and hof1Δ screens but in neither of the cyk3Δ and aselΔ screens and those 39 genes, that were synthetic lethal with myo1Δ and hof1Δ but not with cyk3Δ cells formed the pathway that we call "synthetic lethal with aselΔ". Since each of these genes was identified four times independently in our screens but not in the control screens, we concluded that the likelihood that these genes are false positive is extremely low. Furthermore, as a proof of principle, all genes, expected to be identified in our screens, were indeed found. For example, the cyk3Δ and aselΔ mutations showed up (REF Norden/Barral 2006) in both
myo1Δ and both hof1Δ screens but not in the cyk3Δ and ase1Δ screens. The bni1Δ mutation indeed showed up in the hof1Δ screen (REF Vallen/Bi 2000) and in the cyk3Δ but not in the myo1Δ screen. The shs1Δ deletion showed up in the cyk3Δ screen (REF Tong/Boone 2004) and so did cla4Δ (REF Tong/Boone 2004) to mention only a few.

Together, our data identify therefore most if not all non-essential genes involved in the three main cytokinesis pathways in yeast. They identified also a small category of genes (32 genes) that showed up in all six screens. This suggests that these genes either act in all three pathways, or in a fourth cytokinesis pathway. Alternatively, they might function in one overarching pathway upstream of all three pathways. This last possibility is however not very likely, since such an overarching pathway would be predicted to be essential. This is for example the case of budding yeast septins, which act upstream of all three of Myo1, Hof1 and Cyk3, and are essential for cytokinesis and cell survival. Altogether, our screens identified 189 genes as being required for proper cytokinesis.

Analysis of the genes identified indicates that most of them are unlikely to act directly in cytokinesis.

**Genes in the Myo1 pathway**

The genes that were mapped into the Myo1 pathway due to the criteria outlined above are depicted in Tab.1
## MYO1 PATHWAY

<table>
<thead>
<tr>
<th>Gene name</th>
<th>GO Biological Process</th>
<th>Cellular Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC1, APC10</td>
<td>Anaphase Promoting Complex</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endoplasmal Reticulum, Plasma</td>
</tr>
<tr>
<td>ATG15</td>
<td>Autophagy</td>
<td>Membrane</td>
</tr>
<tr>
<td>BUD23</td>
<td>Bud Site Selection</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>YEL033W</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Unknown</td>
</tr>
<tr>
<td>ARG82</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>ELM1</td>
<td>Cellular Morphogenesis</td>
<td>Bud neck</td>
</tr>
<tr>
<td>SHS1, SEP7</td>
<td>Cytokinesis</td>
<td>Bud neck</td>
</tr>
<tr>
<td>BNI1, PPF3</td>
<td>Cytoskeletal Organization</td>
<td>Bud neck</td>
</tr>
<tr>
<td>PFK1</td>
<td>Glycolysis, Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>PDX3</td>
<td>Lipid Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>YML030W</td>
<td>Metabolism</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>POG1</td>
<td>Mitosis</td>
<td>Nucleus</td>
</tr>
<tr>
<td>LOC1</td>
<td>mRNA binding</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>MRT4</td>
<td>mRNA binding</td>
<td>Nucleus</td>
</tr>
<tr>
<td>ADK1</td>
<td>Nucleotide phosphorylation</td>
<td>Nucleus</td>
</tr>
<tr>
<td>PHO5</td>
<td>Phosphate Metabolism</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>MRPS35</td>
<td>Protein Biosynthesis</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>VMA22</td>
<td>Protein Complex Assembly</td>
<td>Vacuole</td>
</tr>
<tr>
<td>VMA21</td>
<td>Protein Complex Assembly</td>
<td>Vacuole</td>
</tr>
<tr>
<td>VMA2</td>
<td>Protein Complex Assembly</td>
<td>Vacuole</td>
</tr>
<tr>
<td>SAM37</td>
<td>Protein Complex Assembly</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus,</td>
</tr>
<tr>
<td>DOA1</td>
<td>Protein Degradation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleus,</td>
</tr>
<tr>
<td>UMP1</td>
<td>Protein Degradation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>NPL6</td>
<td>Protein Import Into Nucleus</td>
<td>Nucleus</td>
</tr>
<tr>
<td>MNN10, BED1,</td>
<td>Protein Mannosylation</td>
<td>Endoplasmal Reticulum</td>
</tr>
<tr>
<td>SLC2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YIL064W</td>
<td>rRNA processing</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>RAI1</td>
<td>rRNA processing</td>
<td>Nucleus</td>
</tr>
<tr>
<td>TFP1, CLS8,</td>
<td>Stress Response</td>
<td>Vacuole</td>
</tr>
<tr>
<td>VMA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUD27</td>
<td>TOR pathway</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>TCO89</td>
<td>TOR pathway</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>VPS54</td>
<td>Trafficking</td>
<td>Golgi</td>
</tr>
<tr>
<td>ANT1</td>
<td>Transporter</td>
<td>Peroxisome</td>
</tr>
<tr>
<td>TAT2</td>
<td>Transporter</td>
<td>Plasma Membrane</td>
</tr>
</tbody>
</table>
### Genes of the Hof1 pathway

The genes that were mapped into the Hof1 pathway due to the criteria outlined above are depicted in **Tab.2**

### HOF1 PATHWAY

<table>
<thead>
<tr>
<th>Gene name</th>
<th>GO Biological Process</th>
<th>Cellular Compartement</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARO2</td>
<td>Amino Acid Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>RXT2</td>
<td>Cell Cycle Control</td>
<td>Nucleus</td>
</tr>
<tr>
<td>BNI4</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>SMI1</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>YAF9</td>
<td>Chromatin Organization</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>ERV2</td>
<td>Di-sulfate Bond Formation</td>
<td>Endoplasmatic Reticulum</td>
</tr>
<tr>
<td>FOB1, HRM1</td>
<td>DNA Metabolism</td>
<td>Nucleus</td>
</tr>
<tr>
<td>RAD52</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>RAD23</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>MMS22</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>GSH2</td>
<td>Glutathione Synthase</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>POS5</td>
<td>Kinase activity</td>
<td>Mitochondrion</td>
</tr>
</tbody>
</table>

**Tab.1:** The list of genes that are synthetic lethal with baits *hof1Δ, cyk3Δ* and *ase1Δ* therefore act genetically in the Myo1-pathway of cytokinesis.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDB1</td>
<td>Metabolism</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>ZTA1</td>
<td>Metabolism</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>TRK1</td>
<td>Metabolism</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>ADE12</td>
<td>Nucleotide Biosynthesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>NVJ1</td>
<td>Nucleus Vacuole Insertion</td>
<td>Nucleus</td>
</tr>
<tr>
<td>ERF2</td>
<td>Palmitoyltransferase</td>
<td>Endoplasmatic Retriculium</td>
</tr>
<tr>
<td>PEX28</td>
<td>Peroxisome Biogenesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>INO4</td>
<td>Phospholipid Biosynthesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>EPT1</td>
<td>Phospholipid Biosynthesis</td>
<td>Endoplasmatic Retriculium</td>
</tr>
<tr>
<td>RPS27B</td>
<td>Protein Biosynthesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>RIM21</td>
<td>Protein Folding</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>PMT5</td>
<td>Protein Glycosylation</td>
<td>Endoplasmatic Retriculium</td>
</tr>
<tr>
<td>MAP1</td>
<td>Protein Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>PRB1</td>
<td>Protein Metabolism</td>
<td>Vacuole</td>
</tr>
<tr>
<td>VPS66</td>
<td>Protein Targeting to Vacuole</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>NOP6</td>
<td>RNA Metabolism</td>
<td>Nucleus</td>
</tr>
<tr>
<td>PIG1</td>
<td>Signalling</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>LTE1</td>
<td>Signalling</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>MED2</td>
<td>Transcription</td>
<td>Nucleus</td>
</tr>
<tr>
<td>MEF2</td>
<td>Translation</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>MRPL49</td>
<td>Translation</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>TPS1,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BYP1,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIF1,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDP1,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGS1</td>
<td>Trehalose Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>SOY1</td>
<td>Unknown</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>ACO2</td>
<td>Unknown</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>YNL100W</td>
<td>Unknown</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>FMP34</td>
<td>Unknown</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>PSY2</td>
<td>Unknown</td>
<td>Nucleus</td>
</tr>
<tr>
<td>YDL144C</td>
<td>Unknown</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>YML095C-A</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YGL041C</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YGL149W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Tab.2:** The list of genes that are synthetic lethal with baits myo1Δ, cyk3Δ and ase1Δ and therefore act genetically in the Hof1-pathway of cytokinesis.
Genes of the Cyk3 pathway

The genes that were mapped into the Cyk3 pathway due to the criteria outlined above are depicted in Tab.3

**CYK3 PATHWAY**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>GO Biological Process</th>
<th>Cellular Compartement</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP1</td>
<td>Actin Cytoskeletal Organization</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>MET6</td>
<td>Amino Acid Biosynthesis</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>CHS5, CAL3</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Golgi</td>
</tr>
<tr>
<td>PKH1</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>GAS1, CWH52, GGP1</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>YLR194C</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>ECM33</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>SLT2, BYC2, MPK1, SLK2</td>
<td>Cell Wall Stress Response</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>IWR1</td>
<td>Cell Wall Stress Response</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>RHO2</td>
<td>Cytokinesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>CYK3</td>
<td>Cytokinesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>ARC18</td>
<td>Actin Cytoskeletal organization</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>MRE11, RAD58, XRS4</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>FYV6</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>TOF1</td>
<td>DNA Replication/Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>TOP3, EDR1</td>
<td>DNA Topoisomerase</td>
<td>Nucleus</td>
</tr>
<tr>
<td>ERG24</td>
<td>Ergosterol Biosynthesis</td>
<td>Endoplasmatic Reticulum</td>
</tr>
<tr>
<td>STP22</td>
<td>ESCRT-I Trafficking</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>MOH1</td>
<td>ESCRT-I Trafficking</td>
<td>Unknown</td>
</tr>
<tr>
<td>IST1</td>
<td>ESCRT-III Trafficking</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>SWI6, PSL8, SDS11</td>
<td>G1/S Transition</td>
<td>Nucleus</td>
</tr>
<tr>
<td>WHI3</td>
<td>G1/S Transition</td>
<td>Cytoplasm</td>
</tr>
</tbody>
</table>
HSP82, HSP83, HSP90
Heat Shock Response
Plasma Membrane

HCH1
Heat Shock Response
Nucleus, Cytoplasm

Mnn11
Mannosyltransferase
Golgi

SSO2
Membrane Fusion
Plasma Membrane

SSO1
Membrane Fusion
Plasma Membrane

ARO1
Metabolism
Cytoplasm

FSH2
Metabolism
Cytoplasm

IDH2
Metabolism
Mitochondrion

TPS2, HOG2, PFK3
Metabolism
Mitochondrion

COX12
Metabolism
Mitochondrion

GCR2
Metabolism
Nucleus

CUP5, CLS7, GEF2, VMA3
Metabolism
Vacuole

ITR1
Metabolism
Plasma Membrane

MTQ2
Methyl Transferase
Nucleus, Cytoplasm

MDM34
Mitochondrial Morphology
Mitochondrion

NAB6
mRNA binding
Cytoplasm

MAK10
N-Acetylation
Cytoplasm

OST4
N-linked Glycosylation
Endoplasmatic Retriculium

ALG8
N-linked Glycosylation
Endoplasmatic Retriculium

ESC1
Nuclear Envelope Organization
Nucleus

URA1
Nucleotide Biosynthesis
Cytoplasm

PEX1, PAS1
Peroxisome Biogenesis
Cytoplasm

CPT1
Phosphatidylcholine Biosynthesis
Endoplasmatic Retriculium

INO1, APR1
Phospholipid Biosynthesis
Cytoplasm

UAF30
Pol I Transcription
Nucleus

TYE7
Pol I Transcription
Nucleus

SRB8
Pol II Transcription
Nucleus

SRB2
Pol II Transcription
Nucleus

KTI12
Pol II Transcription
Nucleus, Cytoplasm

PET111
Protein Biosynthesis
Mitochondrion

SEL1
Protein Degradation
Endoplasmatic Retriculium

ALF1
Protein Folding
Nucleus, Cytoplasm

OXA1, PET-TS1402
Protein Metabolism
Mitochondrion

IRA2
Ras Mediated Signalling
Cytoplasm

RIB1
Riboflavin Biosynthesis
Nucleus, Cytoplasm

MSS116
RNA Metabolism
Mitochondrion

UBP8
SAGA Complex
Nucleus

SGF73
SAGA Complex
Nucleus
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Function</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>STE20</td>
<td>Signalling</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>TUS1</td>
<td>Signalling</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>BEM1</td>
<td>Signalling</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>PPZ1</td>
<td>Signalling</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>LCB4</td>
<td>Sphingolipid Biosynthesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>ASE1</td>
<td>Spindle Stability</td>
<td>Nucleus</td>
</tr>
<tr>
<td>MSN2</td>
<td>Stress Response</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>APL3</td>
<td>Trafficking</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>SEC72</td>
<td>Trafficking</td>
<td>Endoplasmatic Retriculium</td>
</tr>
<tr>
<td>APL4</td>
<td>Trafficking</td>
<td>Endoplasmatic Retriculium</td>
</tr>
<tr>
<td>CHS6</td>
<td>Trafficking</td>
<td>Golgi</td>
</tr>
<tr>
<td>YPT7, AST4, VAM4</td>
<td>Trafficking</td>
<td>Golgi</td>
</tr>
<tr>
<td>RUD3</td>
<td>Trafficking</td>
<td>Golgi</td>
</tr>
<tr>
<td>TVP15</td>
<td>Trafficking</td>
<td>Golgi</td>
</tr>
<tr>
<td>PEP3, VAM8, VPS18, VPT18</td>
<td>Trafficking</td>
<td>Vacuole</td>
</tr>
<tr>
<td>VAM7</td>
<td>Trafficking</td>
<td>Vacuole</td>
</tr>
<tr>
<td>RPS19B, RP55B</td>
<td>Translation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>DIA4</td>
<td>Translation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>RPL21A</td>
<td>Translation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>RPL22A</td>
<td>Translation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td><strong>YOL070C</strong></td>
<td>Unknown</td>
<td>Bud Neck</td>
</tr>
<tr>
<td><strong>YPL066W</strong></td>
<td>Unknown</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>YMR253C</td>
<td>Unknown</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>YOR059C</td>
<td>Unknown</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>NRP1</td>
<td>Unknown</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>YOR251C</td>
<td>Unknown</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>FMP21</td>
<td>Unknown</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>YLR193C</td>
<td>Unknown</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>PHM8</td>
<td>Unknown</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>YNL095C</td>
<td>Unknown</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>YJR146W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YML035C-A</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YML003W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YMR206W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YNL324W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YNL296W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YNL198C</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YLR358C</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gene name</td>
<td>GO Biological Process</td>
<td>Cellular Compartment</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>SKT5, CAL2,</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>CHS4, CSD4</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>G23 Plastid</td>
</tr>
<tr>
<td>CHS3, CAL5,</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>CSD2, DIT101,</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>KTI2</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>ECM40, ARG7</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>YLR338W</td>
<td>Cell Wall Organization and Biogenesis</td>
<td>Unknown</td>
</tr>
<tr>
<td>IES1</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>CLA4, ERC10</td>
<td>Cytokinesis</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>VRP1, END5,</td>
<td>Cytoskeletal Organization</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>MDP2</td>
<td>Cytoskeletal Organization</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>RVS161</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>RAD50</td>
<td>DNA Repair</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>RAD6, UBC2</td>
<td>DNA Repair</td>
<td>Nucleus</td>
</tr>
</tbody>
</table>

**Tab.3**: The list of genes that are synthetic lethal with baits myo1Δ, hof1Δ and ase1Δ and therefore act genetically in the Cyk3-pathway of cytokinesis.

**Genes present in all six screens**

The genes that showed up in all 6 screens are depicted in **Tab.4**.

**A FOURTH CYTOKINETIC PATHWAY**
<table>
<thead>
<tr>
<th>Genes</th>
<th>Functions</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAX4, CWH8</td>
<td>Dolichyl Metabolism</td>
<td>Endoplasmatic Reticulium</td>
</tr>
<tr>
<td>FAB1, SVL7</td>
<td>Lipid Biosynthesis</td>
<td>Vacuole</td>
</tr>
<tr>
<td>DLD3</td>
<td>Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>ADO1</td>
<td>Metabolism</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>VMA10</td>
<td>Metabolism</td>
<td>Vacuole</td>
</tr>
<tr>
<td>ALP1</td>
<td>Plasma Membrane Transporter</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>FEN2</td>
<td>Plasma Membrane Transporter</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>GLN3</td>
<td>Pol II Transcription</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>DIA2</td>
<td>Protein Degradation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>TRF4, PAP2</td>
<td>RNA Quality Control</td>
<td>Nucleus</td>
</tr>
<tr>
<td>SPT20, ADA5</td>
<td>SAGA Complex</td>
<td>Nucleus</td>
</tr>
<tr>
<td>BEM2, IPL2, SUP9</td>
<td>Signalling</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>STE50</td>
<td>Signalling</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>KCS1</td>
<td>Signalling</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>PLC1</td>
<td>Signalling</td>
<td>Nucleus</td>
</tr>
<tr>
<td>SNF4</td>
<td>Signalling</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>CIK1</td>
<td>Spindle Organization</td>
<td>SPB</td>
</tr>
<tr>
<td>VPS9, VPL31, VPT9</td>
<td>Trafficking</td>
<td>Vacuole</td>
</tr>
<tr>
<td>PCP1</td>
<td>Trafficking</td>
<td>Mitochondrion</td>
</tr>
<tr>
<td>TIF4631</td>
<td>Translation</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>YEL057C</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>YLR402W</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Tab.4:** The list of genes that are synthetic lethal with baits myo1Δ, hof1Δ, cyk3Δ and ase1Δ and therefore act either in a common upstream pathway or in a fourth cytokinetic pathway. The first possibility is however not very likely as genes that act upstream of Myo1-, Hof1-, and Cyk3-depenen pathwày are expected to be most likely essential.
Genes in the "synthetic lethal with ase1Δ" pathway

The list of genes that are potential candidates to play a role in the NoCut checkpoint, either in sensing, signaling or as effectors is listed in Tab.5

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>GO biological process</th>
<th>Cellular compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUB3</td>
<td>Spindle Checkpoint</td>
<td>Kinetochore</td>
</tr>
<tr>
<td>BUB1</td>
<td>Spindle Checkpoint</td>
<td>Kinetochore</td>
</tr>
<tr>
<td>CSM1</td>
<td>Spindle Checkpoint</td>
<td>Kinetochore</td>
</tr>
<tr>
<td>MAM1</td>
<td>Spindle Checkpoint</td>
<td>Kinetochore</td>
</tr>
<tr>
<td>PTC1</td>
<td>Phosphatase</td>
<td>Nucleus, Cytoplasm</td>
</tr>
<tr>
<td>RTS1</td>
<td>Phosphatase</td>
<td>Bud Neck</td>
</tr>
<tr>
<td>FIN1</td>
<td>Microtubule Binding</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>CIN8</td>
<td>Kinesin</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>BIM1</td>
<td>Microtubule Binding</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>KAR3</td>
<td>Microtubule Motor</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>BIK1</td>
<td>Microtubule Binding</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>SPO7</td>
<td>Nuclear Organizaition</td>
<td>Nucleus, Endoplasmic Reticulum</td>
</tr>
<tr>
<td>SCS2</td>
<td>Phospholipid Metabolism</td>
<td>Nucleus, Endoplasmic Reticulum</td>
</tr>
<tr>
<td>CTF8</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>CTF18</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>TRF4</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>TOF1</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>CSE2</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>AHC1</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>SKP2</td>
<td>Protein Metabolism</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>PIF1</td>
<td>Chromatin Organization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>DOT1</td>
<td>DNA repair</td>
<td>Nucleus</td>
</tr>
<tr>
<td>YJL217w</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>YOL131w</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>
LGE1  unknown  unknown
AVL9  unknown  unknown
MLF3  unknown  unknown
YJL211c unknown  unknown
YAR030c unknown  unknown
YLR235c unknown  unknown
OPI11  unknown  unknown
YDL073w unknown  unknown
YDR042c unknown  unknown
YDR149c unknown  unknown
YGL088w unknown  unknown
LDB18  unknown  unknown
YDR332w unknown  unknown

| Tab.5: The list of genes that are synthetic lethal with baits ase1Δ, hof1Δ, myo1Δ but not with kar9Δ (REF Grava et al, Dev. Cell, 2006 Apr; 10(4):425-39 and therefore are candidates that might act in the NoCut pathway. One has to consider that genes involved in mitotic spindle assembly and spindle elongation have been shown to be synthetic lethal with ase1Δ as well, so this list has to be taken with caution and candidates have to be tested for their involvement in NoCut very thoroughly. |

Discussion

The Myo1 pathway

Out of the 46 non-essential genes that our interaction genetic network assigns to the Myo1-dependent pathway, only 4 of the corresponding products (Elm1, Bni1, Shs1 and the uncharacterized ORF Ydr248c, which we rename here Cyk7) have been previously found to localize to the bud-neck. The products of the majority of the other genes localize to places as different as the nucleus, the endoplasmic reticulum, the cytoplasm and the vacuole. Apart from known players in the Myo1-pathway, e.g., Elm1, and Bni1, the Myo1-dependent pathway showed a strong dependence on metabolic genes for function. Indeed, 10 out of the 44 genes identified function in various aspects of the metabolism, such as the phospho-fructose kinase gene Pfk1 involved in glycolysis. In line with these findings, proper function of the Myo1-pathway relies on TOR function, since our screens assigned the two TORC1 and TORC2 components Bud27 and Tco89.
to the Myo1 pathway. Interestingly, our network puts the Ump1, Doc1 and Doa1 genes in the Myo1 pathway. These three genes are involved in ubiquitin-dependent protein degradation. Additionally proper function of the Myo1 pathway seems to require the Protein Complex assembly machinery of Vma21, Vma22 and Sam37.

The Hof1 pathway

Recent studies mostly in *S. pombe* have established a role of Hof1 in remodeling the actin cytoskeleton and facilitating membrane curvature during the process of CAR contraction (REF Tsujita/Takenawa 2006). In line with this, deletion of the *HOF1* gene leads to defects in CAR contraction (REF Vallen/Bi 2000) and morphological defects. According to our definition we could map 43 genes in the Hof1 pathway. In this light it is interesting to find two genes clearly involved in phospho-lipid biosynthesis (Ino4, Ept1), both localizing to the endoplasmic reticulum. Additionally protein glycosylation (Pmt5), di-sulfite bond formation(Erv2) and palmitoylation(Erf2) seem to be vital for proper function of the Hof1 pathway, as well as cell wall biogenesis (Bni4 and Smi1). Remarkably, our network assigns the *LTE1* gene, involved in the activation of the mitosis exit network (MEN), more specifically to the Hof1-pathway, suggesting that Hof1 function strongly depends on MEN function. This observation is in line with recent data that components of the mitotic exit network are required to re-polymerize the actin cytoskeleton on both sides of the bud neck, which corresponds to the timing of contractile ring activation and that translocation of Dbf2/Dbf20 to the bud neck is required to promote Hof1 phosphorylation, which is vital to trigger acto-myosin ring contraction (REF Corbett/Price 2006). Lte1 is one of the few members of the Mitotic Exit Nework, which are non-essential and therefore it is not possible to find more hits from the MEN in our screens. However, there is more and more evidence accumulating that the Mitotic Exit Network acts on the cytokinetic machinery via the Hof1-dependent pathway.

Remarkably also, our study indicates that three DNA repair genes (*Rad23, Rad52, Mms22*) are also required for proper function of the Hof1-pathway. These data suggest that this pathway might be down regulated in response to DNA damage.
The Cyk3 pathway

As for the other pathways, the proteins identified by our screens to function in the Cyk3 pathway localized to all different compartments of the cell. The most prominent compartment was the cytoplasm to which 21 Cyk3-pathway proteins were found to localize. Among those were at least three ribosomal proteins, a few metabolic enzymes (8), such as Met6, Aro1, Fsh2, Ino1, and Ura1. One protein, Whi3, is an RNA-binding protein involved together with the nuclear protein Swi6 (also identified as a Cyk3-pathway protein) in the regulation of the G1/S transition. A number of 8 components of the mitochondrion were also identified. Components of the secretory pathway were strongly represented in the Cyk3-pathway. These hits distributed throughout the endoplasmic reticulum, golgi complex and the exocytic machinery (Sec72). These included Chs5 and Chs6, two proteins of the late Golgi that have been involved in vesicular trafficking to the bud neck plasma membrane. These proteins are essential for the delivery of the chitin synthase Chs3 and the formation of a ring of chitin in the cell wall at the bud neck. However, Chs3 was not identified as a specific component of the Cyk3 pathway (see discussion).

Interestingly, the yeast ortholog of the mammalian protein Tsg101, Stp22, was also mapped into the Cyk3 pathway. Tsg101 is known to act in abscission in mammalian cells (Carlton/Martin-Serrano 2007, Morita/Sundquist 2007). Additionally, our screens also identified the components of the ESCRTI/III complex Moh1 and Ist1 as Cyk3-pathway components, strengthening the idea that components of the machinery involved in endosomal vesicle formation may play an active and direct role in abscission, most probably via membrane fission (REF Morita/Sundquist 2007). Furthermore, the syntaxin 1 homologues Sso1 and Sso2 were found to act in the Cyk3 pathway as well. The sso1Δ sso2-1 temperature sensitive double mutant is impaired in bud neck closure at the end of cytokinesis, as already reported (REF Jäntti/Ronne 2002). Remarkably however, neither SNC1 nor SNC2 were identified in our screen. These two v-SNAREs are supposed to work together with Sso1 and Sso2 in vesicle fusions with the plasma membrane. Possibly, during cytokinesis Sso1 and Sso2 are involved in membrane remodeling events without involving vesicles.

Most remarkably, our interaction network involved the proteins Ste20 (a PAK-like kinase), Tus1 (a Rho GEF), Apl3 (an adaptor for clathrin mediated vesicle formation),
Ypl066w, Yol070c and Ypl158c (three open reading frame of unknown function). What is remarkable about these six different proteins is that previous studies have indicated independently for each of them that they localize to the bud neck at least during part of the budding cycle. They are therefore excellent candidates to be fairly directly involved in cytokinesis. As discussed in the introductory section we would like to suggest renaming Ypl066w into Cyk4, Yol070c into Cyk5 and Ypl158c into Cyk6 due to their role in cytokinesis. Cyk4, Cyk5 and Cyk6 are conserved only in the fungi kingdom. CYK4 encodes a protein of 479 amino acid, which seems to be of unique nature in *S. cerevisiae* as no similar proteins can be found by homology search(source: String-EMBL). However, Cyk4 interacts with very prominent proteins like the Rho1 and Rho2 GEF Tus1(REF Schmelzle/Hall 2002) and the inorganic pyro-phosphatase Ipp1(REF Gavin/Superti-Furga 2002). In contrast to published literature we also found Ipp1 to localize to the bud neck(see Fig.13), which is consistent with its association with a bud neck localized complex. However, which function the essential gene Ipp1 fulfills at the bud neck is not clear.

![Ipp1-GFP](image)

**Fig. 13:** The inorganic pyro-phosphatase Ipp1 localizes to the bud neck and in a punctated pattern to the cell cortex in late mitotic stages.

Additionally Cyk4 has been found to form a complex with Cdc48(REF Krogan/Greenblatt 2006). This is particularly interesting as the p97 homolog Cdc48 is implicated in ubiquitin dependent portein catabolism and vesicle fusion. The possible role of Cdc48 is furthermore backed up by the fact that the non-essential Cdc48 adaptor protein Sel1 has been mapped into the Cyk3 pathway by our screens.
The CYK5 gene encodes a 501 amino acid protein that also localizes to the bud neck. Upon its deletion, cells do not exhibit any obvious phenotypes. Cyk5 interacts with Boi1 in a complex, which might suggest probably a role during the NoCut response. These hypotheses however still have to be tested.

The CYK6 gene encodes a 758 amino acid protein, whose function will be discussed extensively in Result Section III.

Taking all these results together, out of the three pathways, our screens suggested that the Cyk3 pathway is the most richly populated in terms of number of genes involved, the number of potentially new genes directly involved in cytokinesis, and in the frequency of membrane remodeling components.

The fourth cytokinetic pathway?

A fourth group of 32 genes was identified, that showed synthetic lethal interactions with all four baits, myo1Δ, hof1Δ, cyk3Δ and ase1Δ. This group comprised genes involved in cell wall biogenesis, such as the chitin synthase gene Chs3, and Chs4, which encodes the main chitin synthase activatory protein, and genes encoding the putative cell wall componens Ecm40 and Ylr338w. Chitin is the main constituent of the primary septum. However, as for the other pathways, this category comprised genes involved in DNA damage response (Rad6 and Rad50), in lipid metabolism (Cax4, Plc1 and Fab1), vesicle trafficking and the cytoskeleton (Vps9, Vma10, Pcp1, Rvs161, Vrp1), and signaling (Cla4, Ste50, Bem2, Kcs1). Vrp1 and Rvs161 have already been previously shown to be important for cytokinesis (REF Thanabalu/Munn 2001). Consistent with this report a deletion in the gene VRP1 affects both Myo1- and Hof1-dependent pathways.

This class of genes that show synthetic lethal interactions with each of myo1Δ, hof1Δ, and cyk3Δ might represent a fourth pathway in cytokinesis, perhaps the pathway involved in primary septum formation.

For the reminder of this study, we focused our attention to the genes identified in the Cyk3-pathway and encoding bud neck proteins, i.e., the product of which do localized to the actual site of abscission. As mentioned above, these corresponded to the 8 proteins
Cyk3, Bem1, Ste20, Tus1, Apl3, Cyk4, Cyk5 and Cyk6. Cyk6 will be described in more details elsewhere (REF Rauter/Richter/Seufert in preparation 2009).

The "synthetic lethal with \textit{ase1\textDelta}" pathway

As a result of this extraction using Boolean comparison over multiple pathways we were able to extract a comprehensive list of genes that might possibly play a role in sensing, signaling or as effectors within the NoCut checkpoint response. However, as Ase1 is involved in multiple processes, a careful elimination of genes that act in other cellular processes like spindle assembly, spindle stabilization and nuclear positioning, is absolutely vital.

When this study was started, no comprehensive genetic interaction network of \textit{ASE1} existed. In the meantime however, several groups performed comparable experiments to ours and came to comparable results that underline the validity of the screening data presented here. I will discuss the congruent datasets and discuss their possible physiological relevance in a cellular context.

Others and we have found that many kinetochores mutants exhibit synthetic lethal interactions with \textit{ase1\textDelta}. Independent studies confirmed parts of these interactions at least for \textit{csm1\textDelta} (REF Pan/Boeke 2006) and \textit{bub3\textDelta} (REF Daniel 2006). This study also unraveled \textit{mad2\textDelta} as synthetic lethal with \textit{ase1\textDelta} and our study unraveled \textit{mam1\textDelta} and \textit{bub1\textDelta} as additional synthetic lethal interactors of \textit{ase1\textDelta}. In fission yeast it has been shown that loss of Ase1 leads to a delay in anaphase onset that is dependent on the Spindle Assembly Checkpoint proteins most likely due to the loss of tension during bipolar attachment(REF Meadows/Millar 2008), which has its origin in reduced spindle stability. As SAC components are conserved between fission and budding yeast, it is very likely, that \textit{S.cerevisiae} has evolved comparable mechanisms before anaphase onset. The synthetic lethal interaction of kinetochore genes with \textit{ase1\textDelta} is therefore most likely not due to their participation in the NoCut pathway.

A second organelle that appears to become essential in the absence of the Ase1 protein is the Spindle Pole Body. Fin1, Cin8, Bim1, Kar3 and Bik1 localize at least partially during the cell cycle to the Spindle Pole Body and exhibit a synthetic lethal interaction with \textit{ase1\textDelta}. Excitingly, parallel studies confirmed all these proteins as true hits. Cin8 and
Bim1 (REF Pan/Boeke 2004), Fin1 (REF Woodbury/Morgan 2007), Kar3 (REF Tong/Boone 2004) and Bik1 (REF Pellman/Tu 1995) have come to the same conclusion as we did in our study. These confirmations are of great value as they support the stringency and accuracy of our genetic approach as well as underline the physiological significance of the Spindle Pole Body in absence of a mitotic spindle midzone. It has been shown that the spindle midzone contributes to the organization of the astral microtubule network, which allows proper positioning of the nucleus. Therefore these genetic interactions have more to be seen in the light of spindle positioning in metaphase than abscission (REF deGramont/Cohen-Fix 2007). Additionally to that, Kar3 and Bim1 are required for spindle stability during Anaphase B, so unlikely to act in NoCut (REF Gardner/Bloom 2008).

The current model of the NoCut pathway states that the integrity of the spindle midzone is monitored by the NoCut checkpoint (REF Norden/Barral 2006). In Mendoza/Barral 2009 - in press in Nature Cell Biology (see chapter manuscripts) we provide evidence that in fact what is sensed by the NoCut checkpoint is the proximity of chromatin to the Aurora B kinase Ipl1. In line with these results, the group of genes responsible for chromatin organization and dynamics becomes particularly interesting. Ctf8, Ctf18, Trf4, Tof1, Cse2, Pif1 and Ahc1 all localize to the nucleus, have a role in chromatin dynamics and organization and gene deletions of them show synthetic lethal interaction with ase1Δ. Ctf8 (REF Pan/Boeke 2006) and Ctf18 (REF Pan/Boeke 2006) have been confirmed in independent studies and all the other hits are specific to our screens. In the meantime we could show that Ahc1 plays a role in sensing the proximity of chromatin and the Aurora B kinase Ipl1 as cells that are mutant for AHC1 are not any more able to mount a NoCut response (REF Mendoza/Barral 2009 - see Chapter Manuscripts). These results are encouraging, as this group of genes involved in chromatin organization and dynamics seems to be synthetic lethal with ase1Δ out of NoCut reasons specifically.

Rts1 and Ptc1 are parts of phosphatase complexes and upon their deletions they exhibit a synthetic lethal interaction with ase1Δ. Rts1 localizes to the bud-neck and has high potential to function as effectors in the NoCut pathway as it is known that Ipl1 never exits the nucleus and only sends Boi1 and Boi2 to the bud neck to inhibit abscission (REF Norden/Barral 2006). It has been shown that cells depleted for RTS1 exhibit an abscission defect (REF Dobbelaeere/Barral 2003). As Rts1 is a phosphatase and the NoCut pathway involves the Aurora B kinase and the Polo kinase Cdc5, Rts1 might play
a role in the reversion of the NoCut response. The deletion of classical NoCut genes does not exhibit a cytokinetic defect because of their nature acting as inhibitory genes. However, it has been observed that \textit{rts1}Δ cells exhibit an abscission delay, which is consistent with the idea that \textit{rts1}Δ cells are incapable to revert the NoCut-dependent abscission delay, which would lead to a cytokinetic defect as well(REF Dobbeltaere/Barral 2003). Whether this holds true, remains to be elucidated.

At last it would be important to explore the group of unknown genes that is the by far predominant population within the hits found to be synthetic lethal with \textit{ase1}Δ. As I have discussed in the introductory section of this chapter synthetic biology is a powerful tool to unravel "hidden" phenotypes. I have named this to be one of the reasons why 25\% of the yeast genome are still not functionally annotated. This is particularly important for inhibitory pathways like the NoCut checkpoint, as depletions in this pathway do not exhibit a dramatic phenotype \textit{per se}. Only once the cells are stressed in the right way, these inhibitory pathways become essential and dramatic phenotypes are the consequence(REF Norden/Barral 2006). This might be one of the potential reasons why the group of uncharacterized ORFs is so huge within the total amount of hits. If this were true, this group of uncharacterized ORFs would be the group with high biological potential regarding NoCut participation. Secondly, due to the fact that several groups were able to reproduce at least parts of our synthetic interaction map of \textit{ase1}Δ, one can look at these uncharacterized ORFs as true hits with very high confidence. A possibility to check whether these candidates play a role in the NoCut response would be to check, whether depletion of these genes restore the NoCut dependent inhibition of abscission in the \textit{ndc10-1} background. This assay proved suitable to investigate, whether or not a gene plays a role in NoCut(REF Norden/Barral 2006, Mendoza/Barral 2009).

\textbf{Confirmation of the Cyk3-pathway candidates by manual tetrad analysis}

As a first approach, we re-tested, whether the disruption of the corresponding genes indeed caused synthetic lethal interactions with the \textit{myo1}Δ and \textit{hof1}Δ mutations but not with \textit{cyk3}Δ. Therefore, diploid strains heterozygously disrupted for either of the \textit{MYO1}, \textit{HOF1} and \textit{CYK3} genes were constructed, where the targeted gene was replaced with the NAT cassette, conferring resistance to the drug nourseothricin(REF Goldstein/McCusker 1999). In these heterozygous diploids, the \textit{CYK3}, \textit{BEM1}, \textit{STE20},
TUS1, APL3, CYK4, and CYK5 genes were targeted for heterozygous disruption, using a PCR-based approach to replace them with the kanamycin resistance gene, KAN\(^R\), conferring resistance to the drug G418, using high fidelity PCR-based methods of genomic DNA preparations of haploid knock-out strains, one-step transformations and homologue recombination as described in Materials and Methods. Disruption was verified by PCR and the correct heterozygous double mutants were subjected to sporulation and the tetrads were subsequently dissected. We could confirm all genetic interactions as we have previously obtained them in the screens except for ste20\(\Delta\). For the full genetic analysis be referred to the supplementary material Fig.S1 and Fig.S2. This assay confirmed that cyk3\(\Delta\), cyk4\(\Delta\), cyk5\(\Delta\), apl3\(\Delta\) and tus1\(\Delta\) are all strongly synthetic lethal with both myo1\(\Delta\) and hof1\(\Delta\) but not with the cyk3\(\Delta\) mutation. In case of ste20\(\Delta\) the genetic interaction with hof1\(\Delta\) was fully confirmed as well as the full viability in combination with cyk3\(\Delta\). However, the genetic interaction with myo1\(\Delta\) could not be fully confirmed as only 4 out of 23 spores - double mutant for myo1\(\Delta\) and ste20\(\Delta\) - formed inviable progenies. As expected, in the same assays myo1\(\Delta\) and hof1\(\Delta\) were also synthetic lethal with each other. In this assay, the bem1\(\Delta\) mutation proved difficult to score, as it caused extensive spore lethality already as a single mutation. Therefore we dropped this gene already at this stage. In summary, for 5 out of 6 hits tested, the synthetic lethality observed during screening was confirmed by tetrad analysis, thereby validating our multiple screen approach.
Results II

The NoCut checkpoint targets the GTPase Rho2 to inhibit the completion of abscission
Front image: A series of z-aquisitions of a rho2Δ mutant strain exhibiting an abscission defect; note the contracted but not resolved membrane stained with PH-GFP; SPB stained with Spc72-GFP
NoCut interacts with abscission

In both mammalian and yeast cells, a regulatory pathway, called NoCut, ensures next that abscission is not initiated right upon the completion of actomyosin ring contraction, as long as chromosome segregation is not completed (Norden/Barral 2006; Steigemann/Gerlich 2009, Mendoza/Barral 2009). Indeed, in cells with spindle elongation or chromatin segregation defects a dedicated pathway inhibits abscission. In both mammalian and yeast cells, this pathway involves the kinase Aurora B and the chromosome-passenger complex. While the effectors of Aurora B have not yet been identified in animal cells, the anillin-related proteins Boi1 and Boi2 mediate aurora B/Ipl1-dependent inhibition of abscission in budding yeast. Remarkably, defects in NoCut function show radically different outcomes between yeast and animal cells. Inactivation of NoCut in budding yeast leads to inappropriate abscission even in cells with chromatin remaining in the cleavage plane, and hence to chromosome breakage. By contrast, NoCut inactivation in Hela cells with chromosome bridges leads to abscission failure, furrow regression and tetraploidization (REF Steigemann/Gerlich 2009).

However, how NoCut interferes with the abscission machinery is unknown. At least in budding yeast, the proteins Boi1 and Boi2 ensure the communication between the spindle midzone, where the chromosome passenger kinase Aurora B/Ipl1 monitors chromosome segregation (REF Mendoza/Barral 2009, in press Nature Cell Biology), and the bud neck, where abscission takes place. These two proteins shuttle between the nucleus and the cell cortex during the cell cycle, and their export from the nucleus requires Ipl1 activity. How Boi proteins reversibly inhibit abscission is not known.

Tus1 and Cyk4 are displaced from the bud neck during NoCut response

As previously shown the NoCut pathway reversibly inhibits abscission in response to defects in mitotic spindle function (Norden/Barral 2006) and to failure of proper chromosome segregation, such as those due to topoisomerase II defects or expression of a non-cleavable form of cohesin (Mendoza/Barral 2009 submitted). To determine how NoCut impinges on abscission, we next investigated whether the localization of any bud neck components of the Cyk3 pathway changed upon NoCut activation. Therefore, we monitored the localization of Tus1-GFP, Ste20-GFP, Cyk3-GFP, Cyk4-GFP and Cyk5-
GFP reporter constructs in the \textit{n}dc\textit{10-1} mutant and the isogenic wild type cells released from pheromone induced G1 arrest into fresh medium at the restrictive temperature (37°C). Indeed, the \textit{n}dc\textit{10-1} mutant cells show robust and NoCut-dependent inhibition of cytokinesis already after the first cycle at restrictive temperature. After 90, 120 and 150 minutes at the restrictive temperature, the fraction of large budded cells with GFP fluorescence at the bud neck was determined for each of the reporters. Since each of these markers localize only transiently to the bud neck (see below), only a fraction of the large budded cells showed some signal already in the wild type cells, ranging from 5\% ± 1.5\% for Ste20-GFP to 53\% ± 4.8\% Cyk5-GFP. Compared to the wild type control, the \textit{n}dc\textit{10-1} mutation had no effect on Cyk3-GFP and Ste20-GFP localization. In contrast, it had a small (1.4 fold decrease) but reproducible effect on the localization of Cyk5-GFP, and a strong effect on the localization of both Cyk4-GFP and Tus1-GFP (3 to 5 fold decrease, respectively). Thus, spindle defects affected the localization to the site of cleavage of at least three out of these five components of the Cyk3 pathway.

\begin{center}
\includegraphics[width=\textwidth]{figure14.png}
\end{center}

\textbf{Fig. 14:} The localization of Tus1-GFP, Cyk4-GFP and Cyk5-GFP to the bud neck is dramatically reduced in an \textit{n}dc\textit{10-1} mutant when the NoCut checkpoint is activated. Cyk3-GFP and Ste20-GFP localization to the bud neck appear to be independent of the NoCut checkpoint.
Thus, we next asked whether any of these effects was caused by NoCut activity. The localization of the same reporter was therefore investigated also in the ndc10-1 ipl1-321 double mutant cells treated in the same manner. The Aurora kinase Ipl1 acts very upstream in NoCut signaling such that ndc10-1 ipl1-321 double mutant fail to properly inhibit abscission in response to chromosome segregation defects. Aurora inactivation extensively restored the localization of both Tus1- and Cyk4-, but not that Cyk5-GFP. It had no significant effect on the localization of either Cyk3- or Ste20-GFP. We concluded that the reduced localization of Tus1 and Cyk4 to the bud neck in ndc10-1 cells was indeed a response to NoCut activation. The localization of both proteins was also restored in ndc10-1 mutant cells lacking the two proteins Boi1 and Boi2, indicating that the effect of the ndc10-1 mutation on Tus1 and Cyk4 localization depended on these two NoCut effectors (data not shown).

![Fig. 15: The localization of Tus1-GFP and Cyk4-GFP to the bud neck is restored to levels unsignificantly different from wild type levels in ndc10-1 ipl1-321, where the NoCut checkpoint is artificially inhibited. We conclude, that localization of Tus1-GFP and Cyk4-GFP to the bud neck depends on the activation state of the NoCut checkpoint.](image)

The localization of Cyk3-GFP, Cyk5-GFP and Ste20-GFP to the bud neck appears to be independent of the activation state of the NoCut checkpoint.

These results suggest that NoCut impinges on abscission at least in part through the inhibition of Tus1 and Cyk4 localization to the site of cleavage.
Cytokinetic roles of the Cyk3-pathway components

Together, our results suggested that at least Tus1 and Cyk4 play some specific role in abscission. To determine whether this is the case, we next characterized and compared the cytokinetic roles of the different Cyk3-pathway components that localized to the bud neck. To characterize their involvement in acto-myosin ring contraction, we first monitored the dynamics of the contractile acto-myosin ring (CAR) in late mitosis, using a GFP-labeled version of the type II myosin, Myo1, as reporter. Thus, the Myo1-GFP allele was introduced into the tus1Δ, cyk3Δ, cyk4Δ, cyk5Δ, apl3Δ and ste20Δ single mutants, and CAR contraction was monitored by time-lapse microscopy (see Fig.16). The 14 optical z-sections with Δz=0.3μm were maximum projected and analyzed. As controls, actomyosin ring contraction was also characterized in the hof1Δ and wild type strains. These assays were carried out at room temperature.

In wild type cells, CAR contraction took in average 5.75min ±0.7min, in accordance to the literature (REF Vallen/Bi 2000). As previously described (REF Vallen/Bi 2000), CAR contraction was heavily slowed down in the hof1Δ mutant cells, where it took 10.4min ±2.1min in average. Remarkably, a number of Cyk3-pathway components were found to impinge on actomyosin ring contraction. Indeed, CAR contraction was slowed down in cyk3Δ (average time of contraction= 13min ± 0.5min), tus1Δ (average time of contraction= 9.9min ± 1.1min) and cyk4Δ (average time of contraction= 8.8 ± 3.2) single mutant cells. In contrast, the cyk5Δ, apl3Δ and ste20Δ mutations had no significant effect on actomyosin ring contraction(see Fig. 16).
Next, we investigated whether any of these mutations has also an effect on the resolution of the plasma membrane after contraction. The plasma membrane of dividing cells was visualized using as a reporter protein the plextrin-homology domain of *Rattus norvegicus* fused to GFP (REF Christianson/Hieter 1992). In the same cells, the spindle pole bodies (SPB) were visualized using the SPB protein Spc72 fused to GFP (Spc72-GFP) as reporter. In anaphase and post anaphase cells, i.e., with one SPB in the bud and one SPB in the mother cell, we scored the state of the plasma membrane at the bud neck as previously described (REF Norden/Barral 2006; Mendoza/Barral 2009 - submitted). In short, three distinct physiological states were distinguished: (1) the bud neck membrane was open, indicating CAR contraction had not yet occurred, (2) it was contracted but not resolved into two separate membranes, indicating that the cell had successfully completed CAR contraction but not abscission, or (3) the plasma
membrane was resolved into two, indicating that abscission had taken place (see Fig. 27a).

Using this assay, we observed that hof1Δ cells completed abscission soon after contraction, since they did not accumulate in the contracted state, compared to wild type. Thus, slow contraction does not systematically cause a delay in abscission upon contraction. In contrast to hof1Δ cells, lack of any of Cyk3, Cyk4, Cyk5, Tus1, and ApL3 caused cells to linger in the contracted state significantly longer than wild type cells (p = 2.2958E-05), with the strongest effect being observed with Tus1. Lack of the Ste20 kinase caused also some delay in abscission, yet this effect was not highly significant (p = 0.14219755). Thus, Cyk3, Cyk4, Cyk5, Tus1 and ApL3 were all required for timely
abscission after completion of actomyosin ring contraction. In the case of Cyk3, Cyk4 and Tus1, these effects on abscission came in addition to their respective effects on actomyosin ring contraction.

**Tus1 and Cyk4 are interdependent for localization and depend on myosin-II for localization**

Next we asked whether any of the bud neck proteins of the Cyk3 pathway functioned together in cytokinesis. To assay functional relationships between these proteins we first determined whether they showed any interdependency for localization. Thus, the localization of Cyk3-, Cyk4-, Cyk5-, Tus1-, Myo1- and Hof1-GFP was compared between wild type cells and cells carrying either of the myo1Δ, hof1Δ, cyk3Δ, cyk4Δ, cyk5Δ, tus1Δ and ste20Δ mutation individually. In addition, the localization of the same reporters was investigated in cells carrying the cdc15-1 mutation and shifted to the restrictive temperature for this temperature sensitive mutation for 2.5 hours. The Cdc15 kinase is part of the mitotic exit network (MEN) and mediates mitotic exit and cytokinesis onset at the end of telophase. Therefore, this last test determined, which of the Cyk3 components required exit from the mitotic state and/or cytokinesis onset for proper localization to the bud neck.

![Table](image)

**Fig. 18:** Cyk3, Cyk4, Cyk5 and Tus1 depend on successful mitotic exit for their localization to the bud neck. Additionally Cyk4 and Tus1 depend on Myo1 as well as on each other for their localization to the bud neck.

Strains were crossed manually and of each cross the GFP signal of the protein of interest was verified in wild type background before checked in the mutant background.
These studies indicated that all Cyk3 components tested here, i.e., Cyk3, Cyk4, Cyk5 and Tus1, depended on Cdc15 function for proper localization to the bud neck. Furthermore, neither Myo1 nor Hof1 depended on any of the Cyk3-pathway components for proper localization, as one would have expected. Also, Myo1 and Hof1 localization to the bud neck did not require mitotic exit, as reported (REF Vallen/Bi 2000, Lippincott/Li 1998). Surprisingly however, both Tus1 and the Cyk4 proteins were found to depend on Myo1 presence for proper localization to the bud neck. The fact that myo1Δ mutant cells become inviable upon inactivation of either TUS1 or CYK4 genes indicates though that both Tus1 and Cyk4 can still fulfill some function in the absence of Myo1. Hence, proper localization is not fully necessary for these proteins to contribute to cytokinesis.

Within the Cyk3 components, two observations emerge. First, most proteins localized independently of each other to the bud neck, i.e., Cyk3 and Cyk5 localized to the site of cleavage independently of each other and of Ste20, Cyk4 and Tus1. In reverse, Cyk4 and Tus1 localization did not depend on the presence of Ste20, Cyk3 or Cyk5.

**Genetics and localization studies implicate the GTPase Rho2 in abscission**

Tus1 is a well described Rho GEF (REF Schmelzle/Hall 2000) involved in multiple cellular processes like cell wall and plasma membrane salvage pathways (REF Schmelzle/Hall 2000) and for this study more importantly also in cytokinesis (REF Yoshida/Pellman 2006). We rationalized that Rhos might be the direct target of Tus1 that actually function in abscission. To approach this question we manually constructed RHO1, RHO2, RHO3, RHO4 and RHO5 deletions using classical gene disruption methods described in Materials and Methods. We found that in accordance with literature, rho1Δ and rho3Δ yielded only dead spores (REF Winzeler/Davis 1999). When we combined the viable rho2Δ, rho4Δ and rho5Δ deletions with the bait strains myo1Δ, hof1Δ and cyk3Δ we found that exclusively the rho2Δ deletion interacted synthetically lethal with myo1Δ and hof1Δ and thus we confirmed the initial screen results of Rho2 acting in the Cyk3 pathway. The rho1-112 allele showed synthetic interaction with hof1Δ and cyk3Δ (myo1Δ not tested), which prompted us that Rho1 might as well function in abscission but that it is going to be technically extremely challenging to prove this as Rho1 is required for formation (REF Yoshida/Pellman 2006) and probably also contraction of the contractile acto-myosin ring (REF Glotzer 2004).
We rationalized that if Rho2 acts as an effector in abscission it should co-localize with its activating GEF at the bud neck. We constructed N-terminal GFP constructs of Rho1, Rho2, Rho3, Rho4 and Rho5 (see Materials and Methods). C-terminal tagging would have led to a loss-of-function allele as Rho proteins interact with their C-terminus with the membrane. The GFP-Rho2 construct retained the functional properties of wild type Rho2 as it was not found to be synthetically lethal with neither myo1Δ nor hof1Δ. Given the structural similarities of Rhos we concluded that N-terminal GFP tagging of Rhos preserves their functional capabilities.

![Fig. 19: N-terminal GFP constructs under the control of the Ade2 promotor localized differentially throughout the cell cycle: GFP-Rho1-Rho5 were filmed around the time of bud emergence, metaphase, early anaphase, late anaphase, telophase and after cell separation. Images presented are single slices. The SPBs are labeled with red asterisks. GFP-Rho1 and GFP Rho4 localized to the bud neck around the time of late anaphase whereas GFP-Rho2 localized to the bud neck around the time of cytokinesis. GFP-Rho3 and GFP-Rho5 never localized to the bud neck.](image-url)
We found that GFP-Rho1, GFP-Rho2 and GFP-Rho4 localized amongst other cellular compartments in a very pronounced fashion to the bud-neck. GFP-Rho3 and GFP-Rho5 did not do so. Interestingly, GFP-Rho1 and GFP-Rho4 localized to the bud-neck in late anaphase as judged by the dumbbell shape of the nucleus and the distance between distal and proximal spindle pole body (see Fig. 19). However, GFP-Rho2 localized to the bud-neck after spindle breakdown, which corresponds with the timing of CAR contraction and abscission. When we examined the viable RHO deletions for defects in CAR contraction using Myo1-GFP as reporter we found that neither rho2Δ, rho4Δ nor rho5Δ delayed CAR contraction in respect to wild type.

**Fig. 20:** Acto-myosin ring contraction is not impaired in cells carrying deletions of rho2Δ, rho4Δ and rho5Δ. Symbol in black indicate the amount of cells counted per experiment; error bar in red indicates mean and standard deviation over all counted cells.
Thus Rho2 and Rho4 act subsequent to CAR contraction at the bud neck. Probing for defects in abscission using a PH-GFP marker the \( \text{rho2}\Delta \) deletion exhibited exclusively a defect in membrane resolution.

![Graph showing abscession index contracted/total for wt, rho2\( \Delta \), and rho4\( \Delta \) strains](image)

**Fig. 21:** Cells mutant for \( \text{RHO2} \) are slowed down in the process of resolution of the plasma membrane during post actomyosin ring contraction abscission. The illustrated value for the \( \text{rho2}\Delta \) strain mutant strain is a calculated ratio of cells with contracted but non-resolved membranes divided by the total number of cells that are in post anaphase cell cycle stage as judged by Spindle Pole Body in the daughter compartment. In contrast, cells mutant for \( \text{RHO4} \) progress through abscission comparably to wild type cells.

Taking all these results together we concluded that Rho2 is required to promote abscission via its GEF Tus1 and that NoCut targets the GTPase Rho2 to reversibly inhibit abscission.
Results III

Cyk6 supports cytokinesis in *S. cerevisiae*
**Front image:** The Cyk6-GFP localization during the cell cycle in *S. cerevisae*
Swi5 is needed for cell integrity

The transcription factor Swi5 of budding yeast induces a wave of gene expression late in the cell division cycle (REF Knapp/Nasmyth 1996). By upregulating the Cdk-inhibitor Sic1, Swi5 contributes to completion of mitosis (REF Toyn/Johnston 1997). To find additional players of the M-G1 transition we screened a collection of yeast deletion mutants for synthetic growth defects associated with a swi5Δ deletion. One of the candidates identified in the screen was Slt2, which encodes a mitogen-activated protein kinase (MAPK) of the cell integrity pathway (REF Levin 2005, Chen/Thorner 2007). This pathway is stimulated in response to defects in the cell wall or plasma membrane and consequently induces appropriate repair functions. We confirmed the genetic interaction of swi5Δ and slt2Δ by comparing the haploid progeny obtained by sporulation and tetrad dissection of a heterozygote diploid strain. Unlike wild type or single mutants, the swi5Δ slt2Δ double mutant strain formed small colonies at 30°C. Moreover, double mutants failed to form colonies at 37°C unless an osmostabilizer was added to the medium (see Fig.S7). These data suggest that swi5Δ mutants suffer from cell integrity defects and require Slt2 to prevent cell lysis. Consistent with this view we found that Slt2 activity is apparently elevated in a swi5Δ mutant strain. Slt2 activation involves dual phosphorylation at a conserved T-x-Y motif. When protein extracts of wild type and swi5Δ mutant cells were probed in a Western analysis with antibodies specific to the phosphorylated T-x-Y motif, a more intense signal was detected in the swi5Δ mutant. Unlike T-x-Y phosphorylation, Slt2 protein levels were unchanged as determined by the myc epitope-specific antibody (REF Rauter/Richter/Seufert 2009 - in preparation) (see Fig.S7).

To start to define the cell integrity defect of swi5Δ mutants, we looked at cell morphologies and determined the DNA content of cell populations. In growing cultures around 10% of the swi5Δ and swi5Δ slt2Δ mutant cells formed aggregates of 3 or 4 cell bodies and the number of unbudded cells was reduced. In line with this, flow cytometry revealed cells containing more than the regular 2C amount of DNA and a reduced fraction of cells with 1C DNA. Cell aggregates and cells with elevated DNA contents were absent in case of wild type and slt2Δ strains. These results suggest that swi5Δ mutants are defective in cytokinesis or cell separation. To examine the defect in more detail, the plasma membrane was visualized by means of a GFP-fusion to the glucose transporter Hxt1-GFP. Confocal microscopy of 3-bodied swi5Δ mutant cells showed that
a continous plasma membrane has formed at the neck of the mother cell and the larger bud (see Fig. S7). Physical separation of the membrane into two distinct structures could, however, not be observed. This indicates that Swi5 is needed for the timely resolution of the plasma membrane during cytokinesis.

The Swi5 target Cyk6 is needed for cell integrity

The role of Swi5 in cytokinesis is most likely explained by transcriptional activation of genes. Among the transcriptional targets of Swi5 is the gene YPL158C, which we suggest now to rename to Cyk6 for its role in cytokinesis (see below). The promoter region of the CYK6 (YPL158C) gene contains 4 binding sites for Swi5 and Cyk6 transcript levels peak in the M-G1 cell cycle phase (REF Cho/Davis 1998, Spellman/Futcher 1998). Deletion of the CYK6 gene is reported to cause synthetic growth defects when combined with the slt2Δ deletion (REF Tong/Boone 2004). We therefore decided to study the phenotype of cyk6Δ mutants. Similar to the swi5Δ slt2Δ mutant, cyk6Δ slt2Δ mutants formed small colonies following tetrade dissection and colony formation at 37°C required an osmostabilizer (see Fig. S8). Moreover, T-x-Y phosphorylation but not protein levels of the MAPK Slt2 were elevated in cyk6Δ mutants. Finally, cyk6Δ mutants formed cell aggregates with unresolved plasma membrane structures between mother cells and older buds. The phenotypic similarity of swi5Δ and cyk6Δ double mutants fits in with the idea that Swi5 activates transcription of the CYK6 gene whose product is needed for cell integrity and cytokinesis (see Fig. S8). Consistent with this view, combining swi5Δ and cyk6Δ deletions did not cause a synthetic effect as indicated by the fact that single and double mutant strains formed colonies of wild type size in a tetrade dissection analysis. Interestingly we found that the cyk6Δ mutant strain showed full synthetic lethality when combined with the hof1Δ mutation. When combined with the cyk3Δ no additive effects could be observed. Upon combination of the cyk6Δ with the myo1Δ we observed that 50% of the double mutant spores cyk6Δ myo1Δ formed inviable progenies (see Fig. S5). Conversely 50% of the progenies were fully viable and showed no exacerbated phenotype to the myo1Δ haploid strain. This observation is most likely due to a suppressor mutation that in the absence of Cyk6 either kills or allows myo1Δ mutant cells to live. These results suggest that Swi5 targets, at least partially, the Cyk3 pathway of cytokinesis.
To determine the relevance of Cyk6 for the cellular functions of Swi5, we placed the CYK6 gene under control of a Swi5-independent promoter. As expected, this construct complemented a cyk6Δ deletion mutant and, interestingly, it also suppressed swi5Δ mutant phenotypes. Constitutive expression of Cyk6 in a swi5Δ mutant prevented the appearance of cell aggregates and shifted the ratio of cells with 1C and 2C DNA content back to wild type values. Moreover, Cyk6 suppressed the slow growth phenotype and osmosensitivity of swi5Δ slt2Δ double mutant so that these cells were able to form colonies at 37°C on medium lacking an osmostabilizer. These data indicate that Cyk6 is the major target of Swi5 with respect to cell integrity and cytokinesis.

**Cyk6 accumulates at the bud-neck in late mitosis**

To characterize Cyk6 in more detail, we looked at its abundance and localization in the cell division cycle. To this end, we followed levels of a myc epitope tagged version of Cyk6 (Cyk6\textsuperscript{m13}) by Western analysis in cells released from a pheromone-induced arrest in G1. Cyk6 levels were very low in early cell cycle stages and increased strongly in late mitosis, when most cells had separated their chromosomes and levels of the mitotic cyclin Clb2 declined (see Fig.22). This fits together with the notion of Swi5-induced expression of the CYK6 gene.

![Image](image.png)

**Fig. 22**: Levels of myc-tagged Cyk6 strongly increase upon the exit of mitosis, which is indicated by cyclin Clb2 degradation. This observation is consistent with the strong accumulation of Cyk6-GFP at the bud neck upon the exit of mitosis (see Fig.23). Image taken from Rauter/Richter et al, 2009 in preparation.
Cyk6-GFP has been reported to localize to the bud neck (REF Huh/O'Shea 2003). Consistent with these data and our observation on protein expression levels, we observed an intensified signal of Cyk6-GFP at the bud neck in a fraction of large-budded wild type cells but not in swi5Δ deletion cells. These and wild type cells of earlier cell cycle stages showed a low intensity signal of Cyk6-GFP at the bud neck (see panel G and H in Fig. 23). Thus, Cyk6 is expressed at a low constitutive level in addition to its upregulation by Swi5 in late mitosis.

Fig. 23: Different cell cycle stages were examined for the cell cycle dependent localization of Cyk6 using different methods to differentially confirm the validity of the data obtained. Panel (A) indicates the cell cycle stage using DAPI staining for chromatin mass; Panel (B) shows S. cerevisiae in transmission during the cell cycle; Panel (C) shows the bi-polar budding pattern of diploid cells; Myc-tagged Cyk6 stays at the old bud scar and disappears from there when accumulating at the bud neck late in mitosis; Panel (D) septins localize to the bud neck as previously reported; Panel (E) Cyk6 localizes between the splitted septin ring and Panel (F) localizes at the bud neck in a septin dependent manner; Panel (G) Cyk6-GFP localizes at the bud neck identically to Myc-tagged Cyk6; Panel (H) the signal at the bud neck gets 3 fold enhanced once the cells have exited mitosis, as this accumulation is lacking in cells that are mutant for SWI5.
Unlike many other bud neck proteins, Cyk6-GFP remained associated with the division site of mother and daughter cells and appeared to stay there in the ensuing G1 phase. This interpretation is confirmed by immunofluorescence staining of Cyk6m13 in diploid cells whose bipolar budding pattern allows sites of previous division and future bud formation to be easily distinguished. Cyk6m13 localized to the bud neck soon after association of septins when the bud was still very small. At this and later stages, Cyk6m13 colocalized with septins. Co-localization ended in late mitosis when septins split into two rings and Cyk6m13 remained in between as a single ring. Localization of Cyk6 to the bud neck is septin-dependent since the Cyk6-GFP signal disappeared from the neck in a temperature sensitive septin mutant strain within a few minutes after temperature shift-up.

**Cyk6 supports acto-myosin ring contraction**

To learn more about the role of Cyk6 in cytokinesis, we followed contraction of the actomyosin ring in wild type and mutant strains by time-lapse microscopy of Myo1-GFP (Fig.24). In wild type cells it took about 4 minutes for the ring to contract from its original diameter of 1.5 μm to 0.3 μm. The signal then disappeared due to disassembly of the contractile ring. In cyk6Δ deletion mutant strains the Myo1-GFP signal had normal appearance, but ring contraction was slowed down and it took about 8 minutes to go from 1.5 μm to 0.3 μm. This difference was confirmed by each 4 independent measurements. Unlike contraction, disassembly of the ring was apparently unaffected in cyk6Δ mutants. These data show that Cyk6 facilitates contraction of the actomyosin ring.

**Fig. 24:** Acto-myosin ring contraction was followed in wt cells and cells mutant for CYK6(Ypl158c), for the glucan synthase **FKS1** and for the endochitinase **CTS1**. Red stars mark the onset of contraction. Time interval between each frame was 30sec.
In cytokinesis of budding yeast, ring contraction and septum formation are coupled (REF Schmidt/Cabib 2002). To evaluate the consequences of defects in septation, we analyzed ring contraction in an \textit{fks1}\Delta mutant strain that is partially defective in glucan synthesis and formation of the secondary septum. We also looked at a \textit{cts1}\Delta mutant strain, which lacks an endochitinase required for cell separation. Time-lapse series of Myo1-GFP were performed. There was a substantial delay of ring contraction in the \textit{fks1}\Delta mutant, but somewhat less severe compared to the \textit{cyk6}\Delta defect. No effect was observed in the \textit{cts1}\Delta. Thus, we could confirm that defects in septation make an impact on actomyosin ring contraction. Additionally and more importantly we could show, that cells carrying a \textit{cyk6}\Delta mutation are 2,2x slower in contracting the acto-myosin ring.

When we investigated the dynamics of the acto-myosin ring contraction we found that the ring contracts less homogenously in cells that take longer to contract the ring. This is true for cells that are defective in septum formation machinery e.g. \textit{fks1}\Delta and is even exacerbated in cells carrying a deletion of \textit{CYK6} (see \textbf{Fig.25}).

\textbf{Fig. 25}: Acto-myosin ring contraction gets proportionally less homogenous the longer the ring takes to contract. In average the ring contracts to a diameter of 0.3\,\mu m before it disappears. Representative samples are illustrated here for each mutant.
As a cyk6Δ mutant strain is severely impaired in acto-myosin ring contraction we were interested whether this effect is direct and Cyk6 is a constituent of the contractile ring structure or whether it is effect is of secondary nature.

To ask if Cyk6 is a physical constituent of the contractile ring or affects ring contraction indirectly, we determined the localization of Cyk6-GFP and Myo1-CFP during cytokinesis. Cyk6-GFP co-localized with Myo1-CFP prior to ring contraction. Unlike Myo1-CFP, the Cyk6-GFP signal did not contract and remained at the bud neck after disassembly of the actomyosin ring. These data indicate that Cyk6 supports actomyosin contraction without being a physical component of the contractile ring structure.

**Fig. 26:** Myo1-CFP and Cyk6-GFP showed co-localization at the bud neck, however the Cyk6-GFP signal persisted beyond the time-point when the Myo1-CFP signal has already disappeared due to contraction of the ring. This is consistent with the observation that Cyk6 does not depend on Myo1 for its localization to the bud neck (see Fig. S6).

**Cyk6 is required for plasma membrane resolution**

Recent advances in cytokinetic research established that membrane resolution, known as abscission, is a distinct and individually controlled step within cytokinesis (REF Norden, Mendoza et.al 2006, Gromley et al 2005, Echard 2008) from yeast to animal
cells. To characterize the possible role of Cyk6 in abscission we visualized the plasma membrane using a construct of GFP coupled to a PH domain, which, upon palmitoylation, is directly targeted to the plasma membrane (REF Christianson/Hieter 1992). We scored for cells that underwent anaphase, judged by the localization of a Spindle Pole Body (SPB) both in the mother and in the daughter cell indicated by the red arrow (see Fig. 27). The SPBs were visualized using the well established marker Spc72-GFP (REF Wigge/Kilmartin 1998). In these telophase cells we could distinguish three stages of the plasma membrane at the mother-bud neck. Open configuration indicates that cells have not exited mitosis and have not yet contracted the acto-myosin ring. Contracted configuration indicates that the acto-myosin ring has already contracted but the resolution of the plasma membrane has not yet taken place. In the third state the two individual plasma membranes of mother and daughter cells are clearly differentiable indicating that abscission has taken place but Cts1 dependent degradation of the primary septum, cell separation, has not yet happened (REF Colman-Lerner/Brent 2001). This assay proved suitable to assess defects in abscission (REF Norden/Barral 2006) in budding yeast. To determine whether cyk6Δ mutant cells are deficient in membrane resolution we quantified the frequency of these three different stages in asynchronous wt and mutant cultures and plotted it as a ratio of cells in contracted state versus the total score of post anaphase cells. We refer to this ratio as the abscission index (see Fig. 27).
Fig. 27: (A) The three cytokinetic cell cycle stages scored were open, contracted and resolved. The plasma membrane is stained with PH-GFP and the telophase cell cycle stage was assessed by proper SPB localization in the bud by using Spc72-GFP. (B) cyk6Δ mutant cells require 3.2x more time to progress from contracted stage to resolved stage; fks1Δ cells take 2.2x longer indicating that septum formation also influences the process of abscission. As expected, cts1Δ mutant cells progress through abscission with comparable kinetics to wild type cells. 

14.2%(±0.4%) of total wild type post anaphase cells were found in the contracted state. In cells mutant for cyk6, this number was more than three times elevated to 45.5%(±1.7%) indicating that cyk6Δ mutant cells are severely delayed in abscission. Independent results from several groups reported mutual influence of septum formation and acto-myosin ring contraction(REF Schmidt/Cabib 2002, this study). To assess whether defects in septum formation also feed back on abscission we examined again
Discussion

Formation of two daughter cells during cytokinesis requires reorganization of the plasma membrane. This involves contraction of the actomyosin ring linked to the plasma membrane at the cleavage site and targeted membrane delivery. In budding yeast, a septum of cell wall material is deposited at the cleavage site and there is an interdependence of primary septum formation and acto-myosin contraction. The final steps by which the invaginated plasma membrane is resolved into two separate membrane structures remain ill defined. Here we introduce the budding yeast Cyk6 protein, a novel cytokinesis factor needed for timely resolution of the plasma membrane at the cleavage site following actomyosin ring contraction. Thus, Cyk6 might function in a recently defined pathway of membrane abscission. Since membrane resolution is influenced by defects of the secondary septum, a primary role of Cyk6 in septum formation would also be conceivable. Deletion of CYK6 caused a moderate delay in actomyosin ring contraction. Since Cyk6 is not a physical part of the contractile apparatus (see Fig. 26), this delay might stem from defects in membrane reorganization or septation.

Like other cytokinesis proteins, Cyk6 supports cell integrity and localizes to the bud neck in a septin-dependent fashion. Expression of the CYK6 gene is controlled in part by the
cell cycle transcription factor Swi5 and, as a consequence, Cyk6 levels are upregulated during cytokinesis. This upregulation is critical for Cyk6 function as indicated by phenotypic similarities of swi5Δ and cyk6Δ mutants and the suppression of cytokinesis defects in swi5Δ mutants by Swi5-independent expression of Cyk6. The Swi5-Cyk6 connection reported here may therefore contribute to the cell cycle regulation of cytokinesis. Swi5 is inhibited by cyclin-dependent kinases, which phosphorylate the transcription factor and interfere with its nuclear import (REF Knapp/Nasmyth 1996). The Swi5-Cyk6 module thus marks a novel pathway by which cyclin-dependent kinases keep a check on cytokinesis.
Chapter III

Conclusions and Perspectives

(c) Tracey says

\[(\sqrt{2} + \sqrt{8})\] is an irrational number.

\[(\sqrt{2} + \sqrt{8})^2 = 18\]

I think that if you square any number you always get a rational number.

Tracey is wrong. Use an example to show that Tracey is wrong.

*She’s a woman*
Front image: The world's funniest exam answers
at http://www.masalatime.com/?p=419
**General Conclusion:**

Recent advances in the cytokinetic research have pushed our understanding on how cytokinesis and abscission are accomplished further. However these results presented isolated pieces of evidence and a comprehensive and complete picture is still missing. In our attempt to characterize cytokinesis and abscission in a genome wide comprehensive manner we chose yeast as model system and screened genetically for genes involved in cytokinesis with particular interest in genes involved in abscission.

We succeeded in identifying 105 unique hits that genetically act in the Cyk3 pathway. We subsequently identified the Cyk3 pathway to be at least partially required for proper abscission and characterized the interplay of the Cyk3 pathway with the NoCut pathway and its consequence on activation or inhibition of abscission.

**Differential and specific activation of Rho2 is required to promote abscission in *S.cerevisiae***

Among the 6 Rho family members in budding yeast Rho1, Rho2, Rho3, Rho4, Rho5 and Cdc42 we have tested 5 of them genetically and 4 cytologically for their involvement in cytokinesis and more precisely in abscission. Here we describe a novel function of Rho2 during the plasma membrane fission, or abscission, during yeast cytokinesis.

The *rho3Δ* deletion yielded only dead spores as already reported(REF Matsui/Toh-e 1992, Winzeler/Davis 1999), which can be rescued by addition of 1,2M Sorbitol. We could find that GFP-Rho3 localizes to several membranes during the entire cell cycle but never localizes at the bud-neck(see Fig.19). As there are no ts - alleles available we dropped Rho3 for further investigation at this point.

Consistent with literature we could not discover or unravel any significant phenotype for *rho4Δ* mutants(REF Matsui/Toh 1992), although we could find that GFP-Rho4 localizes to the bud neck at the end of anaphase suggesting a yet to be elucidated role at the bud neck after mitotic exit(see Fig.19). Genetically, the *rho4Δ* deletion does not interact with any of the three cytokinetic pathways Myo1, Hof1 nor Cyk3. Neither our functional analysis showed any consequence of the *rho4Δ* deletion on the timing or efficiency of contractile ring assembly, contraction nor abscission. Our functional assays didn’t
address the function of Rho4 function at the bud neck or the Rho4 function is "buffered" by parallel pathways covering phenotypic consequences upon the deletion of the RHO4 gene. It has been suggested that Rho3 and Rho4 act in concert to promote nucleation of Actin fibers (REF Matsui/Toh-e 1992). This might be true, however, our data suggest that the roles are probably more divergent than previously anticipated. Rho5 was the last to be discovered (REF Roumanie/Doignon 2001). In line with literature we could not detect any obvious phenotype for the rho5Δ mutant cells. One report states that Rho5 might function to downregulate the cell wall integrity pathway (REF Schmitz/Heinisch 2002) as the rho5Δ displays elevated basal and stress induced Mpk1/Slt2 activity and increased resistance to cell wall stressors. This would explain why our assays do not detect any phenotypes for the rho5Δ mutation as only problems in cell wall synthesis interfere with contractile ring contraction (REF Schmidt/Cabib 2002) and partially influence the abscission assay using PH-GFP (REF this study).

The rho1-112 allele could only be investigated genetically and exhibited severe synthetic lethality already at room temperature with the cyk3Δ and also with hof1Δ. In parallel, we observed that the myo1Δ rho1-112 heterozygous diploid strain was not able to undergo meiosis and therefore could not be analyzed. Reports from S.pombe state that Myosin is partially required for efficient sporulation (REF Itadani/Shimoda 2006) and that the additional perturbation by using Rho1 hypomorphs leads to a full meiotic defect. Maybe this is conserved in budding yeast. All together these genetic data indicate that Rho1 does indeed play more roles during cytokinesis.

Rho2 does not affect contractile ring contraction as judged by the Myo1-GFP contraction assay (see Fig.20) but Rho2 is required to split the plasma membrane during abscission (see Fig.21) as the deletion of the RHO2 gene strongly delays this process, specifically.

This study raises a couple of questions:

- What are targets of Rho2 within the abscission pathway?

- Are these targets distinct for their function in abscission or are these conserved molecules that also function in cell wall and plasma membrane integrity pathways, which would functionally link these two processes.

- Does this reflect a possible mechanism conserved from yeast to higher eukaryotes?
- if yes, how could this look like?

**Outlook on Rho2 function in abscission**

It is important to state that at this point we know very little about how Rho2 in promotes abscission. Neither can we precisely explain, how the NoCut checkpoint controls abscission, a question that will be discussed later. However, our findings are remarkably in line with numerous reports from other groups from different organisms showing that ras-like GTPases are essential for abscission. In the following section I would like to discuss, which experiments and findings would still be important to be able to propose a firm model of yeast abscission and its regulation by the NoCut checkpoint.

To confirm the requirement of Rho2 for abscission more directly, it is first important to find the downstream effectors of Rho2. Unfortunately Rho2 is the "terra incognita" of Rho biology and virtually nothing is known about the function of this protein during cell division. The few reports available implicated that Rho2 has overlapping functions with Rho1 (REF Madaule/Myers 1987, Ozaki/Takai 1996), which is backed up by the fact that Rho1 and Rho2 to some extend share common GEFs (REF Schmidt/Hall 1997) and GAPs (REF Roumanie/Doignon 2001). Rho1 and Rho2 have been well described for their role in the cell wall integrity (CWI) pathway, which ensures that despite the gross morphological changes the cell wall experiences throughout the cell cycle, polarized growth, and mating, the integrity of the cell wall is maintained, and if cell wall injuries occur, proper responses are initiated. These so-called salvage pathways are of great importance, because disturbance of the cell wall are the major threat to the viability of yeast cells (REF Levin 2005).

These salvage pathways consist of a coordinated transcriptional program that induces the appropriate response genes to counteract the damages.
Fig. 27: The signal cascade that leads to transcription in response to cell wall damage is called the Cell Wall Integrity pathway or Cell Wall salvage pathway. It allows the cell to monitor cell wall integrity and respond to damages of the cell wall. Rho1 is involved in transducing the signal to a MAP kinase cascade, which ultimately allows activation of transcription. A prominent candidate transcribed through this program is Fks2; image taken from Levin, Microbiol Mol Biol Rev. 2005 Jun;69(2):262-91

Rho1 is a key regulator of the CWI salvage pathway (REF Levin 2005). On top of this well described role in CWI, Rho1 is also required for proper cell wall biosynthesis itself and during cytokinesis for the formation of the acto-myosin contractile ring structure in budding yeast (REF Yoshida/Pellman 2006). These multiple roles of Rho1 might explain why the deletion of the RHO1 gene is fully lethal. In contrast, the rho2Δ deletion is viable and shows no obvious phenotypes. Also, the CWI salvage pathway responds to damage (REF Levin 2005) despite the absence of Rho2 suggesting that Rho1 is the master regulator of CWI signaling. It is interesting to note that there is increasing evidence that the specificity of Rho function seems to be determined by the specific GEF that dictates it. Rho1 activation by Rom1/Rom2 has been reported to be essential for transduction of the signal during cell wall integrity response (REF Ozaki/Takai 1996) in turn, Rom1/Rom2 stimulation seems to be of inferior importance during acto-myosin ring formation (REF Yoshida/Pellman 2006). Our data suggest that this might also be the case for Rho2 as
our data show that Rho2 activation by Tus1 is essential for abscission, since inactivation of these two proteins shows the most exacerbated abscission delay compared to all other candidates that we have investigated. Taking these findings together Tus1 might represent the GEF that specifically promotes cytokinetic functions of Rho1 and Rho2.

It will be important but also technically challenging to dissect the different roles of Rho1 and Rho2 stimulated by Rom1/Rom2 GEFs or Tus1 GEF during abscission events. However, the deletion of the RHO2 gene is not lethal, which suggests that Rho2 function might be more specialized, that its major contribution is to promote abscission, and that its role in the CWI salvage pathways is of inferior importance.

It will be important to determine, whether the abscission defect, that we see in rho2Δ mutant cells is independent of its role in the CWI salvage pathway, such as to test to which extend those two processes - cell wall synthesis/homeostasis and abscission, are mutually dependent.

Sequence homology searches reveal that Rho2 shows striking similarities with prominent small GTPases like Cdc42 and especially Rab11 in mammals, whose requirement in abscission has already been demonstrated (REF Simon/Prekeris 2008, Pohl/Jentsch 2008). It will be important to determine, which function Rho2 indeed fulfills and whether these functions are in any manner related to those of Cdc42 and Rab11.

Yet, there is another line of observations that could shed interesting light on the function of Rho2 during abscission. Sec3 is a component of the exocyst, which is required for targeted secretion to the plasma membrane. It has been shown that Sec3, as well as all the other exocyst components, localize to the bud neck (REF Barral/Snyder 2000) and are required for abscission in yeast (REF Dobbelaere/Barral 2004) but also in mammalian cells (REF Gromley/Doxsey 2005). Rho1, Rho2, Rho3 and Rho4 interact biochemically with exocyst components by co-precipitation, and Sec3 mis-localizes in certain rho1- mutant alleles (REF Zhang/Guo 2001). As our phenotypic analysis would predict, it has been shown that Sec3 localization to the bud-neck is unperturbed in sorbitol stabilized rho3Δ and is also unperturbed in rho4Δ cells (REF Zhang/Guo 2001) as we saw no phenotype for rho4Δ mutant cells tested. It would be very important to check exocyst localization in rho2Δ mutant cells, as the function of Rho2 might well be to serve as a spatial landmark at the bud-neck for essential abscission factors like the exocyst.
Our studies suggest a model for how abscission is regulated both from an abscission promotion perspective, via Rho2, as well as from an abscission inhibiting perspective via the NoCut checkpoint. They did not yet provide major clues about the molecular mechanism of abscission. To address this issue, high-resolution electron microscopy studies might shed more light on the mechanism of abscission by providing precise information about the structure of the bud neck in \( \textit{rho2}\Delta \) and other abscission deficient mutants, such as \( \textit{ndc10-1} \) and \( \textit{ase1}\Delta \) mutant cells (REF Norden/Barral 2006).

**A comprehensive model for the inhibition of abscission in response to spindle and chromosome segregation defects**

The key principle in the classical model of how NoCut inhibits abscission is, that this inhibition is reversible and only delays abscission. This inhibition transduction cascade depends on Ipl1, Boi1 and Boi2 (REF Norden/Barral 2006).

The two important questions raised by this hypothesis are

First, whether it is indeed the spindle midzone defect \textit{per se} that triggers the NoCut response or whether it is a different kind of signal, which is only mimicked by the spindle midzone defect? We advanced on this question in a joint effort with Dr. Manuel Mendoza at the Center for Genomic Regulation (Barcelona, Spain) and I will summerize the most intriguing results in the next section and will discuss their functional implications.

Secondly, how does NoCut mount the inhibitory signal at the bud neck? In a second section I will discuss the functional implications of the work presented in results section II.

In the following conclusions and perspective chapter I would like to discuss our major advances in the understanding of these two crucial question and present an extended model on our current understanding of NoCut.
What "really" triggers NoCut

Together with Dr. Manuel Mendoza we examined the question whether it is indeed the spindle midzone defect that triggers a NoCut response or whether it is another event that is only mimicked by a defective spindle midzone. In Mendoza et al, 2009 in press at Nature Cell Biology (see section Manuscripts) we found in short, that

(1) NoCut is triggered by the presence of unsegregated chromatin lagging over the spindle midzone, even in the absence of spindle defects. Therefore both in yeast (REF Mendoza/Barral 2009) and animal cells (Steigemann/Gerlich 2009), the CPC coordinates abscission with chromosome segregation.

(2) NoCut function requires targeting of the CPC to the central spindle during anaphase, indicating that the NoCut signal is generated at this location and time.

(3) The histone acetylase component Ahc1 contributes to NoCut function

In our synthetic lethal screen against the bait ase1Δ we have identified a significant amount of genes that act in cellular context of chromatin organization and chromatin homeostasis (see Tab.5 for detail). It was a long-standing suspicion that the event that is really triggering a NoCut response is in fact proximity of chromatin to the spindle midzone and therefore to Ipl1, that localizes there. Therefore, this group of genes involved in chromatin organization was a promising class to be examined for the involvement in the NoCut pathway. One of the genes identified was AHC1, encoding a component of the ADA histone acetyltransferase complex and required for integrity of the complex (REF Eberharter/Workman 1999).

Indeed, inhibition of cytokinesis in the ndc10-1 mutant was abrogated in the absence of Ahc1, as shown by the reduced number of ndc10-1 ahc1Δ multibudded cells following treatment with zymolyase. Further, the double mutant ahc1Δ ase1Δ had reduced viability compared to either single mutant, which confirmed our initial screen results. Because ase1Δ cells rely on NoCut to prevent chromosome damage (REF Norden/Barral 2006), this observation suggests that Ahc1 is required to delay abscission in cells with anaphase spindle defects. To test this possibility, we examined the status of the plasma membrane in post-anaphase ase1Δ and ahc1Δ ase1Δ cells expressing PH-GFP and Spc42-CFP. Abscission was delayed in the absence of Ase1 as previously reported (REF Norden/Barral 2006), as shown by an increase in the pre-abscission index in
ase1Δ compared to wild type cells; however, this delay was significantly reduced in the absence of Ahc1. Therefore, Ahc1 was required for proper NoCut function in cells with anaphase spindle defects.

This result is exciting in two ways:

(1) The successful identification of a novel NoCut pathway gene confirms the valid assumption that NoCut genes must become essential in the absence of the ASE1 gene. Therefore, it is very likely that our genetically identified hits in Tab.5 still contain more possible NoCut candidates that still need to be tested.

(2) The identification of Ahc1 to act in NoCut strengthens the idea that the actual trigger of NoCut is not the central spindle per se, but in fact the chromatin and its associated proteins. In Mendoza et al, 2009 we provide evidence that the proximity of chromatin to the Aurora B kinase at the spindle midzone serves as a trigger for NoCut activation. Although the precise mechanism is not known up to this point, this initial result will allow to precisely ask how the chromatin communicates with NoCut.

In addition to that it will be important to learn more about the dynamics of the checkpoint through the cell cycle in future. As the SAC in metaphase one would expect that NoCut is a checkpoint of very dynamic nature (REF Nelson/Pine 2008) as it has to be reverted once chromatin is not in proximity of Ipl1 any more. We also know little about the requirements for the reversion of NoCut but one might assume that genes that are responsible for the reversion of NoCut might be "hidden" within our screen data as well. If we assume that cells are incapable of reverting NoCut, this genes will become essential in a NoCut activated state like in an ase1Δ background, and thus such genes might have been identified in our ase1Δ screen. As NoCut employs very prominent kinases like Aurora B (REF Norden/Barral 2006) and Cdc5 polo kinase (see later) it is tempting to speculate that phosphorylation events play a prominent role in transducing the signal from the place of its generation to the place of action at the bud neck. In order to revert these phosphorylation signals and thus revert NoCut-dependent inhibition of abscission, phosphatases are good candidates. Rts1 and Ptc1 are subunits of the PP2A and PP2C phosphatase complexes, respectively. Both rts1Δ and ptc1Δ mutations are synthetically lethal with ase1Δ and are therefore likely to act in the NoCut pathway. Rts1
is a particularly good candidate as Rts1-GFP localizes to the bud neck (REF Gentry/Hallberg 2002). Excitingly, cells depleted for RTS1 exhibit a cytokinetic phenotype specifically at 37°C whereas at room temperature, rts1Δ myo1Δ as well as rts1Δ hof1Δ cells are not sicker than the respective single mutants. This indicates that Rts1 does not act in one of the classical cytokinetic pathways Myo1, Hof1 nor Cyk3 (REF Dobbelaeere/Barral 2003). Additionally, this cytokinetic defect of rts1Δ cells can be reverted by simultaneous inactivation of Ipl1, indicating that the cytokinetic defect of rts1Δ cells is indeed NoCut dependent (Dr. Caren Norden - personal communication). It suggests that cells grown at 37°C mount a NoCut response that in absence of RTS1 are not able to revert it any more, and thus arrest in a NoCut active - abscission inhibited state.

Additionally to exploring the NoCut candidate genes identified in our ase1Δ screen, it will be important to explore the "cut" mutant library (REF Samejina/Yanagida 1993), which will provide a fantastic pool of candidate genes that might act in NoCut like it has been shown for separase, securin and topoisomerase 2 (REF Mendoza/Barral 2009).

This combined strategy will allow the identification of many more genes that act in NoCut and learn more about the dynamic nature of the checkpoint. Only then will we be able to draw a conclusive map of how the NoCut response is mounted and how it dynamically behaves during the cell cycle progression.

**How is the NoCut signal conveyed to the bud neck**

For reasons of completeness I would like to very briefly summarize our current knowledge of how the NoCut signal is conveyed to the bud neck. This is still work in progress and the data presented here are personal communications of Caren Norden and Martin Dinkel.

Already when the first model of NoCut was established (REF Norden/Barral 2006), the question was raised of how a signal, which is mounted in the nucleus is able to inhibit abscission, which happens at the bud neck outside the nucleus. A partial answer to this question lies in the fact that Ipl1 is able to send Boi1 and Boi2 out of the nucleus into the cytoplasm, as temperature sensitive alleles of Ipl1 sequester Boi1 and Boi2 in the nucleus. However the mechanism is not as straightforward as it seems to be. Both Boi1
and Boi2 have Aurora B consensus phosphorylation sites but they are still able to exit the nucleus even upon the site directed mutation of all consensus phosphorylation sites on the Boi proteins. Either Ipl1 dependent phosphorylation is required but not on these consensus sites, or Ipl1 engages additional players to send Boi1 and Boi2 out of the nucleus. The results of another graduate student in the lab (Martin Dinkel - personal communication) suggests, that this second option is most probably true.

Martin Dinkel in the Barral lab found that proper localization of Boi1 and Boi2 to the bud neck requires the Polo kinase Cdc5. Cdc5 seems to play a dual role in cytokinesis as it promotes contractile ring assembly (REF Yoshida/Pellman 2006) and acts as an inhibitor of abscission within the NoCut checkpoint, at the bud neck. The role of Cdc5 as inhibitor of abscission is supported by the fact that overexpression of Cdc5 leads to inhibition of abscission (REF Song/Lee 2001). These findings about the dual role of Cdc5 in cytokinesis are in striking similarity to the role of the mammalian Aurora B kinase, which has been shown to be required for furrow ingression in higher eukaryotes (REF Hu/Mitchison 2008) but has also been characterized as a potent inhibitor of abscission in the mammalian NoCut pathway (REF Steigemann/Gerlich 2009).

It will be important to dissect the multiple roles of the polo kinase Cdc5 in the regulation of the different cytokinetic steps, its recruitment to the bud neck and its function. This is technically challenging, because Cdc5 inactivation at this location leads to multiple mitotic phenotypes and cause cell cycle arrest before cytokinesis can occur. At last, Cdc5 is required for exit of mitosis (REF Hu/Elledge 2001). But this would ultimately allow studying the exclusive role of Cdc5 in abscission and most importantly to determine,

- whether Cdc5 directly regulates Boi1 and Boi2 proteins at the bud neck, and

- if so, how Cdc5 regulates Boi1 and Boi2 function at the bud neck, and

- whether there are additional NoCut players that are regulated by Cdc5 and ultimately lead to inhibition of abscission.
**NoCut prevents activating factors of abscission from localizing to the bud neck**

Our data suggest a model where Tus1 stimulates Rho2, which in turn promotes abscission by a yet unknown mechanism. We have found that Rho2 localizes to the bud neck in a Tus1 independent manner (data not shown here).

We know from previous experiments that *ndc10-1* cells delay abscission in a NoCut dependent manner and that this abscission delay can be reverted by the simultaneous deletion of either the Aurora B kinase Ipl1 or the effector proteins Boi1 and Boi2 (Ref Norden/Barral 2006). To extend this model we can show that in *ndc10-1* cells, at least the novel protein Cyk4, former Ypl066w, and the Rho GEF Tus1 are de-localized from the bud neck in a NoCut-dependent manner. This effect is reversible, and Cyk4 and Tus1 re-localize to the bud neck upon further inactivation Ipl1 (see Fig. 14 and Fig. 15).

These findings suggest that one possible mechanism for how NoCut interferes with abscission. NoCut causes essential abscission factors to be reversibly kept away from the bud neck, such as to put the entire abscission machinery on hold. This model makes sense from a cell's physiological perspective, as it would allow a very rapid response and reversion of the inhibitory signal independent of transcription and also of translocation of entire complexes to the bud neck.

Although this interpretation is intuitive and is consistent with the predictions of the standing NoCut model, it will be important to confirm and elaborate it further through additional experiments. At this point we have evidence that the displacement of Tus1 and Cyk4 is Ipl1 dependent. In order to be able to call it fully NoCut-dependent the localization to the bud neck in *ndc10-1* should also be Boi1-, and Boi2-dependent, meaning that the localization frequency of Tus1 and Cyk4 to the bud neck should be restored to wild type levels in *ndc10-1 boi1Δ boi2Δ*. Very recent data indicate that this is indeed the case for Tus1; it still has to be tested for Cyk4.

We show that Rho2 localizes to the bud neck independently of Tus1 and we predict that Tus1 activates Rho2 and thereby promotes abscission. It will be vital to show that this is indeed the case. Martin Dinkel in the lab is currently constructing GTP-locked versions of Rho2, which should bypass the requirements for Tus1 at the bud neck. The implications of this experiment would be two fold:

a) It would show that Tus1 directly activates Rho2 and thereby promotes abscission
b) GTP-locked Rho2 should be constitutively active and should be able to promote abscission independently of Tus1. If this was true, ndc10-1 cells should exhibit no abscission defect although Tus1 should be still de-localized from the bud neck and therefore this construct would have the potential to overwrite the mounted NoCut signal.
Chapter IV

Materials and Methods
Strains, plasmids and Growth conditions:

All strains are derivatives of S288C if not stated otherwise. As parental wild type strains served YYB384 mat a, and YYB386 mat alpha with the genotype: his3-200 ura3-52 trp1-Δ63 leu2Δ lys2-801 ade2-101. All strains were grown in rich medium (YPD) at 30°C unless indicated otherwise. Gene deletions and tagging was performed using a single step PCR based approach as described in Janke et al, Yeast Aug;21(11):947-62. Gene deletion was accomplished by PCR amplification of the pYM plasmid carrying the deletion marker of interest with primers S1 and S2 plus 50bp of homologues sequence to the target region and homologues recombination on the chromosomal locus. Gene tagging was accomplished by PCR amplification of the pYM plasmid carrying the tag of interest with primers S3 and S2 plus 50bp of homologues sequence to the target region and homologues recombination on the chromosomal locus.

GFP-Rho1, GFP-Rho2, GFP-Rho3, GFP-Rho4, GFP-Rho5 were generated using PCR amplification of the pYM-N9 plasmid with primers S1 and S4 plus 50bp of homologues sequence to the target region and homologues recombination on the chromosomal locus. The constitutive expression of the protein was under the control of the ade2 promotor. To verify full functionality of the GFP tagged Rho2 protein GFP-Rho2 was backcrossed with myo1Δ and hof1Δ strains and no synthetic effects were observed (data not shown).

The Tus1-GFP:His3 was a kind gift of Prof. Dr. David Pellman from the Dana Faber Cancer Institute at Harvard Medical School, USA. The generation of the strain and the genotype of the strain is published in Yoshida S. et al, Science 2006 Jul 7, 313(5783): 108 - 11.

The bait strains for the SGA screens were obtained by directed chromosomal gene deletion in the strain Y8205 mat alpha can1::Ste2pr-Sp_his5 lyp1::Ste3pr-Leu2 his3Δ leu2Δ ura3Δ met15Δ. The Nourseothricin cassette (NAT) was amplified from a pAG25 plasmid(REF Goldstein/McCusker 1999) using primers

NAT_1F CAGATCTGTTTAGCTTGCTTG and NAT_1R AATCGAGCTCGTTTTTGAC plus 50bp of homologues sequence to the target region and homologues recombination on the chromosomal locus.
Correct knock-out of the targeted genes was verified by PCR and correct tagging of the genes of interest was verified by fluorescence microscopy.

The plasmid used for the abscission assay was a kind gift of Prof. Dr. Scott Emr from the Howard Hughes Medical Institute, San Diego, USA and contains the pleckstrin homology domain of Rattus norvegicus phospholipase C \( \beta 1 \) fused to GFP(PH-GFP) based on a pRS426 plasmid (REF Christianson/Hieter 1992).

YPD (yeast extract, peptone, glucose media) and SD (synthetic dextrose media) were prepared as previously described.

One step transformations were carried out as described in Knop et. al, Yeast Jul;15(10B): 963 - 72.

"Bust n' Grab" protocol for preparation of yeast genomic DNA was carried out as described in Kang T.J. et. al, BMC Biotechnology 2004 Sep 2; 4:20.

**Synthetic Lethal Screens**

The Synthetic lethal screens were carried out essentially as described in Tong et al, Science 2001, Dec 14; 294(5550): 2364-8. The screens were performed in a semi-automated manner on a BD robotic handling unit. Sanitation procedure included water wash followed by 75% ethanol and 96% ethanol sterilization steps of pins.

Plates were video recorded after final pinning and each spot (=colony) was individually visually inspected. Classification of synthetic effects was sl for synthetic lethal, ss for synthetic sick and wt for no synthetic interaction. A hit was considered true in case it showed up two times in each screen at least as synthetic sick. Boolean pathway comparison was performed using Excel function "countif".

**Genetic Analysis**

To verify the hits of the Cyk3 pathway described in this manuscript heterozygous diploid \( myo1\Delta, hof1\Delta \) and \( cyk3\Delta \) strains were constructed. These bait strains were used to disrupt the gene of interest by PCR based gene disruption techniques as described
above. Successful gene disruption was verified by PCR and plating on selective media. Diploid strains were sporulated in standard sporulation medium containing 1.5% KAc. The meiotic spores were dissected using micro-manipulation techniques and plated on selective media after colony size reached a size that allowed replica plating.

**Fluorescence Microscopy**

**Fig.16 and Fig.20** (CAR contraction)

was performed using strains carrying a chromosomal kanMX6 deletion in the gene locus of interest and a Myo1-GFP:His3 to visualize the contracting ring. Cells were filmed using a Visitech Spinning Disk Microscope equipped with a 100x 1.4 NA Oil DIC Plan-Apochromat objective. Cells were grown o/n in liquid YPD culture + extra adenine, diluted to OD0.3 and grown for 3 hours in liquid YPD medium on RT. Cells were collected by centrifugation at 2500rpm for 1.5min in a table centrifuge and washed 2x with Non Fluorescence Medium (NFM) as described in Waddle et. al, J. Cell Biol 132, 861-870. Cells were fixed in a LabTek 8 well chamber using Concavalin A fixation method. Surface of LabTek 8 well chamber was incubated with 1mg/ml Concavalin A solution in PBS for 2 hours. LabTeks were washed 2x with PBS and cells, suspended in non-fluorescent media (NFM), were incubated with the LabTek surface 30min before filming. Cells were imaged over time with a frame interval of 30 sec with 250ms exposure time per slice and 14 slices with 0.3μm steps in the z-dimension.

**Fig.17, Fig.21 and Fig.27** (Membrane resolution assay)

was performed using strains carrying a chromosomal kanMX6 deletion in the gene locus of interest, a chromosomal copy of Spc72-GFP:His3 and a constitutively expressed PH-GFP:Ura3 on a pRS426 plasmid. Cells were filmed using an Olympus BX50 wide field fluorescence microscope equipped with a piezo motor and a 100x oil emulsion objective (REF Kusch/Barral 2002). Cells were grown o/n in liquid SD-Uracil medium, diluted to OD0.3 and grown for 3 hours in liquid YPD medium on RT. Cells were collected by centrifugation at 2500rpm for 1.5min in a table centrifuge and washed with 2x with Non Fluorescence Medium as described in Waddle et. al, J. Cell Biol 132, 861-
Cells were imaged on regular microscopy slides using z-stack acquisition with 600ms exposure time per slice over 10 slices with 0.3μm steps in the z-dimension.

**Fig.26** (Co-localization of Cyk6-GFP and Myo1-CFP)

was performed using stains carrying an endogenous copy of Cyk6-GFP:His3 from Euroscarf (REF Huh/O'Shea 2003) and Myo1-CFP:kanMX6 (REF Dobbelaere/Barral 2004). Cells were filmed using a 100x 1.4 NA oil UplanSApo oil objective on an Olympus CellR system equipped with a piezo motor in the CFP and YFP filter. Cells were grown o/n in liquid YPD culture, diluted to OD0.3 and grown for 3 hours in liquid YPD medium on RT. Cells were collected by centrifugation at 2500rpm for 1.5min in a table centrifuge and washed 2x with Non Fluorescence Medium as described in Waddle et. al, J. Cell Biol 132, 861-870. Cells were fixed in a LabTek 8 well chamber using Concavalin A fixation method. Surface of LabTek 8 well chamber was incubated with 1mg/ml Concavalin A solution in PBS for 2 hours. LabTeks were washed 2x with PBS and cells, suspended in non-fluorescent media (NFM), were incubated with the LabTek surface 30min before filming. Cells were imaged over time with a frame interval of 120 sec with 250ms exposure time per slice and channel and 14 slices with 0.3μm steps in the z-dimension.

Image processing was done using ImageJ version 10.2 with various plugins from the ImageJ website.

Unpaired t-test was used to assess the statistical significance of the data in Fig.16, Fig17, Fig.20, Fig.21 and Fig.27 using Excel function "TTEST".

**Cell synchronization:**

**Fig.14** and **Fig.15**: Cells were arrested with α-factor (10μg/ml) for 2 hours at RT. Cells were washed 2x with YPD medium and released at restrictive temperature for the temperature sensitive mutant strains ndc10-1 as well as ndc10-1 ipl1-321 and wt
controls respectively. Cells were imaged as described above using a Olympus BX50 wide field microscope.
References


20) Buttery et al, Mol Biol Cell. 2007 May;18(5):1826-38 - Yeast formins Bni1 and Bnr1 utilize different modes of cortical interaction during the assembly of actin cables.


48) Canman et al, Science. 2008 Dec 5;322(5907):1543-6 - Inhibition of Rac by the GAP activity of centralspindlin is essential for cytokinesis.


55) Pohl et al, *Cell*. 2008 Mar 7;132(5):832-45 - Final stages of cytokinesis and midbody ring formation are controlled by BRUCE.


71) Ohkura et al, Genes Dev. 1995 May 1;9(9):1059-73 - The conserved Schizosaccharomyces pombe kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells.


73) Bement, Dev Cell. 2007 May;12(5):663-4 - Cytokinetic pyrotechnics.


75) Citterio et al, Exp Cell Res. 2006 Apr 15;312(7):1050-64 - Alfalfa Mob1-like proteins are involved in cell proliferation and are localized in the cell division plane during cytokinesis.


79) Barral et al, Genes Dev. 1999 Jan 15;13(2):176-87 - Nim1-related kinases coordinate cell cycle progression with the organization of the peripheral cytoskeleton in yeast.

compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier.


96) Hall et al, *Clin Cancer Res*. 2005 Oct 1;11(19 Pt 1):6780-6 - The septin-binding
protein anillin is overexpressed in diverse human tumors.


108) Zhang et al, J Biol Chem. 2000 Nov 10;275(45):35600-6 - Phosphatidylinositol 4-phosphate 5-kinase Its3 and calcineurin Ppb1 coordinately regulate cytokinesis in fission yeast.


111) Kouranti et al, Curr Biol. 2006 Sep 5;16(17):1719-25 - Rab35 regulates an
endocytic recycling pathway essential for the terminal steps of cytokinesis.


115) Zhao et al, Mol Biol Cell, 2006 Sep;17(9):3881-96. Epub 2006 Jun 21 - Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis.


121) Schmidt et al, J Cell Sci, 2002 Jan 15;115(Pt 2):293-302 - In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other.


125) O'Conallain et al, Mol Gen Genet, 1999 Sep;262(2):275-82 - Regulated nuclear localisation of the yeast transcription factor Ace2p controls expression of chitinase
(CTS1) in Saccharomyces cerevisiae.


134) Nakajima et al, J Cell Sci. 2007 Dec 1;120(Pt 23):4188-96 - The complete removal of cohesin from chromosome arms depends on separase.


137) Norden et al, Cell. 2006 Apr 7;125(1):85-98 - The NoCut pathway links completion of cytokinesis to spindle midzone function to prevent chromosome breakage.

138) Steigemann et al, accepted in Cell

139) Hirano et al, EMBO J. 1986 Nov;5(11):2973-2979 - Isolation and characterization of Schizosaccharomyces pombe cutmutants that block nuclear division but not cytokinesis.


142) Bouck et al, Biochem Cell Biol. 2005 Dec;83(6):696-702 - The role of centromere-
binding factor 3 (CBF3) in spindle stability, cytokinesis, and kinetochore attachment.


170) Ozaki et al, *EMBO J*, 1996 May 1;15(9):2196-207 - Rom1p and Rom2p are GDP/GTP exchange proteins (GEPs) for the Rho1p small GTP binding protein in *Saccharomyces cerevisiae*.


180) Lippincott et al, *J Cell Sci*. 2001 Apr;114(Pt 7):1379-86 - The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis


185) Ono et al, *Cancer Res*. 2002 Jan 15;62(2):333-7 - SEPTIN6, a human homologue to mouse Septin6, is fused to MLL in infant acute myeloid leukemia with complex chromosomal abnormalities involving 11q23 and Xq24


190) Thanabalu et al, *EMBO J*. 2001 Dec 17;20(24):6979-89 - Functions of Vrp1p in...
cytokinesis and actin patches are distinct and neither requires a WH2/V domain


192) Toyn et al, Genetics. 1997 Jan;145(1):85-96 - The Swi5 transcription factor of Saccharomyces cerevisiae has a role in exit from mitosis through induction of the cdk-inhibitor Sic1 in telophase


196) Dijkgraaf et al, Yeast. 2002 Jun 15;19(8):671-90 - Mutations in Fks1p affect the cell wall content of beta-1,3- and beta-1,6-glucan in Saccharomyces cerevisiae

197) de Gramont et al, Cell Cycle. 2007 May 15;6(10):1231-41 - The spindle midzone microtubule-associated proteins Ase1p and Cin8p affect the number and orientation of astral microtubules in Saccharomyces cerevisiae

198) Gardner et al, J Cell Biol. 2008 Jan 14;180(1):91-100 - The microtubule-based motor Kar3 and plus end-binding protein Bim1 provide structural support for the anaphase spindle


**APPENDIX:**

**Abbreviations:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPC</td>
<td>Chromosomes Passenger Complex</td>
</tr>
<tr>
<td>MEN</td>
<td>Mitotic Exit Network</td>
</tr>
<tr>
<td>SIN</td>
<td>Septation Initiation Network</td>
</tr>
<tr>
<td>PP2A</td>
<td>Protein Phosphatase 2A</td>
</tr>
<tr>
<td>SPB</td>
<td>Spindle Pole Body</td>
</tr>
<tr>
<td>PTIns</td>
<td>Phosphatidylinositols</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin Dependent Kinase</td>
</tr>
</tbody>
</table>
Supplementary Material:

The most promising hits obtained in the synthetic lethal screens to act in the Cyk3-pathway have been confirmed by independent methods as described in the Results Section II. The raw data are presented here:

**Fig. S1:** Summary of the confirmation of the genetic interaction of the genes further investigated in Result Section II. On the x-axis are the bait strains, on the y-axis the genes of interest. Color-coding indicates the degree of genetic interaction from green: does not interact to red: does fully interact; numbers in the box indicate the amount of double mutants scored.

**Tab. S1** describes the absolute scores in a non-graphical manner. Per row the three numbers mean: total number of genotype scored/fraction of dead scored/fraction of alive scored; ΔΔ: double mutant, bait Δ and prey Δ.

**Tab. S1:** Summary of the confirmation of the genetic interaction of the genes further investigated in Result Section II. This is a non-graphical illustration of the absolute scores that are graphically illustrated in Fig.S1
The characterization of the viable \( \text{rho4} \Delta \) and \( \text{rho5} \Delta \) deletion mutants that were not found as hits in the synthetic lethal screens:

![Fig. S2: Summary of the tested genetic interaction for the viable \( \text{rho2} \Delta \), \( \text{rho4} \Delta \) and \( \text{rho5} \Delta \) with bait strains \( \text{myo1} \Delta \), \( \text{hof1} \Delta \) and \( \text{cyk3} \Delta \) as they are discussed in Result Section II. On the x-axis are the bait strains, on the y-axis the genes of interest. Color-coding indicates the degree of genetic interaction from green: does not interact to red: does fully interact; numbers in the box indicate the amount of double mutants scored.](image)

\[
\begin{array}{|c|c|c|c|}
\hline
 & \text{myo1} \Delta & \text{hof1} \Delta & \text{cyk3} \Delta \\
\hline
\text{rho2} \Delta & 8 & 13 & 11 \\
\text{rho4} \Delta & 12 & 6 & 5 \\
\text{rho5} \Delta & 21 & 15 & 14 \\
\hline
\end{array}
\]

Tab. S2 describes the absolute scores in a non-graphical manner. Per row the three numbers mean: total number of genotype scored/fraction of dead scored/fraction of alive scored; \( \Delta \Delta \): double mutant, \( \text{bait} \ \Delta \) and \( \text{prey} \ \Delta \).

\[
\begin{array}{|c|c|c|c|c|c|c|c|c|}
\hline
 & \text{myo1} \Delta & & & & \text{hof1} \Delta & & & \text{cyk3} \Delta \\
\hline
 & \Delta \Delta & \text{myo1} \Delta & \text{prey} \Delta & & \Delta \Delta & \text{hof1} \Delta & \text{prey} \Delta & & \Delta \Delta & \text{cyk3} \Delta & \text{prey} \Delta \\
\hline
\text{rho2} \Delta & 8/8/0 & 24/0/24 & 24/0/24 & & 13/1/2 & 11/1/10 & 11/0/11 & & 11/0/11 & 9/0/9 & 9/0/9 \\
\text{rho4} \Delta & 12/0/12 & 20/0/20 & 20/0/20 & & 6/0/6 & 12/0/12 & 12/0/12 & & 5/0/5 & 6/0/6 & 6/0/6 \\
\text{rho5} \Delta & 21/0/21 & 15/0/15 & 15/0/15 & & 15/0/15 & 12/0/12 & 12/0/12 & & 14/0/14 & 21/0/21 & 21/0/21 \\
\hline
\end{array}
\]

Tab. S2: Summary of the confirmation of the genetic interaction of the genes further investigated in Result Section II. This is a non-graphical illustration of the absolute scores that are graphically illustrated in Fig.S2

**Abscission index calculation:**

We define the abscission index as the ratio between those cells that are in contracted stage of cytokinesis versus all cells in telophase in an asynchronous culture of the strain of interest. In practice, prior to calculating the abscission index, we score all cytokinetic stages individually and calculate the abscission index according to these counts.
Tab. S3 and Fig. S3 gives an overview over the raw data of these cytokinetic stage counts (open neck, contracted neck and resolved neck) as they have been presented in the Results Section II in Fig. 17 and Fig. 21.

<table>
<thead>
<tr>
<th></th>
<th>open</th>
<th>contracted</th>
<th>resolved</th>
<th>stdev open</th>
<th>stdev contr</th>
<th>stdev res</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>37%</td>
<td>14.31%</td>
<td>48.88%</td>
<td>1.67%</td>
<td>1.55%</td>
<td>3.12%</td>
</tr>
<tr>
<td>tus1Δ</td>
<td>39%</td>
<td>47%</td>
<td>15%</td>
<td>2.34%</td>
<td>2.57%</td>
<td>0.72%</td>
</tr>
<tr>
<td>cyk4Δ</td>
<td>40.00%</td>
<td>28.51%</td>
<td>35.24%</td>
<td>3%</td>
<td>1.77%</td>
<td>2.55%</td>
</tr>
<tr>
<td>cyk5Δ</td>
<td>29.00%</td>
<td>18.95%</td>
<td>52.40%</td>
<td>3%</td>
<td>1.87%</td>
<td>1.85%</td>
</tr>
<tr>
<td>cyk3Δ</td>
<td>40.40%</td>
<td>33.09%</td>
<td>26.55%</td>
<td>2.51%</td>
<td>1.13%</td>
<td>3.56%</td>
</tr>
<tr>
<td>rho2Δ</td>
<td>41.95%</td>
<td>39.73%</td>
<td>18.00%</td>
<td>1.83%</td>
<td>0.87%</td>
<td>3.22%</td>
</tr>
<tr>
<td>rho4Δ</td>
<td>41.16%</td>
<td>17.16%</td>
<td>41.68%</td>
<td>2%</td>
<td>1.15%</td>
<td>3.10%</td>
</tr>
<tr>
<td>apl3Δ</td>
<td>42%</td>
<td>24%</td>
<td>35%</td>
<td>2.10%</td>
<td>1.20%</td>
<td>3.40%</td>
</tr>
<tr>
<td>ste20Δ</td>
<td>38.00%</td>
<td>16.83%</td>
<td>44.89%</td>
<td>2.10%</td>
<td>3.20%</td>
<td>2.40%</td>
</tr>
<tr>
<td>hof1Δ</td>
<td>48.00%</td>
<td>16.71%</td>
<td>36.20%</td>
<td>3.90%</td>
<td>2.10%</td>
<td>0.23%</td>
</tr>
</tbody>
</table>

Tab. S3: Summary of the raw data scored for the three cytokinetic stages open, contracted and resolved. Values represent a mean of three independent measurements and stdev represents the standard deviation of these three values.

Fig. S3: Graphical representation of the values summarized in Tab. S3
**Abscission index calculation for cyk6Δ, fks1Δ, cts1Δ and wt:**

For the evaluation of the cytokinetic defect of cells mutant for CYK6, we have analyzed the abscission index identically to Tab.S3 and Fig.S3. The abscission index is illustrated in Results Section III in Fig.27.

<table>
<thead>
<tr>
<th></th>
<th>open</th>
<th>contracted</th>
<th>resolved</th>
<th>stdev open</th>
<th>stdev con</th>
<th>stdev res</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>36%</td>
<td>14.22%</td>
<td>49.95%</td>
<td>1.95%</td>
<td>0.40%</td>
<td>2.30%</td>
</tr>
<tr>
<td>cyk6Δ</td>
<td>24.22%</td>
<td>44.95%</td>
<td>30.83%</td>
<td>2.85%</td>
<td>2.28%</td>
<td>0.70%</td>
</tr>
<tr>
<td>fks1Δ</td>
<td>36.75%</td>
<td>32.07%</td>
<td>31.48%</td>
<td>2.80%</td>
<td>2.10%</td>
<td>4.50%</td>
</tr>
<tr>
<td>cts1Δ</td>
<td>32.04%</td>
<td>14.69%</td>
<td>53.28%</td>
<td>3.20%</td>
<td>0.53%</td>
<td>2.70%</td>
</tr>
</tbody>
</table>

**Tab. S4:** Summary of the raw data scored for the three cytokinetic stages open, contracted and resolved. Values represent a mean of three independent measurements and stdev represents the standard deviation of these three values.

**Fig. S4:** Graphical representation of the values summarized in Tab.S4
Genetic Interaction of cyk6Δ with cytinesis genes

We found cyk6Δ to interact genetically with hof1Δ, myo1Δ but not with cyk3Δ therefore we have tried to confirm this interaction by manual tetrad analysis:

Fig. S5: The cyk6Δ mutant strain exhibits full synthetic lethality with hof1Δ and is fully viable in combination with the cyk3Δ mutation. In combination with the myo1Δ mutation 50% of the double mutant spores scored showed full lethality and 50% of the double mutant spores were fully alive indicating that a possible suppressor mutation that becomes essential or toxic in the absence of CYK6, n referres to the double delete spores scored after dissection

Fig. S6: Cyk6-GFP requires septins for its localization to the bud neck but localizes there independently of Myo1, Hof1 or Cyc3
**Fig. S7:** (A) swi5Δ genetically interacts with slt2Δ; (B) swi5Δslt2Δ double mutant exhibits a severe growth defect at 37°C which can be rescued by addition of the osmo-stabilizer sorbitol; (C) The fraction of phosphorylated Slt2, but not the total protein level increases in swi5Δ background; (D) swi5Δ slt2Δ cells form multibudded cell bodies, which implicates a cytokinetic defect; (E) The plasma membrane is not resolved although a new bud has already emerged strengthening the idea that swi5Δ cells suffer from a cytokinetic defect.
Fig. S8: (A) cyk6Δ genetically interacts with slt2Δ; (B) cyk6Δ slt2Δ double mutant exhibits a severe growth defect at 37°C which can be rescued by addition of the osmo-stabilizer sorbitol similarly to the swi5Δ slt2Δ phenotype presented in Fig. S7; (C) The fraction of phosphorylated Slt2 but not the total protein level increases in cyk6Δ background; (D) cyk6Δ slt2Δ cells form multibudded cell bodies which implicates a cytokinetic defect; (E) The plasma membrane is not resolved in cyk6Δ slt2Δ double mutant cells although a new bud has already emerged strengthening the idea that cyk6Δ cells suffer from a cytokinetic defect. (F) cyk6Δ and swi5Δ cells exhibit no synthetic genetic defects suggesting that Swi5 and Cyk6 act in the same genetic pathway and in the same cellular process.
Cytokinesis Goes Polo

Temporal and spatial coordination of cytokinesis with chromosome segregation is key for successful cell division, but it is poorly understood. A recent article in *Science* by Pellman and coworkers (Yoshida et al., 2006) reveals how the yeast polo-like kinase Cdc5 triggers Rho1/RhoA activation and the assembly of the contractile actin ring during anaphase.

Shortly after the segregation of sister chromatids, cytokinesis completes the actual division of the cell. In animal cells and fungi, cytokinesis starts with the formation of a cleavage furrow, where the plasma membrane is pinched down through actin-dependent contraction at the cortex. The contractile actin ring (CAR) involved in this process consists of antiparallel actin cables cross-linked by myosin II (Glotzer, 2001). Although the mechanism of CAR contraction and its regulation are beginning to be fairly well understood, less is known about CAR assembly and how it is coordinated with mitosis. In animal cells, nonmuscle myosin II localizes to the entire cell cortex until anaphase. At that point, the contractile tension exerted by myosin on the cortex is relaxed around the spindle poles, causing the contracting actin-myosin network to focus into a ring at the spindle equator. Therefore, in these organisms CAR assembly and contraction are strongly interdependent. The situation is different in fungi like the budding yeast *S. cerevisiae*, where myosin II assemblies as a ring at the mother-bud neck during spindle assembly in a septin-dependent but actin-independent manner. Later in anaphase/early telophase, this myosin ring recruits actin to form the CAR. A number of studies have shown that components of the mitotic exit network (MEN), such as the protein kinases Cdc15 (Mennsen et al., 2001) and Mob1/Dbf2 (Luca et al., 2001), trigger CAR contraction upon mitotic exit. In cells where these MEN components do not reach the cleavage site and contraction fails, the CAR still assembles properly, demonstrating that contractile activity is not required for CAR formation. A comparable situation is observed in the fission yeast *S. pombe* where the septation initiation network (SIN), to which the MEN is highly similar, triggers CAR contraction. Perturbation of the Sid2p-Mob1p (Hou et al., 2004) protein kinase prevents CAR contraction but not its assembly. Thus, in both yeasts, how CAR formation is linked to mitotic progression remained unknown.

In yeast, as in animal cells, CAR assembly involves a number of players. In addition to Myosin II and septins, CAR assembly also requires the activity of the Rho GTPase Rho1 and the formin Bni1 (Tolliday et al., 2002). Rho1 appears to stimulate the Bni1-dependent nucleation of actin cables, which can in turn be incorporated into the CAR. In support of the idea that Rho1 controls Bni1 function, a constitutively active allele of Bni1 partially bypasses the requirement for Rho1 in CAR assembly. However, little was known about how the recruitment and function of Rho1 and Bni1 at the bud neck are coordinated with anaphase.

In a study recently published in *Science*, David Pellman and coworkers show that Rho1 and CAR assembly is under the direct control of the polo-like kinase Cdc5. First, the authors wondered whether any of the well-known late mitotic regulators control CAR assembly. Comparing the late anaphase arrest of cdc5-2 and cdc15-2 temperature-sensitive cells, the authors confirmed that MEN activity is not required but found that polo activity is. Further controls demonstrated that this requirement does not depend on Cdc5 function in early mitosis or its function in Cdc14 activation during late anaphase, suggesting that polo might act very directly in CAR assembly. In support to this idea, the authors demonstrate that polo directly binds, and activates, probably through phosphorylation, several GEFs for Rho1 and, in particular, Tus1 and Rom2. Furthermore, Cdc5 activity and the ability of both of these GEFs to bind to Cdc5 were all required for the recruitment of these molecules and the accumulation of Rho1-GTP at the cleavage site. Conversely, cells expressing Tus1...
and Rom2 phosphomimicking mutants became at least partially independent of Cdc5 for CAR assembly. Thus, Cdc5 is directly involved in the activation of Rho1 at the site of cleavage, and this aspect of its function is both necessary and sufficient for CAR formation.

With this study, Pellman and coworkers have added a new function to the long list of Cdc5 roles in mitosis. In conjunction with Cdk1-cyclin B, Cdc5 regulates APC/c activation and thus contributes to anaphase onset. At the same time, Cdc5 phosphorylates the cohesin subunit Scc1 and facilitates its cleavage and sister chromatid segregation (Alexandru et al., 2001). During anaphase, Cdc5 translocates to the bud neck and promotes Tus1 and Rom2 recruitment, Rho1 activation, and CAR assembly. At spindle poles, Cdc5 contributes to MEN activation and hence indirectly to mitotic exit and CAR contraction. The organization of Cdc5 functions therefore ensures that cytokinetic events take place in the right order and in synchrony with spindle function (Figure 1). For example, it means that CAR assembly takes place before contraction.

This study provides important hints for the analysis of CAR assembly in animal cells, where both CAR assembly and contraction depend on RhoA. Like yeast Rho1, RhoA acts through the formin mDia1 (Narumiya and Yasuda, 2006). In addition, it promotes CAR contraction through ROCK-dependent phosphorylation of the myosin light chain MRLC (Glotzer, 2001). Thus, it is tempting to speculate that polo-like kinases might also control cytokinesis of mammalian cells through phosphorylation and activation of RhoA GEFs.

This study also opens new questions. It is unclear how Cdc5 triggers different mitotic events at different times in mitosis. In short, we still know little about how Cdc5/polo itself is regulated. Is it through its localization? If so, then is Cdc5 recruitment to the bud neck required for CAR assembly? More generally, we still need to understand how the cell controls Cdc5/polo localization throughout mitosis.

Harald Rauter 1 and Yves Barral 1
1 Institute of Biochemistry
Eidgenössische Technische Hochschule Zurich
8093 Zurich
Switzerland

Selected Reading

Figure 1. Cdc5 Plays an Essential Role in Linking Car Assembly and Contraction to Mitotic Progression
Cdc5 promotes Cdk1 activation at the G2/M transition and Rho1-dependent CAR assembly during anaphase. At that stage, Cdk1 inhibits CAR contraction. Once sister chromatids have been successfully segregated between mother and bud, the MEN promotes Cdk1 inactivation and mitotic exit, as well as CAR contraction through targeting of the MEN kinase Dbf2/Mob1 to the site of cleavage.
Role of the Ipl1/aurora protein kinase and the spindle midzone in monitoring chromatin segregation during anaphase

Manuel Mendoza$^{1,3}$, Caren Norden$^{1,4}$, Kathrin Durrer$^1$, Harald Rauter$^1$, Frank Uhlmann$^2$ and Yves Barral$^{1,5}$

1- Institute of Biochemistry, Biology Department, ETH Zurich, 8093 Zurich, Switzerland
2- Chromosome Segregation Laboratory, Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom
3- Current address: Centre for Genomic Regulation (CRG), C/ Dr. Aiguader, 88, 08003 Barcelona, Spain.
4- Current address: Physiology Development and Neuroscience, Cambridge University, Downing St., Cambridge CB2 3DY, UK
5- Corresponding author:

Email: yves.barral@bc.biol.ethz.ch

Tel: +41 (0) 44 632 0678

Fax: +41 (0) 44 632 1591
In budding yeast\textsuperscript{1} and HeLa cells\textsuperscript{2}, the NoCut checkpoint, which involves the passenger kinase aurora B, delays the completion of cytokinesis in response to anaphase defects. However, how NoCut monitors anaphase progression was unknown. Here, we show that retention of chromatin in the plane of cleavage is sufficient to trigger NoCut, provided that aurora/Ipl1 localizes properly to the spindle midzone, and that the ADA histone acetyltransferase complex is intact. Furthermore, forcing aurora onto chromatin was sufficient to elicit NoCut, independently of anaphase defects. These findings provide first evidence that NoCut is triggered by the interaction of acetylated chromatin with the passenger complex at the spindle midzone.
During mitosis, anaphase onset is controlled by the spindle assembly checkpoint, which inhibits separase activation and chromosome separation until sister-chromatid bi-orientation is achieved\(^3\). Once sister chromatids migrate to opposite spindle poles, the cleavage furrow starts to pinch the cell. However, the final cleavage of the cell, called abscission, awaits until all chromatids have been pulled out of the cleavage plane\(^5,6\). How cells coordinate abscission with chromosome segregation is poorly understood.

In budding yeast, inactivation of spindle midzone components such as Ase1 or Ndc10, causes premature spindle breakage and compromises chromosome segregation. In these cells, furrow ingression proceeds properly but abscission is delayed by the NoCut pathway, preventing that stranded chromosomes are damaged by cytokinesis\(^1\). The chromosome passenger complex (CPC), comprising the aurora kinase Ipl1 and its regulator INCENP/Sli15\(^7,8\), acts at the top of the NoCut pathway. It conveys the NoCut signal by targeting the NoCut effectors Boi1 and Boi2, two anillin-like proteins, to the site of cleavage at the bud neck\(^1\). CPC-dependent targeting of Boi1 and Boi2 to the bud neck occurs in every anaphase, but is relieved at cytokinesis onset upon proper completion of anaphase. In cells with spindle defects, however, Boi1 and Boi2 stay at the bud neck and abscission is delayed. The exact event triggering the NoCut response is, however, unknown. Interestingly, NoCut function is conserved in HeLa cells, where human aurora B delays abscission in cells with chromosome bridges\(^2\). To investigate how Aurora kinase monitors anaphase completion in budding yeast, we first asked whether NoCut responds to lagging chromatin in the absence of spindle defects.
The topoisomerase II mutant *top2-4* fails to decatenate sister chromatids at the restrictive temperature (30°C), and chromatin lags over the spindle midzone throughout anaphase (Ref. 9 and Supplementary Figure S1). Inactivation of Top2 was not associated with spindle midzone damage. Indeed, the midzone reporters Ase1-GFP and Ipl1-3GFP showed the same localization pattern on anaphase spindles in wild type and in *top2-4* mutant cells at 30°C (Fig. 1a) and no increase in broken spindles was observed in these cells. To test whether lagging chromatin can trigger NoCut, we first compared cytokinesis progression between *top2-4* and wild type cells. Using the pleckstrin homology domain of phospholipase C fused to GFP (PH-GFP) as a reporter, we inspected the plasma membrane at the bud neck of anaphase and telophase cells, i.e., of cells with one spindle pole body (SPB; visualized using Spc42-CFP as a reporter) in the bud. We then scored whether neck membranes were open, contracted or resolved into two separate membranes (Fig. 1b). The fraction of cells with an open bud neck was comparable between *top2-4* (mean = 39%, standard deviation = 5%) and wild type cells (39%±2%) indicating that mitotic exit and onset of furrowing were not delayed. In contrast, the pre-abscission index, defined as the ratio of cells with contracted versus resolved plasma membrane, was increased almost four-fold in the *top2-4* mutant culture compared to wild type (Fig. 1c; p<0.005), indicating that completion of abscission was delayed in this mutant. Remarkably however, this delay was no-longer observed when the NoCut effectors Boi1 and Boi2 had been inactivated (Fig. 1c, *top2-4 boi1Δ boi2Δ*). We concluded that topoisomerase II defects leads to a NoCut-dependent delay of abscission.
These data suggest that lagging chromatin triggers NoCut in the absence of spindle defects. To test this notion further, we next examined whether failure to cleave cohesin and separate chromosome arms also affected cytokinesis. Expression of the non-cleavable cohesin Scc1-RRDD under the control of the inducible GAL1-10 promoter blocks nuclear division in the first cycle following release from G1 phase\textsuperscript{10}, yet these cells assemble a robust spindle midzone, as shown by the accumulation of Ase1-GFP and Ipl1-3GFP (Fig. 1d). Strikingly, these cells failed to separate from their buds even after they started a new cycle, and became bi-budded (Fig. 1e). Calcofluor staining showed that primary septa were incomplete at the neck of the first buds, indicating that cytokinesis aborted (Fig. 1e). Consistently, the bibudded cells could not be resolved through digestion of their cell walls by zymolyase (Fig. 1f, p<0.001 compared to wild type). By contrast, the same assays revealed that the majority of the GAL:SCC1-RRDD boi1Δ boi2Δ mutant cells had completed cytokinesis similarly to wild type (Fig. 1e; f, p<0.002 compared to GAL:SCC1-RRDD). Unlike the GAL:SCC1-RRDD single mutant, these triple mutant cells developed a penetrant cut phenotype, with nuclei cleaved into unequal masses (Fig. 1e). Thus, cells that failed to separate chromosome arms during anaphase triggered a NoCut-dependent inhibition of cytokinesis, in the absence of midzone damage.

Strikingly, while expression of non-cleavable cohesin impaired cytokinesis progression, preventing chromosome separation through inactivation of separase/Esp1 did not. Four hours after release from a G1 block at the restrictive temperature (37°C), the esp1-1 cells accumulated as bibudded mothers. However, digestion of the cell wall separated most of them readily into unbudded and single-budded cells, showing that cytokinesis was
completed (Fig. 2a). Accordingly, calcofluor staining established that they had also completed septation (Fig. 2b). Because the *esp1-l* mutant is defective in chromosome segregation but not in cytokinesis, cell division should lead to DNA damage in this mutant. As shown in Fig. 2c, foci of DNA damage reporter protein Ddc1-GFP\textsuperscript{11} indeed accumulated in the nuclei of dividing *esp1-l* cells between 150 and 200 minutes after release at the restrictive temperature from G\textsubscript{i} arrest. This timing is consistent with damage occurring at a late stage of division. Moreover, Ddc1-GFP foci accumulation was suppressed in the *esp1-l cdc12-6* double mutant cells. The *cdc12-6* septin mutation causes rapid septin ring disassembly\textsuperscript{12}, and impairs cytokinesis. We concluded that separase inactivation lead to a "cut" phenotype, where cytokinesis occurred in spite of defective chromosome segregation, and damaged DNA.

Together, these results suggest that coordination of cytokinesis with chromosome segregation might be impaired in the *esp1-l* mutant. Thus, we investigated whether separase contributes to the inhibition of cytokinesis in cells carrying the *ndc10-l* mutation\textsuperscript{1}. When these cells are released from G\textsubscript{i} arrest at the restrictive temperature (37°C), midzones fail to form, anaphase spindles break prematurely and the cells restart budding without completing cytokinesis. They therefore accumulate as bibudded cells that are not resolved upon cell wall digestion\textsuperscript{1} (Fig. 2d, p=0.01 compared to wild type). Strikingly, the bibudded, *ndc10-l esp1-l* double mutant cells were effectively resolved into unbudded and single-budded cells by zymolyase treatment. Furthermore, plasma membrane imaging and cell wall staining demonstrated that these cells properly completed abscission and septation, unlike the *ndc10-l* single mutant cells (Fig. 2e).
Thus, separase function was required for the inhibition of abscission in cells with fragile spindle midzones. We concluded that separase is required for mounting a NoCut response.

Budding yeast separase mediates both the resolution of sister-chromatid cohesion, by cleaving cohesin\textsuperscript{13}, and the activation of the protein phosphatase Cdc14 in early anaphase\textsuperscript{14, 15}. This latter function is independent of the proteolytic activity of separase, such that the protease-dead form, Esp1-C1531A, successfully mediates Cdc14 activation in early anaphase, but not cohesin cleavage\textsuperscript{16}. Remarkably, cultures of esp1-1 single and ndc10-1 esp1-1 double mutant cells expressing Esp1-C1531A under the control of the \textit{GAL1,10} promoter, and released from G\textsubscript{1} arrest at 37°C in galactose medium aborted cytokinesis, as shown by calcofluor staining and zymolyase digestion (respectively, p<0.0001 and p<0.01 in the zymolyase assay compared to wild type; Fig. 3a, b). In contrast, expression of Esp1-C1531A did not abrogate cytokinesis when expressed in wild type cells. Thus, the non-catalytic activity of separase is required for cytokinesis inhibition in response to both spindle and chromosome segregation defects.

In addition to separase activity, the FEAR network, which includes the partially redundant nucleolar proteins Bns1 and Spo12, and the spindle-associated factor Slk19 is also required for Cdc14 activation in early anaphase\textsuperscript{15}. Remarkably, zymolyase digestion, septum staining and imaging of the plasma membrane (Fig. 3c, d) all showed that unlike the \textit{ndc10-1} single, the \textit{ndc10-1 bns1Δ spo12Δ} triple mutant released from G\textsubscript{1} arrest at 37°C completed cytokinesis efficiently within 4 hours. Inactivation of Slk19 in
the \textit{ndc10-1} background also restored membrane resolution and septation, as determined by imaging of the plasma membrane and the septum. Deletion of either \textit{BNS1} and \textit{SPO12} or of \textit{SLK19} did not suppress the spindle stability defects due to the \textit{ndc10-1} mutation (Supplementary Information, Fig. S2). Similarly, after release from G\textsubscript{1} arrest in galactose medium, the \textit{GAL:SCC1-RRDD bns1Δ spo12Δ} mutant cells underwent two rounds of budding within 4 hours without cytokinesis failure, and unlike the \textit{GAL:SCC1-RRDD} single mutant cells did not accumulate as multibudded upon zymolyase digestion (Fig. 3c). Thus, both in cells with fragile spindles and with sister chromatid resolution defects, the NoCut response depended on FEAR function.

Because Cdc14 is required for both mitotic exit and the onset of cytokinesis\textsuperscript{17}, it is not possible to directly test whether it plays a role in inhibiting cytokinesis completion. To investigate the potential function of Cdc14 in NoCut, we asked instead whether any one of its known targets needs to be dephosphorylated for NoCut to function. Interestingly, Cdc14 regulates the most upstream NoCut component known, the chromosome passenger complex (CPC). Indeed, the INCENP/Sli15 protein is heavily phosphorylated by Cdk1 during metaphase and dephosphorylated by Cdc14 at anaphase onset; Sli15 dephosphorylation depends on FEAR and targets CPC to the central spindle\textsuperscript{18}. To investigate whether Sli15 dephosphorylation is required for NoCut, we tested whether the constitutively dephosphorylated allele of Sli15, Sli15-6A\textsuperscript{19}, could restore NoCut in FEAR-defective cells. Contrasting with \textit{esp1-1} and \textit{ndc10-1 esp1-1} mutant cells (see above), calcofluor staining, plasma membrane staining with PH-GFP and cell wall digestion all indicated that a large fraction of the \textit{esp1-1 SLI15-6A} (p=0.01 compared to
esp1-1) and ndc10-1 esp1-1 SLI15-6A (p=0.0001 compared to ndc10-1 esp1-1) mutant cells aborted cytokinesis (Fig. 3e, f). Like for Esp1-C1531A, expression of Sli15-6A did not cause abortion of cytokinesis in wild type cells. Thus, non-phosphorylatable Sli15 restored NoCut function in FEAR defective cells. We concluded that Cdc14-dependent regulation of CPC accounted for most, if not all, of FEAR requirement in the NoCut pathway.

As mentioned above, a consequence of Sli15 dephosphorylation is the relocation of the CPC to the spindle midzone. Consistent with this, the CPC component Ipl1 localized to the spindle midzone in cells expressing non-cleavable cohesin (Fig. 1d), but not in the esp1-1 mutant cells, which lacked FEAR activity (Fig. 3g, h). Furthermore, expression of SLI15-6A restored Ipl1-3GFP recruitment to the spindle in the esp1-1 and ndc10-1 esp1-1 cells, as predicted\textsuperscript{18} (Fig. 3g, and data not shown). It also restored Ase1 localization to the spindle in ndc10-1 esp1-1 mutant cells, consistent with the CPC playing an important role in anaphase spindle organization (Fig. 3i).

Since expression of Sli15-6A did not cause inhibition of cytokinesis in wild type cells, but only in cells with lagging chromatin and spindle defects (Fig. 3e), we also concluded that dephosphorylation of Sli15 is required for NoCut ability to sense anaphase defects, but does not trigger NoCut on its own. Thus, CPC relocalization to the spindle midzone is required for sensing of spindle and sister-chromatid resolution defects.
Intriguingly, a screen for NoCut components (H.R. and Y.B., in preparation) independently identified several chromatin components, among which Ahc1, a scaffolding element of the ADA histone acetyltransferase complex\textsuperscript{20}. In support of Ahc1 functioning in NoCut, inhibition of cytokinesis was not observed in the ndc10-1 ahl1\Delta cells, as shown by the reduced number of multibudded cells following zymolyase treatment (Fig. 4a). Further, the double mutant ahl1\Delta ase1\Delta showed reduced viability compared to either single mutant (Fig. 4b). Because ase1\Delta cells rely on NoCut to prevent chromosome damage\textsuperscript{1}, this observation suggests that Ahc1 is required to delay abscission in cells with anaphase spindle defects. To test this possibility, we examined the status of the plasma membrane in ana- and telophase ase1\Delta and ahl1\Delta ase1\Delta cells expressing PH-GFP and Spc42-CFP. As previously reported\textsuperscript{1}, the pre-abscission index was increased in ase1\Delta compared to wild type cells; however, the timing of abscission was restored in the ase1\Delta ahl1\Delta double mutant cells (Fig. 4c). Consistent with this, localization of the NoCut component Boi2 to the site of cytokinesis was perturbed in ahl1\Delta mutant cells (fig. 4d-e). Therefore, the chromatin component Ahc1 was required for proper NoCut function in cells with anaphase defects.

These observations raised the possibility that chromatin is directly involved in NoCut sensing. Furthermore, chromatin is a potent activator of aurora B in vitro\textsuperscript{21}. Thus, we rationalized that CPC on the midzone might respond to the presence of chromatin. We rationalized that if this is the case, preventing the segregation of the CPC away from chromosome arms should trigger NoCut independently of chromosome segregation defects or spindle damage. To test this possibility, Ipl1 was fused to the Tet repressor and
YFP (Ipl1-TetR-YFP) to tether it to chromatin in cells carrying Tet operator (TetO) repeats. Expression of the Ipl1-TetR-YFP construct under the control of the GAL1-10 promoter did not affect growth of wild type cells, and fully complemented the ipl1-321 mutant (Fig. 5a). Furthermore, like wild type Ipl1 the fusion protein localized to two dots located between the two spindle poles of metaphase cells, probably the kinetochores, and to the spindle midzone of anaphase cells (Fig. 5b). In cells containing TetO repeats, one or two supplementary YFP foci were also observed in metaphase and anaphase nuclei, respectively (Fig. 5c). Thus, a fraction of Ipl1-TetR-YFP successfully attached to chromatin in these cells.

We determined the pre-abscission index of cells co-expressing Ipl1-TetR-YFP, Spc42-CFP and PH-GFP, and containing TetO arrays on the sub-telomeric region of chromosome XII (tetO:TEL12R) or near the centromere of chromosome IV (tetO:TRP1). The fraction of cells with an open bud neck was comparable in TetO:TEL12R cells carrying an empty plasmid or expressing Ipl1-TetR-YFP (control: 41.5\%\pm 6.1\%; Ipl1-TetR-YFP: 37.8\%\pm 6.3\%). In contrast, the pre-abscission index was increased 2.5-fold in IPL1 TetO:TEL12R cells expressing the Ipl1-TetR-YFP fusion, relative to TetO cells carrying an empty plasmid (p=0.005) (Fig. 5d). The extent of this increase was independent of the position of the TetO array on the chromosome, since Ipl1-TetR elicited a comparable effect in both tetO:TRP1 and tetO:TEL12R (Supplementary Information, Fig. S3). Since these effects were observed in the presence of wild type, endogenous Ipl1, the Ipl1-TetR construct acted in a dominant fashion to delay completion of cytokinesis.
Strikingly, the inhibition of abscission caused by Ipl1-TetR expression was abrogated in the *IPL1 boi1Δ boi2Δ tetO:TEL12R* strain (Fig. 5e). Thus, Ipl1 tethering to chromatin lead to a NoCut-dependent abscission delay. This delay was not due to spindle stabilization or destabilization by Ipl1-TetR, since the fraction of cells undergoing anaphase was not altered upon Ipl1-TetR expression (Supplementary Information, Fig. S4). Moreover, Ipl1-TetR kinase activity was required in order to delay abscission; no delay was observed when TetR was fused to a kinase-dead allele of Ipl1 (Ipl1-D227A)22 (Fig. 5e). Furthermore, Ipl1-TetR activated NoCut also in *slk19Δ TetO* cells (Fig. 5e). Thus, whereas under normal conditions the abscission delay requires the simultaneous occurrence of Ipl1 on the spindle midzone and lagging chromatin around it, both requirements were bypassed when Ipl1 is forced to interact with chromatin. Similarly, Ipl1-TetR fully bypassed the requirement for Ahc1 in NoCut activation (Fig. 5e), indicating that Ahc1 contributes to NoCut upstream of Ipl1 function. Clustering of aurora-B can lead to its activation, as observed when two CPC complexes are brought together with specific antibodies21. However, the effect of "clustering-mediated" activation of Ipl1 is probably limited here. Indeed, TetR mediated dimerization23 caused only a mild abscission delay on its own, i.e., in cells lacking TetO sequences (Fig. 5d, p=0.008). We concluded that tethering Ipl1 to chromatin mimicked the events required for the activation of the NoCut response in cells with chromosome segregation and spindle defects.
In summary, our data show that 1- NoCut is triggered by the presence of unsegregated chromatin lagging over the spindle midzone, even in the absence of spindle defects, supporting the notion that NoCut coordinates abscission with chromatin segregation both in yeast (this study) and animal cells. 2- NoCut function requires targeting of the CPC to the central spindle during anaphase, indicating that the NoCut signal is generated at this location and time. 3- The chromatin component Ahc1 contributes upstream of Ipl1 to proper NoCut activation, suggesting that chromatin itself acts as signal in NoCut. Since Ahc1 is a core component of the histone acetylation complex ADA, acetylation events might trigger or be required for the activation of Ipl1 by chromatin. Remarkably, ahc1Δ mutant cells not only fail to inhibit abscission in response to anaphase defects, but also show a high incidence of DNA damage. We suggest that at least part of these damages are a consequence of NoCut inactivation. 4- Holding Ipl1 in contact to chromatin throughout anaphase elicits the NoCut response independently of anaphase defects, Ahc1 function, FEAR and proper localization of Ipl1 to the spindle midzone. Altogether, these data suggest that the CPC acts as a sensor that activates NoCut in response to the presence of chromatin around the spindle midzone (Fig. 5f). This model explains why abscission is delayed in response to situations as distinct as the presence of chromosome bridges and premature spindle breakage.

Classic studies in fission yeast identified the cut mutants, which block anaphase yet proceed through cytokinesis, cutting the undivided nucleus. These findings led to the conclusion that cytokinesis is not coordinated with chromosome segregation. Remarkably, several S. pombe "cut" mutations identified separase and its regulator
securin\textsuperscript{26} as well as components of the CPC\textsuperscript{27} and topoisomerase II. These observations suggest two possibilities to reconcile our and the fission yeast data. First, the cut phenotype might stem from abscission being only delayed by NoCut and not fully prevented, as we observe in the \textit{top2-4} mutant. Indeed, at the restrictive temperature these mutant cells do eventually complete cytokinesis, damaging DNA\textsuperscript{28}, and causing cell death. Second, other \textit{cut} mutants might impair both chromosome segregation and NoCut. Indeed, the budding yeast separase mutant \textit{esp1-1} also develops a cut phenotype, but as we show, this is due to separase being required for mounting the NoCut response. Thus, not only NoCut appears to be conserved in evolution, but the \textit{cut} gene collection will probably provide an excellent resource for further dissection of the NoCut checkpoint.
METHODS

**Strains and plasmids.** All yeast strains are derivatives of S288C. *SPC72-GFP* and *ASE1-GFP* strains have been described\(^2\). The *slk19Δ, bns1Δ, spo12Δ, ase1Δ* and *ahc1Δ* strains were obtained from EUROSCARF\(^3\) or were generated by PCR-based gene disruption. The Boi2-GFP plasmid has been described\(^1\). The *tetO* strains were kind gifts of Luis Aragón (MRC, London) and Duncan Clarke (University of Minnesota). Ipl1 constructs were cloned into pRS416\(^3\); the kinase dead Ipl1 allele was generated by site-directed mutagenesis (QuickChange, Stratagene). The pleckstrin homology domain of *Rattus norvegicus* phospholipase C 61 fused to GFP (PH-GFP), a kind gift from Scott Emr (HHMI, San Diego), was expressed from pRS426-based plasmids\(^3\). The Sli15-6A allele is identical to the one described in\(^1\) except in the choice of one phosphorylation site: serines 335, 427, 437, 448, 462 and threonine 474 were mutated to alanine.

**Growth Conditions and staining procedures.** Cells were grown in rich medium (YPD) at room temperature, unless indicated otherwise. For synchronization experiments, cells were arrested with α-factor (Sigma) at 10 μg/ml for 2-4 hours at 22°C, washed twice in fresh medium and released at the restrictive temperature (for temperature sensitive mutants) or at 22°C. For galactose induction, cells were arrested with α-factor in YP + 2% raffinose, released in YP + raffinose + 2% galactose and examined after 4-5 hours (8 h for *ahc1Δ*). Expression of Ipl1 constructs was induced by addition of galactose (2%) to exponentially growing cultures in selective raffinose media at 22°C; cells were examined
4-5 hours after galactose addition. For DAPI staining, cells were fixed for 30 min in 70% ethanol, washed in PBS, and resuspended in PBS containing 1 μg/ml DAPI. For calcofluor staining and septum digestion, cells were fixed with 3.7% formaldehyde for 30 min, and washed twice with PBS. Calcofluor (SIGMA) was used at 0.01 mg/ml in PBS.

**Microscopy.** Imaging was performed on Olympus BX50 and Leica AF7000 fluorescence microscopy systems equipped with a piezo motor, essentially as described\(^{33}\). Spindle images are maximum projections of Z-stacks; plasma membrane status was evaluated on single non-confocal Z axis slices (9 stacks spaced 300 nm).

**Cytokinesis assays.** The frequency of multibudded (cytokinesis-defective) cells was evaluated by light microscopy after 30 min digestion with Zymolyase (2 mg/ml) in 1M sorbitol at 22°C. Pre-abscission indexes of cells expressing PH-GFP and Spc42-CFP were calculated as (fraction of cells with contracted bud neck membranes) / (fraction of cells with resolved membranes). Only cells in which one SPB had entered the bud were considered for analysis. In all cases, the fraction of cells with open bud necks did not change significantly (p > 0.05). Results of abscission and zymolyase assays are given as mean of at least three independent experiments; error bars represent standard deviations. More than 100 cells were counted for condition.

**Statistical analysis.** Unpaired two-tailed t-tests allowing for unequal variance were used (Microsoft Excel).
ACKNOWLEDGMENTS

We are grateful to Patrick Steigemann, Daniel Gerlich, Patrick Meraldi, Hemmo Meyer and all members of the Barral lab for fruitful discussions and critical reading of the manuscript, as well as Dominik Theler, Trinidad Sanmartin and Joelle Sasse for technical assistance. We are thankful to the ETH Light Microscopy Center for their invaluable support. This work was supported by SNF Grant 2-77542-04 to YB.
REFERENCES


FIGURE LEGENDS
**Figure 1:** Defects in chromosome segregation trigger NoCut-dependent inhibition of cytokinesis in the absence of midzone damage. (a) Localization of the central spindle components Ase1 and Ip11 in wild type and *top2-4* mutants. (b) Configuration of the plasma membrane (PH-GFP) in anaphase and post-anaphase cells with one SPB (Spc42-CFP; red arrows) segregated into the bud. White symbols indicate open (arrowhead), contracted (arrow) or resolved (asterisk) bud neck membranes. (c) Quantification of the pre-abscission index (fraction of cells with contracted / resolved membranes) in cells of the indicated strains. Except when indicated otherwise, in this and following graphs statistically significant differences from wild type (p<0.02) are highlighted with an asterisk. Error bars represent standard deviations. (d) Localization of Ase1 and Ip11 in cells expressing the non-cleavable cohesin Scc1-RRDD. (e) DAPI / phase and calcofluor white cell wall staining of cells expressing Scc1-RRDD in wild type and *boi1Δ boi2Δ* strains. Arrowheads point to open bud necks; the arrow points to a completed septum. (f) Fraction of bibudding cells in the indicated strains following septum digestion with zymolyase. In a-c, wild type and *top2-4* cells were grown at 30°C for 4 hours. Strains expressing Scc1-RRDD and cells in D-F were released from a G1 arrest in galactose media at 37°C and examined after 90 minutes (d) or 4 hours (e-f).

**Figure 2:** Separese is required for the NoCut response. (a) Wild type and *esp1-l* cells were released from G1 arrest at 37°C for 4 hours, and the fraction of multibudding cells was determined before and after digestion of the cell wall with zymolyase. (b) Cell wall and septum staining with calcofluor white of wild type and *esp1-l* mutant cells. Open bud necks are marked with arrowheads; arrows point to completed septa. (c) Time-course of
Ddc1-GFP foci formation. Nuclear foci of Ddc1-GFP, corresponding to DNA double strand breaks, are observed in esp1-1 cells but absent in esp1-1 cdc12-6 cells. (d) Fraction of bibudding cells in the indicated strains following septum digestion with zymolyase. (e) Status of the division septum (stained with calcofluor) and plasma membrane (PH-GFP) in cells of the indicated strains. Arrowheads point to open septa or open bud neck membranes; arrows point to complete septa or contracted membranes; asterisks mark resolved membranes. Cells of the indicated strains were arrested in G₁ with alpha-factor, released in fresh media at 37°C, and analyzed every 30 min. (e) or after 4 hours.

**Figure 3:** FEAR is required for the NoCut response through Ipl1 targeting to spindle microtubules. (a, c, e) Fraction of bibudding cells following septum digestion with zymolyase. (b, d, f) Status of the division septum (stained with calcofluor) and of the plasma membrane (PH-GFP) in cells of the indicated strains. Arrowheads point to open septa or open bud neck membranes; arrows point to complete septa; asterisks mark resolved membranes. (g-i) Recruitment of Ipl1-3GFP and Ase1-GFP to the spindle midzone in large budded cells of the indicated strains after 2 hours at 37°C. A small-budded, metaphase wild type cell is shown in (g) for comparison; SCC1-RRDD midzones are shown in Fig. 1d. In all panels, cells were arrested in G₁ with alpha-factor and then released in fresh galactose (for Sec1-RRDD and Esp1-C1531A induction) or glucose media at 37°C for 4 hours before processing.

**Figure 4:** The ADA histone acetyltransferase component Ahc1 is required for the NoCut
response. (a) Fraction of bibudded cells following septum digestion with zymolyase. Cells were released from G₁ arrest at 37°C for 4 hours before processing. (b) 3-fold serial dilutions of cells of the indicated strains were plated on YPD and grown for 2-3 days at the indicated temperature. (c) Quantification of the pre-abscission index (fraction of cells with contracted / resolved membranes) in cells of the indicated strains expressing Spc42-CFP and PH-GFP. (d-e) Localization of Boi2-GFP (green) in wild type and ahl1Δ cells expressing Spc42-CFP (red). In c-e, cells were grown in minimal medium at 23°C.

Figure 5: Tethering of Ipl1 to chromosome arms triggers the NoCut response. (a) 3-fold serial dilutions of wild type or ipl1-32I cells bearing control, Ipl1-YFP or Ipl1-TetR-YFP encoding plasmids were plated on galactose medium and grown for 3 days at 35°C. (b-c) Localization of Ipl1-TetR-YFP (green) and Spc42-CFP (red) in wild type (b) or TetO strains expressing the membrane marker PH-GFP (green) (c). (b) Arrowhead, nuclear focus likely corresponding to kinetochores in metaphase cell; arrow, spindle midzone in anaphase cell. (c) Green arrows, Ipl1 foci at chromosomal TetO:TEL12R arrays in anaphase and telophase cells; white symbols indicate open (arrowhead), contracted (arrow) or resolved (asterisk) bud neck membranes. (d-e) Pre-abscission index (fraction of cells with contracted / resolved membranes) in cells of the indicated strains expressing Ipl1-TetR fusions. (e) A model for how aurora monitors chromatin segregation during anaphase. Left: In early anaphase, separase and FEAR-dependent activation of Cdc14 targets the CPC (the Ipl1 and Sli15 subunits are depicted in red and blue, respectively) to spindle midzone microtubules (in orange). There, we speculate that Ipl1 is activated through interaction with chromatin-associated factors (in green; chromosomes are
depicted in purple), which require Ahc1 function to interact with midzone-bound CPC.
As a result, abscission is inhibited. Right: Upon completion of chromosome segregation,
the CPC is no longer activated by chromatin and the NoCut signal is turned off;
abscission ensues.
**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Fig. 1.** *top2-4* mutants incompletely segregate their chromosomes, visualized by DAPI. Arrowheads point at post-anaphase nuclei; arrows point at elongated anaphase nuclei in wild type (left) and to chromosome bridges in *top2-4* mutant cells. Cells (N>100) were grown at 30°C for 4 hours.

**Supplementary Fig. 2.** Quantification of spindle elongation, as determined by the distance between SPBs, in Spc42-CFP expressing cells of the indicated strains. Cells were arrested in G1 with alpha-factor, released in fresh media at 37°C, and analyzed every 30 min.

**Supplementary Fig. 3.** Pre-abscission index (fraction of cells with contracted / resolved membranes) in *tetO:TRP1* cells expressing Ipl1-TetR-YFP. Expression was induced in galactose media for 4 hours.

**Supplementary Fig. 4.** Ipl1-TetR-YFP does not delay spindle disassembly. The fraction of cells with elongated spindles (green arrow) was quantified in a *TetO:TEL12R CFP-TUB1 SPC42-CFP* strain bearing either a control or Ipl1-TetR-YFP encoding plasmid. Cells were grown in galactose media for 4 hours to induce Ipl1-TetR-YFP expression (red arrows). N > 300.
Mendoza et al.
Figure 1

- **a**: wt top2-4 top2-4 boi1 ∆ boi2 ∆
- **b**: open contracted resolved
- **c**: abscission index

- **d**: wt top2-4 top2-4 boi1 ∆ boi2 ∆

- **e**: GAL-SCC1-RRDD GAL-SCC1-RRDD boi1∆ boi2∆

- **f**: % of multibudded cells after zymolyase digestion
Figure 2

a) Percentage of multibudded cells after zymolyase digestion. The graph shows a comparison between untreated and zymolyase-treated samples for wild-type (wt) and esp1-1 strains.

b) Immunostaining for Ddc1-GFP with calcofluor. Images depict wild-type (wt) and esp1-1 strains.

c) Fraction of cells with Ddc1-GFP foci over time after G₁ release. The graph illustrates the time course of Ddc1-GFP foci formation in wild-type (wt), esp1-1, and esp1-1 cdc12-6 strains.

d) Percentage of multibudded cells after zymolyase digestion. The chart compares wild-type (wt), esp1-1, ndc10-1, and ndc10-1 esp1-1 strains.

e) Immunostaining for PH-GFP with calcofluor. Images show ndc10-1 and ndc10-1 esp1-1 strains.
Figure 3

% of multibudded cells after zymolyase digestion

- wt
- esp1-1
- esp1-1 SLI5-6A
- ndc10-1 esp1-1
- ndc10-1 esp1-1 SLI5-6A

% of large-budded cells with Ip1 at the midzone

- wt
- esp1-1
- esp1-1 SLI5-6A
- SCC1-RRDD

ASE1-GFP SPC42-CFP
PL1-3GFP SPC72-GFP

% of multibudded cells after zymolyase digestion

- wt
- esp1-1
- ESP1(C1531A)
- ndc10-1 esp1-1
- ndc10-1 esp1-1 SLI5-6A

- wt
- esp1-1
- ESP1(C1531A)
- ESP1(C1531A)

Mendoza et al.
**Fig. 4**

(a) % of multibudded cells after zymolyase digestion.

(b) Pre-abscission index of multibudded cells at 25°C and 37°C.

(c) Boi2-GFP localization in ana- and telophase (%).

(d) Spc42-CFP localization in bud neck and bud cortex.

(e) Boi2-GFP localization in ana- and telophase (%) at 25°C and 37°C.
Mendoza et al.
Figure 5

(a) yeast strains

(b) immunofluorescence images showing Spc42-CFP, Ipl1-TetR-YFP, and PH-GFP in metaphase and anaphase

(c) Ipl1-TetR-YFP, PH-GFP, and SPC42-CFP images showing open, contracted, and resolved states

(d) bar graph showing pre-abscission index in wild type (wt) and Ipl1-TetR strains

(e) bar graph showing pre-abscission index in wild type (wt), Ipl1-TetR, boi1Δ, boi2Δ, silk19Δ, and ahc1Δ strains

(f) schematic diagram showing chromatin localization and pre-abscission index in wild type and mutant strains

Chromatin in midzone
Ipl1 active
No abscission

Chromatin away from midzone
Ipl1 inactive
Abscission
Curriculum Vitae

Harald Rauter
born the 03.11.1978 in Klagenfurt
unmarried
citizenship - Austria

Gamperstrasse 7
8004 Zürich, Schweiz
Mobil: +41 76 33 01 320
Email: Harald.Rauter@gmail.com

Education

04.2005 - 02.2009  Dissertation at the Swiss Federal Institute of Technology
Zurich (ETH Zurich) entitled:
“Genetic characterization of the abscission pathway and of its
regulation by the NoCut checkpoint in the budding yeast
Saccharomyces cerevisiae”

06.2006 - 02.2009  Attendance at courses on selected topics in the field of
macroeconomics, patent law, international politics, ethics, and
art at the ETH Zurich

09.1997 - 04.2004  Studies of genetics and microbiology at the University of
Vienna, Austria
Graduation with average grade of 1.6 [highest grade = 1]

01.1999 - 12.2000  Studies of jazz and popular music at the Vienna Music
Institute, Vienna
Graduation 12.2000

09.1990 - 06.1997  Gymnasium (A-level equivalent) with particular consideration
of Information Technologies, Spittal/Drau, Carinthia, Austria
Training and Employment

04.2005 - 02.2009  *Scientific Staff* at the ETH Zurich with responsibilities in research and teaching of students of all levels; organisation of practica, student courses and diploma thesis

02.2006 - 02.2009  *Instructor* at the Life Science Learning Center (LSLC) of ETH Zurich and Interpharma Switzerland to promote Life Sciences, extend public awareness and scout for talented students for the degree program Life Sciences of ETH and University of Zurich

05.2004 - 12.2004  *Scientific Assistant* at Attophotonics Biosciences Inc. Vienna, with responsibilities in the area of nanotechnological product development

04.2003 - 02.2004  *Project Manager* at SAUR S.A, Paris, France as developer and consultant in the industrialization phase of the robotic water analysis system GenPlus®

01.2002 - 09.2002  *Research Sabbatical* at the Institute of Analytical Biotechnology at the Technical University Delft, Netherlands

02.2001 - 12.2001  *Research Associate* at the Institute of Forensic Medicine at the Medical University Vienna, Austria

Publications

02.2009  The NoCut checkpoint targets the GTPase Rho2 to inhibit the completion of abscission, *Rauter H.*, Dinkel M. and Barral Y. in preparation

Role of the Ipl1/aurora protein kinase and the spindle midzone in monitoring chromatin segregation during anaphase, Mendoza M., Norden C., Durrer K., Rauter H., Uhlmann F., and Barral Y. re-submitted to Nature Cell Biology


External Presentations and activities

Rauter H., Dinkel M. and Barral Y. “The NoCut pathway prevents activating factors of abscission from localizing to the site of cytokinesis and thereby protects the chromatin from being cut by the cytokinetic machinery in *S.cerevisiae*” Translational cancer medicine, Jerusalem, Israel

Rauter H. and Barral Y “Genome wide synthetic lethal screens reveal potential novel candidates for the abscission pathway in *S.cerevisiae*” Mechanisms and Control of cytokinesis, Edinburgh, United Kingdom

Rauter H., Mendoza M. and Barral Y “The NOCUT pathway coordinates chromosome segregation with cytokinesis and protects the chromatin from being cut by the cytokinetic machinery”, EMBO and FEBS advances lecture course on signal transduction and cancer, Spetses, Greece

Rauter H. and Barral Y “Identification of novel genes involved in abscission” 3rd international conference on molecular mechanisms of fungal cell wall biogenesis, Heidelberg, Germany

*Organizer of the MLS Ph. D Students Lecture Series 2007 of ETH and University of Zürich*

Languages

German (mother-tongue)
Englisch (fluent written and spoken)
Französisch (advanced)