Initiating the Spindle Assembly Checkpoint signal: Checkpoint protein Mad1 associates with outer kinetochore protein Ndc80 in Budding Yeast

by

Alexandra Weirich

Department of Biochemistry, Microbiology, and Immunology
Submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Graduate Studies
The University of Ottawa
Ottawa, ON

THE UNIVERSITY OF OTTAWA

ABSTRACT

The spindle assembly checkpoint (SAC) is an evolutionarily conserved mechanism that delays the initiation of anaphase by inhibiting the Anaphase Promoting Complex (APC) until all kinetochores have achieved bipolar attachment on the mitotic spindle. Mad1-3, Bub1, and Bub3, components of the SAC are conserved from yeast to humans. These proteins localize to unattached kinetochores, though it is unknown with which kinetochore proteins they interact and how these interactions transduce information about microtubule attachment. Here, purification of the checkpoint proteins from Saccharomyces cerevisiae suggests that Mad1 interacts with the outer kinetochore protein Ndc80 in a SAC, cell cycle, and DNA dependent manner. Ndc80 is thought to mediate attachment of kinetochores to microtubules, so the interaction between Mad1 and Ndc80 suggests a mechanism by which cells sense kinetochore-microtubule attachment. The SAC is of special importance in some types of cancer where genetic damage and aneuploidy is correlated with mutated SAC genes. A better understanding of the SAC mechanism will aid in the development of targeted cancer therapeutics.

ACKNOWLEDGEMENTS

No one succeeds alone. I owe much gratitude to many outstanding people without whose help this would not have been possible:

To my supervisor, Dr. Adam Rudner, for giving space to learn.

To all members of the Rudner lab for scientific help and advice, especially: Elizabeth Williams for her patience and generosity, Carole Dore for great friendship, Sophie Pilon for an inspiring example, and Katherine Harding for Tim Bits. Thank you for supporting me through highs and lows.

To all members of the Baetz lab, the Kearn lab, and the Figeys lab for invaluable scientific help, advice, and many laughs.

Special thanks to the Scott Gerber Lab at Norris Cotton Cancer Center for completing mass spectrometry spectra on purifications of checkpoint proteins and to the Sue Biggins lab at the Fred Hutchinson Cancer Research Center for providing yeast strains containing mutations of kinetochore proteins.

To Andy Stewart and Herb Thompson for teaching me perseverance and discipline.

To my mom for being my refuge.

To William Beswick for exercising great patience and for being my light at the end of the tunnel.

Table of Contents:

List of Abbreviations:

APC	anaphase promoting complex
Ben	benomyl
CCAN	constitutive centromere-associated network
CDC	cell division cycle
CDE	centromere DNA element
CDK	cyclin dependent kinase
D box	R-X-X-L-X-X-X-X-N "Destruction box" APC binding sequence motif
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	dithiothreitol
EGTA	ethylene glycol tetraacetic acid
EDTA	ethylenediaminetetraacetic acid
EM	electron micrograph
Gal	galactose
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IgG	immunoglobulin G
KEN box	K-E-N-X-X-X-N/D sequence APC binding motif
KMN network	KNL1-Mis12-Ndc80 network
MCC	mitotic checkpoint complex
MS	mass spectrometry
NP-40	nonyl phenoxypolyethoxylethanol 40
ts	temperature sensitive
PCR	polymerase chain reaction
PMSF	phenylmethanesulfonyl fluoride
SAC	spindle assembly checkpoint
SPB	spindle pole body
TAP	tandem affinity purification
TEV	tobacco etch virus
TPR	tetratrico-peptide repeat (TPR)
YEP	yeast extract peptone
YPD	yeast extract peptone dextrose
YPGal	yeast extract peptone galactose
YPRaf	yeast extract peptone raffinose

List of Figures:

List of Tables:

Chapter 1: Introduction

An essential step in the life cycle of all living organisms is replication, thus passing their genetic information on to subsequent generations. At the cellular level this means the cell must replicate its entire genome – coded in DNA and organized into densely packed chromosomes – into two complete copies, and faithfully partition exactly one complete copy to each of two daughter cells. The cellular consequence of committing errors in segregation of chromosomes is severe genetic damage in the form of chromosome breakage, chromosome loss, and aneuploidy. Sufficient genetic damage can trigger cell death by apoptosis in vertebrate cells. Aneuploidy (the presence of an abnormal number of chromosomes) is also the hallmark of certain genetic diseases, including Down syndrome, and some types of cancer(Rajagopalan et al. 2003; Gordon, Resio, and Pellman 2012; Heller 1969).

The processes regulating segregation of chromosomes are medically relevant to humans and evolutionarily conserved in all eukaryotes. We therefore employ a unicellular organism, the budding yeast *Saccharomyces cerevisiae*, to model processes controlling chromosome segregation in eukaryotic cells.

BUDDING YEAST AS A EUKARYOTIC MODEL SYSTEM OF THE CELL DIVISION CYCLE

Yeast is a unicellular eukaryotic fungus. The budding yeast *Saccharomyces cerevisiae* is the same species that has been used by humans for thousands of years for fermentation and baking bread. Budding yeast has a very small genome of around 12.1 Mb packaged into 16 chromosomes. They are exceptionally amenable to genetic manipulation and exist in both a haploid and diploid state. The entire *S. cerevisiae* genome has also been

sequenced. All of these factors contribute to the power of this organism as a model for studying eukaryotic cells.

Much of our understanding of the processes regulating cell growth and division has come from studies in yeast. The main drivers of the cell cycle in all eukaryotic cells, Cyclin-Dependent Kinases (CDKs) were first discovered in budding and fission yeast as mutations of the *CDC28* and *cdc2*$^{+}$ genes, respectively. Yeast was also the first eukaryotic genome to be sequenced. Studies in yeast have provided us with information on the mechanisms of some current cancer treatments. (Jonathon Pines, personal communication).

Budding yeast was used as a model for early investigations into the nature of cell division. Early work tried to differentiate between two models of cell cycle; one where a central "clock" drives different events, and one where passage from one stage to another is strictly dependent on completion of the prior stage. Passage out of a yeast cell's G1 phase and commitment to division corresponds to the emergence of a bud whose growth is indicative of the cell's position along the cell division cycle (**Figure 1a**). This inherent visual cue and yeast's short doubling time made *S. cerevisiae* a useful system for investigating the cell cycle. To this end, several Cell Division Cycle (*CDC*) genes were characterized from a screen in budding yeast that identified mutations causing a visual defect in progress through the cell cycle(Hartwell, Culotti, and Reid 1970). The logical ordering of the dependence of *CDC* genes for cell cycle progression, along with biochemical studies, provided some clues towards *CDC* gene function and the mechanism of cell cycle progression. For example, the functions of all other *CDC* genes were found to rely on *CDC28*. Thus *CDC28* defined the beginning of the cell cycle (START) in yeast(Hartwell et al. 1974).

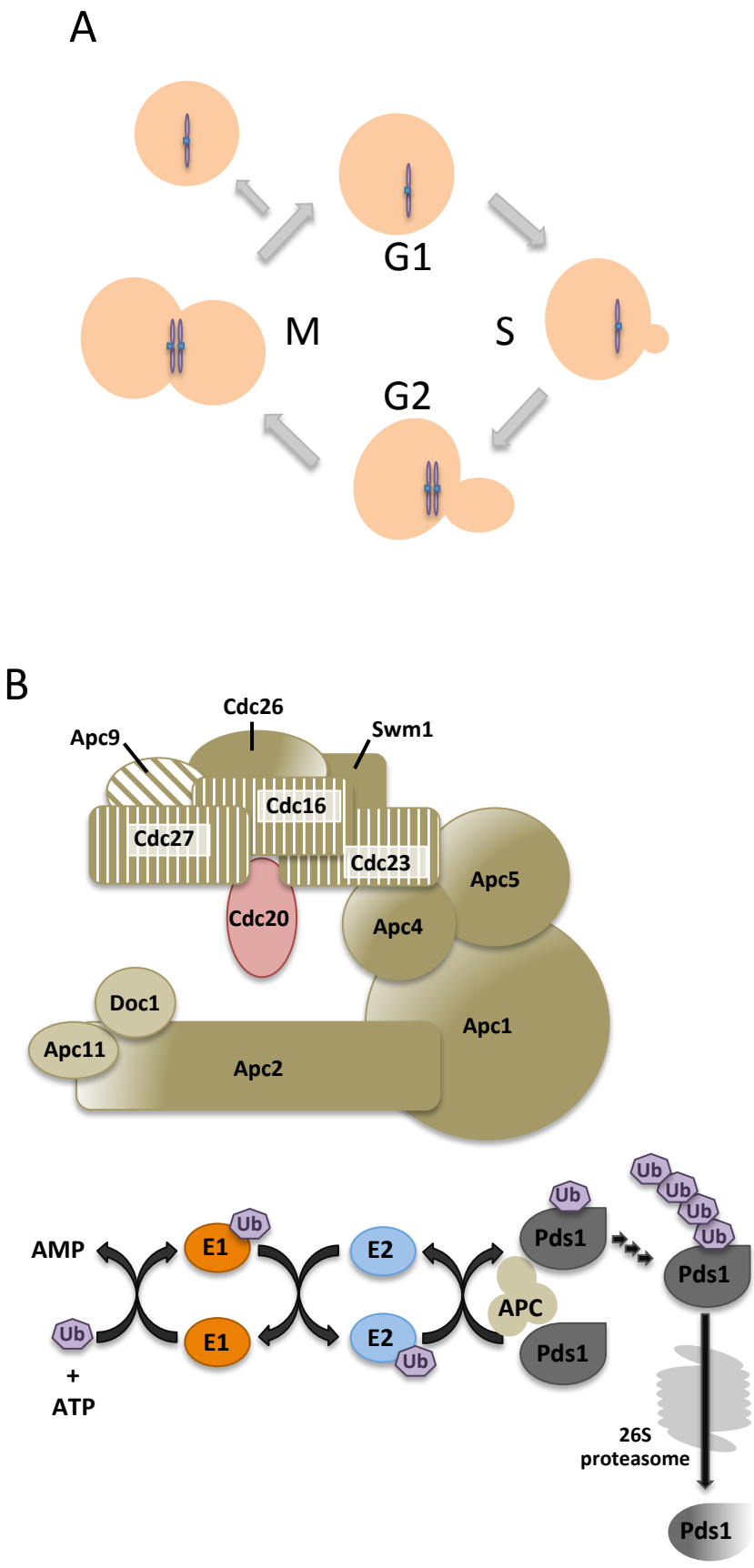

A
G1
S
G2
M
B
Cdc26
Apc9
Swm1
Cdc16
Cdc27
Cdc23
Apc5
Cdc20
Apc4
Doc1
Apc1
Apc11
Apc2
AMP
E1
Ub
E2
Pds1
APC
+
ATP
26S
proteasome

Figure 1. Budding yeast and control mechanisms of the cell division cycle.
(**A**) The cell cycle of budding yeast. (**B**) A schematic diagram of the Anaphase Promoting Complex showing the 'arc-lamp' configuration. The active site is located within the concave face where Cdc20 helps to form the substrate-binding site and Apc11 is the catalytic subunit. Adapted from{Peters:2006cj}. Ubiquitination of Pds1 requires three enzymes: an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2), and an ubiquitin ligase (E3). The APC in complex with its activator acts as the E3 ligase.

The discovery of cyclins, so called for the cyclical nature levels of abundance corresponding to divisions of sea urchin embryos, was a key finding towards determining the biochemical identity of the mechanism controlling cell division(Evans et al. 1983). Experiments in *Xenopus laevis* egg extracts showed that, in the absence of all other protein synthesis, cyclin synthesis alone could drive cell extracts into mitosis(Murray and Kirschner 1989). Subsequent research showed that cyclins are cell cycle regulated targeting subunits for Cyclin-Dependent Kinases (CDKs). Cdk1 (Cdc28 in *S. cerevisiae*) is the primary CDK in yeast. It phosphorylates and activates proteins required for the initiation of DNA replication, mitotic spindle assembly, chromosome condensation, and anaphase onset(Rudner and Murray 2000; Rahal and Amon 2008; Zegerman and Diffley 2007; Kimura et al. 1998). Cyclins target Cdk1 activity to the proper substrates according to their timed cellular abundance(Loog and Morgan 2005; F. W. Pagliuca et al. 2011; Holt et al. 2009). The pattern of cyclin expression and degradation, therefore, drives the cell through its divisions by controlling the timing of activation of Cdk1 substrates. The degradation of cyclin is also critical for turning off Cdk1 kinase activity, which is essential for exit from the mitotic cycle and resetting origins of DNA replication(Amon, Irniger, and Nasmyth 1994; Ghiara et al. 1991; Nasmyth 1993; Mimura et al. 2004; Dahmann, Diffley, and Nasmyth 1995). The precise timing of cyclin destruction is thus an integral feature of a central cellular ‘clock’.

This revelation led to investigations into the mechanism controlling cyclin degradation. Because oscillations in cyclin levels regulate the activity of the cellular clock, disruption of their degradation prevents proper cell cycle progression. In particular, non-degradable forms of cyclin B constitutively activate CDK and prevent exit from mitosis(Murray, Solomon, and Kirschner 1989). In addition to cyclin, destruction of an anaphase inhibitor is also required to trigger anaphase and is mediated by a similar

proteolytic pathway(Glotzer, Murray, and Kirschner 1991; Holloway et al. 1993; Hershko et al. 1991). Genetic and biochemical experiments determined that the destruction of both cyclin and the anaphase inhibitor was found to depend on an evolutionarily conserved protein complex with ubiquitin conjugating activity(Sudakin et al. 1995; Irniger et al. 1995; Tugendreich et al. 1995; King et al. 1995).

What triggers the sudden separation of sister chromatids during mitosis? How are mitotic cyclins targeted for destruction? These questions in the cell cycle field were answered by the discovery of a 20S protein complex that could ubiquitinate the mitotic anaphase inhibitor and cyclins, thus targeting them for degradation by the 26S proteasome(Tugendreich et al. 1995). The complex found in clam extracts was called the cyclosome(Sudakin et al. 1995) whereas that found in budding yeast and frogs was called the Anaphase-Promoting Complex (APC) due to its role in allowing cells to enter anaphase(Zachariae and Nasmyth 1996; Peters et al. 1996; Zachariae et al. 1998; Irniger et al. 1995; Peters et al. 1996; Zachariae et al. 1998; Tugendreich et al. 1995; King et al. 1995). Subsequent immuno-purification and mass spectrometric studies uncovered a highly conserved protein complex with ubiquitination activity(Kurasawa and Todokoro 1999; Yu et al. 1998; Yoon et al. 2002).

SUBUNITS AND ARCHITECTURE OF THE ANAPHASE PROMOTING COMPLEX

Purification and electron microscope studies revealed a 13 subunit core complex in an ‘arc-lamp’ configuration (**Figure 1b and Table 2**)(Thornton et al. 2006; Schreiber et al. 2011; Herzog et al. 2009; Passmore et al. 2005). The major catalytic subunit, Apc11 contains a RING H2 domain common to other E3 ubiquitin ligases(Leverson et al. 2000). Doc1/Ap10

Table 2. Anaphase-Promoting Complex Subunits

	S. cerevisiae	S. Pombe	Mammals	Comments
core subunits:				
Apc1	Apc1	Cut4	Apc1/ Tsg24	
Apc2	Apc2/ Rsi2	Apc2	Apc2	Cullin domain, substrate binding
Apc3	Cdc27	Nuc2	Cdc27	TPRs, activator binding
Apc4	Apc4	Lid1	Apc4	
Apc5	Apc5	Apc5	Apc5	
Apc6	Cdc16	Cut9	Cdc16	TPRs, activator binding
Apc7	-	-	Apc7	TPRs, activator binding
Apc8	Cdc23	Cut23	Cdc23	TPRs, activator binding
Apc9	Apc9	-	-	
Apc10	Doc1	Apc10	Apc10	Doc domain, substrate binding
Apc11	Apc11	Apc11	Apc11	RING finger, catalytic site
Cdc26		Cdc26	Hcn1	
Apc13	Swm1	Apc13	-	
Mnd2	Mnd2	Apc15	Apc15	SAC turnover
Apc14	-	Apc14	-	
Activating subunits:				
Cdc20	Cdc20	Sip1	Cdc20/ $p55^{CDC}$	WD40 repeats, substrate binding
Cdh1	Hct1/ Cdh1	Srw1/ Ste9	Cdh1	WD40 repeats, substrate binding
Ama1	Ama1	Mir1		meiosis-specific activator, substrate binding

* Adapted from Morgan, David O. The Cell Cycle: Principle of Control. New Science Press.

has been shown to promote substrate binding and to support enzyme processivity(Carroll, Enquist-Newman, and Morgan 2005; Carroll and Morgan 2002). Some core proteins of the APC contain multiple repeat motifs that support protein-protein interactions and are thought to function as a scaffold. The largest of these are Apc1 and Apc2(Peters 2006). Cdc27, Cdc16, Cdc23 contain multiple repeats of a 34 amino acid tetratricopeptide motif (TPR) and are thought to form the binding site of the APC activator subunits(Sikorski et al. 1990; Sikorski et al. 1990; Matyskiela and Morgan 2009; Matyskiela and Morgan 2009; Dube et al. 2005; Rudner and Murray 2000). Recently, the Mnd2 (mammalian Apc15) subunit has been shown to function in substrate turnover, which may provide a link between anaphase onset and kinetochore attachments (see section on the spindle assembly checkpoint)(Foster and Morgan 2012; Uzunova et al. 2012; Mansfeld et al. 2011). The exact functions of all the core APC subunits are still not known and a complete crystal structure has yet to be obtained.

The APC functions from the initiation of anaphase until the G1 to S transition, so it is active during periods of high Cdk1 activity (in mitosis), and during low Cdk1 activity (in G1). Two sub-stoichiometric components, Cdh1 and Cdc20, function as activators of the APC during these two periods in the cell cycle. Cdc20 is the mitotic activator of the APC, while Cdh1 functions after mitotic Cdk1 activity falls in anaphase(Visintin, Prinz, and Amon 1997; Schwab, Lutum, and Seufert 1997). The APC is heavily phosphorylated in mitosis(King et al. 1995; Peters et al. 1996). Phosphorylation of the TPR subunits Cdc27, Cdc16, and Cdc23 by Cdk1 promotes the association of the APC with its mitotic activator Cdc20, degradation of mitotic substrates, and exit from mitosis(Rudner and Murray 2000; Rudner, Hardwick, and Murray 2000; Kraft et al. 2003; Shteinberg et al. 1999).

The activators are required to form the substrate binding-site as well as provide some degree of substrate specificity through interaction with substrate destruction box (D-box) and

KEN-box motifs(Kraft et al. 2005; Hilioti et al. 2001; Pfleger, Lee, and Kirschner 2001; Eytan et al. 2006; da Fonseca et al. 2010). RXXLXXXXN, the D-box, was the first sequence shown to be important in targeting APC substrates for degradation(Glotzer, Murray, and Kirschner 1991). The KEN-box motif (KENXXN) was later shown to be another destruction domain that predominantly promotes association of mitotic substrates with APC^{Cdc20}(Pfleger and Kirschner 2000). The APC regulates the abundance of many proteins throughout the cell cycle but Pds1 and Clb5 are the most important substrates for exit from mitosis and the only essential substrates of the mitotically active APC^{cdc20} in *S. cerevisiae*(Shirayama et al. 1999; Cohen-Fix et al. 1996; Thornton and Toczyski 2003; Zachariae and Nasmyth 1999).

The metaphase to anaphase transition is characterized by the concerted segregation of replicated and condensed sister chromosomes to opposite spindle poles. A key inhibitor of this transition is a multi-protein complex called cohesin which holds sister chromatids together, opposing the pulling forces of the spindle. Cleavage of the cohesin complex by the targeted proteolysis of its Mcd1/Scc1 subunit is promoted by the APC. APC targets Pds1 (securin), and inhibitor of Esp1 (separase), for degradation. The released Esp1 (separase) is a protease that cleaves Mcd1/Scc1 and releases chromosomes from their cohesin bonds(Uhlmann et al. 2000; Uhlmann, Lottspeich, and Nasmyth 1999).

KINETOCHORE ARCHITECTURE

The spindle microtubules attach to chromosomes via a large protein structure assembled at their centromere called the kinetochore. In order that the integrity of the genome and the chromosome number be maintained, sister chromatids must develop a bi-polar attachment to the spindle so that when sister chromatid cohesion is dissolved,

chromosomes will segregate accurately to opposite poles of the cell during anaphase (**see Figure 2**).

Kinetochores are large protein scaffold structures that mediate the connection of chromosomes to microtubules. The simplest kinetochore architecture is found in budding yeast where a single DNA sequence in the centromere region of each chromosome promotes the assembly of a kinetochore that attaches to a single spindle microtubule (**Figure 3a**)(Murphy, Fowlkes, and Fitzgerald-Hayes 1991; Winey et al. 1995). Because kinetochore proteins and subcomplexes are highly conserved, kinetochores in higher organisms are thought to be assemblies of repeated units of the basic yeast kinetochore(Joglekar, Bloom, and Salmon 2009; Blower, Sullivan, and Karpen 2002) (also reviewed in (Santaguida and Musacchio 2009)). The kinetochore is made up of purifiable subcomplexes whose associations with the centromere depend on one another, suggesting an assembly hierarchy: the inner complexes bind DNA, the central kinetochore links the inner and outer complexes, and outer complexes bind to microtubules. However, the architecture and stoichiometry of the more than 40-60 proteins that make up the yeast kinetochore has not been exactly elucidated. Significant progress has recently been made by studying electron micrograph (EM) scans of purified budding yeast kinetochores(Gonen et al. 2012). The EM structures are consistent with the model presented here.

Most components of the kinetochore are essential so yeast strains harboring deletions of the kinetochore genes are not viable. Conditional mutations allow us to observe what happens when proteins that are otherwise essential for the survival of the cell are removed. Temperature sensitive (ts) mutants of many kinetochore components display a ‘kinetochore

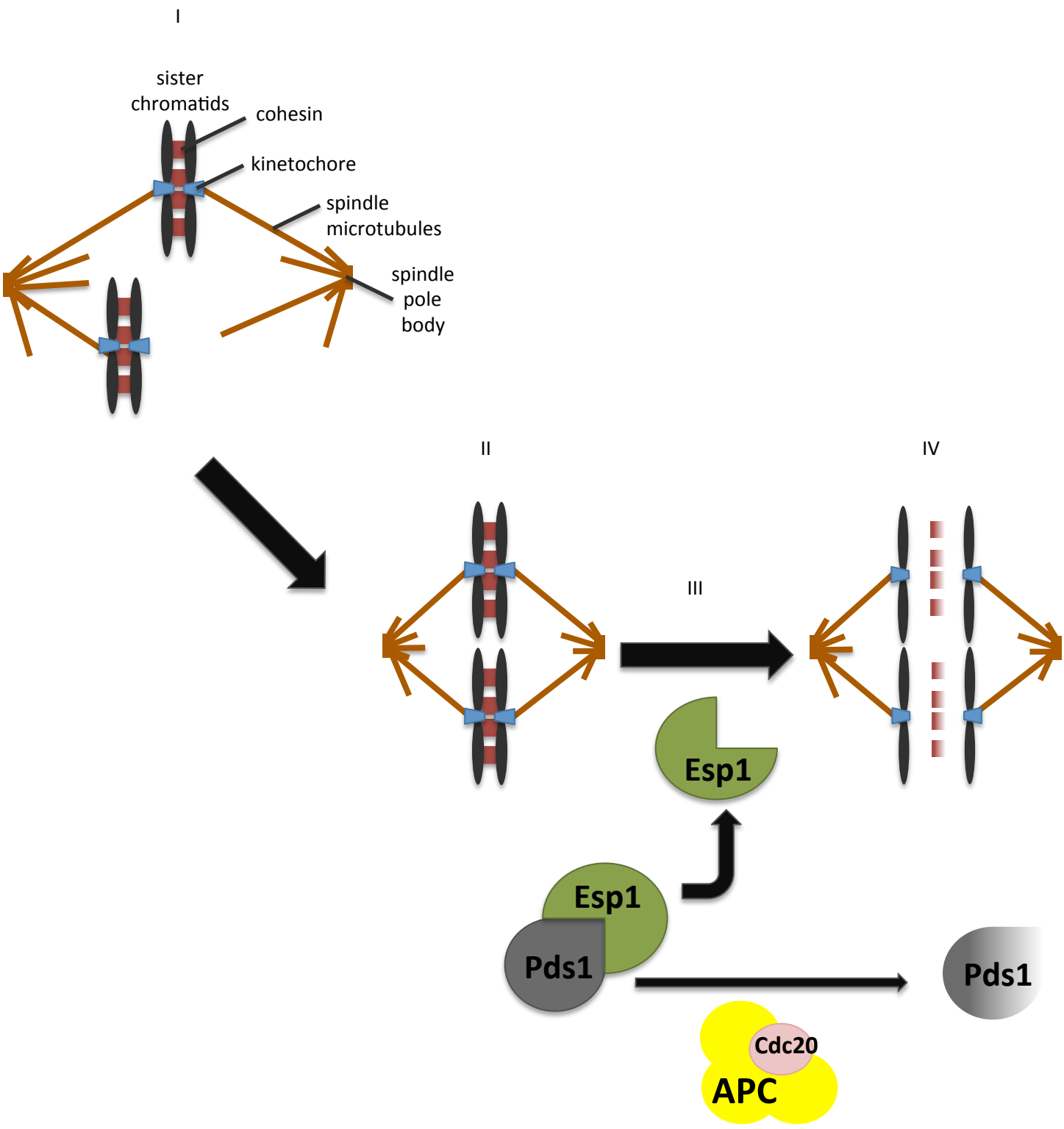
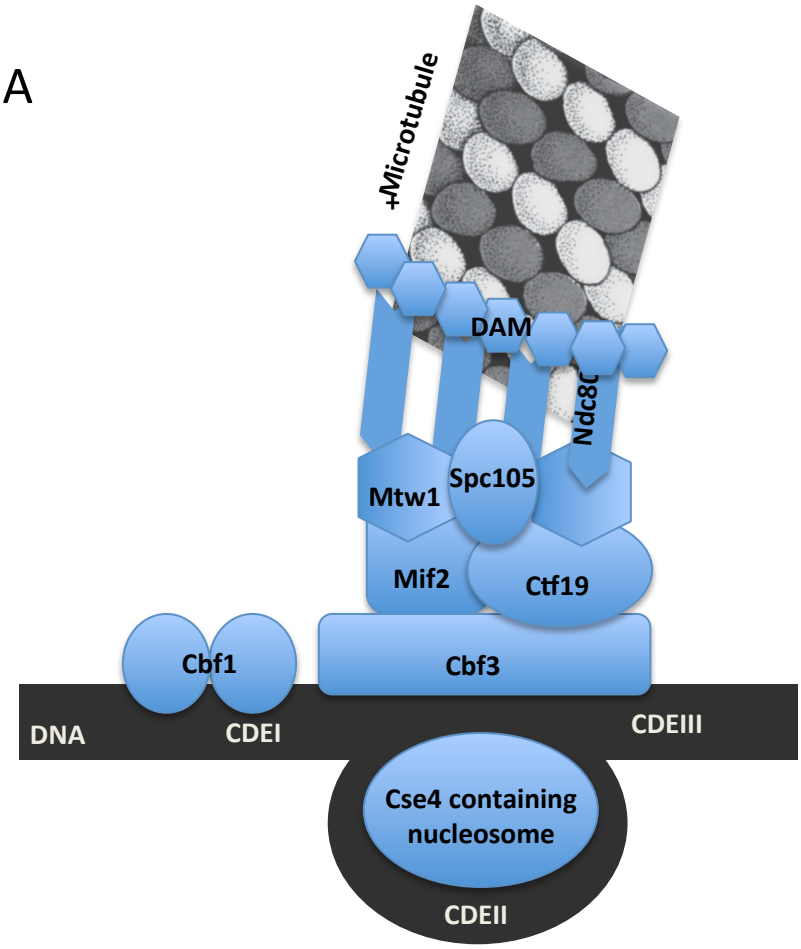

I
sister
chromatids
cohesin
kinetochore
spindle
microtubules
spindle
pole
body
II
III
IV
Esp1
Esp1
Pds1
Pds1
Cdc20
APC

Figure 2. The Anaphase Promoting Complex triggers the metaphase to anaphase transition

During metaphase, replicated and condensed chromosomes attach to the mitotic spindle via a large protein structure called the kinetochore (I). Sister chromatids must develop a bi-polar attachment to the spindle so that the sister chromatids will segregate to opposite poles of the cell during anaphase (II). The Anaphase Promoting Complex, in complex with its mitotic activator Cdc20, activates the initiation of anaphase when all chromosomes are attached to the mitotic spindle by ubiquitinating Pds1. Ubiquitinated Pds1 is degraded by the 26S proteasome, releasing the protease Esp1 to cleave the Mcd1 subunit of the cohesin complex that holds sister chromatids together (III). Released chromosomes are pulled to each of two daughter cells by the pulling force of depolymerizing spindle microtubules (IV).

A


+Microtubule
DAM
Ndc80
Mtw1
Spc105
Mif2
Ctf19
Cbf1
Cbf3
DNA
CDEI
CDEIII
Cse4 containing nucleosome
CDEII

B

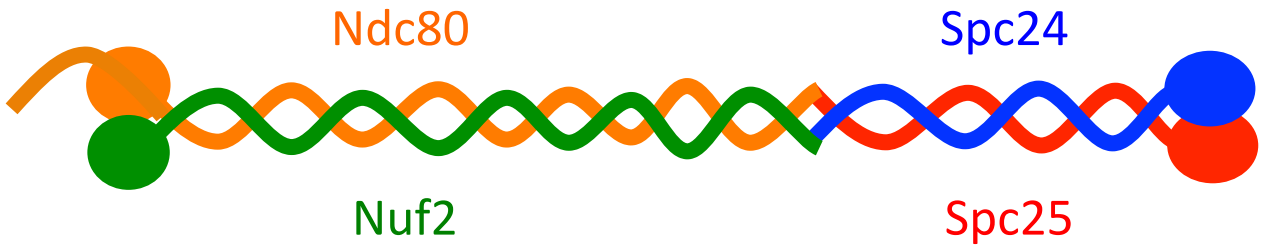

Ndc80
Spc24
Nuf2
Spc25

Figure 3. Kinetochore architecture
(**A**) A schematic diagram of the yeast kinetochore showing the proposed architecture of its sub-complexes. Adapted from (Santaguida and Musacchio 2009) (**B**) A schematic of the NDC80 complex, located in the outer kinetochore. The Ndc80 complex consists of four coiled coil proteins: Ndc80, Nuf2, Spc24, and Spc25. Each has a globular head domain that is involved in forming its binding interactions. The Ndc80/Nuf2 head is oriented towards outwards from the kinetochore and is involved in microtubule binding. Spc24/Spc25 is oriented towards the inner kinetochore where it forms interactions with the Mtw1 Complex.

null' phenotype when grown at the restrictive temperature; cells continue to bud and replicate DNA even though DNA is not partitioned to daughter cells, or chromosomes attach to microtubules but the DNA is not segregated evenly, resulting in genetic damage(Goh and Kilmartin 1993; Ortiz et al. 1999; Ghosh et al. 2001; Wigge and Kilmartin 2001). Other mutants cause cells to arrest in mitosis as a result of an inability to form proper chromosomes-spindle attachments
(see section on Spindle Assembly Checkpoint)(Meluh and Koshland 1995; Osborne et al. 1994). The varied phenotypes of these mutants may be a function of their location and role within the larger kinetochore structure.

In yeast, the centromere is defined by an essential 125 bp DNA sequence that is made up of three parts; CDEII flanked by the shorter CDEI and CDEIII elements(Cottarel et al. 1989). The CDEII sequence wraps around a centromere specific histone H3 variant called Cse4, forming the core of the kinetochore(Meluh et al. 1998). Cse4 is the yeast homologue of human CENP-A, which is also found at centromere regions of human chromosomes(Sullivan, Hechenberger, and Masri 1994). Cbf1 protein and the Cbf3 complex (Cep3, Ctf13, Ndc10, Skp1) bind the centromere DNA sequences CDEI and CDEIII respectively(Baker, Fitzgerald-Hayes, and O'Brien 1989; Lechner and Carbon 1991). The Cbf3 complex is required for localization of all other kinetochore proteins to the centromere(Ortiz et al. 1999). For example, *ndc10-1* ts mutants grown at the restrictive temperature continue to replicate DNA, divide, and form proper spindles in the absence of a functional Cbf3 complex. However, their DNA does not associate properly with microtubule ends and accumulates asymmetrically in the mother cell(Goh and Kilmartin 1993). Furthermore, higher order kinetochore proteins do not assemble at the centromere in *ndc10-1* cells(Janke et al. 2001). This phenotype is consistent with a model where outer kinetochore

complexes build on complexes that assemble more proximal to DNA so that the microtubule-binding complexes require the previous localization of DNA binding and linker complexes.

Another member of the inner kinetochore is Mif2, a centromere DNA binding protein that bridges Cse4 to the central kinetochore(Westermann 2003). The *mif2-3* ts mutant has a point mutation in the C-terminal region that shares the highest degree of homology with human and mouse CENP-C but whose exact function is not known(Meluh and Koshland 1995). Studies have noted that cells harboring the *mif2-3* mutation mostly arrest in mitosis when shifted to the restrictive temperature. Interestingly, longer incubation time at the restrictive temperature results in a less uniform arrest. Although *mif2-3* cells do not have a strong arrest phenotype, they show a marked increase in chromosome segregation defects, even when grown at the permissive temperature(Brown, Goetsch, and Hartwell 1993). Higher order proteins still localize to kinetochores in *mif2-3* strains(Pinsky et al. 2003). These studies suggest that the *mif2-3* mutation either retains some function or else does not completely disrupt kinetochore assembly.

The central kinetochore is composed of mostly conserved linker complexes that connect the DNA binding proteins of the inner kinetochore to the microtubule capturing machinery of the outer kinetochore(Westermann 2003). The main linker complexes in the yeast kinetochore are the Ctf19/COMA, Mtw1/MIND, and Spc105. The Ctf19/COMA contains over 12 proteins (including Ctf19, Mcm16, Mcm21, Ame1, Okp1, Chl4) most of which are non-essential, in contrast to most other kinetochore proteins. There is no strongly defined function of this complex, although mutants have increased rates of chromosome missegregation(Ortiz et al. 1999; Ghosh et al. 2001). The yeast Spc105 Complex contains just two proteins, Spc105 and Kre28. Mutations in this complex show an increase in chromosome loss and decreased viability(Nekrasov et al. 2003; C. Pagliuca et al. 2009). The

Mtw1/MIND complex consists of four proteins (Mtw1, Nnf1, Nsl1, and Dsn1) and is homologous to the Mis12 complex in human cells. The human Mis12 complex is thought to function with KNL1 (Spc105) and Ndc80 Complexes as part of the larger KNL1-Mis12-Ndc80 (KMN) network that links the inner centromere directly to the microtubules(Goshima and Yanagida 2000; Kline et al. 2006). The yeast Spc105 Complex co-purifies purified with both the Mtw1 and Ndc80 complexes, placing it within the yeast version of the KMN network of the central kinetochore(Nekrasov et al. 2003).

Although CENP-A is highly conserved, CBF3 is not, and is functionally replaced by a network of centromere proteins in higher eukaryotes. The specialization of the CBF3 in budding yeast probably reflects the DNA sequence (CDEI, CDEII, CDEII) specificity of yeast centromeres. In contrast, the vertebrate centromere is comprised mainly of repeating alpha satellite DNA sequences, which are also present throughout the vertebrate genome. In the absence of a unique centromere targeting sequence, the centromere must be defined epigenetically. In vertebrate cells, a complex of proteins called the constitutive centromere-associated network (CCAN) plays a role in propagating centromere chromatin through consecutive cell divisions(Santaguida and Musacchio 2009). CENP-B is known to bind directly to the CENP-B box sequence of α satellite repeats at centromeres and is found in complex with other CENP proteins at the centromere(Muro et al. 1992; Masumoto et al. 1989). It has been shown that some of the subunits of the CCAN (CENP-B, CENP-C and CENP-N) bind directly to CENP-A nucleosomes and these interactions are important for the assembling of other centromere and kinetochore proteins(Carroll et al. 2009; Carroll, Milks, and Straight 2010; Foltz et al. 2006). Furthermore, several CCAN subunits share homology to subunits of the yeast Ctf19/COMA complex, indicating that although DNA sequences and DNA binding proteins are not evolutionarily conserved, yeast and vertebrate inner

kinetochore proteins share conserved functions(Santaguida and Musacchio 2009).

Both composition and function of the Ndc80 complex are conserved in yeast and vertebrates(Janke et al. 2001; Wigge and Kilmartin 2001; Zheng, Chen, and Lee 1999). The Ndc80 protein shows high sequence homology to the human Hec1 protein (Highly Expressed in Cancer) that is required for normal chromosome alignment and segregation(Wigge et al. 1998; Y. Chen et al. 1997). It is a rod-shaped complex comprised of four coiled coil proteins (Ndc80-Nuf2 and Spc24-Spc25 dimers) capped by globular head domains at both ends (**Figure 3b**)(Wang et al. 2008; Ciferri et al. 2008). The Spc24-Spc25 dimer binds the inner kinetochore Mtw1 Complex, while the Ndc80-Nuf2 dimer has microtubule binding capability. Ndc80 protein can also undergo biased diffusion to track with plus ends of shrinking microtubules showing that, in addition to its ability to link kinetochores to microtubules, Ndc80 Complex might also play a major role in coupling microtubule dynamics to chromosome motion(McIntosh et al. 2008; Powers et al. 2009). Mutations in Ndc80 result in kinetochores that are mostly defective at binding microtubules, although localization of inner kinetochore proteins to the centromere is unaffected(Wigge and Kilmartin 2001). The majority of *nuf2-61* cells arrest in mitosis with large buds and missegregated DNA(Osborne et al. 1994). Even though most *spc24-1* cells cannot maintain microtubule attachments, many of these mutant cells are unable to arrest the cell cycle, resulting in abnormal DNA accumulation in daughter cells and cell death(Wigge and Kilmartin 2001). The varied phenotypes of mutations in this complex suggest that its role is multi-functional.

DAM/DASH is a 10 protein complex of the outer kinetochore (includes Dam1p, Duo1p, Dad1p, Spc19p, Spc34p, Dad2p, and Ask1p)(Y. Li et al. 2002). This complex has the interesting ability to bind microtubules and oligomerize to form ring structures around

microtubules *in vitro*. This suggests an attractive mechanism for the ability of kinetochores to track to the end of shrinking microtubules during chromosome segregation(Cheeseman, Brew, Wolyniak, Desai, Anderson, Muster, Yates, Huffaker, Drubin, and Barnes 2001; Westermann et al. 2005; Santaguida and Musacchio 2009). DAM/DASH also promotes microtubule assembly, prevents disassembly, and supports end-on microtubule attachment to centromeres(Westermann et al. 2005; Cheeseman, Enquist-Newman, Müller-Reichert, Drubin, and Barnes 2001). Interestingly, Ask1 also requires intact microtubules to localize to kinetochores and *ask1-2* mutant strains arrest as large budded cells with short spindles and replicated DNA(Y. Li et al. 2002). These results suggest a specific role for this complex in kinetochore attachment to microtubules and chromosome segregation. Since DAM/DASH Complex homologues have not been found in higher eukaryotes, the Ndc80 Complex is likely the more conserved microtubule binding mechanism.

MECHANICS OF CHROMOSOME ATTACHMENT AND SEGREGATION

Yeast experience a 'closed' mitosis where the nuclear envelope remains intact(Heath 1980). The commitment of a yeast cell to division is marked by duplication of the microtubule organizing centre or centrosome – called the spindle-pole body (SPBs) in yeast – and migration of SPBs to opposite ends of the nucleus. The location of the SPBs defines the poles to which chromosomes will segregate at anaphase. Spindle microtubules emanate from the poles with their growing ends (plus ends) directed towards the nuclear centre and minus ends anchored in the SPBs(Mitchison 1989). Direct experimental observation in newt lung cells describes rapid growth and shrinkage of microtubules that allows for the search and capture of chromosomes. The successful contact of a searching microtubule with a

centromere is followed by movement of its chromosome toward the spindle where it originates(Hayden, Bowser, and Rieder 1990; Kirschner and Mitchison 1986).

Chromosomes are initially captured by the plus ends of growing microtubules through attachment of their kinetochore to the lateral side of a single spindle microtubule(Tanaka et al. 2005). Side-on attachments are thought to be converted to more stable end-on attachments that have been observed to promote microtubule elongation(Tanaka et al. 2007). Chromosomes congress at the midpoint between spindle poles due to an equilibrium in 'polar ejection force' created by the pushing forces of polymerizing microtubules from each SPB. The back and forth movement of chromosomes as they alternately form connections to one spindle pole and are pushed back towards the centre by the polar ejection force is referred to as the metaphase 'Tug-of-War'(Rieder and Salmon 1994).

As the cohesion between sister chromosomes is dissolved, releasing the tension of bi-polar attachments, sister chromatids are pulled to the opposite poles. This may occur by the active transport of kinetochores along microtubules by minus-end directed microtubule motor proteins(Tanaka et al. 2005). The observation that the DAM/DASH complex forms rings around microtubules lead to the alternative theory that the DAM ring might carry kinetochores towards the pole by passive diffusion. In this model, fraying plus ends of depolymerizing microtubules exert a pushing force on the DAM/DASH ring(Asbury et al. 2006; Grishchuk, Spiridonov, Volkov, Efremov, Westermann, Drubin, Barnes, Ataullakhanov, and McIntosh 2008; Grishchuk, Efremov, Volkov, Spiridonov, Gudimchuk, Westermann, Drubin, Barnes, McIntosh, and Ataullakhanov 2008). Neither hypothesis occludes the other, so it is possible that both events take place when chromosomes are pulled to the poles.

THE SPINDLE ASSEMBLY CHECKPOINT

In wild type cells, the failure of chromosomes to form proper kinetochore-spindle attachments will cause a delay in the progress of chromosome segregation until proper attachments can form. This cell checkpoint, called the Spindle Assembly Checkpoint (SAC), is a sensitive and robust mechanism for monitoring the proper bi-polar attachment of chromosomes. The presence of even a single unattached kinetochore will activate the SAC(Rieder et al. 1994; X. Li and Nicklas 1995).

The SAC is a signaling pathway comprised of the proteins Mad1(Hardwick and Murray 1995), Mad2(R. H. Chen et al. 1996), Mad3 (BubR1 in mammals)(Hardwick et al. 2000), Bub3(Hoyt, Totis, and Roberts 1991), and the kinases Bub1(Roberts, Farr, and Hoyt 1994), Mps1(Weiss and Winey 1996), and Ipl1(Biggins et al. 1999). The *MAD* (mitotic arrest deficient) and *BUB* (budding uninhibited by benzimidazole) genes were discovered in screens for mutations that prevented the arrest of budding yeast in the presence of drugs that interfere with microtubule polymerization, preventing proper kinetochore-spindle attachments(R. Li and Murray 1991; Hoyt, Totis, and Roberts 1991). The SAC and the proteins involved are evolutionarily conserved from yeast to humans(Musacchio and Salmon 2007).

The checkpoint proteins physically interact with one another to form complexes, they localize to unattached kinetochores, and dissociate from kinetochores as proper attachments are made to the spindle(Brady and Hardwick 2000; Gillett 2004; R. H. Chen et al. 1999). Current models propose that the SAC functions in the following way (**see Figure 4**): Bub1 is associated with kinetochore early in the cell cycle and is thought to function as a scaffold for SAC proteins. Upon SAC activation, Bub1 dimerizes with Bub3 and recruits it to kinetochores. Mad1 also localizes to unattached kinetochores and forms a complex with

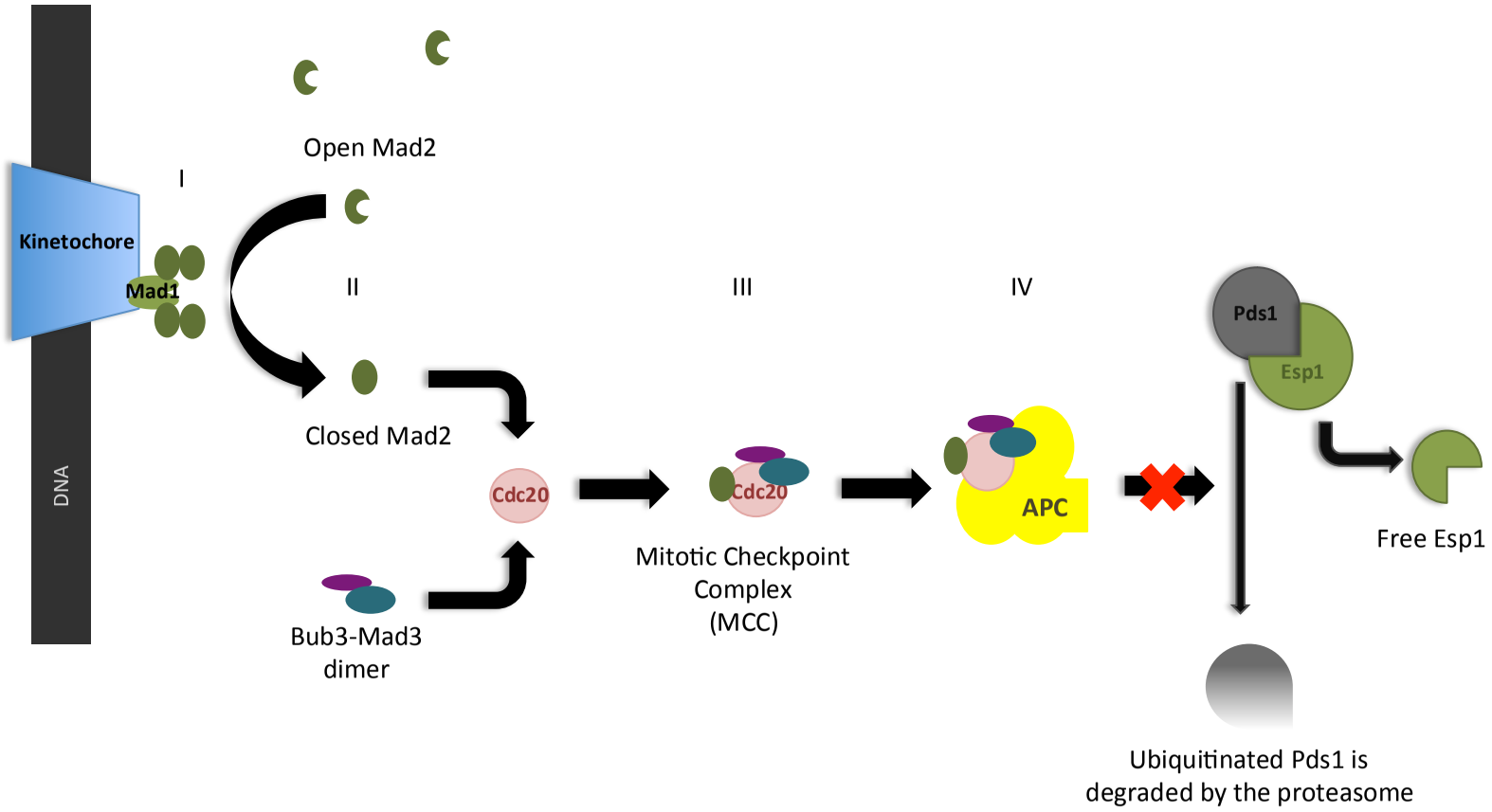

Open Mad2
I
Kinetochore
Mad1
II
III
IV
Pds1
Esp1
DNA
Closed Mad2
Cdc20
Cdc20
APC
Free Esp1
Mitotic Checkpoint
Complex
(MCC)
Bub3-Mad3
dimer
Ubiquitinated Pds1 is
degraded by the proteasome

Figure 4. The Spindle Assembly Checkpoint
Dimerized Mad1 is localized to unattached kinetochores when the Spindle Assembly Checkpoint (SAC) is activated (I). Soluble Mad2 exists in an 'open' configuration. Open-Mad2 binds to 'closed' Mad2 in complex with Mad1 at the kinetochore, causing a conformational change from open to closed (II). Closed-Mad2 dissociates from unattached kinetochores, and together with Mad3 and Bub3, binds Cdc20 to form the Mitotic Checkpoint Complex (MCC) (III). The MCC binds to the APC and occludes substrates, like Pds1, from the APC active site, thus preventing degradation of mitotic substrates and halting progression into anaphase. In the case of Pds1, preventing its degradation keeps Esp1 – the protease responsible for dissolving the physical linkages between sister chromatids - sequestered and inactive (IV).

Bub1 and Bub3 that is required for checkpoint function(Brady and Hardwick 2000). Mad1 dimerizes with Mad2 and the recruitment of Mad2 to unattached kinetochores is dependent on Mad1(R. H. Chen et al. 1999). Thus the assembly of the SAC proteins at unattached kinetochores is a signaling cascade dependent on the upstream functions of Bub1 and Mad1.

The "Templating Model" proposes that Mad2 is the soluble messenger that amplifies the signal from unattached kinetochores and transfer it to the target of the SAC, the APC^{Cdc20}(Mapelli and Musacchio 2007; Visintin, Prinz, and Amon 1997; Hwang 1998; Kim 1998). Soluble Mad2 exists in an 'open' and a 'closed' conformation(Luo et al. 2004). A pool of 'open' Mad2 binds to the 'closed' Mad2 that is in complex with Mad1 at the kinetochore, causing a conformational change from open Mad2 to closed Mad2(De Antoni et al. 2005). Closed Mad2 dissociates from unattached kinetochores, and together with Mad3 and Bub3, binds Cdc20 to form the Mitotic Checkpoint Complex (MCC)(Sudakin 2001; Tipton et al. 2011; Kulukian, Han, and Cleveland 2009).

The MCC translates the signal from unattached kinetochores to stall the cell cycle machinery. The MCC binds to the APC and occludes substrates, like Pds1, from the APC active site, thus preventing degradation of mitotic substrates and halting progression into anaphase. A recent crystal structure of the MCC shows that the Mad3 KEN box domain serves as pseudo substrate inhibitor, blocking access of Cdc20 to KEN box degrons in APC substrates(Chao et al. 2012).

In yeast, an observed physical separation between centromere regions of cohered sister chromosomes is thought to be caused by tension forces created by the centromeres being pulled toward opposite spindle poles when bi-polar attachments are formed(Goshima and Yanagida 2000). Tension has been shown to stabilize kinetochore-microtubule

attachments, reinforcing formation of proper bi-polar attachments(Akiyoshi et al. 2010; Nicklas and Koch 1969). Therefore tension is a likely mechanism by which the cell senses proper bi-polar attachments as opposed to improper ones where both sisters can attach to microtubules emanating from the same spindle pole body. In addition to unattached kinetochores, the SAC also senses these improper tensionless attachments(Skibbens, Skeen, and Salmon 1993; Stern and Murray 2001). Ipl1, the budding yeast homologue of the mammalian Aurora B kinase, is thought to be the molecule responsible for sensing and correcting tensionless attachments(Biggins and Murray 2001). The mechanism is thought to function by removing improper attachments, activating the SAC and creating time for bi-polar connections to form(Pinsky et al. 2006).

SAC protein localization to, and retention at, kinetochores has been shown to depend on each other and on the Mps1, Bub1 and Ipl1 kinases(Brady and Hardwick 2000; Rischitor, May, and Hardwick 2007; Weiss and Winey 1996; Palframan et al. 2006), providing more clues as to the mechanism of checkpoint function. Mad1 is highly phosphorylated upon SAC activation, and this is thought to be due to the kinase activity of Mps1(Hardwick and Murray 1995; Hardwick et al. 1996). In addition, Mps1 has been implicated in Mad2 phosphorylation which regulates incorporation of Mad2 into the MCC(Zich et al. 2012). Mps1 is also an upstream regulator of the checkpoint, as its overexpression results in a checkpoint arrest. Removing downstream signaling proteins like Mad2 allows cells to bypass the Mps1 mediated arrest, indicating that the arrest induced by Mps1 overexpression is dependent on a functioning checkpoint. Cells overexpressing Mps1 in a *mad2Δ* background segregate chromosomes normally, demonstrating that overexpressing Mps1 induces inappropriate SAC activation(Hardwick et al. 1996). Hyper-activation of Bub1 kinase activity in budding yeast has also been shown to activate the SAC although it is unclear what

proteins are important substrates(Farr and Hoyt 1998). The role of Ipl1 seems to be mainly part of the tension correction mechanism described above.

QUENCHING THE SPINDLE ASSEMBLY CHECKPOINT SIGNAL

The Spindle Assembly Checkpoint inhibits the APC^{Cdc20} when chromosomes are not properly attached to the spindle, arresting cells in mitosis (prior to anaphase, or at pro-metaphase) and allowing correction mechanisms to ensure chromosomes are bi-oriented before segregation. Once proper attachments are made, the checkpoint signal is turned off and inhibition of APC^{Cdc20} is relieved(Musacchio and Salmon 2007).

Since simple diffusion off of the kinetochore of checkpoint proteins like Bub1 and Mad1 is slow and may not be sufficient to quench the SAC, active stripping of these proteins off of the kinetochore may be required to effectively turn off the signal. Dyneins are motor proteins associated with the outer kinetochore. In mammalian Ptk1 cells, disrupting dynein function causes retention of Mad2 and BubR1 (Mad3) at kinetochores and induces a metaphase arrest even though proper chromosome attachments seem to be made(Howell et al. 2001). This suggests that dyneins could have a function in removing checkpoint proteins from kinetochores and facilitate the silencing of kinetochore signals.

Another model of checkpoint quenching is based on conformational changes that occur in kinetochores when they are under tension of bi-polar attachments. Kinetochores under tension have been shown to stretch by almost 25 nm(Joglekar, Bloom, and Salmon 2009). This could conceivably pull outer kinetochore proteins away from a checkpoint maintenance signal concentrated at the inner kinetochore or make them accessible to silencing molecules. A phosphatase is an attractive candidate as a silencing molecule for its ability to reverse phosphorylation events required to activate the checkpoint. $PP1^{Dis2}$ phosphatase activity has

been shown to help reverse Aurora- and Mps1-dependent phosphorylation at kinetochores and contribute to checkpoint silencing(VANOOSTHUYSE and Hardwick 2009; Pinsky, Nelson, and Biggins 2009). Fin1 is also a PP1 regulatory subunit that helps localize PP1 to kinetochores. Constitutive targeting of PP1 to kinetochores by Fin1 results in checkpoint silencing and increased chromosome segregation defects(Akiyoshi et al. 2009). Thus the targeting of PP1 by Fin1 to kinetochores may oppose SAC activation by removing activating phosphorylation marks at the kinetochore. Further evidence is emerging that Cdc20 itself is also regulated by phosphorylation in mitosis. Phosphorylation of Cdc20 makes it more sensitive to SAC inhibition and then its dephosphorylation is required to activate it again(Chung and Chen 2003; Tang et al. 2004; Labit et al. 2012).

Recently, the characterization of the APC subunit Mnd2/Apc15 has provided insights on how the APC is released from the inhibition of the MCC(Foster and Morgan 2012; Uzunova et al. 2012; Mansfeld et al. 2011). The MCC prevents ubiquitination of Pds1 (securin) but increases ubiquitination of Cdc20. Mnd2/Apc15 catalyzes MCC turnover at the APC by contributing to Cdc20 auto-ubiquitination and subsequent destruction. Mnd2/Apc15 is not an essential subunit of the APC – when Mnd2 is deleted in yeast, both Cdc20 and Pds1 are ubiquitinated at wild type levels. However, *mnd2Δ* cells have a defect in Cdc20 ubiquitination *in vitro* when the proteins of the MCC are present and are slower to recover from a SAC arrest *in vivo*(Foster and Morgan 2012). Thus, it is becoming clear that APC mediated proteolysis of an MCC component contributes to turning the SAC off after chromosomes are properly attached to the mitotic spindle.

IMPORTANCE OF MAD1 AND NDC80

Despite advances in our understanding of the mechanism underlying the Spindle Assembly Checkpoint, it is still poorly understood how checkpoint proteins localize to kinetochores, where they bind on kinetochores, and what is the mechanism by which checkpoint proteins help sense attachment.

Mad1 is an integral part of the SAC signaling pathway. Mad1 is highly phosphorylated when the checkpoint is activated, but not in cells arrested in G1 or cells lacking Bub1, Mad2, or Bub3 genes(Hardwick and Murray 1995). The functional consequence of Mad1 phosphorylation is not known although it seems to happen early in SAC signaling. Artificially tethering Mad1 to centromeres by fusing it to Mis12, a member of the central kinetochore, causes localization of other SAC proteins and a mitotic arrest(Maldonado and Kapoor 2011). This study provided further evidence of the importance of Mad1 localization to kinetochores as an upstream SAC signaling event. Overexpressing Mad1 causes cells to bypass a SAC arrest, potentially by titrating Mad2 away from unattached kinetochores(Kim et al. 2012). Although we know that Bub1 and Mad1 are upstream signaling components that localize to the kinetochore and are released as microtubule attachments are made, we do not yet know where they bind or why they dissociate when proper attachments are made.

The Ndc80 complex is an attractive candidate as a docking site for checkpoint proteins. It is an outer linker complex that binds microtubules in vitro and can track disassembling tips. It is therefore the primary centromere protein complex responsible for forming kinetochore-microtubule attachments. Ndc80 is the yeast homologue of a human protein that is highly expressed in cancer (Hec1), and so dis-regulation of Ndc80 protein levels is associated with a disease that displays increased chromosome segregation

defects(Zheng, Chen, and Lee 1999; Y. Chen et al. 1997). Ndc80 complex integrity is important for checkpoint control and has suggested that it might also play a role in recruiting Mad1 and Bub1 proteins to kinetochores(Janke et al. 2001; McCleland et al. 2003; Gillett 2004). Depletion of Ndc80 in human cells diminished Mad1 kinetochore localization but not proper formation of kinetochores(Martin-Lluesma, Stucke, and Nigg 2002; Kline et al. 2006; DeLuca et al. 2003). Other experiments implicate phosphorylation as a regulator of checkpoint protein recruitment to kinetochores in *S. cerevisiae*. For example, non-phosphorylatable Spc105 mutants fail to recruit Bub1 to the kinetochore and are checkpoint defective(London et al. 2012). Mps1 can phosphorylate Ndc80 *in vitro*. Non-phosphorylatable mutants of Ndc80 are also checkpoint defective and phospho-mimetic mutants constitutively activate the SAC(Kemmler et al. 2009). This suggests that Mps1 phosphorylation of Ndc80 could be directly involved in the activation of the SAC signal, although how this contributes to signaling is unclear.

GENERAL HYPOTHESIS

Genetic damage has long been associated with development of malignant tumours(Perez de Castro, de Carcer, and Malumbres 2006; Bakhoum and Compton 2012). Chromosome instability (the loss or gain of chromosomes or aneuploidy) has been shown in many forms of cancer and is associated with mutations in Spindle Assembly Checkpoint (SAC) genes(Cahill et al. 1998). Normal functioning of the SAC, therefore, likely contributes to suppressing tumour formation by protecting against chromosome instability. Understanding the mechanisms by which the SAC ensures fidelity of chromosomes segregation could reveal therapeutic targets where this mechanism is disrupted.

SAC proteins relay the signal to the APC that kinetochores have achieved bi-polar attachments on the mitotic spindle. In order to monitor proper attachments, some SAC proteins might bind to the kinetochore at or close to the microtubule binding sites. Mad1 is an upstream signaling protein of the SAC and requires Ndc80 for its localization to kinetochores. Since the Ndc80 Complex is involved in kinetochore binding to microtubules, required for Mad1 localization, and functioning components of this complex seem to be required for checkpoint arrest, a direct interaction between Mad1 and Ndc80 may be a mechanism for sensing kinetochore-microtubule attachments.

SPECIFIC HYPOTHESIS

We propose that the Ndc80 complex acts as a scaffold for the association of Mad1 with the kinetochore, allowing them to sense unattached chromosomes and trigger a signaling cascade to arrest the cell cycle prior to anaphase onset.

STATEMENT OF OBJECTIVES

1) To identify kinetochore protein(s) that interact with SAC proteins by protein purification and mass spectrometric analysis.
2) To identify, validate, and characterize protein interactions that could be involved in SAC signaling.

Chapter 2: Material and Methods

Strain construction

Yeast strains used in this study are listed in Table 1. All strains are derivatives of the W303 strain background (W303-1a) and all manipulations were done in a ADR4006 strain background. Epitope tag integrations were made by PCR (polymerase chain reaction) amplification off plasmid from standard libraries (EUROSCARF) followed by standard gene integration technique as described (Janke et al. 2004). All deletions and replacements were confirmed by immunoblotting or PCR. Standard genetic techniques were used to manipulate yeast strains (Sherman, Fink, and Lawrence). Briefly, haploid strains with opposite mating type (MATa and MATα) were allowed to mate on nutrient rich media. Successfully mated diploids were sporulated on nutrient depleted media, and haploid spores were segregated and allowed to grow on media rich plates (yeast extract peptone agar + 2% dextrose). Replica plating onto selective media allowed for selection of a haploid strain with the desired genotype. The bacterial strains TG1 and DH5α were used for amplification of DNA.

TAP-tagged strains were made by PCR amplification of the TAP tag off genomic DNA from the yeast TAP collection (Thermo Scientific) and gene specific primers. PCR product was integrated into ADR4006. *NDC80-myc9x-KANr* strains were created using PCR-targeted recombination using pYM18 (EUROSCARF) and gene specific primers. Full length *NDC80, SPC24, SPC25, NUF2* genes were amplified from genomic DNA and cloned into pAR123 or pAR63 as Xho1/NotI or SalI/BamHI fragments respectively. pAR123 and pAR63 is pRS305 and pDK20 respectively with the *GAL1-10* promoter cloned between Kpn1/Xho1. *MAD2* was disrupted by transforming HindIII and XhoI digested PRC10.1 (gift from Andrew Murray, UCSF, San Francisco, CA) at the genetic locus.

Kinetochore mutant strains were made by crossing SBY164, SBY7289, SBY1119, SBY2499, SBY1439 (gifts from Sue Biggins, Fred Hutchinson Cancer Research Center, Seattle, WA) to the appropriate strains.

Cell growth assays

Strains were grown in nutrient rich media at 25°C for two days. A 48 pin frogger (Dan-Kar Corp. New Boston, MA) was used to spot 10-fold dilutions onto media plates. Plates were grown for two days at 25 °C and photographed using epi-white imaging on the ImageQuant LAS4000 (GE Healthcare Bio-sciences). Cell growth assay experiments were repeated in triplicate using different isolates of each strain.

Analysis of mitotic substrates in cycling cells

Cells were grown in yeast extract peptone + 2% raffinose (to A600 ~0.5 – 1.0). 1 µg/mL α-factor was added to arrest cells and after 2.5 hrs, 2% galactose was added for 30 min to activate expression of the gene under control of the GAL promoter. Cells were then washed three times in YEP and released from arrest into the experimental condition. 3 x 10^7 cells were harvested from cultures at 30 min intervals and flash frozen in liquid nitrogen. α-factor was added back after 60 min to re-arrest cells after one cell cycle. Frozen samples were lysed in 250 µl 1X loading buffer (80 mM Tris-Cl, pH 6.8, 2% SDS, 0.1% bromphenol blue, 10% glycerol, 10mM EDTA, 5% BME, 1mM PMSF) and proteins were separated on a 12.5% denaturing polyacrylamide gel. Protein levels were assessed by immunoblotting.

Immunoblotting

These methods have been described previously (Rudner, Hardwick, and Murray 2000; Rudner and Murray 2000).

The following antibodies were used for Western blots: Rabbit polyclonal α-Cdk1, α-Pds1, α-Clb5, α-Mcd1, were used at 1:1000, in TBS-T with 4% Fat Free Milk Powder, 5%

glycerol, 0.02% NaN_3. An autoclaved solution of 5% milk was used to make the 4% milk dilution buffer to increase the longevity of the antibody solution. Membranes were pre-blocked with TBS-T with 4% Fat Free Milk Powder, 5% glycerol before incubation with all primary antibodies.

α-Pds1, α-Clb5, α-Mcd1 antibodies were generated as follows. Coding sequences for the truncated proteins $Clb5_{2\text{-}137}$, $Pds1_{178\text{-}373}$ and $Mcd1_{201\text{-}301}$ were amplified using PCR and cloned into pGEX6P-1 (Promega) as BamH1/EcoR1 fragments to create pAR627, pAR624 and pAR742 respectively. 1mg of each GST fusion protein was injected into rabbits every 4 weeks for 8 to 16 weeks (uOttawa animal facility). Rabbit serum was harvested, clarified by centrifugation and loaded on Affigel-10 (Bio-rad) columns coupled to purified malE- $Clb5_{2\text{-}137}$, malE- $Pds1_{178\text{-}373}$ or $Mcd1_{201\text{-}301}$ respectively. malE-fusion proteins were expressed from the plasmids pAR651, pAR652 and pAR1117, which contain the same fragments listed above cloned as BamH1/Sal1 fragments into pMAL-c2 (NEB). Antibody was eluted from Affigel columns with either 100mM triethylamine pH 11.5 or 100mM glycine pH 2.3. The triethylamine and glycine elutions were neutralized, dialyzed in PBS + 50% glycerol and stored at -80°C.

Preparation of IgG dynabeads

M270 epoxy dynabeads (Invitrogen) were resuspended in dry DMF at a concentration of $2x10^9$ beads/mL. Using a magnet, DMF was removed and beads were washed in 0.1M sodium phosphate buffer pH 7.4 in a volume such that beads were at $1x10^9$ beads/mL. Beads were vortexed, washed again, and incubated at 25˚C for 10 min with end-over-end rotation. Beads were resuspended in sodium phosphate buffer at $3x10^9$ beads/mL, vortexed, and an equal volume of 1mg/mL IgG (Sigma-Aldrich) was added. Beads were vortexed again, and equal volume of 3M ammonium sulfate was added. IgG is made fresh in 0.1M sodium

phosphate buffer. Incubate beads 16hr at 37˚C with end-over-end rotation. The following morning, one quick wash was done with equal volume sodium phosphate buffer at 25˚C, followed by two washes with 10 min incubations. Beads were then washed in sodium phosphate buffer with 1% Triton-X-100 for 10 min at 37˚C, with rotation. Again one quick wash was done with an equal volume sodium phosphate buffer at 25˚C, followed by two washes with 10 min incubations. To remove loosely associated IgG, beads were washed four times in 0.1M citric acid, pH 3.1 (at a volume of $1x10^8$ beads/mL). Beads were again washed quickly once with equal volume sodium phosphate buffer followed by three washes with 10 min incubations. Beads were stored at a final concentration of $1x10^9$ beads/mL.

Physiology

Cells were grown in 400 mL of yeast extract peptone + 2% dextrose (YPD) at 30°C to mid-log phase (A_{600} ~0.6– 0.8). Cultures were spun down, split, and resuspended in 400 ml YPD + 30 μg/mL Benomyl or YPD + 0.001% DMSO. Cells were grown at 25°C and were harvested when YPD + Ben cultures were >95% arrest as large budded cells (approximately 3 hrs).

Kinetochore mutant strains were grown in YPD at 25°C to mid-log phase (A_{600} ~0.6– 0.8). Cultures were arrested with 25-100ng/mL α-factor (Biosynthesis) for 3 hrs before being washed three times in YPD before release into YPD + 0.001% DMSO at 37°C or YPD + 30 μg/mL Benomyl at 25°C for 3 hrs.

Protein purifications

Cell pellets (from above) were thawed in equal volume 1.5X lysis buffer (100 mM HEPES, pH 8.5, 10% glycerol, 10 mM EGTA, 0.1 mM EDTA, 0.4% NP-40, 2 mM $Mg(OAc)_2$, 75 mM NaOAc, 150 mM NaF, 150 mM Na-β-glycerophosphate pH 8.3, 1 mM DTT, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM benzamidine, and leupeptin, bestatin, pepstatin A and

chymostatin all at 1 mM (Sigma), split into 1.5 ml screw cap tubes with an equal volume of glass beads (BioSpec Products, 11079105), and lysed by bead beating (Mini Beadbeater, Biospec) for 25 s. Holes were poked in the bottom of tubes and centrifuged briefly at 3000 rpm at 4°C (Sorvall RC6 Plus,Thermo Electric Corporation) to collect whole-cell extract. Pooled lysate was clarified by centrifugation at full speed on a desktop centrifuge at 4°C. Samples were normalized by protein concentration and incubated end-over-end for 1 h at 4°C with magnetic Dynabeads (Dyna, Invitrogen, 143.02D) coupled to rabbit immunoglobulin G (IgG) (Sigma, I5006-10MG) or for 2 h with magnetic protein A Dynabeads (Invitrogen, 10001D) bound to mouse 9E10 monoclonal antibody (Babco). Dynabeads were bound to a magnet, washed three times with 1 ml of cold wash buffer, and resuspended in 50 µl of 1X loading buffer (80 mM Tris-Cl, pH 6.8, 2% SDS, 0.1% bromphenol blue, 10% glycerol, 10mM EDTA, 5% BME, 1mM PMSF). Bound proteins were eluted from the beads at 65°C for 10 min and transferred to a new tube. Immunoprecipitates were subjected to SDS-PAGE and Western blotting. Primary antibodies used are as follows: anti-Myc 9E10 (Babco)(Kari et al. 1986), and anti-Mad1p (Hardwick and Murray 1995). Secondary antibodies were HRP linked, goat anti-rabbit IgG (Bio-Rad, 170-6515 1:5000), and goat anti-mouse IgG (Bio-Rad, 170–6516, 1:5000).

Mass spectrometry analysis

Cells were grown in 400 mL of yeast extract peptone + 2% dextrose (YPD) at 30°C to mid-log phase (A_{600} ~0.6– 0.8). Cultures were spun down, split, and resuspended in 400 ml YPD + 30 µg/mL Benomyl or YPD + 0.001% DMSO. Cells were grown at 25°C and were harvested when YPD + Ben cultures were >95% arrest as large budded cells (approximately 3 hrs). Protein purification from pellets was performed as described above. Whole protein purifications were subject to mass spectrometric analysis. Mass spectrometry was performed

by Arminja N. Kettenbach in the laboratory of Dr. Scott Gerber at the Norris Cotton Cancer Centre, NH, USA. Each purification was analyzed by nanoscale microcapillary LC-MS/MS essentially as described (Kettenbach et al. 2012)on a LTQ-Orbitrap (Thermo Electron). "LC-MS/MS analysis was performed on a LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an Agilent 1100 HPLC and LC-Packings FAMOS auto sampler. Peptides were redissolved in 6% ACN/1% formic acid and loaded onto an in-house pulled and packed fused silica column (18 cm length, 100 mm inner diameter, ReproSil, C18 AQ 3 mm). The peptides were eluted with a 50 min gradient of 0%–29% B solvent (Buffer A: 0.0625% FA, 3% ACN; Buffer B: 0.0625% FA, 95% ACN). Peptide precursor ions were measured in the Orbitrap at a resolution of 60,000, and fragmentation spectra were collected in the LTQ. Up to ten of the most intense peptides were selected in each MS scan for MS/MS fragmentation. Raw data were searched using SEQUEST".

Chapter 3: Results

Purification of SAC proteins suggests an association with the outer kinetochore

To address if the yeast SAC proteins assemble into an MCC complex and how interactions between the proteins change when the checkpoint is activated, I created strains where endogenous checkpoint genes were C-terminally tagged with a TAP peptide so that they could be purified from cell extracts along with any associated proteins. Since the tagged checkpoint genes are the only copy of the gene in the cell, I tested if the tag interferes with the wild type function of the genes by examining their sensitivity to the microtubule destabilizing drug benomyl (**Figure 5a**). Unlike loss of function mutations in *MAD* or *BUB* genes, the tagged checkpoint genes do not exhibit a sensitivity to spindle disruption. In order to preserve delicate interactions that might be disrupted under high salt lysis conditions, TAP-tagged proteins and associated complexes were isolated from whole cell lysates on IgG-coated magnetic Dynabeads using a low salt buffer. Magnetic Dynabeads are simple to manipulate and can be collected and concentrated easily, allowing for quick an efficient purification of proteins compared to sepharose columns. Purified samples were subjected to trypsin digestion and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Raw data were searched against the SEQUEST protein sequence database and SAC proteins were detected in both benomyl treated and untreated samples. Results are summarized in **Table 3.** Similar to what has been found in vertebrate cells, all members of the MCC (Mad2, Mad3, Bub3, and Cdc20) and some APC components were detected only in samples where the SAC was activated by treatment with benomyl.

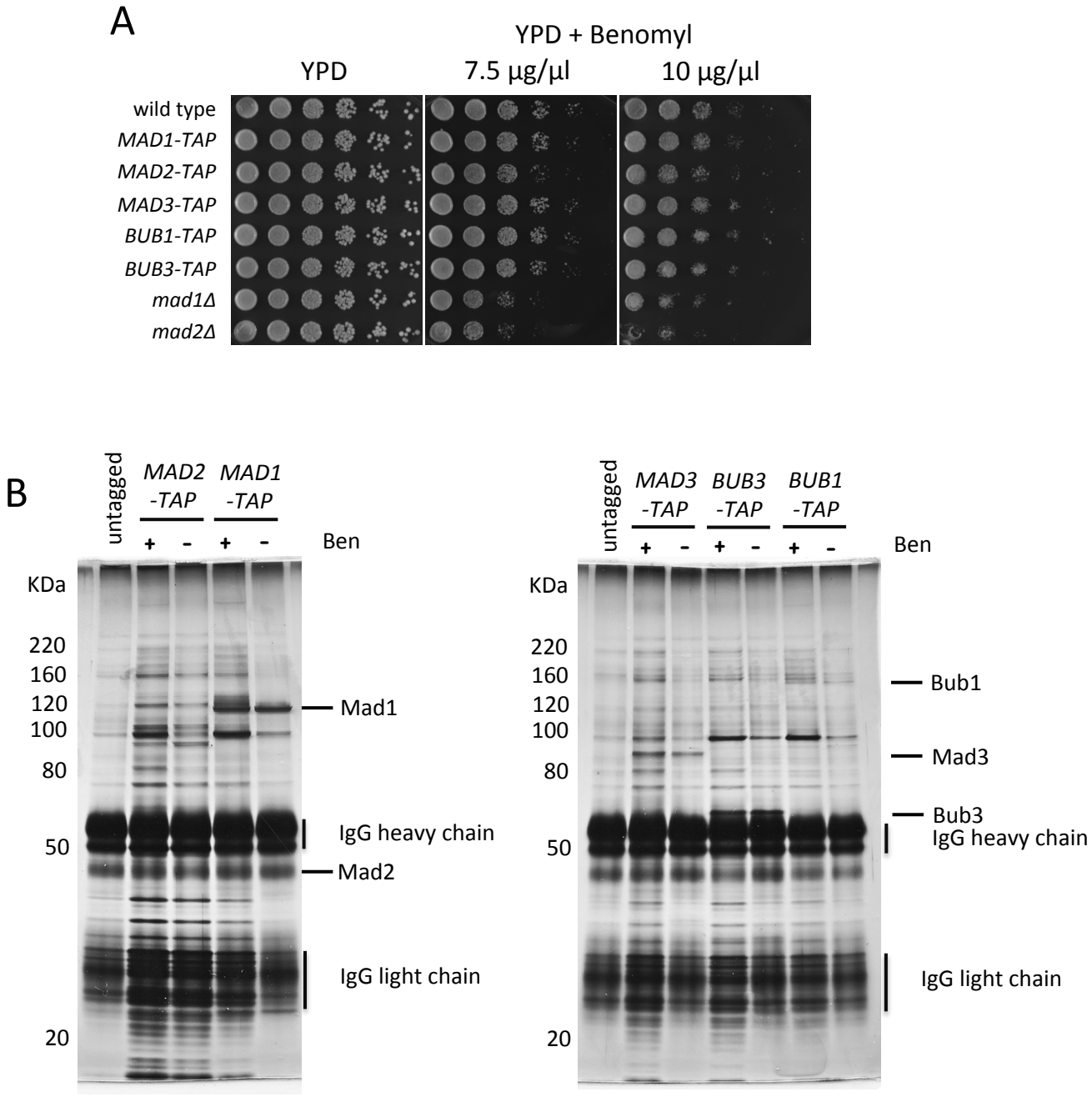

A
YPD
YPD + Benomyl
7.5 µg/µl
10 µg/µl
wild type
MAD1-TAP
MAD2-TAP
MAD3-TAP
BUB1-TAP
BUB3-TAP
mad1Δ
mad2Δ
B
untagged
MAD2 -TAP
MAD1 -TAP
+ - + -
Ben
KDa
220
160
120
100
80
50
20
Mad1
IgG heavy chain
Mad2
IgG light chain
untagged
MAD3 -TAP
BUB3 -TAP
BUB1 -TAP
+ - + - + -
Ben
KDa
220
160
120
100
80
50
20
Bub1
Mad3
Bub3
IgG heavy chain
IgG light chain

Figure 5. Purification of Checkpoint Complexes
(**A**) Tagging SAC proteins does not interfere with their function. Serial dilutions of cells with TAP-tagged SAC proteins were tested for growth at 25°C on YPD plates containing the indicated concentrations of benomyl. (**B**) TAP-tagged SAC proteins were purified from cells arrested in mitosis with an active SAC by treatment with benomyl or from cycling cells using IgG conjugated magnetic Dynabeads. Proteins were purified in mild conditions using a lysis buffer containing 75 mM NaOAc in order to preserve delicate interactions. Purifications eluted with SDS sample buffer and visualized by silver stain on a 10-20% polyacrylamide gel. Purified complexes were analyzed by LC-MS/MS mass spectrometry to identify components (see Table 3).

Table 3. Mass spectrometric analysis of purified checkpoint complexes

	Mad1	Mad1	Mad2	Mad2	Mad3	Mad3	Bub1	Bub1	Bub3	Bub3
Benomyl	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
MCC	Mad1 *(56/69) Mad2 (16/17) Bub1 (3/3) Bub3 (1/1)	Mad1 (50/67) Mad2 (12/14) Bub1 (32/34) Bub3 (11/12)	Mad1 (47/58) Mad2 (12/13) Bub3 (1/1) Cdc20 (2/2)	Mad1 (46/52) Mad2 (11/13) Mad3 (9/9) Bub1 (15/15) Bub3 (9/9) Cdc20 (15/19)	Mad1 (13/14) Mad2 (3/3) Mad3 (17/19) Bub3 (10/10)	Mad2 (5/5) Mad3 (16/17) Bub3 (9/10) Cdc20 (15/17)	Mad1 (5/5) Bub1 (25/26) Bub3 (7/7)	Mad1 (31/31) Mad2 (3/3) Bub1 (42/46) Bub3 (12/12)	Mad3 (15/16) Bub1 (20/20) Bub3 (12/16) Cdc20 (1/1)	Mad1 (29/30) Mad2 (8/8) Mad3 (15/16) Bub1 (25/26) Bub3 (12/16) Cdc20 (14/16)
APC				Cdc16 (6/6) Cdc23 (2/2) Cdc27 (1/1) Apc1 3(/3)		Cdc16 (7/7) Cdc23 (3/3) Cdc27 (1/1) Apc1 (3/3) Apc5 (1/1)				Cdc16 (4/4) Cdc27 (1/1) Apc1 (1/1) Apc5 (1/1)
Kinetochore		Ndc80 (6/6) Spc24 (1/1) Spc105 (5/5) Mtw1 (2/2) Dsn1 (1/1) Nnf1 (1/1)						Ndc80 (13/13) Spc25 (1/1) Nuf2 (2/2) Spc105 (7/7) Mtw1 (2/2) Dsn1 (1/1) Nnf1 (2/2) Mif2 (1/1)		

Interestingly, components of the outer kinetochore Ndc80 complex and some inner kinetochore proteins were also detected in Mad1 and Bub1 purified samples where the SAC was activated. These proteins were not detected in Mad1 or Bub1 samples purified from cycling cells.

Overexpressing Ndc80 elicits a SAC-mediated cell cycle arrest

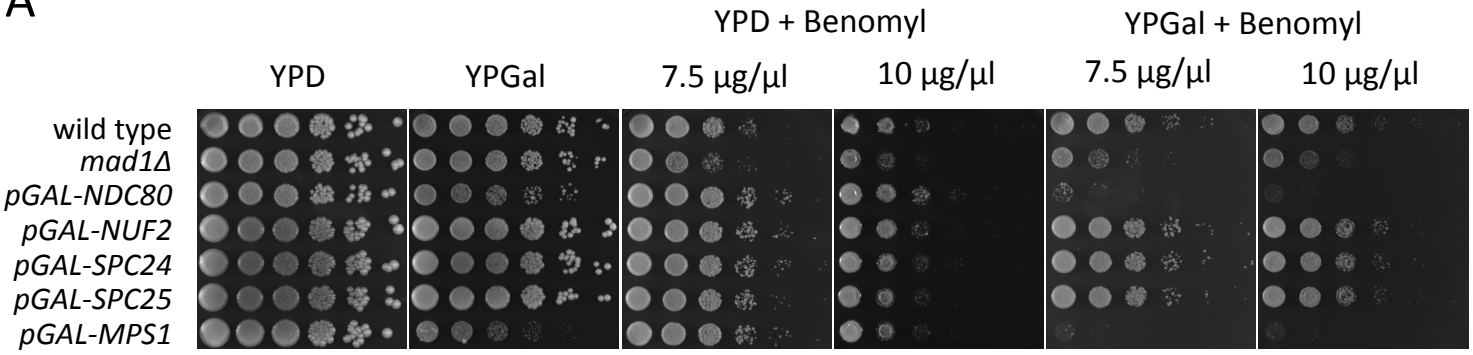
The SAC signal originates from kinetochores that are either unattached to spindle microtubules or from kinetochores whose attachments lack tension(Stern and Murray 2001; Biggins and Murray 2001; R. Li and Murray 1991; Nicklas and Koch 1969; Rieder et al. 1994). The kinetochore proteins that SAC proteins directly bind to have not been isolated, and my MS data suggested that Mad1 and Bub1 may interact directly with the Ndc80 complex, which assembles at the outer kinetochore and plays a role in microtubule binding (Powers et al. 2009). The detection of kinetochore proteins in samples of purified Mad1 or Bub1 from cells with an activated checkpoint suggested that the Ndc80 Complex could be an important docking site for the upstream SAC signaling proteins Bub1 and Mad1. I wondered if an excess of these outer kinetochore proteins could monopolize any free Mad1 or Bub1 molecules, effectively titrating the signal away from unattached kinetochores and interrupting the SAC signaling cascade, causing cells to bypass an activated checkpoint.

To test this hypothesis, an extra copy of each of the Ndc80 Complex protein genes (*NDC80*, *NUF2*, *SPC24*, *SPC25*) under control of the *GAL1* promoter were integrated into wild type strains at the *URA3* gene locus. In normal growth conditions the *GAL* promoter is repressed, but the cells still express the endogenous proteins. Adding 2% galactose to the growth media induces overexpression of the protein.

Only the strain harboring a copy of *NDC80* under control of the *GAL1* promoter showed a modest decrease in growth, in the presence of galactose. When overexpression of the protein was simultaneously induced with SAC activation by the presence of both galactose and benomyl, only the overexpression of Ndc80 showed a significant growth defect compared to the other strains (**Figure 6a**). This data suggested that Ndc80 overexpression might indeed be interrupting SAC signaling under conditions where it is required and causing cell death, and Ndc80 might provide an important docking site for Mad1 at the kinetochore.

The APC is the target of the SAC, and its mitotic substrates are stabilized during SAC arrest (Hwang 1998; Kim 1998; Minshull et al. 1996). Checkpoint-deficient cells cannot inhibit the APC, and prematurely exit mitosis and degrade APC substrates. To examine SAC function in cells overexpressing *NDC80* more closely, APC substrate levels were monitored in cells synchronized in G1 and released from this arrest into media containing benomyl and galactose. Mcd1 and Pds1, two proteins that are degraded when cells exit mitosis (Cohen-Fix et al. 1996; Guacci, Koshland, and Strunnikov 1997; Michaelis, Ciosk, and Nasmyth 1997), were stabilized after overexpression of *NDC80* suggesting that high levels of Ndc80 does not disrupt SAC signaling, in contrast to a strain where *MAD2* was deleted (**Figure 6b**). In fact, *NDC80* overexpression in the absence of checkpoint activation stabilizes mitotic APC substrates suggesting that this overexpression may cause a checkpoint arrest. The stabilization of mitotic substrates after *NDC80* overexpression depends on *MAD2*, confirming that overexpression of *NDC80* triggers a SAC arrest (**Figure 7a**).

A


YPD
YPGal
YPD + Benomyl
7.5 µg/µl
10 µg/µl
YPGal + Benomyl
7.5 µg/µl
10 µg/µl
wild type
mad1Δ
pGAL-NDC80
pGAL-NUF2
pGAL-SPC24
pGAL-SPC25
pGAL-MPS1

B

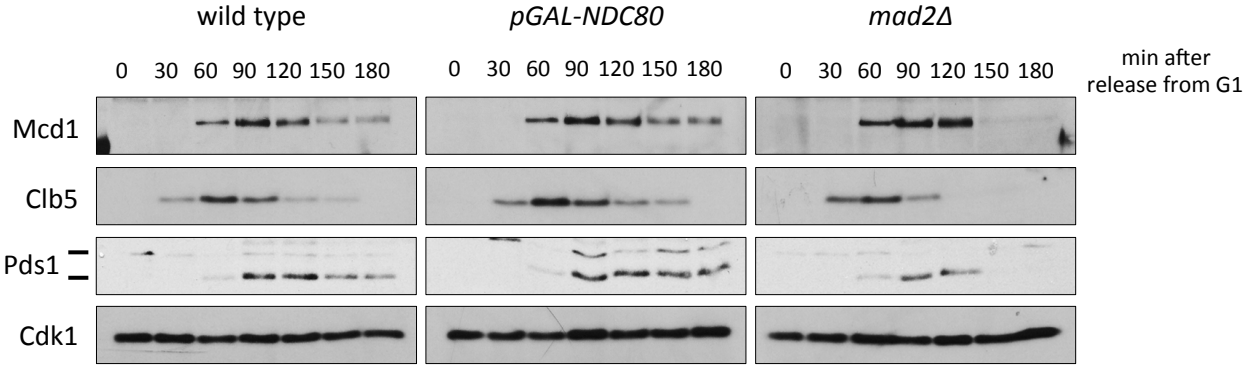
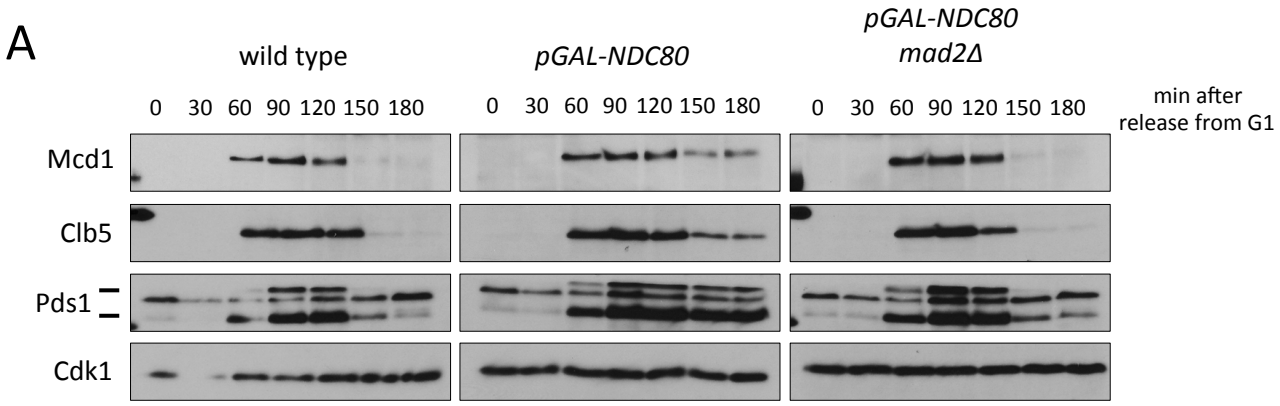

wild type
pGAL-NDC80
mad2Δ
0 30 60 90 120 150 180
0 30 60 90 120 150 180
0 30 60 90 120 150 180
min after release from G1
Mcd1
Clb5
Pds1
Cdk1

Figure 6. Effect of overexpressing Ndc80 complex proteins
(**A**) Overexpressing Ndc80 causes sensitivity to microtubule inhibitors. Serial dilutions of strains harbouring an integrated plasmid with a kinetochore protein under the control of the *GAL1* promoter were tested for growth at 25°C on YPD (2% glucose), YPGal (2% galactose), or YPD/YPGal (containing the indicated concentrations of benomyl) plates for growth. (**B**) Overexpressing Ndc80 does not bypass the SAC. Cultures were synchronized in G1 phase with α-factor for 3 hrs. Cultures were released into YPGal + benomyl at 25°C and samples for immunoblotting were harvested at the given timepoints. α-factor was re-added at 60 minutes to re-arrest the cells in the subsequent G1.


A
wild type
pGAL-NDC80
pGAL-NDC80
mad2Δ
0 30 60 90 120 150 180
0 30 60 90 120 150 180
0 30 60 90 120 150 180
min after
release from G1
Mcd1
Clb5
Pds1
Cdk1

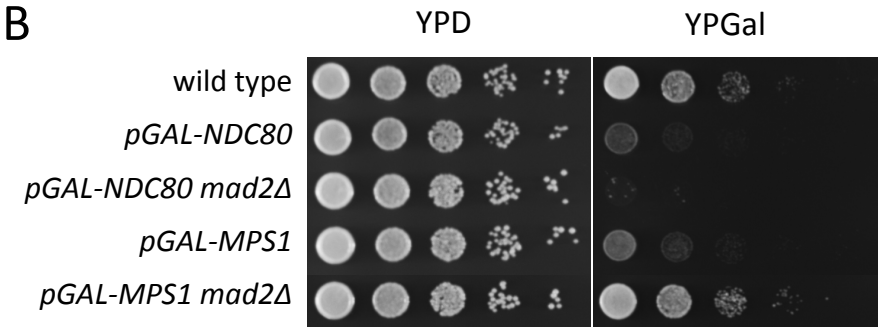

B
YPD
YPGal
wild type
pGAL-NDC80
pGAL-NDC80 mad2Δ
pGAL-MPS1
pGAL-MPS1 mad2Δ

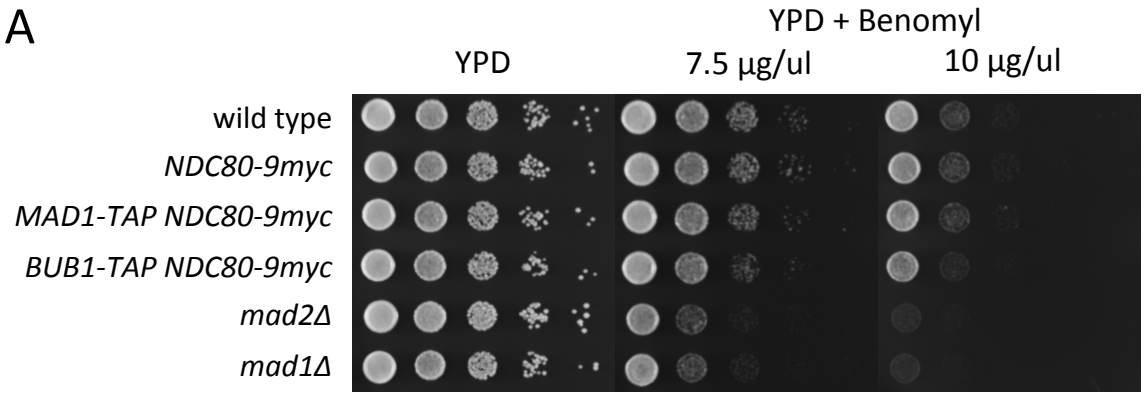
Figure 7. Ndc80 overexpression activates the Spindle Assembly Checkpoint
(**A**) Overexpressing Ndc80 causes cells to arrest with high levels of mitotic substrates. Cultures were synchronized in G1 phase with α-factor for 3 hrs. Cultures were released from their arrest into YPGal (2% galactose) at 25°C. Cultures were re-arrested after one cell cycle by the re-addition of α-factor at 60 min. Whole cell extracts were derived at the indicated time points after release from α-factor and subjected to western blot. (**B**) Cells that have a defective SAC and are overexpressing Ndc80 are non-viable. Serial dilutions of strains were tested for growth at 25°C on YPD (2% glucose), YPGal (2% galactose).

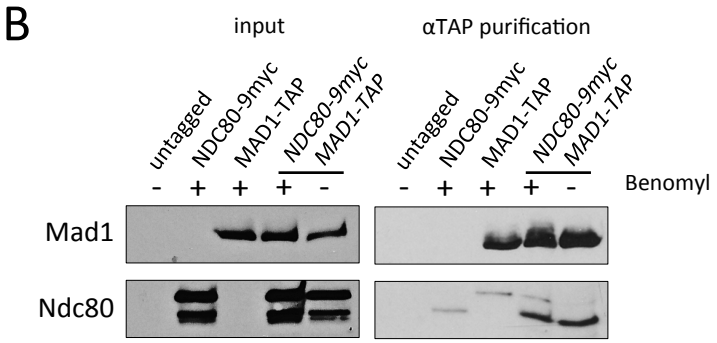
To test if *NDC80* overexpression is appropriately activating the SAC, strains were grown on solid media containing galactose (YPGal plates) to induce genes that are under control of the *GAL1* promoter. In *mad2Δ* strains, cells overexpressing *NDC80* were unable to grow (**Figure 7b**) suggesting that these cells mis-segregate chromosomes and die in the absence of a functional checkpoint. Overexpression of the upstream SAC kinase Mps1 also caused checkpoint activation, but in contrast to overexpression of *NDC80*, deleting *MAD2* improves growth showing that Mps1 activation of the checkpoint is inappropriate.

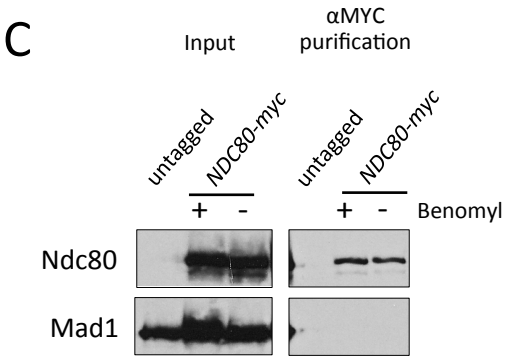
In contrast to my hypothesis, these data show that overexpression of *NDC80* does not bypass the checkpoint. Because an abundance of Ndc80 likely disrupts kinetochore-microtubule attachments, which activates the checkpoint, it is unclear whether there is also an effect of Ndc80 interacting with Mad1. Overexpressed Ndc80 may bind much of the cellular pool of Mad1, but its effect could be masked by the activation of the checkpoint.

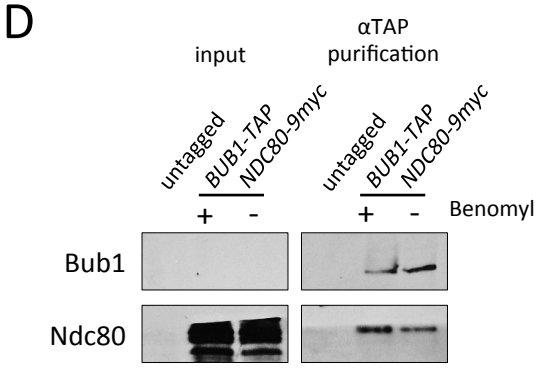
Ndc80 co-purifies with Mad1

To further characterize the role of Ndc80 in SAC signaling, I investigated whether its association with SAC proteins could be confirmed by immunoblotting after purification. To allow detection of the protein, nine myc tags were fused to the C-terminus of the endogenous *NDC80* gene in strains where Mad1 and Bub1 had been TAP tagged. *NDC80* is an essential gene and the tagged version shows no growth defect suggesting that the tags do not interfere with normal protein function (**Figure 8a**). Ndc80-myc is detected by immunoblotting in purifications of Mad1 and Bub1 from benomyl-arrested cells and is detected at lower levels when purified from cycling cells


A
YPD
YPD + Benomyl
7.5 µg/ul
10 µg/ul
wild type
NDC80-9myc
MAD1-TAP NDC80-9myc
BUB1-TAP NDC80-9myc
mad2Δ
mad1Δ


B
input
αTAP purification
untagged
NDC80-9myc
MAD1-TAP
NDC80-9myc
MAD1-TAP
Benomyl
Mad1
Ndc80


C
Input
αMYC purification
untagged
NDC80-myc
Benomyl
Ndc80
Mad1


D
input
αTAP purification
untagged
BUB1-TAP
NDC80-9myc
Benomyl
Bub1
Ndc80

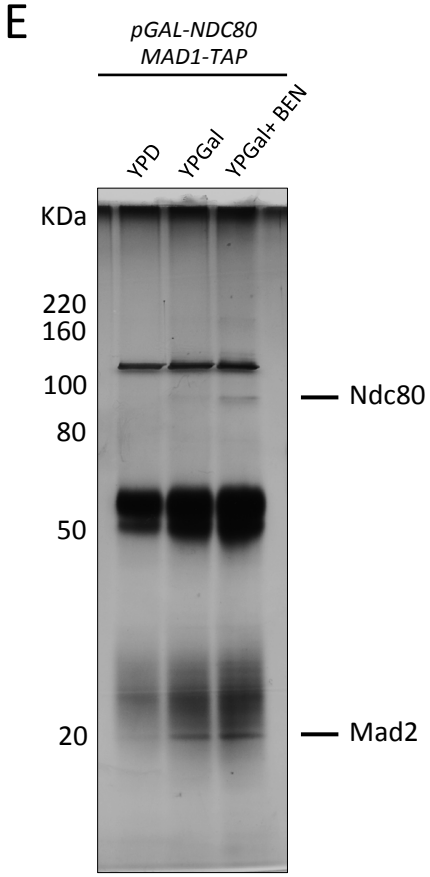

E
pGAL-NDC80
MAD1-TAP
YPD
YPGal
YPGal+ BEN
KDa
220
160
100
80
50
20
Ndc80
Mad2

Figure 8. Ndc80 co-purifies with Mad1
(**A**) TAP and myc tags do not interfere with protein function. Serial dilutions of cells with endogenously tagged proteins were tested for growth at 25°C on YPD plates containing the indicated concentrations of benomyl. (**B, C**) Proteins were purified from cultures arrested in mitosis with an active SAC by treatment with benomyl or from cycling cells using IgG conjugated magnetic Dynabeads in a mild lysis buffer containing 75 mM NaOAc. Purified complexes were eluted off beads by cleaving the TAP tag with TEV protease overnight. Samples were immunoblotted for the indicated proteins. (**B**) Ndc80 can be purified with Mad1. TAP-tagged Mad1 was purified from cultures of *MAD1-TAP NDC80-9myc* strain (**C**) Mad1 is not purified with Ndc80-myc. myc-tagged Ndc80 was purified from cultures of a *NDC80-9myc* strain using protein A Dynabeads coated with anti-myc antibodies. (**D**) Ndc80-9myc can be purified with Bub1-TAP. TAP-tagged Bub1 was purified from cultures of *BUB1-TAP NDC80-9myc* strain. (**E**) Levels of Ndc80 binding to Mad1 increases with expression and SAC activation. A culture of a *MAD1-TAP* strain with an integrated plasmid containing and extra copy of *NDC80* under control of the *GAL1* promoter was grown overnight in YPRaf (2% raffinose). The culture was split and grown in indicated media for 3 hrs before harvesting. TAP-tagged Mad1 was purified in 75 mM NaOAc containing lysis buffer, beads were heated in sample buffer, and eluted complexes visualized on a 10-20% gradient gel by silver stain. For all TAP purifications, 4% of the eluate was loaded in the TAP blot, and 40% of the eluate was loaded in the myc blot.

(**Figure 8b and d**). Conversely, when Ndc80 is purified, endogenous Mad1 protein is not detected (**Figure 8c**).

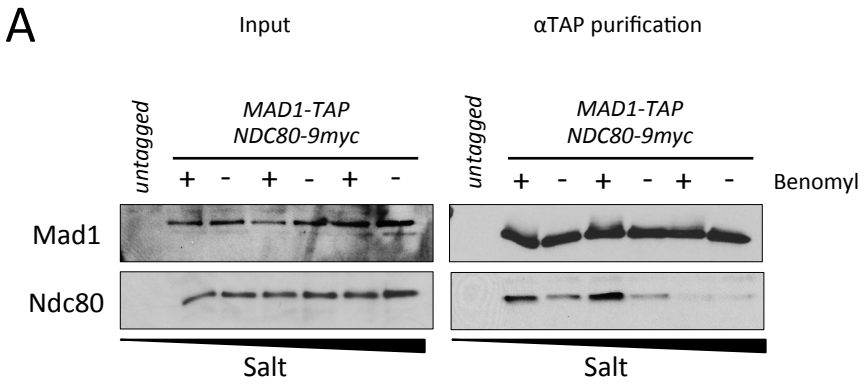
By silver stain, purifications of Mad1 from cells overexpressing Ndc80 show a faint band that runs at the predicted size of Ndc80. Upon SAC activation by the addition of benomyl, the intensity of this band markedly increased (**Figure 8e**).

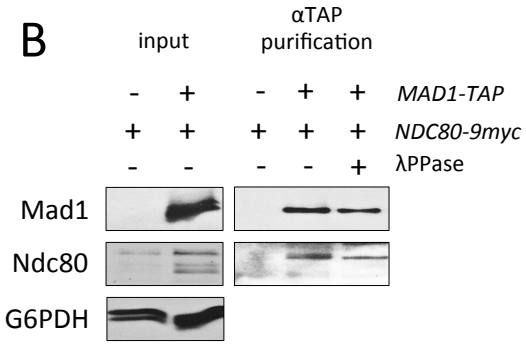
Ndc80-Mad1 interaction is cell cycle and DNA dependent

To test the strength of the association between Mad1 and Ndc80, Mad1 was purified in increasingly stringent conditions by increasing the salt concentration of purification buffers. Ndc80-myc is purified with Mad1 at salt concentrations as high as 300mM NaOAc but not at 700mM NaOAc (**Figure 9a**). For comparison, kinetochore sub-complexes are purified with neighbouring kinetochore subcomplexes at 300 mM but at salt concentrations of 600mM, the subcomplexes are purified alone(Westermann 2003).

Ndc80 and Mad1 are both phosphorylated during SAC arrest. Phosphorylation of Ndc80 is also cell cycle dependent and required for the recruitment of Mad1/Mad2 to kinetochores (Kemmler et al. 2009; Hardwick and Murray 1995). To test whether phosphorylation might be important for Mad1 association to Ndc80, purifications from cells with an active SAC were treated with lambda protein phosphatase. Lambda phosphatase treatment increases the electrophoretic mobility of Mad1 indicating that most of the protein phosphorylation was removed(Hardwick and Murray 1995); however, Ndc80-Mad1 interactions are not disrupted (**Figure 9b**).

Since the SAC functions during chromosome segregation in mitosis, I wondered whether Mad1 and Ndc80 interacted exclusively during mitosis. Mad1-TAP was


A
Input
αTAP purification
untagged
MAD1-TAP
NDC80-9myc
+ - + - + -
Benomyl
Mad1
Ndc80
Salt
Salt


B
input
αTAP
purification
- + - + + MAD1-TAP
+ + + + + NDC80-9myc
- - - - + λPPase
Mad1
Ndc80
G6PDH

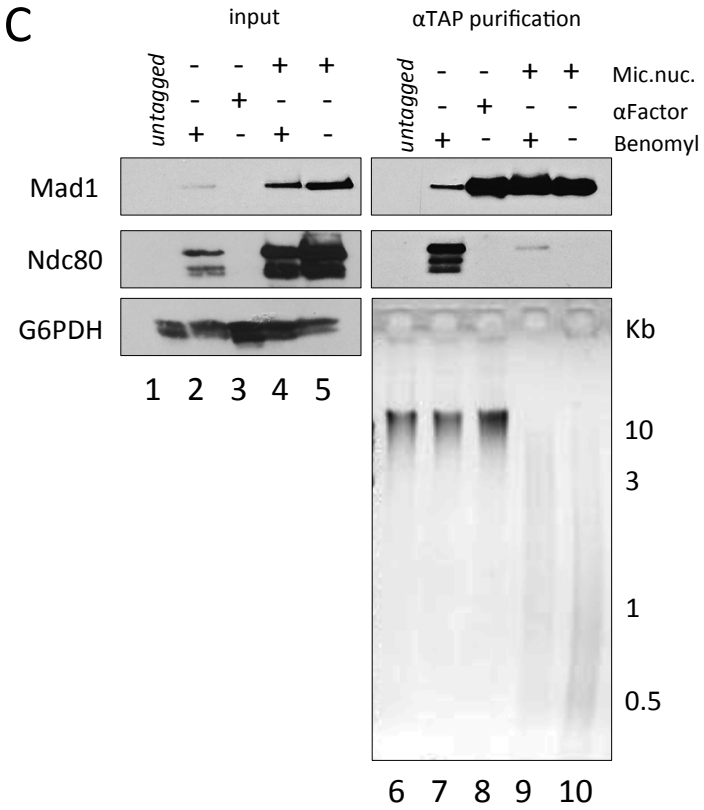

C
input
αTAP purification
untagged
Mic.nuc.
αFactor
Benomyl
Mad1
Ndc80
G6PDH
1 2 3 4 5
Kb
10
3
1
0.5
6 7 8 9 10

Figure 9. Cell cycle position plays a role in Ndc80-Mad1 binding
(**A-C**) Mad1-TAP was purified from cultures of a *MAD1-TAP NDC80-9myc* strain arrested in mitosis with an active SAC by treatment with benomyl or from cycling cells using IgG conjugated magnetic Dynabeads in a mild lysis buffer containing 75 mM NaOAc. Purified complexes were eluted off beads by cleaving the TAP tag with TEV protease overnight. Samples were immunoblotted for the indicated proteins. (**A**) Ndc80 dissociates from Mad1 at higher stringencies. TAP-tagged Mad1 was purified from cultures using lysis buffer at varying salt concentrations (150 mM, 300 mM, 700 mM NaOAc). (**B**) Protein phosphorylation does not affect Ndc80 interaction with Mad1. Purifications were treated with lambda protein phosphatase to remove all phosphorylation, then washed before cleaving complexes off beads. (**C**) Ndc80 binding to Mad1 is cell cycle and DNA dependent. Normalized lysates prepared as above from benomyl, α-factor arrested, or cycling cells were pre-treated with micrococcal nuclease before TAP-tagged Mad1 was purified. DNA from lysates was separated on agarose gel to verify efficacy DNAse treatment. For all TAP purifications, 4% of the eluate was loaded in the TAP blot, and 40% of the eluate was loaded in the myc blot.

purified from cells arrested in G1 phase with the mating pheromone α-factor. Although expression of both Mad1-TAP and Ndc80-myc was reduced in αfactor-arrested cells (**Figure 9c lane 3**), Ndc80-myc does not co-purify with Mad1-TAP from whole cell extracts of these cells (**Figure 9c lane 6**).

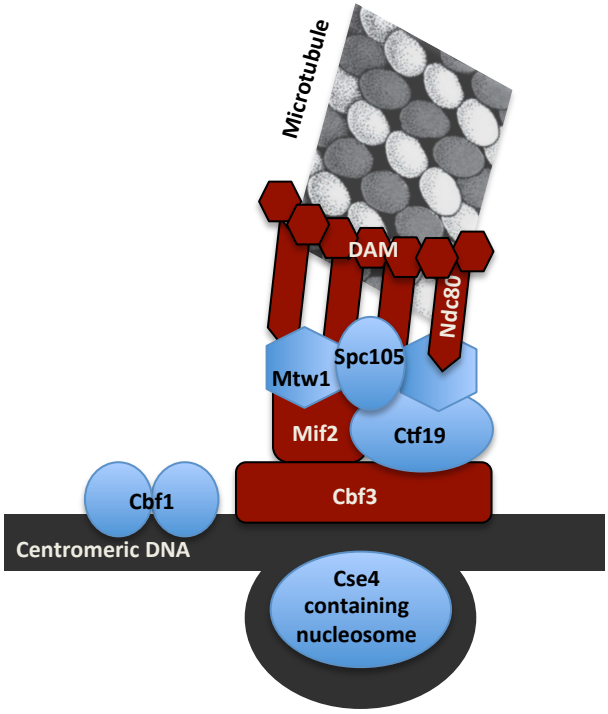
The absence of a detectable interaction during G1 suggested that the association between Mad1 and Ndc80 might be mitosis specific. Since the SAC signal originates from kinetochores assembled on replicated and condensed chromosomes during mitosis, I tested whether Ndc80 and Mad1 form a complex on kinetochores and therefore would require the presence of intact chromosomal DNA. I treated whole cell lysates with micrococcal nuclease to digest DNA before adding magnetic beads to purify Mad1-TAP. The amount of Ndc80-myc that co-purifies with Mad1-TAP is drastically reduced in the absence of intact chromatin and is undetectable in lysates of cycling cells treated with nuclease (**Figure 9c, compare lane 7, to lanes 9 & 10**).

Ndc80-Mad1 association does not depend on kinetochore integrity

The absence of a Ndc80/Mad1 complex in G1 arrested cells and the requirement of DNA for co-purification of these proteins supports the hypothesis that the association of these two proteins during mitosis is important for the function of the SAC. If the kinetochore serves as the platform for Mad1-Ndc80 interaction, then disrupting its assembly might disrupt association of Mad1 with Ndc80. Since most kinetochore proteins are essential in yeast, I introduced tagged *MAD1* and *NDC80* genes into strains harboring temperature sensitive (ts) mutations in kinetochore components (**Figure 10a**). The proteins display normal function when grown at the permissive temperature (25°C) but when grown at the restrictive temperature (37°C) causes

A

Mutant	Kinetochore sub-complex
ask2-1	DAM
spc24-1 nuf2-61	Ndc80
mif2-3	Mif2
ndc10-1	Cbf3



B

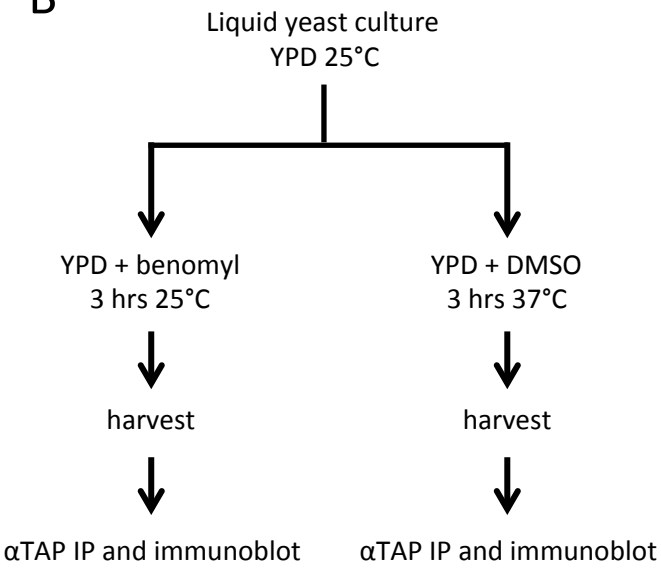
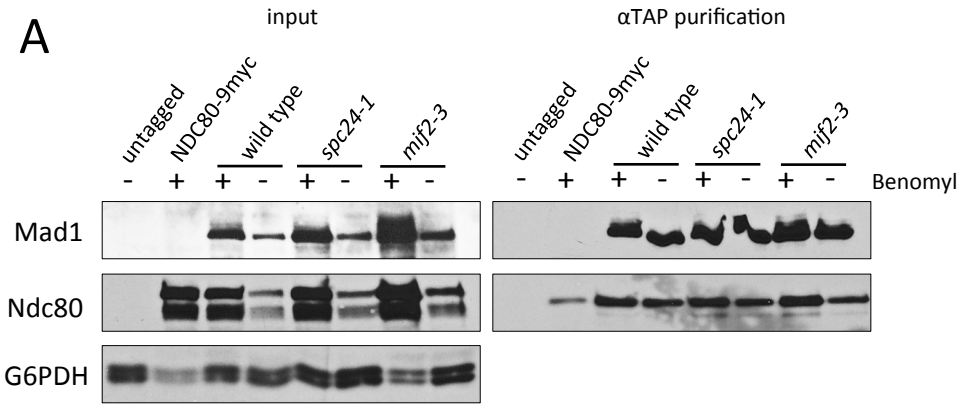
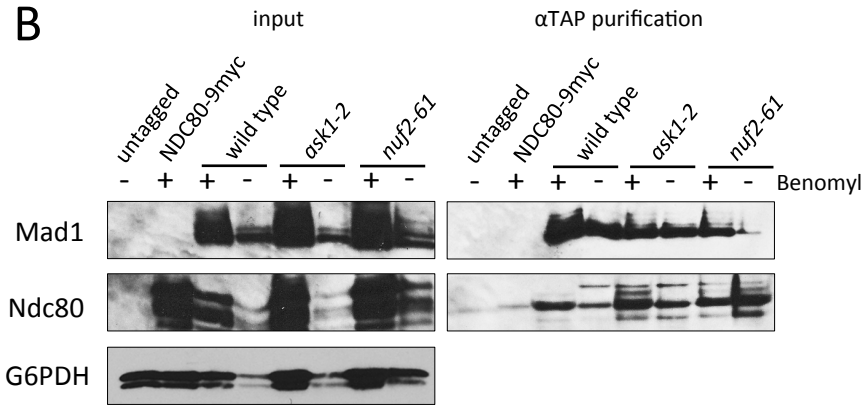


Figure 10. Experimental design for testing the dependence of Ndc80-Mad1 associations on the kinetochore

(**A**) Yeast strains contained temperature sensitive mutations in endogenous kinetochore genes. A schematic showing locations of mutated proteins within kinetochore subcomplexes and the larger kinetochore structure. Adapted from (Santaguida and Musacchio 2009). (**B**) Work flow of purifications from strains harboring mutations in kinetochore proteins. Asynchronous cultures were grown in YPD at the permissive temperature (25°C) to mid-log phase. Cultures were split and arrested in YPD + benomyl at 25°C or in YPD + DMSO at the restrictive temperature (37°C) for 3 hrs before harvesting.

lethality due to improper kinetochore function. Many of the ts mutants arrest in mitosis at the restrictive temperature because they activate the SAC. To control for cell cycle position I compared the association of Mad1 and Ndc80 in these mutants grown at 37˚C and wild type cells arrested in benomyl (**Figure 10b**). Ndc80-myc co-purifies with Mad1 in *ask1-2*, *nuf2-61*, and *spc24-1* strains in both benomyl and restrictive temperature conditions (**Figure 11a and b**). Ndc80-myc also co-purifies with Mad1 in *mif2-3* and *ndc10-1* strains grown at the restrictive temperature even though these cells do not activate the SAC and do not form functional kinetochores (**Figure 11b and c**). These data suggest that interaction between Mad1 and Ndc80 is not dependent on the Ndc10, Mif2, Spc24, Nuf2, or Ask1 proteins.


A
input
αTAP purification
untagged
NDC80-9myc
wild type
spc24-1
mif2-3
Benomyl
Mad1
Ndc80
G6PDH


B
input
αTAP purification
untagged
NDC80-9myc
wild type
ask1-2
nuf2-61
Benomyl
Mad1
Ndc80
G6PDH

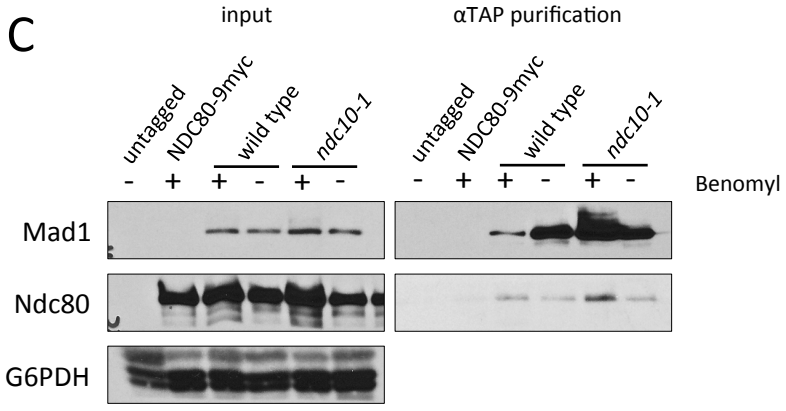

C
input
αTAP purification
untagged
NDC80-9myc
wild type
ndc10-1
Benomyl
Mad1
Ndc80
G6PDH

Figure 11. Ndc80 and Mad1 association does not depend on kinetochore integrity
(**A-C**) Mad1-TAP was purified from cultures of a MAD1-TAP NDC80-myc strain arrested in mitosis with an active SAC by treatment with benomyl or from cycling cells using IgG conjugated magnetic Dynabeads in a mild lysis buffer containing 75 mM NaOAc. Purified complexes were eluted off beads by cleaving the TAP tag with TEV protease overnight. Samples were immunoblotted for the indicated proteins. For all TAP purifications, 4% of the eluate was loaded in the TAP blot, and 40% of the eluate was loaded in the myc blot.

Chapter 4: Discussion

Past studies show that Mad1 localization to kinetochores depends on the Ndc80 complex, one of the protein complexes responsible for kinetochore-microtubule interaction(McIntosh et al. 2008; Powers et al. 2009; Ciferri et al. 2008; Martin-Lluesma, Stucke, and Nigg 2002; DeLuca et al. 2003; McCleland et al. 2003; Gillett 2004). However, a direct docking site for Mad1 on the kinetochore has not been identified. In this study, I sought to determine the proteins mediating Mad1 interaction with the kinetochore using protein purification and mass spectrometric analysis. My results support a model where Mad1 localizes to kinetochores via Ndc80.

Purification of checkpoint complexes and mass spectrometric analysis

The TAP tag allows for two-step purification of fusion proteins, however we found that a single step yielded sufficiently clean preparations of checkpoint complexes. We used conditions of relatively low stringency in order to detect delicate or transient protein interactions. When analyzed by silver stain, we could detect successful purification of the target proteins by predicted molecular weight shifts of tagged proteins. We could also detect slower migrating forms of phosphorylated Mad1 when the SAC is activated, as previously observed(Hardwick and Murray 1995). There were differences visible by silver stain in the composition of complexes purified from SAC arrested versus cycling cells (**Figure 4**).

Proteins that were previously shown to form complexes were also isolated together in the purifications of checkpoint components performed in this study (**Table 3**)(R. H. Chen et al. 1996; Brady and Hardwick 2000; Hardwick et al. 2000; R. H. Chen et al. 1999; Fraschini et al. 2001). We saw interactions between proteins that associate

throughout the cell cycle such as Mad1-Mad2 and Bub1-Bub3 in both checkpoint-arrested and cycling cells. However, as predicted from work in vertebrate cells, the MCC proteins (Mad2, Mad3, Bub3, Cdc20) were isolated only in samples purified from checkpoint-arrested cells(Sudakin 2001). We also detected subunits of the APC including the TPR subunits Cdc16, Cdc27 and Cdc23 in these samples. This is not surprising as the TPR subunits make up the binding site for Cdc20 and the MCC on the APC(Sikorski et al. 1990; Izawa and Pines 2011). The detection of proteins known to associate with components of the checkpoint only during SAC activation indicates that my purifications were successful and lends support for the conservation of the MCC in budding yeast.

Kinetochore proteins were detected only in Mad1 and Bub1 purifications when the SAC was activated. Isolation of Ndc80, Mtw1, and Spc105 complexes, members of the yeast KMN (KNL1-Mis12-Ndc80) network is consistent with the function of this network in binding to both microtubule and checkpoint proteins and detecting microtubule occupancy at the kinetochore(Goshima and Yanagida 2000; Kline et al. 2006; Pinsky et al. 2003; C. Pagliuca et al. 2009). This is also consistent with the upstream signaling function of Mad1 and Bub1 reported in previous studies(Maldonado and Kapoor 2011; Farr and Hoyt 1998; Gillett 2004; Sharp-Baker and Chen 2001). Some protein interactions may not have been detected in this study. For example, it is curious that I did not co-purify Mps1 or Fin1, which have been detected in purifications of kinetochores and are thought to associate with the KMN networks of kinetochore proteins(Akiyoshi et al. 2009). The mass spectrometric analysis performed here suffers from at least two limitations; small peptides are often

underrepresented and the presence of large amounts of IgG in the sample preparations masks the detection of peptides with properties similar to IgG peptides. Either the limitations of this experiment occluded detection of Mps1 and Fin1, or else these proteins do not interact sufficiently strongly with kinetochores to be detected in the purifications performed here.

Ndc80 expression affects SAC signaling

The detection of checkpoint protein interactions with Ndc80 Complex components was particularly interesting. Ndc80 is important for kinetochore-microtubule binding, has previously been implicated in SAC signaling and has been proposed to be responsible for Mad1 localization to kinetochores(McIntosh et al. 2008; Powers et al. 2009; Ciferri et al. 2008; McCleland et al. 2003; Gillett 2004). Hec1, the human homologue of yeast Ndc80, was identified as a Mad1 interacting protein in a yeast-two-hybrid assay. This group also found that Mad1 association with kinetochores was dependent on both Hec1 and Mps1 (Martin-Lluesma, Stucke, and Nigg 2002). It has also been shown that Nuf2 and Hec1 are required for retention of Mad1 at kinetochores during a SAC arrest(DeLuca et al. 2003). I reasoned that overexpressing Ndc80 in yeast should cause a checkpoint bypass by titrating Mad1 away from kinetochores thus disabling the checkpoint signal pathway when chromosomes are not properly aligned. Consistent with this hypothesis, cells displayed decreased viability in the presence of benomyl when Ndc80 was overexpressed. However, overexpressing Ndc80 appears to activate, not bypass the SAC as mitotic markers are stabilized when cells are released from a G1 arrest into conditions that both activate the SAC and induce Ndc80 overexpression (**Figure 6**). The *pGAL-Ndc80* strain behaves similarly to a *pGAL-Mps1*

strain that also activates the checkpoint and causes slow growth on media inducing its overexpression. In the case of Mps1 overexpression, activation of the SAC is inappropriate. Removal of a downstream signaling component, like Mad2, relieves SAC arrest and restores healthy growth (**Figure 7b**) (Hardwick et al. 1996). In contrast, Ndc80 overexpression triggers an appropriate checkpoint response. High Ndc80 levels cause lethality when *MAD2* is deleted (**Figure 7b**). This result suggests that when Ndc80 is overexpressed, microtubule-kinetochore connections are disrupted. When the checkpoint is impaired, by removing Mad2, cells continue to segregate chromosomes despite improper attachments and this results in chromosome missegregation and cell death (**Figure 7**). High levels of Ndc80 may interfere with kinetochore-microtubule binding by disrupting the Ndc80 Complex itself or disrupting the localization of the DAM/DASH Complex to kinetochores.

Although my simple titration model was incorrect, the appropriate activation of the checkpoint after Ndc80 overexpression suggests that Mad1 is still localized normally to kinetochores.

Confirmation that Ndc80 can be purified with Mad1

I was able to confirm that Ndc80 associates with Mad1 through co-immunoprecipitation experiments (**Figure 8**). Although it appears that some Ndc80-myc binds directly to IgG coated Dynabeads, this amount is considerably less than is purified with Mad1-TAP. Previous fluorescence studies in yeast have concluded that Mad1, Mad2, and Mad3 do not associate with kinetochores at an appreciable amount in cycling cells but Mad1 and Mad2 assemble at kinetochores in the presence of microtubule depolymerizing drugs(Gillett 2004). My results suggest that Mad1

associates with the kinetochore protein Ndc80 both in benomyl-arrested and cycling cells. There are two likely explanations for this observation: either Ndc80 and Mad1 interact independently of SAC activation or a population of cycling cells has an active SAC. It is thought that the MCC is formed during mitosis in unperturbed cell cycles in mammalian cells, indicating that the SAC may be activated during every cell cycle(Musacchio and Hardwick 2002). I favor the explanation that Ndc80 co-purifies with Mad1 from cycling cells because a subset of the population of cycling cells will be undergoing mitosis. The low levels of Mad1 associated with Ndc80 in cycling cells, increases substantially when cells are arrested in benomyl. Furthermore, the salt stringency experiments show that the Ndc80 association with Mad1 is stronger in benomyl-arrested cells as more Ndc80-Mad1 interaction was observed at high salt concentrations from benomyl-arrested cells than from cycling cells (**Figure 9a**). This indicates that activating the checkpoint might increase Mad1 localization to the kinetochore by strengthening Mad1-Ndc80 interactions. Conformational changes in Mad2 upon SAC activation promote its binding to Cdc20, but evidence for a conformational change in Mad1 or Ndc80 upon SAC activation is lacking(Tipton et al. 2011). Upstream phosphorylation events play an important role in SAC arrest and possibly in establishing kinetochore localization of Mad1(Hardwick et al. 1996; Chi et al. 2008).

Ndc80 and Mad1 are both phosphorylated during SAC arrest(Hardwick and Murray 1995; Kemmler et al. 2009). So far, the function of the phosphorylation is poorly understood. Phosphorylation of Ndc80 facilitates the recruitment of Mad1-Mad2 to kinetochores and Phospho-mimetic mutants of Ndc80 activate the SAC (Kemmler et

al. 2009). It is unknown which kinases are responsible for Ndc80 phosphorylation, although it has been proposed to be Mps1 in yeast or Nek2 in mammalian cells (Wei et al. 2011; Kemmler et al. 2009). (Nek2 and Npk1 (non-essential protein kinase) in *S. cerevisiae* both show sequence homology to NIMA in *Aspergillus nidulans*(Lou et al. 2004; Schweitzer and Philippsen 1992). The function of Npk1 is uncharacterized but if its function is conserved, it may be the yeast homologue of Nek2 and be an unidentified yeast SAC kinase.) The kinase activity of Bub1 has been shown to activate the SAC but its substrates are not known(Farr and Hoyt 1998). Although other studies showed that phosphorylation of Ndc80 and Mad1 is important for checkpoint activation, treating Mad1 purifications with phosphatase did not appreciably diminish the amount of Ndc80 detected (**Figure 9**).

These results suggest that maintenance of Ndc80-Mad1 interaction does not require phosphorylation. It is possible that the establishment of Ndc80-Mad1 interaction requires phosphorylation or that phosphorylation sites important for Mad1-Ndc80 association could not be de-phosphorylated by phosphatase treatment. Other approaches to study the contribution of phosphorylation to Mad1-Ndc80 interaction may be more direct. The checkpoint kinase Mps1 has been shown to control upstream SAC signaling events and to phosphorylate Mad1 in vitro(Hardwick et al. 1996). It would be interesting to determine whether inhibiting Mps1 affects Mad1 association with Ndc80 and if high levels Mps1 promote the interaction. Since phospho-mimic mutants of Ndc80 activate and non-phosphorylatable mutants bypass the checkpoint, studies of Mad1 association with these mutants could provide better

insight into the contribution of phosphorylation to localization of Mad1 to the kinetochore(Kemmler et al. 2009).

Mutant analysis

Studying the SAC with temperature sensitive (ts) mutants can be difficult as microtubule destabilizing drugs function best at low temperature and the ts mutants used here are inactivated at high temperature therefore, it is difficult to evaluate the effect of activating the SAC and disrupting the kinetochore at the same time. Ts mutant analysis has provided conflicting data regarding the link between kinetochore proteins and checkpoint signaling (**Figure 8**). On the one hand, some kinetochore mutants can support checkpoint signaling. Studies characterizing the *mif2-3* mutation have noted a weak metaphase arrest and the assembly of higher order kinetochore structure which can support a SAC response(Brown, Goetsch, and Hartwell 1993; Pinsky et al. 2003), *nuf2-61* cells arrest in mitosis without segregating DNA (Osborne et al. 1994), and *ask1-2* mutants also support Ndc80 complex assembly on the kinetochore but do not make proper microtubule attachments and arrest in mitosis. Since these mutants support SAC signaling, it not surprising that that they do not interfere with a SAC dependent Mad1-Ndc80 association.

On the other hand, I still detect Ndc80 in Mad1 purifications from the *ndc10-1* and *spc24-1* strains. These results are surprising because *ndc10-1* has been shown to destroy the kinetochore (including mis-localization of the Ndc80 complex) and neither *ndc10-1* nor *spc24-1* cells at the restrictive temperature can support a checkpoint response(Goh and Kilmartin 1993; Ortiz et al. 1999; Janke et al. 2001; Wigge and Kilmartin 2001).

When a population of ts mutant cells is shifted to the restrictive temperature, the majority of the population will display a dominant phenotype, but not all of it. For example, the group that characterized *mif2-3* mutant noted a particularly weak phenotype(Brown, Goetsch, and Hartwell 1993).

The purifications from ts mutant strains done here may not be the best assay for observing contributions of kinetochore proteins to Ndc80-Mad1 association because of their non-uniform arrest phenotypes. The Ndc80 detection in this assay is sensitive to even small amount of Ndc80-Mad1 complex, as shown from the detection of Ndc80 from cycling cells by immunoblotting (**Figure 8**). So I may be detecting the small amount of Ndc80-Mad1 complex that forms in cells that don't display the majority phenotype. Even though the purifications performed here make it difficult to detect decreases in Mad1-Ndc80 binding, it is possible to detect increases in Mad1-Ndc80 association. *nuf2-61* mutants have been shown to activate the checkpoint(Osborne et al. 1994). These mutants display an increase in slower migrating phosphorylated Mad1 as well as a marked increase in Ndc80 protein purified with Mad1, consistent with an activated checkpoint. Therefore the purifications performed here may be better at detecting SAC dependent increases in Mad1-Ndc80 interaction than decreases. Increasing the stringency of the purifications might alleviate this problem.

I was not able to assess the effect of ts mutations in Ndc80 itself on Ndc80-Mad1 interaction. Studies of *ndc80-1* mutants are not fully consistent with a model where Mad1 localizes to kinetochores via Ndc80. Some studies report that *ndc80-1* mutants arrest at metaphase and engage the checkpoint(Janke et al. 2001; Wigge and Kilmartin 2001). Others have shown that the *ndc80-1* ts and ^{DT}*Nuf2* degron double mutant is

completely checkpoint deficient and either mutation alone is not(McCleland et al. 2003). Some researchers have proposed that because the fate of ts mutant proteins like *ndc80-1* is uncertain, some mutations may result in partial loss of function while others might be completely inactivated(Gillett 2004). Therefore poorly characterized ts mutants appear to be a poor tool to analyze kinetochore protein contribution to SAC signaling and checkpoint protein localization to the kinetochore.

Models and future experiments

The objective of this study was to attempt to characterize the association of Mad1 at the kinetochore. Taken together, the experiments shown in this work support a model where Mad1 localizes to kinetochores by binding to Ndc80. These data do not rule out the possibility of other kinetochore proteins strengthening this interaction, or phosphorylation of Ndc80 and/or Mad1 being critical for checkpoint signaling or Ndc80/Mad1 localization to the kinetochore.

These data do not support a simple model of unregulated binding of Mad1 to Ndc80 (**Figure 12a**). In this model, overexpression of Ndc80 would titrate Mad1 away from kinetochore causing a checkpoint defect. The Ndc80 overexpression experiments presented here demonstrate that this is not the case, but do not rule out the possibility that Mad1 binds to Ndc80 directly. It would be interesting to test whether high levels of Ndc80 activate the SAC because they disrupt the formation of the Ndc80 Complex or localization of the DAM/DASH Complex.

Two results in this study seem to contradict each other: The micrococcal nuclease (MNase) experiment shows suggests that Ndc80-Mad1 depends on intact DNA and therefore a kinetochore while mutant experiments suggest that the interaction is

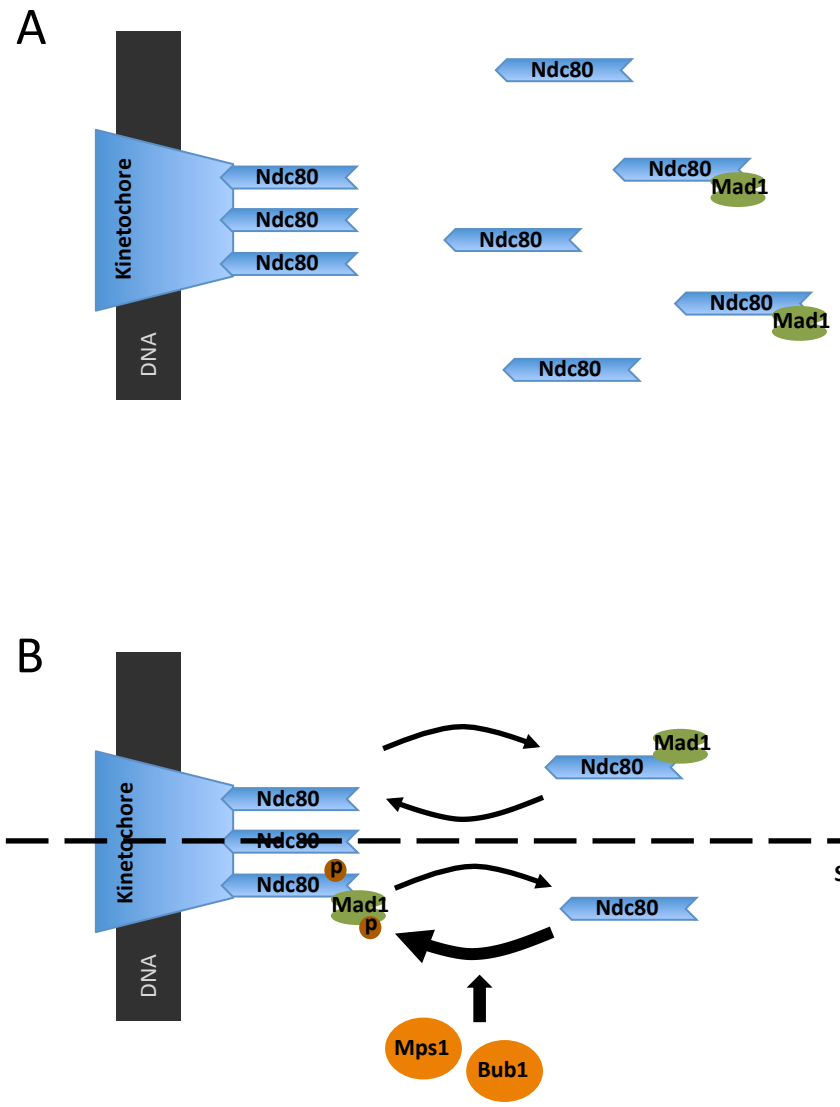

A
Kinetochore
DNA
Ndc80
Ndc80
Ndc80
Ndc80
Ndc80
Mad1
Ndc80
Ndc80
Mad1
Ndc80
B
Kinetochore
DNA
Ndc80
Ndc80
Ndc80
P
Mad1
P
Mad1
Ndc80
Cycling
SAC activated
Mps1
Bub1
C
Kinetochore
DNA
Bub1
Mad1
Bub1
Mad1
Ndc80
Ndc80
Ndc80

Figure 12. Models for Mad1 localization to the kinetochore
(**A**) Titration model. Mad1 localization to the kinetochore depends on its regulated binding to Ndc80. Overexpression of Ndc80 would titrate the pool of Mad1 off the kinetochores and cause a bypass of the SAC. (**B**) Soluble complex model. A soluble Mad1-Ndc80 has low affinity for kinetochores in cycling cells. SAC activation promotes Ndc80-Mad1 binding to the kinetochore, displacing any non-Mad1-bound Ndc80 Complex. Phosphorylation of Mad1 and Ndc80 by checkpoint kinases (Mps1 and/or Bub1) could be the SAC specific event that increases the affinity of Mad1-complexed Ndc80 for the kinetochore. (**C**) Multi-protein binding site model. Multiple proteins at the kinetochore form a docking site for Mad1 and contribute to its localization at the kinetochore.

not dependent on an intact kinetochore. Either the mutant experiments are misleading due to limitations discussed above, or the MNase result is misleading. If the MNase is contaminated by a protease, a phosphatase, or other molecule and interferes with Ndc80-Mad1, then we must conclude that the Ndc80-Mad1 interaction does not depend on the kinetochore. If this is the case, there could be a soluble pool of Ndc80 Complex alone and a pool of Ndc80 Complex bound to Mad1. Activating the SAC increases the binding of Ndc80 to Mad1 and also increases the affinity of this complex for the kinetochore. So when the SAC is activated, the Mad1-bound Ndc80 Complex outcompetes the Ndc80 Complex alone for the kinetochore binding spot (**Figure 12b**). This is consistent with my results that show Ndc80 interactions with Mad1 are stronger with SAC activation and do not require DNA or an intact kinetochore. To verify this model, we would have to confirm that the Ndc80-Mad1 complex still forms when Ndc80 is overexpressed and determine its localization. The soluble complex model also does not rule out the possibility that phosphorylation helps establish Mad1 at the kinetochore. Overexpression of Mps1 or Bub1 kinases activates the SAC and may promote Mad1-Ndc80 interaction(Farr and Hoyt 1998; Hardwick et al. 1996). A more delicate way to investigate this possibility would be to test whether phospho-mimetic mutants of Ndc80 could strengthen Mad1 interaction with Ndc80 and thus the localization of Mad1 to the kinetochore. This might also explain why the SAC is activated by phospho-mimetic mutants in yeast(Kemmler et al. 2009).

A multi-protein binding site for Mad1 at kinetochores has been suggested by other groups (**Figure 12c**) and may explain the association of Mad1 with Ndc80 seen here even after phosphatase treatment and mutation of kinetochore proteins. Bub1 is

constitutively associated to the kinetochore and is thought to be a scaffold protein for more transient checkpoint signaling proteins like Bub3, Mad3 and Mad1. This may account for the dependence of Mad1 on Bub1 for association with the kinetochore(Gillett 2004). A recent study in vertebrate cells showed that mutating important residues in its Centromere Targeting Domain only reduces Mad1 at the kinetochores by about 50%. This group's structural analysis of human Mad1 reveals the presence of other protein interacting domains in the coiled-coil stem of Mad1 suggesting that Mad1 might contact several proteins at the kinetochore (Kim et al. 2012). Bub1 may contribute to a Mad1 docking site at the kinetochore as it is required for localization of downstream checkpoint proteins like Mad1, Mad2, and Mad3(Rischitor, May, and Hardwick 2007; Sharp-Baker and Chen 2001; Gillett 2004). The effect on the interaction between Mad1 and Ndc80 of deleting or overexpressing Bub1 would be simple to test in yeast. Alternatively, mutation analysis of conserved targeting domains in Mad1 would be useful to evaluate their contributions to both Mad1 binding to Ndc80 and Mad1 localization to the kinetochore.

Neither model proposed here excludes the other. It is possible that a soluble Mad1-bound Ndc80 Complex outcompetes a 'naked' Ndc80 Complex when the SAC is activated through phosphorylation of a mediating protein. Further experimentation could tease out the contribution of these models to SAC signaling. A full understanding of how Mad1 localizes will be critical to understanding how the signal from a single unattached kinetochore can be amplified to halt the cell cycle.

References

Akiyoshi, B, C R Nelson, J A Ranish, and S Biggins. 2009. "Quantitative Proteomic Analysis of Purified Yeast Kinetochores Identifies a PP1 Regulatory Subunit." *Genes & Development* 23 (24) (December 15): 2887–2899. doi:10.1101/gad.1865909.

Akiyoshi, Bungo, Krishna K Sarangapani, Andrew F Powers, Christian R Nelson, Steve L Reichow, Hugo Arellano-Santoyo, Tamir Gonen, Jeffrey A Ranish, Charles L Asbury, and Sue Biggins. 2010. "Tension Directly Stabilizes Reconstituted Kinetochore-Microtubule Attachments." *Nature* 468 (7323) (November 16): 576–579. doi:10.1038/nature09594.

Amon, A, S Irniger, and K Nasmyth. 1994. "Closing the Cell Cycle Circle in Yeast: G2 Cyclin Proteolysis Initiated at Mitosis Persists Until the Activation of G1 Cyclins in the Next Cycle.." *Cell* 77 (7) (July 1): 1037–1050.

Asbury, Charles L, Daniel R Gestaut, Andrew F Powers, Andrew D Franck, and Trisha N Davis. 2006. "The Dam1 Kinetochore Complex Harnesses Microtubule Dynamics to Produce Force and Movement.." *Proceedings of the National Academy of Sciences of the United States of America* 103 (26) (June 27): 9873–9878. doi:10.1073/pnas.0602249103.

Baker, R E, M Fitzgerald-Hayes, and T C O'Brien. 1989. "Purification of the Yeast Centromere Binding Protein CP1 and a Mutational Analysis of Its Binding Site.." *The Journal of Biological Chemistry* 264 (18) (June 25): 10843–10850.

Bakhoum, Samuel F, and Duane A Compton. 2012. "Chromosomal Instability and Cancer: a Complex Relationship with Therapeutic Potential.." *The Journal of Clinical Investigation* 122 (4) (April 2): 1138–1143. doi:10.1172/JCI59954.

Biggins, S, and A W Murray. 2001. "The Budding Yeast Protein Kinase Ipl1/Aurora Allows the Absence of Tension to Activate the Spindle Checkpoint.." *Genes & Development* 15 (23) (December 1): 3118–3129. doi:10.1101/gad.934801.

Biggins, S, F F Severin, N Bhalla, I Sassoon, A A Hyman, and A W Murray. 1999. "The Conserved Protein Kinase Ipl1 Regulates Microtubule Binding to Kinetochores in Budding Yeast.." *Genes & Development* 13 (5) (March 1): 532–544.

Blower, Michael D, Beth A Sullivan, and Gary H Karpen. 2002. "Conserved Organization of Centromeric Chromatin in Flies and Humans.." *Developmental Cell* 2 (3) (March): 319–330.

Brady, D M, and K G Hardwick. 2000. "Complex Formation Between Mad1p, Bub1p and Bub3p Is Crucial for Spindle Checkpoint Function.." *Current Biology : CB* 10 (11) (June 1): 675–678.

Brown, M T, L Goetsch, and L H Hartwell. 1993. "MIF2 Is Required for Mitotic Spindle Integrity During Anaphase Spindle Elongation in Saccharomyces Cerevisiae.." *The Journal of Cell Biology* 123 (2) (October): 387–403.

Cahill, D P, C Lengauer, J Yu, G J Riggins, J K Willson, S D Markowitz, K W Kinzler, and B Vogelstein. 1998. "Mutations of Mitotic Checkpoint Genes in Human Cancers.." *Nature* 392 (6673) (March 19): 300–303. doi:10.1038/32688.

Carroll, Christopher W, and David O Morgan. 2002. "The Doc1 Subunit Is a Processivity Factor for the Anaphase-Promoting Complex.." *Nature Cell Biology* 4 (11) (November): 880–887. doi:10.1038/ncb871.

Carroll, Christopher W, Kirstin J Milks, and Aaron F Straight. 2010. "Dual Recognition of CENP-a Nucleosomes Is Required for Centromere Assembly.." *The Journal of Cell Biology* 189 (7) (June 28): 1143–1155. doi:10.1083/jcb.201001013.

Carroll, Christopher W, Maria Enquist-Newman, and David O Morgan. 2005. “The APC Subunit Doc1 Promotes Recognition of the Substrate Destruction Box..” *Current Biology : CB* 15 (1) (January 11): 11–18. doi:10.1016/j.cub.2004.12.066.

Carroll, Christopher W, Mariana C C Silva, Kristina M Godek, Lars E T Jansen, and Aaron F Straight. 2009. “Centromere Assembly Requires the Direct Recognition of CENP-a Nucleosomes by CENP-N..” *Nature Publishing Group* 11 (7) (July): 896–902. doi:10.1038/ncb1899.

Chao, William C H, Kiran Kulkarni, Ziguo Zhang, Eric H Kong, and David Barford. 2012. “Structure of the Mitotic Checkpoint Complex..” *Nature* (March 21). doi:10.1038/nature10896.

Cheeseman, I M, C Brew, M Wolyniak, A Desai, S Anderson, N Muster, J R Yates, T C Huffaker, D G Drubin, and G Barnes. 2001. “Implication of a Novel Multiprotein Dam1p Complex in Outer Kinetochore Function..” *The Journal of Cell Biology* 155 (7) (December 24): 1137–1145. doi:10.1083/jcb.200109063.

Cheeseman, I M, M Enquist-Newman, T Müller-Reichert, D G Drubin, and G Barnes. 2001. “Mitotic Spindle Integrity and Kinetochore Function Linked by the Duo1p/Dam1p Complex..” *The Journal of Cell Biology* 152 (1) (January 8): 197–212.

Chen, R H, D M Brady, D Smith, A W Murray, and K G Hardwick. 1999. “The Spindle Checkpoint of Budding Yeast Depends on a Tight Complex Between the Mad1 and Mad2 Proteins..” *Molecular Biology of the Cell* 10 (8) (August): 2607–2618.

Chen, R H, J C Waters, E D Salmon, and A W Murray. 1996. “Association of Spindle Assembly Checkpoint Component XMAD2 with Unattached Kinetochores..” *Science* 274 (5285) (October 11): 242–246.

Chen, Y, D J Riley, P L Chen, and W H Lee. 1997. “HEC, a Novel Nuclear Protein Rich in Leucine Heptad Repeats Specifically Involved in Mitosis..” *Molecular and Cellular Biology* 17 (10) (October): 6049–6056.

Chi, Ya-Hui, Kerstin Haller, Michael D Ward, O John Semmes, Yan Li, and Kuan-Teh Jeang. 2008. “Requirements for Protein Phosphorylation and the Kinase Activity of Polo-Like Kinase 1 (Plk1) for the Kinetochore Function of Mitotic Arrest Deficiency Protein 1 (Mad1)..” *The Journal of Biological Chemistry* 283 (51) (December 19): 35834–35844. doi:10.1074/jbc.M804967200.

Chung, Eunah, and Rey-Huei Chen. 2003. “Phosphorylation of Cdc20 Is Required for Its Inhibition by the Spindle Checkpoint..” *Nature Cell Biology* 5 (8) (August): 748–753. doi:10.1038/ncb1022.

Ciferri, Claudio, Sebastiano Pasqualato, Emanuela Screpanti, Gianluca Varetti, Stefano Santaguida, Gabriel Dos Reis, Alessio Maiolica, et al. 2008. “Implications for Kinetochore-Microtubule Attachment From the Structure of an Engineered Ndc80 Complex..” *Cell* 133 (3) (May 2): 427–439. doi:10.1016/j.cell.2008.03.020.

Cohen-Fix, O, J M Peters, M W Kirschner, and D Koshland. 1996. “Anaphase Initiation in Saccharomyces Cerevisiae Is Controlled by the APC-Dependent Degradation of the Anaphase Inhibitor Pds1p..” *Genes & Development* 10 (24) (December 15): 3081–3093. doi:10.1101/gad.10.24.3081.

Cottarel, G, J H Shero, P Hieter, and J H Hegemann. 1989. “A 125-Base-Pair CEN6 DNA Fragment Is Sufficient for Complete Meiotic and Mitotic Centromere Functions in Saccharomyces Cerevisiae..” *Molecular and Cellular Biology* 9 (8) (August): 3342–3349.

da Fonseca, Paula C A, Eric H Kong, Ziguo Zhang, Anne Schreiber, Mark A Williams,

Edward P Morris, and David Barford. 2010. “Structures of APC/CCdh1 with Substrates Identify Cdh1 and Apc10 as the D-Box Co-Receptor.” *Nature* (November 24): 1–7. doi:10.1038/nature09625.

Dahmann, C, J F Diffley, and K A Nasmyth. 1995. “S-Phase-Promoting Cyclin-Dependent Kinases Prevent Re-Replication by Inhibiting the Transition of Replication Origins to a Pre-Replicative State..” *Current Biology : CB* 5 (11) (November 1): 1257–1269.

De Antoni, Anna, Chad G Pearson, Daniela Cimini, Julie C Canman, Valeria Sala, Luigi Nezi, Marina Mapelli, et al. 2005. “The Mad1/Mad2 Complex as a Template for Mad2 Activation in the Spindle Assembly Checkpoint..” *Current Biology : CB* 15 (3) (February 8): 214–225. doi:10.1016/j.cub.2005.01.038.

DeLuca, Jennifer G, Bonnie J Howell, Julie C Canman, Jennifer M Hickey, Guowei Fang, and E D Salmon. 2003. “Nuf2 and Hec1 Are Required for Retention of the Checkpoint Proteins Mad1 and Mad2 to Kinetochores..” *Current Biology : CB* 13 (23) (December 2): 2103–2109.

Dube, Prakash, Franz Herzog, Christian Gieffers, Bjoern Sander, Dietmar Riedel, Shirley A Müller, Andreas Engel, Jan-Michael Peters, and Holger Stark. 2005. “Localization of the Coactivator Cdh1 and the Cullin Subunit Apc2 in a Cryo-Electron Microscopy Model of Vertebrate APC/C..” *Molecular Cell* 20 (6) (December 22): 867–879. doi:10.1016/j.molcel.2005.11.008.

Evans, T, E T Rosenthal, J Youngblom, D Distel, and T Hunt. 1983. “Cyclin: a Protein Specified by Maternal mRNA in Sea Urchin Eggs That Is Destroyed at Each Cleavage Division..” *Cell* 33 (2) (June): 389–396.

Eytan, Esther, Yakir Moshe, Ilana Braunstein, and Avram Hershko. 2006. “Roles of the Anaphase-Promoting Complex/Cyclosome and of Its Activator Cdc20 in Functional Substrate Binding..” *Proceedings of the National Academy of Sciences of the United States of America* 103 (7) (February 14): 2081–2086. doi:10.1073/pnas.0510695103.

Farr, K A, and M A Hoyt. 1998. “Bub1p Kinase Activates the Saccharomyces Cerevisiae Spindle Assembly Checkpoint..” *Molecular and Cellular Biology* 18 (5) (May): 2738–2747.

Foltz, Daniel R, Lars E T Jansen, Ben E Black, Aaron O Bailey, John R Yates, and Don W Cleveland. 2006. “The Human CENP-a Centromeric Nucleosome-Associated Complex..” *Nature Cell Biology* 8 (5) (May): 458–469. doi:10.1038/ncb1397.

Foster, Scott A, and David O Morgan. 2012. “The APC/C Subunit Mnd2/Apc15 Promotes Cdc20 Autoubiquitination and Spindle Assembly Checkpoint Inactivation..” *Molecular Cell* (August 29). doi:10.1016/j.molcel.2012.07.031.

Fraschini, R, A Beretta, L Sironi, A Musacchio, G Lucchini, and S Piatti. 2001. “Bub3 Interaction with Mad2, Mad3 and Cdc20 Is Mediated by WD40 Repeats and Does Not Require Intact Kinetochores..” *The EMBO Journal* 20 (23) (December 3): 6648–6659. doi:10.1093/emboj/20.23.6648.

Ghaemmaghami, Sina, Won-Ki Huh, Kiowa Bower, Russell W Howson, Archana Belle, Noah Dephoure, Erin K O'Shea, and Jonathan S Weissman. 2003. “Global Analysis of Protein Expression in Yeast..” *Nature* 425 (6959) (October 16): 737–741. doi:10.1038/nature02046.

Ghiara, J B, H E Richardson, K Sugimoto, M Henze, D J Lew, C Wittenberg, and S I Reed. 1991. “A Cyclin B Homolog in S. Cerevisiae: Chronic Activation of the Cdc28 Protein Kinase by Cyclin Prevents Exit From Mitosis..” *Cell* 65 (1) (April 5): 163–174.

Ghosh, S K, A Poddar, S Hajra, K Sanyal, and P Sinha. 2001. “The IML3/MCM19 Gene of

Saccharomyces Cerevisiae Is Required for a Kinetochore-Related Process During Chromosome Segregation..” *Molecular Genetics and Genomics : MGG* 265 (2) (April): 249–257.
Gillett, E S. 2004. “Spindle Checkpoint Proteins and Chromosome-Microtubule Attachment in Budding Yeast.” *The Journal of Cell Biology* 164 (4) (February 17): 535–546. doi:10.1083/jcb.200308100.
Glotzer, M, A W Murray, and M W Kirschner. 1991. “Cyclin Is Degraded by the Ubiquitin Pathway..” *Nature* 349 (6305) (January 10): 132–138. doi:10.1038/349132a0.
Goh, P Y, and J V Kilmartin. 1993. “NDC10: a Gene Involved in Chromosome Segregation in Saccharomyces Cerevisiae..” *The Journal of Cell Biology* 121 (3) (May): 503–512.
Gonen, Shane, Bungo Akiyoshi, Matthew G Iadanza, Dan Shi, Nicole Duggan, Sue Biggins, and Tamir Gonen. 2012. “The Structure of Purified Kinetochores Reveals Multiple Microtubule-Attachment Sites..” *Nature Structural & Molecular Biology* 19 (9) (September): 925–929. doi:10.1038/nsmb.2358.
Gordon, David J, Benjamin Resio, and David Pellman. 2012. “Causes and Consequences of Aneuploidy in Cancer..” *Nature Reviews. Genetics* 13 (3) (March): 189–203. doi:10.1038/nrg3123.
Goshima, G, and M Yanagida. 2000. “Establishing Biorientation Occurs with Precocious Separation of the Sister Kinetochores, but Not the Arms, in the Early Spindle of Budding Yeast..” *Cell* 100 (6) (March 17): 619–633.
Grishchuk, E L, I S Spiridonov, V A Volkov, A Efremov, S Westermann, D Drubin, G Barnes, F I Ataullakhanov, and J R McIntosh. 2008. “Different Assemblies of the DAM1 Complex Follow Shortening Microtubules by Distinct Mechanisms..” *Proceedings of the National Academy of Sciences of the United States of America* 105 (19) (May 13): 6918–6923. doi:10.1073/pnas.0801811105.
Grishchuk, Ekaterina L, Artem K Efremov, Vladimir A Volkov, Ilia S Spiridonov, Nikita Gudimchuk, Stefan Westermann, David Drubin, Georjana Barnes, J Richard McIntosh, and Fazly I Ataullakhanov. 2008. “The Dam1 Ring Binds Microtubules Strongly Enough to Be a Processive as Well as Energy-Efficient Coupler for Chromosome Motion..” *Proceedings of the National Academy of Sciences of the United States of America* 105 (40) (October 7): 15423–15428. doi:10.1073/pnas.0807859105.
Guacci, V, D Koshland, and A Strunnikov. 1997. “A Direct Link Between Sister Chromatid Cohesion and Chromosome Condensation Revealed Through the Analysis of MCD1 in S. Cerevisiae..” *Cell* 91 (1) (October 3): 47–57.
Hardwick, K G, and A W Murray. 1995. “Mad1p, a Phosphoprotein Component of the Spindle Assembly Checkpoint in Budding Yeast..” *The Journal of Cell Biology* 131 (3) (November): 709–720.
Hardwick, K G, E Weiss, F C Luca, M Winey, and A W Murray. 1996. “Activation of the Budding Yeast Spindle Assembly Checkpoint Without Mitotic Spindle Disruption..” *Science* 273 (5277) (August 16): 953–956.
Hardwick, K G, R C Johnston, D L Smith, and A W Murray. 2000. “MAD3 Encodes a Novel Component of the Spindle Checkpoint Which Interacts with Bub3p, Cdc20p, and Mad2p..” *The Journal of Cell Biology* 148 (5) (March 6): 871–882.
Hartwell, L H, J Culotti, and B Reid. 1970. “Genetic Control of the Cell-Division Cycle in Yeast. I. Detection of Mutants..” *Proceedings of the National Academy of Sciences of the United States of America* 66 (2) (June): 352–359.
Hartwell, L H, J Culotti, J R Pringle, and B J Reid. 1974. “Genetic Control of the Cell

Division Cycle in Yeast..” *Science* 183 (4120) (January 11): 46–51.
Hayden, J H, S S Bowser, and C L Rieder. 1990. “Kinetochores Capture Astral Microtubules During Chromosome Attachment to the Mitotic Spindle: Direct Visualization in Live Newt Lung Cells..” *The Journal of Cell Biology* 111 (3) (September): 1039–1045.
Heath, I B. 1980. “Variant Mitoses in Lower Eukaryotes: Indicators of the Evolution of Mitosis..” *International Review of Cytology* 64: 1–80.
Heller, J H. 1969. “Human Chromosome Abnormalities as Related to Physical and Mental Dysfunction..” *The Journal of Heredity* 60 (5) (August): 239–248.
Hershko, A, D Ganoth, J Pehrson, R E Palazzo, and L H Cohen. 1991. “Methylated Ubiquitin Inhibits Cyclin Degradation in Clam Embryo Extracts..” *The Journal of Biological Chemistry* 266 (25) (September 5): 16376–16379.
Herzog, Franz, Ivana Primorac, Prakash Dube, Peter Lenart, Björn Sander, Karl Mechtler, Holger Stark, and Jan-Michael Peters. 2009. “Structure of the Anaphase-Promoting Complex/Cyclosome Interacting with a Mitotic Checkpoint Complex..” *Science* 323 (5920) (March 13): 1477–1481. doi:10.1126/science.1163300.
Hilioti, Z, Y S Chung, Y Mochizuki, C F Hardy, and O Cohen-Fix. 2001. “The Anaphase Inhibitor Pds1 Binds to the APC/C-Associated Protein Cdc20 in a Destruction Box-Dependent Manner..” *Current Biology : CB* 11 (17) (September 4): 1347–1352.
Holloway, S L, M Glotzer, R W King, and A W Murray. 1993. “Anaphase Is Initiated by Proteolysis Rather Than by the Inactivation of Maturation-Promoting Factor..” *Cell* 73 (7) (July 2): 1393–1402.
Holt, Liam J, Brian B Tuch, Judit Villén, Alexander D Johnson, Steven P Gygi, and David O Morgan. 2009. “Global Analysis of Cdk1 Substrate Phosphorylation Sites Provides Insights Into Evolution..” *Science* 325 (5948) (September 25): 1682–1686. doi:10.1126/science.1172867.
Howell, B J, B F McEwen, J C Canman, D B Hoffman, E M Farrar, C L Rieder, and E D Salmon. 2001. “Cytoplasmic Dynein/Dynactin Drives Kinetochore Protein Transport to the Spindle Poles and Has a Role in Mitotic Spindle Checkpoint Inactivation..” *The Journal of Cell Biology* 155 (7) (December 24): 1159–1172. doi:10.1083/jcb.200105093.
Hoyt, M A, L Totis, and B T Roberts. 1991. “S. Cerevisiae Genes Required for Cell Cycle Arrest in Response to Loss of Microtubule Function..” *Cell* 66 (3) (August 9): 507–517.
Hwang, L H. 1998. “Budding Yeast Cdc20: a Target of the Spindle Checkpoint.” *Science* 279 (5353) (February 13): 1041–1044. doi:10.1126/science.279.5353.1041.
Irniger, S, S Piatti, C Michaelis, and K Nasmyth. 1995. “Genes Involved in Sister Chromatid Separation Are Needed for B-Type Cyclin Proteolysis in Budding Yeast..” *Cell* 81 (2) (April 21): 269–278.
Izawa, Daisuke, and Jonathon Pines. 2011. “How APC/C–Cdc20 Changes Its Substrate Specificity in Mitosis.” *Nature Publishing Group* 13 (3) (February 20): 223–233. doi:10.1038/ncb2165.
Janke, C, J Ortiz, J Lechner, A Shevchenko, A Shevchenko, M M Magiera, C Schramm, and E Schiebel. 2001. “The Budding Yeast Proteins Spc24p and Spc25p Interact with Ndc80p and Nuf2p at the Kinetochore and Are Important for Kinetochore Clustering and Checkpoint Control..” *The EMBO Journal* 20 (4) (February 15): 777–791. doi:10.1093/emboj/20.4.777.
Janke, Carsten, Maria M Magiera, Nicole Rathfelder, Christof Taxis, Simone Reber, Hiromi Maekawa, Alexandra Moreno-Borchart, et al. 2004. “A Versatile Toolbox for PCR-Based Tagging of Yeast Genes: New Fluorescent Proteins, More Markers and Promoter

Substitution Cassettes.." *Yeast (Chichester, England)* 21 (11) (August): 947–962. doi:10.1002/yea.1142.
Joglekar, Ajit P, Kerry Bloom, and E D Salmon. 2009. "In Vivo Protein Architecture of the Eukaryotic Kinetochore with Nanometer Scale Accuracy." *Current Biology* 19 (8) (April): 694–699. doi:10.1016/j.cub.2009.02.056.
Kari, B, N Lussenhop, R Goertz, M Wabuke-Bunoti, R Radeke, and R Gehrz. 1986. "Characterization of Monoclonal Antibodies Reactive to Several Biochemically Distinct Human Cytomegalovirus Glycoprotein Complexes.." *Journal of Virology* 60 (2) (November): 345–352.
Kemmler, Stefan, Manuel Stach, Maria Knapp, Jennifer Ortiz, Jens Pfannstiel, Thomas Ruppert, and Johannes Lechner. 2009. "Mimicking Ndc80 Phosphorylation Triggers Spindle Assembly Checkpoint Signalling.." *The EMBO Journal* 28 (8) (April 22): 1099–1110. doi:10.1038/emboj.2009.62.
Kettenbach, Arminja N, Tuobin Wang, Brendan K Faherty, Dean R Madden, Stefan Knapp, Chris Bailey-Kellogg, and Scott A Gerber. 2012. "Rapid Determination of Multiple Linear Kinase Substrate Motifs by Mass Spectrometry.." *Chemistry & Biology* 19 (5) (May 25): 608–618. doi:10.1016/j.chembiol.2012.04.011.
Kim, S H. 1998. "Fission Yeast Slp1: an Effector of the Mad2-Dependent Spindle Checkpoint." *Science* 279 (5353) (February 13): 1045–1047. doi:10.1126/science.279.5353.1045.
Kim, Soonjoung, Hongbin Sun, Diana R Tomchick, Hongtao Yu, and Xuelian Luo. 2012. "Structure of Human Mad1 C-Terminal Domain Reveals Its Involvement in Kinetochore Targeting.." *Proceedings of the National Academy of Sciences of the United States of America* 109 (17) (April 24): 6549–6554. doi:10.1073/pnas.1118210109.
Kimura, K, M Hirano, R Kobayashi, and T Hirano. 1998. "Phosphorylation and Activation of 13S Condensin by Cdc2 in Vitro.." *Science* 282 (5388) (October 16): 487–490.
King, R W, J M Peters, S Tugendreich, M Rolfe, P Hieter, and M W Kirschner. 1995. "A 20S Complex Containing CDC27 and CDC16 Catalyzes the Mitosis-Specific Conjugation of Ubiquitin to Cyclin B.." *Cell* 81 (2) (April 21): 279–288.
Kirschner, M W, and T Mitchison. 1986. "Microtubule Dynamics.." *Nature* 324 (6098) (November): 621. doi:10.1038/324621a0.
Kline, Susan L, Iain M Cheeseman, Tetsuya Hori, Tatsuo Fukagawa, and Arshad Desai. 2006. "The Human Mis12 Complex Is Required for Kinetochore Assembly and Proper Chromosome Segregation.." *The Journal of Cell Biology* 173 (1) (April 10): 9–17. doi:10.1083/jcb.200509158.
Kraft, Claudine, Franz Herzog, Christian Gieffers, Karl Mechtler, Anja Hagting, Jonathon Pines, and Jan-Michael Peters. 2003. "Mitotic Regulation of the Human Anaphase-Promoting Complex by Phosphorylation.." *The EMBO Journal* 22 (24) (December 15): 6598–6609. doi:10.1093/emboj/cdg627.
Kraft, Claudine, Hartmut C Vodermaier, Sebastian Maurer-Stroh, Frank Eisenhaber, and Jan-Michael Peters. 2005. "The WD40 Propeller Domain of Cdh1 Functions as a Destruction Box Receptor for APC/C Substrates.." *Molecular Cell* 18 (5) (May 27): 543–553. doi:10.1016/j.molcel.2005.04.023.
Kulukian, Anita, Joo Seok Han, and Don W Cleveland. 2009. "Unattached Kinetochores Catalyze Production of an Anaphase Inhibitor That Requires a Mad2 Template to Prime Cdc20 for BubR1 Binding.." *Developmental Cell* 16 (1) (January): 105–117. doi:10.1016/j.devcel.2008.11.005.

Kurasawa, Y, and K Todokoro. 1999. “Identification of Human APC10/Doc1 as a Subunit of Anaphase Promoting Complex..” *Oncogene* 18 (37) (September 16): 5131–5137. doi:10.1038/sj.onc.1203133.

Labit, Helene, Kazuyuki Fujimitsu, N Sumru Bayin, Tohru Takaki, Julian Gannon, and Hiroyuki Yamano. 2012. “Dephosphorylation of Cdc20 Is Required for Its C-Box-Dependent Activation of the APC/C..” *The EMBO Journal* 31 (15) (August 1): 3351–3362. doi:10.1038/emboj.2012.168.

Lechner, J, and J Carbon. 1991. “A 240 Kd Multisubunit Protein Complex, CBF3, Is a Major Component of the Budding Yeast Centromere..” *Cell* 64 (4) (February 22): 717–725.

Leverson, J D, C A Joazeiro, A M Page, H k Huang, P Hieter, and T Hunter. 2000. “The APC11 RING-H2 Finger Mediates E2-Dependent Ubiquitination..” *Molecular Biology of the Cell* 11 (7) (July): 2315–2325.

Li, R, and A W Murray. 1991. “Feedback Control of Mitosis in Budding Yeast..” *Cell* 66 (3) (August 9): 519–531.

Li, X, and R B Nicklas. 1995. “Mitotic Forces Control a Cell-Cycle Checkpoint..” *Nature* 373 (6515) (February 16): 630–632. doi:10.1038/373630a0.

Li, Yumei, Jeff Bachant, Annette A Alcasabas, Yanchang Wang, Jun Qin, and Stephen J Elledge. 2002. “The Mitotic Spindle Is Required for Loading of the DASH Complex Onto the Kinetochore..” *Genes & Development* 16 (2) (January 15): 183–197. doi:10.1101/gad.959402.

London, Nitobe, Steven Ceto, Jeffrey A Ranish, and Sue Biggins. 2012. “Phosphoregulation of Spc105 by Mps1 and PP1 Regulates Bub1 Localization to Kinetochores..” *Current Biology : CB* 22 (10) (May 22): 900–906. doi:10.1016/j.cub.2012.03.052.

Longtine, M S, A McKenzie, D J Demarini, N G Shah, A Wach, A Brachat, P Philippsen, and J R Pringle. 1998. “Additional Modules for Versatile and Economical PCR-Based Gene Deletion and Modification in Saccharomyces Cerevisiae..” *Yeast (Chichester, England)* 14 (10) (July): 953–961. doi:10.1002/(SICI)1097-0061(199807)14:10<953::AID-YEA293>3.0.CO;2-U.

Loog, Mart, and David O Morgan. 2005. “Cyclin Specificity in the Phosphorylation of Cyclin-Dependent Kinase Substrates..” *Nature* 434 (7029) (March 3): 104–108. doi:10.1038/nature03329.

Lou, Yang, Jianhui Yao, Arzhang Zereshki, Zhen Dou, Kashif Ahmed, Hongmei Wang, Junbin Hu, Yuzhen Wang, and Xuebiao Yao. 2004. “NEK2A Interacts with MAD1 and Possibly Functions as a Novel Integrator of the Spindle Checkpoint Signaling..” *The Journal of Biological Chemistry* 279 (19) (May 7): 20049–20057. doi:10.1074/jbc.M314205200.

Luo, Xuelian, Zhanyun Tang, Guohong Xia, Katja Wassmann, Tomohiro Matsumoto, Josep Rizo, and Hongtao Yu. 2004. “The Mad2 Spindle Checkpoint Protein Has Two Distinct Natively Folded States..” *Nature Structural & Molecular Biology* 11 (4) (April): 338–345. doi:10.1038/nsmb748.

Maldonado, Maria, and Tarun M Kapoor. 2011. “Constitutive Mad1 Targeting to Kinetochores Uncouples Checkpoint Signalling From Chromosome Biorientation..” *Nature Publishing Group* 13 (4) (April): 475–482. doi:10.1038/ncb2223.

Mansfeld, Jörg, Philippe Collin, Mark O Collins, Jyoti S Choudhary, and Jonathon Pines. 2011. “APC15 Drives the Turnover of MCC-CDC20 to Make the Spindle Assembly Checkpoint Responsive to Kinetochore Attachment..” *Nature Publishing Group* 13 (10) (October): 1234–1243. doi:10.1038/ncb2347.

Mapelli, Marina, and Andrea Musacchio. 2007. “MAD Contortions: Conformational Dimerization Boosts Spindle Checkpoint Signaling..” *Current Opinion in Structural Biology* 17 (6) (December): 716–725. doi:10.1016/j.sbi.2007.08.011.

Martin-Lluesma, Silvia, Volker M Stucke, and Erich A Nigg. 2002. “Role of Hec1 in Spindle Checkpoint Signaling and Kinetochore Recruitment of Mad1/Mad2..” *Science* 297 (5590) (September 27): 2267–2270. doi:10.1126/science.1075596.

Masumoto, H, H Masukata, Y Muro, N Nozaki, and T Okazaki. 1989. “A Human Centromere Antigen (CENP-B) Interacts with a Short Specific Sequence in Alphoid DNA, a Human Centromeric Satellite..” *The Journal of Cell Biology* 109 (5) (November): 1963–1973.

Matyskiela, Mary E, and David O Morgan. 2009. “Analysis of Activator-Binding Sites on the APC/C Supports a Cooperative Substrate-Binding Mechanism..” *Molecular Cell* 34 (1) (April 10): 68–80. doi:10.1016/j.molcel.2009.02.027.

McCleland, Mark L, Richard D Gardner, Marko J Kallio, John R Daum, Gary J Gorbsky, Daniel J Burke, and P Todd Stukenberg. 2003. “The Highly Conserved Ndc80 Complex Is Required for Kinetochore Assembly, Chromosome Congression, and Spindle Checkpoint Activity..” *Genes & Development* 17 (1) (January 1): 101–114. doi:10.1101/gad.1040903.

McIntosh, J Richard, Ekaterina L Grishchuk, Mary K Morphew, Artem K Efremov, Kirill Zhudenkov, Vladimir A Volkov, Iain M Cheeseman, Arshad Desai, David N Mastronarde, and Fazly I Ataullakhanov. 2008. “Fibrils Connect Microtubule Tips with Kinetochores: a Mechanism to Couple Tubulin Dynamics to Chromosome Motion..” *Cell* 135 (2) (October 17): 322–333. doi:10.1016/j.cell.2008.08.038.

Meluh, P B, and D Koshland. 1995. “Evidence That the MIF2 Gene of Saccharomyces Cerevisiae Encodes a Centromere Protein with Homology to the Mammalian Centromere Protein CENP-C..” *Molecular Biology of the Cell* 6 (7) (July): 793–807.

Meluh, P B, P Yang, L Glowczewski, D Koshland, and M M Smith. 1998. “Cse4p Is a Component of the Core Centromere of Saccharomyces Cerevisiae..” *Cell* 94 (5) (September 4): 607–613.

Michaelis, C, R Ciosk, and K Nasmyth. 1997. “Cohesins: Chromosomal Proteins That Prevent Premature Separation of Sister Chromatids..” *Cell* 91 (1) (October 3): 35–45.

Mimura, Satoru, Takashi Seki, Seiji Tanaka, and John F X Diffley. 2004. “Phosphorylation-Dependent Binding of Mitotic Cyclins to Cdc6 Contributes to DNA Replication Control..” *Nature* 431 (7012) (October 28): 1118–1123. doi:10.1038/nature03024.

Minshull, J, A Straight, A D Rudner, A F Dernburg, A Belmont, and A W Murray. 1996. “Protein Phosphatase 2A Regulates MPF Activity and Sister Chromatid Cohesion in Budding Yeast..” *Current Biology : CB* 6 (12) (December 1): 1609–1620.

Mitchison, T J. 1989. “Polewards Microtubule Flux in the Mitotic Spindle: Evidence From Photoactivation of Fluorescence..” *The Journal of Cell Biology* 109 (2) (August): 637–652.

Muro, Y, H Masumoto, K Yoda, N Nozaki, M Ohashi, and T Okazaki. 1992. “Centromere Protein B Assembles Human Centromeric Alpha-Satellite DNA at the 17-Bp Sequence, CENP-B Box..” *The Journal of Cell Biology* 116 (3) (February): 585–596.

Murphy, M R, D M Fowlkes, and M Fitzgerald-Hayes. 1991. “Analysis of Centromere Function in Saccharomyces Cerevisiae Using Synthetic Centromere Mutants..” *Chromosoma* 101 (3) (December): 189–197.

Murray, A W, and M W Kirschner. 1989. “Cyclin Synthesis Drives the Early Embryonic

Cell Cycle.." *Nature* 339 (6222) (May 25): 275–280. doi:10.1038/339275a0.
Murray, A W, M J Solomon, and M W Kirschner. 1989. "The Role of Cyclin Synthesis and Degradation in the Control of Maturation Promoting Factor Activity.." *Nature* 339 (6222) (May 25): 280–286. doi:10.1038/339280a0.
Musacchio, Andrea, and Edward D Salmon. 2007. "The Spindle-Assembly Checkpoint in Space and Time." *Nature Reviews Molecular Cell Biology* 8 (5) (April 11): 379–393. doi:10.1038/nrm2163.
Musacchio, Andrea, and Kevin G Hardwick. 2002. "The Spindle Checkpoint: Structural Insights Into Dynamic Signalling.." *Nature Reviews Molecular Cell Biology* 3 (10) (October): 731–741. doi:10.1038/nrm929.
Nasmyth, K. 1993. "Control of the Yeast Cell Cycle by the Cdc28 Protein Kinase.." *Current Opinion in Cell Biology* 5 (2) (April): 166–179.
Nekrasov, Vladimir S, Melanie A Smith, Sew Peak-Chew, and John V Kilmartin. 2003. "Interactions Between Centromere Complexes in Saccharomyces Cerevisiae.." *Molecular Biology of the Cell* 14 (12) (December): 4931–4946. doi:10.1091/mbc.E03-06-0419.
Nicklas, R B, and C A Koch. 1969. "Chromosome Micromanipulation. 3. Spindle Fiber Tension and the Reorientation of Mal-Oriented Chromosomes.." *The Journal of Cell Biology* 43 (1) (October): 40–50.
Ortiz, J, O Stemmann, S Rank, and J Lechner. 1999. "A Putative Protein Complex Consisting of Ctf19, Mcm21, and Okp1 Represents a Missing Link in the Budding Yeast Kinetochore.." *Genes & Development* 13 (9) (May 1): 1140–1155.
Osborne, M A, G Schlenstedt, T Jinks, and P A Silver. 1994. "Nuf2, a Spindle Pole Body-Associated Protein Required for Nuclear Division in Yeast.." *The Journal of Cell Biology* 125 (4) (May): 853–866.
Pagliuca, Cinzia, Viji M Draviam, Eugenio Marco, Peter K Sorger, and Peter De Wulf. 2009. "Roles for the Conserved Spc105p/Kre28p Complex in Kinetochore-Microtubule Binding and the Spindle Assembly Checkpoint.." *PLoS ONE* 4 (10): e7640. doi:10.1371/journal.pone.0007640.
Pagliuca, Felicia Walton, Mark O Collins, Agata Lichawska, Philip Zegerman, Jyoti S Choudhary, and Jonathon Pines. 2011. "Quantitative Proteomics Reveals the Basis for the Biochemical Specificity of the Cell-Cycle Machinery.." *Molecular Cell* 43 (3) (August 5): 406–417. doi:10.1016/j.molcel.2011.05.031.
Palframan, William J, Janet B Meehl, Sue L Jaspersen, Mark Winey, and Andrew W Murray. 2006. "Anaphase Inactivation of the Spindle Checkpoint.." *Science* 313 (5787) (August 4): 680–684. doi:10.1126/science.1127205.
Passmore, Lori A, Christopher R Booth, Catherine Vénien-Bryan, Steven J Ludtke, Céline Fioretto, Louise N Johnson, Wah Chiu, and David Barford. 2005. "Structural Analysis of the Anaphase-Promoting Complex Reveals Multiple Active Sites and Insights Into Polyubiquitylation.." *Molecular Cell* 20 (6) (December 22): 855–866. doi:10.1016/j.molcel.2005.11.003.
Perez de Castro, I, G de Carcer, and M Malumbres. 2006. "A Census of Mitotic Cancer Genes: New Insights Into Tumor Cell Biology and Cancer Therapy." *Carcinogenesis* 28 (5) (November 27): 899–912. doi:10.1093/carcin/bgm019.
Peters, J M, R W King, C Höög, and M W Kirschner. 1996. "Identification of BIME as a Subunit of the Anaphase-Promoting Complex.." *Science* 274 (5290) (November 15): 1199–1201.

Peters, Jan-Michael. 2006. “The Anaphase Promoting Complex/Cyclosome: a Machine Designed to Destroy.” *Nature Reviews Molecular Cell Biology* 7 (9) (August 9): 644–656. doi:10.1038/nrm1988.

Pfleger, C M, and M W Kirschner. 2000. “The KEN Box: an APC Recognition Signal Distinct From the D Box Targeted by Cdh1..” *Genes & Development* 14 (6) (March 15): 655–665.

Pfleger, C M, E Lee, and M W Kirschner. 2001. “Substrate Recognition by the Cdc20 and Cdh1 Components of the Anaphase-Promoting Complex..” *Genes & Development* 15 (18) (September 15): 2396–2407. doi:10.1101/gad.918201.

Pinsky, Benjamin A, Charles Kung, Kevan M Shokat, and Sue Biggins. 2006. “The Ipl1-Aurora Protein Kinase Activates the Spindle Checkpoint by Creating Unattached Kinetochores..” *Nature Cell Biology* 8 (1) (January): 78–83. doi:10.1038/ncb1341.

Pinsky, Benjamin A, Christian R Nelson, and Sue Biggins. 2009. “Protein Phosphatase 1 Regulates Exit From the Spindle Checkpoint in Budding Yeast..” *Current Biology : CB* 19 (14) (July 28): 1182–1187. doi:10.1016/j.cub.2009.06.043.

Pinsky, Benjamin A, Sean Y Tatsutani, Kimberly A Collins, and Sue Biggins. 2003. “An Mtw1 Complex Promotes Kinetochore Biorientation That Is Monitored by the Ipl1/Aurora Protein Kinase..” *Developmental Cell* 5 (5) (November): 735–745.

Powers, Andrew F, Andrew D Franck, Daniel R Gestaut, Jeremy Cooper, Beth Gracyzk, Ronnie R Wei, Linda Wordeman, Trisha N Davis, and Charles L Asbury. 2009. “The Ndc80 Kinetochore Complex Forms Load-Bearing Attachments to Dynamic Microtubule Tips via Biased Diffusion..” *Cell* 136 (5) (March 6): 865–875. doi:10.1016/j.cell.2008.12.045.

Rahal, Rami, and Angelika Amon. 2008. “Mitotic CDKs Control the Metaphase-Anaphase Transition and Trigger Spindle Elongation..” *Genes & Development* 22 (11) (June 1): 1534–1548. doi:10.1101/gad.1638308.

Rajagopalan, Harith, Martin A Nowak, Bert Vogelstein, and Christoph Lengauer. 2003. “The Significance of Unstable Chromosomes in Colorectal Cancer..” *Nature Reviews. Cancer* 3 (9) (September): 695–701. doi:10.1038/nrc1165.

Rieder, C L, A Schultz, R Cole, and G Sluder. 1994. “Anaphase Onset in Vertebrate Somatic Cells Is Controlled by a Checkpoint That Monitors Sister Kinetochore Attachment to the Spindle..” *The Journal of Cell Biology* 127 (5) (December): 1301–1310.

Rieder, C L, and E D Salmon. 1994. “Motile Kinetochores and Polar Ejection Forces Dictate Chromosome Position on the Vertebrate Mitotic Spindle..” *The Journal of Cell Biology* 124 (3) (February): 223–233.

Rischitor, Patricia E, Karen M May, and Kevin G HardwickDaniel Lew to. 2007. “Bub1 Is a Fission Yeast Kinetochore Scaffold Protein, and Is Sufficient to Recruit Other Spindle Checkpoint Proteins to Ectopic Sites on Chromosomes.” Daniel LewEd. *PLoS ONE* 2 (12) (December 19): e1342. doi:10.1371/journal.pone.0001342.t001.

Roberts, B T, K A Farr, and M A Hoyt. 1994. “The Saccharomyces Cerevisiae Checkpoint Gene BUB1 Encodes a Novel Protein Kinase..” *Molecular and Cellular Biology* 14 (12) (December): 8282–8291.

Rudner, A D, and A W Murray. 2000. “Phosphorylation by Cdc28 Activates the Cdc20-Dependent Activity of the Anaphase-Promoting Complex..” *The Journal of Cell Biology* 149 (7) (June 26): 1377–1390.

Rudner, A D, K G Hardwick, and A W Murray. 2000. “Cdc28 Activates Exit From Mitosis in Budding Yeast..” *The Journal of Cell Biology* 149 (7) (June 26): 1361–1376.

Santaguida, Stefano, and Andrea Musacchio. 2009. “The Life and Miracles of Kinetochores” 28 (17) (July 23): 2511–2531. doi:10.1038/emboj.2009.173.
Schreiber, Anne, Florian Stengel, Ziguo Zhang, Radoslav I Enchev, Eric H Kong, Edward P Morris, Carol V Robinson, Paula C A da Fonseca, and David Barford. 2011. “Structural Basis for the Subunit Assembly of the Anaphase-Promoting Complex..” *Nature* 470 (7333) (February 10): 227–232. doi:10.1038/nature09756.
Schwab, M, A S Lutum, and W Seufert. 1997. “Yeast Hct1 Is a Regulator of Clb2 Cyclin Proteolysis..” *Cell* 90 (4) (August 22): 683–693.
Schweitzer, B, and P Philippsen. 1992. “NPK1, a Nonessential Protein Kinase Gene in Saccharomyces Cerevisiae with Similarity to Aspergillus Nidulans nimA..” *Molecular & General Genetics : MGG* 234 (1) (July): 164–167.
Sharp-Baker, H, and R H Chen. 2001. “Spindle Checkpoint Protein Bub1 Is Required for Kinetochore Localization of Mad1, Mad2, Bub3, and CENP-E, Independently of Its Kinase Activity..” *The Journal of Cell Biology* 153 (6) (June 11): 1239–1250.
Sherman, Fred, Gerald R. Fink, and Christopher W LawrenceCold Spring Harbor to. *Methods in Yeast Genetics*. Cold Spring HarborEd. 1974th ed. Cold Spring Harbor Laboratory of Quantitative Biology. Cold Spring Harbour.
Shirayama, M, A Toth, M Galova, and K Nasmyth. 1999. “APC(Cdc20) Promotes Exit From Mitosis by Destroying the Anaphase Inhibitor Pds1 and Cyclin Clb5..” *Nature* 402 (6758) (November 11): 203–207. doi:10.1038/46080.
Shteinberg, M, Y Protopopov, T Listovsky, M Brandeis, and A Hershko. 1999. “Phosphorylation of the Cyclosome Is Required for Its Stimulation by Fizzy/Cdc20..” *Biochemical and Biophysical Research Communications* 260 (1) (June 24): 193–198. doi:10.1006/bbrc.1999.0884.
Sikorski, R S, and P Hieter. 1989. “A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces Cerevisiae..” *Genetics* 122 (1) (May): 19–27.
Sikorski, R S, M S Boguski, M Goebl, and P Hieter. 1990. “A Repeating Amino Acid Motif in CDC23 Defines a Family of Proteins and a New Relationship Among Genes Required for Mitosis and RNA Synthesis..” *Cell* 60 (2) (January 26): 307–317.
Skibbens, R V, V P Skeen, and E D Salmon. 1993. “Directional Instability of Kinetochore Motility During Chromosome Congression and Segregation in Mitotic Newt Lung Cells: a Push-Pull Mechanism..” *The Journal of Cell Biology* 122 (4) (August): 859–875.
Stern, B M, and A W Murray. 2001. “Lack of Tension at Kinetochores Activates the Spindle Checkpoint in Budding Yeast..” *Current Biology : CB* 11 (18) (September 18): 1462–1467.
Sudakin, V. 2001. “Checkpoint Inhibition of the APC/C in HeLa Cells Is Mediated by a Complex of BUBR1, BUB3, CDC20, and MAD2.” *The Journal of Cell Biology* 154 (5) (September 3): 925–936. doi:10.1083/jcb.200102093.
Sudakin, V, D Ganoth, A Dahan, H Heller, J Hershko, F C Luca, J V Ruderman, and A Hershko. 1995. “The Cyclosome, a Large Complex Containing Cyclin-Selective Ubiquitin Ligase Activity, Targets Cyclins for Destruction at the End of Mitosis..” *Molecular Biology of the Cell* 6 (2) (February): 185–197.
Tanaka, Kozo, Etsushi Kitamura, Yoko Kitamura, and Tomoyuki U Tanaka. 2007. “Molecular Mechanisms of Microtubule-Dependent Kinetochore Transport Toward Spindle Poles..” *The Journal of Cell Biology* 178 (2) (July 16): 269–281. doi:10.1083/jcb.200702141.

Tanaka, Kozo, Naomi Mukae, Hilary Dewar, Mark van Breugel, Euan K James, Alan R Prescott, Claude Antony, and Tomoyuki U Tanaka. 2005. "Molecular Mechanisms of Kinetochore Capture by Spindle Microtubules.." *Nature* 434 (7036) (April 21): 987–994. doi:10.1038/nature03483.

Tang, Zhanyun, Hongjun Shu, Dilhan Oncel, She Chen, and Hongtao Yu. 2004. "Phosphorylation of Cdc20 by Bub1 Provides a Catalytic Mechanism for APC/C Inhibition by the Spindle Checkpoint.." *Molecular Cell* 16 (3) (November 5): 387–397. doi:10.1016/j.molcel.2004.09.031.

Thornton, Brian R, and David P Toczyski. 2003. "Securin and B-Cyclin/CDK Are the Only Essential Targets of the APC.." *Nature Cell Biology* 5 (12) (December): 1090–1094. doi:10.1038/ncb1066.

Thornton, Brian R, Tessie M Ng, Mary E Matyskiela, Christopher W Carroll, David O Morgan, and David P Toczyski. 2006. "An Architectural Map of the Anaphase-Promoting Complex.." *Genes & Development* 20 (4) (February 15): 449–460. doi:10.1101/gad.1396906.

Tipton, Aaron R, Michael Tipton, Tim Yen, and Song-Tao Liu. 2011. "Closed MAD2 (C-MAD2) Is Selectively Incorporated Into the Mitotic Checkpoint Complex (MCC).." *Cell Cycle (Georgetown, Tex.)* 10 (21) (November 1): 3740–3750. doi:10.4161/cc.10.21.17919.

Tugendreich, S, J Tomkiel, W Earnshaw, and P Hieter. 1995. "CDC27Hs Colocalizes with CDC16Hs to the Centrosome and Mitotic Spindle and Is Essential for the Metaphase to Anaphase Transition.." *Cell* 81 (2) (April 21): 261–268.

Uhlmann, F, D Wernic, M A Poupart, E V Koonin, and K Nasmyth. 2000. "Cleavage of Cohesin by the CD Clan Protease Separin Triggers Anaphase in Yeast.." *Cell* 103 (3) (October 27): 375–386.

Uhlmann, F, F Lottspeich, and K Nasmyth. 1999. "Sister-Chromatid Separation at Anaphase Onset Is Promoted by Cleavage of the Cohesin Subunit Scc1.." *Nature* 400 (6739) (July 1): 37–42. doi:10.1038/21831.

Uzunova, Kristina, Billy T Dye, Hannelore Schutz, Rene Ladurner, Georg Petzold, Yusuke Toyoda, Marc A Jarvis, et al. 2012. "APC15 Mediates CDC20 Autoubiquitylation by APC/C(MCC) and Disassembly of the Mitotic Checkpoint Complex.." *Nature Structural & Molecular Biology* (September 24). doi:10.1038/nsmb.2412.

VANOOSTHUYSE, V, and K G Hardwick. 2009. "Overcoming Inhibition in the Spindle Checkpoint." *Genes & Development* 23 (24) (December 15): 2799–2805. doi:10.1101/gad.1882109.

Visintin, R, S Prinz, and A Amon. 1997. "CDC20 and CDH1: a Family of Substrate-Specific Activators of APC-Dependent Proteolysis.." *Science* 278 (5337) (October 17): 460–463.

Wang, Hong-Wei, Sydney Long, Claudio Ciferri, Stefan Westermann, David Drubin, Georjana Barnes, and Eva Nogales. 2008. "Architecture and Flexibility of the Yeast Ndc80 Kinetochore Complex.." *Journal of Molecular Biology* 383 (4) (November 21): 894–903. doi:10.1016/j.jmb.2008.08.077.

Wei, Randy, Bryan Ngo, Guikai Wu, and Wen-Hwa Lee. 2011. "Phosphorylation of the Ndc80 Complex Protein, HEC1, by Nek2 Kinase Modulates Chromosome Alignment and Signaling of the Spindle Assembly Checkpoint.." *Molecular Biology of the Cell* 22 (19) (October): 3584–3594. doi:10.1091/mbc.E11-01-0012.

Weiss, E, and M Winey. 1996. "The Saccharomyces Cerevisiae Spindle Pole Body Duplication Gene MPS1 Is Part of a Mitotic Checkpoint.." *The Journal of Cell Biology*

132 (1-2) (January): 111–123.
Westermann, S. 2003. “Architecture of the Budding Yeast Kinetochore Reveals a Conserved Molecular Core.” *The Journal of Cell Biology* 163 (2) (October 20): 215–222. doi:10.1083/jcb.200305100.
Westermann, Stefan, Agustin Avila-Sakar, Hong-Wei Wang, Hanspeter Niederstrasser, Jonathan Wong, David G Drubin, Eva Nogales, and Georjana Barnes. 2005. “Formation of a Dynamic Kinetochore- Microtubule Interface Through Assembly of the Dam1 Ring Complex..” *Molecular Cell* 17 (2) (January 21): 277–290. doi:10.1016/j.molcel.2004.12.019.
Wigge, P A, and J V Kilmartin. 2001. “The Ndc80p Complex From Saccharomyces Cerevisiae Contains Conserved Centromere Components and Has a Function in Chromosome Segregation..” *The Journal of Cell Biology* 152 (2) (January 22): 349–360.
Wigge, P A, O N Jensen, S Holmes, S Souès, M Mann, and J V Kilmartin. 1998. “Analysis of the Saccharomyces Spindle Pole by Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry..” *The Journal of Cell Biology* 141 (4) (May 18): 967–977.
Winey, M, C L Mamay, E T O'Toole, D N Mastronarde, T H Giddings, K L McDonald, and J R McIntosh. 1995. “Three-Dimensional Ultrastructural Analysis of the Saccharomyces Cerevisiae Mitotic Spindle..” *The Journal of Cell Biology* 129 (6) (June): 1601–1615.
Yoon, Hyun-Joo, Anna Feoktistova, Benjamin A Wolfe, Jennifer L Jennings, Andrew J Link, and Kathleen L Gould. 2002. “Proteomics Analysis Identifies New Components of the Fission and Budding Yeast Anaphase-Promoting Complexes..” *Current Biology : CB* 12 (23) (December 10): 2048–2054.
Yu, H, J M Peters, R W King, A M Page, P Hieter, and M W Kirschner. 1998. “Identification of a Cullin Homology Region in a Subunit of the Anaphase-Promoting Complex..” *Science* 279 (5354) (February 20): 1219–1222.
Zachariae, W, A Shevchenko, P D Andrews, R Ciosk, M Galova, M J Stark, M Mann, and K Nasmyth. 1998. “Mass Spectrometric Analysis of the Anaphase-Promoting Complex From Yeast: Identification of a Subunit Related to Cullins..” *Science* 279 (5354) (February 20): 1216–1219.
Zachariae, W, and K Nasmyth. 1996. “TPR Proteins Required for Anaphase Progression Mediate Ubiquitination of Mitotic B-Type Cyclins in Yeast..” *Molecular Biology of the Cell* 7 (5) (May): 791–801.
Zachariae, W, and K Nasmyth. 1999. “Whose End Is Destruction: Cell Division and the Anaphase-Promoting Complex..” *Genes & Development* 13 (16) (August 15): 2039–2058.
Zegerman, Philip, and John F X Diffley. 2007. “Phosphorylation of Sld2 and Sld3 by Cyclin-Dependent Kinases Promotes DNA Replication in Budding Yeast..” *Nature* 445 (7125) (January 18): 281–285. doi:10.1038/nature05432.
Zheng, L, Y Chen, and W H Lee. 1999. “Hec1p, an Evolutionarily Conserved Coiled-Coil Protein, Modulates Chromosome Segregation Through Interaction with SMC Proteins..” *Molecular and Cellular Biology* 19 (8) (August): 5417–5428.
Zich, Judith, Alicja M Sochaj, Heather M Syred, Laura Milne, Atlanta G Cook, Hiro Ohkura, Juri Rappsilber, and Kevin G Hardwick. 2012. “Kinase Activity of Fission Yeast Mph1 Is Required for Mad2 and Mad3 to Stably Bind the Anaphase Promoting Complex..” *Current Biology : CB* 22 (4) (February 21): 296–301. doi:10.1016/j.cub.2011.12.049.

Appendix A

Table 1: Strains used in this study

Strain	Relevant Genotype	Ref
*ADR4006	MAT**a** ura3-1 leu2-3,112 trp1-1 his3-11 ade2-1 can1-100 bar1Δ	Rudner lab strain collection
ADR4989	*MAT**a** MAD1-TAP-klHIS3 bar1Δ*	This study
ADR4983	*MAT**a** MAD2-TAP-klHIS3 bar1Δ*	This study
ADR4995	*MAT**a** MAD3-TAP-klHIS3 bar1Δ*	This study
ADR5004	*MAT**a** BUB1-TAP-klHIS3 bar1Δ*	This study
ADR5000	*MAT**a** BUB3-TAP-klHIS3 bar1Δ*	This study
ADR54	*MAT**a** mad1Δ::HIS3*	(Hardwick and Murray 1995)
ADR6568	*MAT**a** mad2Δ-URA3 bar1Δ*	This study
Strains used in GAL overexpression studdies:		
ADR6444	*MAT**a** pGAL-NDC80-LEU2 bar1Δ*	This study
ADR6644	*MAT**a** pGAL-NUF2-URA bar1Δ*	This study
ADR6440	*MAT**a** pGAL-SPC24-LEU2 bar1Δ*	This study
ADR6442	*MAT**a** pGAL-SPC25-LEU2 bar1Δ*	This study
ADR6569	*MAT**a** pGAL-NDC80-LEU2 mad2Δ-URA3 bar1Δ*	This study
ADR853	*MAT**a** pGAL-MPS1*	(Hardwick et al. 1996)
ADR6931	*MAT**a** pGAL-MPS1 mad2Δ*	This study
Strains used in purifications:		
ADR6133	*MAT**a** NDC80-myc9X-HIS bar1Δ*	This study
ADR6194	*MAT**a** NDC80-myc9X-KANR MAD1-TAP-HIS3 bar1Δ*	This study
ADR6570	*MAT**a** BUB1-TAP-HIS3 NDC80-myc9x-KANR bar1Δ*	This study
ADR6759	*MAT**a** pGAL-NDC80-LEU MAD1-TAP-HIS3*	This study
ADR6763	*MAT**a** mif2-3 MAD1-TAP-HIS3 NDC80-myc9x-KANR bar1Δ*	This study
ADR6766	*MAT**a** spc24-1 NDC80-myc9x-KANR MAD1-TAP-HIS3 bar1Δ*	This study

ADR6725	*MAT**a** ask1-2 MAD1-TAP-HIS3 NDC80-myc9x-KANR bar1Δ*	This study
ADR6727	*MAT**a** nuf2-61 MAD1-TAP-HIS3 NDC80-myc9x-KANR bar1Δ*	This study
ADR6768	*MAT**a** ndc10-1 MAD1-TAP-HIS3 NDC80-myc9x-KANR*	This study
ADR852	*MAT**a** mad2Δ-URA3*	Rudner lab strain collection
ADR5771	*MAT**a** LYS2 ndc10-1 bar1+ (gift from Sue Biggins)*	(Hartwell et al. 1974)
ADR5773	*MAT**a** nuf2-61 bar1Δ (gift from Sue Biggins)*	(Osborne et al. 1994)
ADR5774	*MAT**a** spc24-1 bar1Δ (gift from Sue Biggins)*	(Janke et al. 2001)
ADR5775	*MAT**a** LYS2+ mif2-3 bar1Δ (gift from Sue Biggins)*	(Brown, Goetsch, and Hartwell 1993)
ADR5776	*MAT**a** pCUP1-GFP-lacI12-HIS3 256lacO-TRP ask1-2 bar1+ (gift from Sue Biggins)*	(Y. Li et al. 2002)
ADR4912	*MAT**a** LYS2+ MAD2-TAP-HIS3(sp)mx6 (gift from Kristin Baetz)*	(Ghaemmaghami et al. 2003)
ADR4913	*MAT**a** LYS2+ MAD1-TAP-HIS3(sp)mx6 (gift from Kristin Baetz)*	(Ghaemmaghami et al. 2003)
ADR4914	*MAT**a** LYS2+ MAD3-TAP-HIS3(sp)mx6 (gift from Kristin Baetz)*	(Ghaemmaghami et al. 2003)
ADR4915	*MAT**a** LYS2+ BUB3-TAP-HIS3(sp)mx6 (gift from Kristin Baetz)*	(Ghaemmaghami et al. 2003)
ADR4916	*MAT**a** LYS2+ BUB1-TAP-HIS3(sp)mx6 (gift from Kristin Baetz)*	(Ghaemmaghami et al. 2003)
ADR6571	*MAT**α** MAD1-TAP-HIS3 NDC80-myc9X-KANR bar1Δ*	This study
ADR6572	*MAT**α** MAD1-TAP-HIS3 NDC80-myc9X-KANR bar1Δ*	This study
ADR6573	*MAT**α** NDC80-myc9X-KANR bar1Δ*	This study
ADR6574	*MAT**α** NDC80-myc9X-KANR bar1Δ*	This study
ADR6575	*MAT**α** MAD1-TAP-HIS3*	This study
ADR6576	*MAT**α** MAD1-TAP-HIS3*	This study
ADR5459	*MAT**a** pGAL-MPS1-myc-URA3 cdc15-2 CDC16-TAP-klHIS3 bar1Δ*	This study

*All strains used in this study have a W303 background with ADR4006 auxotrophies unless they came from the TAP collection: (Ghaemmaghami et al. 2003)

Table 4: Plasmids used in this study

Plasmid	Original name	Description	Ref
pAR123	pRS305	*pGAL1, LEU (integrating)*	(Sikorski and Hieter 1989)
pAR63	pDK20	*pGAL1, URA (integrating)*	Rudner lab plasmid collection
pAR1078		*pGAL-NDC80-LEU (integrating)*	This study
pAR1079		*pGAL-SPC24-LEU (integrating)*	This study
pAR1080		*pGAL-SPC25-LEU (integrating)*	This study
pAR1096		*pGAL-NUF2-URA3 (integrating)*	This study
	pYM18	*myc9x-KANr*	
pAR946	PRC10.1	*mad2 disruption with URA (integrating)*	(R. H. Chen et al. 1999)
pAR356	pRS314	*TRP-CEN-ARS*	(Sikorski and Hieter 1989)
	pFA6a-kanMX6		(Longtine et al. 1998)
	pFA6a-HIS3MX6		(Longtine et al. 1998)

Table 5: Primers used in this study

Primer	name	Sequence
Primers used for epitope tagging:		
683	Mad2-US	ACAGACGCGGATGCTAAAGT
684	Mad2-DS	TCCACCCCTCAAATACAACA
685	Mad2-US-check	CCTTTCTGCCCGAACTAACA
686	Mad2-DS-check	AGGATGCAAGATCCGAATTG
706	MAD1-US	CGTGCTTTTTGGCAACAATA
707	MAD1-US-check	TGGGTGGAAGATAGAGGTCAA
708	MAD1-DS	AGTGCATTTATGCCCCCTTA
709	MAD1-DS-check	TGATGGAGAGGACAGCAACA
710	Mad3-US	AAAGGGACGAAGCACTGAGA
711	Mad3-US-Check	CCCTCAAACCAGGGAAGAAT
712	Mad3-DS	CGTTAATCGGACAAACGTTCA
713	Mad3-DS-check	GCCTCGAGGTTAAACTTCATT
714	Bub1-US	AATAGCGGTCAAGCTTCCAA
715	Bub1-US-check	GAACAGCACGCAGAAAATCA
716	Bub1-DS	GCGGTAACAAGTGGAGCAAT
717	Bub1-DS-check	TACTGCGAATTGCGAATCAA
718	Bub3-US	CCTACAAACCCGCAAGAAAA
719	Bub3-US-check	TCCTATACACGGCTGGCTCT
720	Bub3-DS	GTGAGGAATTTGGGAGCAGA
721	Bub3-DS-check	AAATGCAGCGTTACCTTGTTG
987	NDC80-plasmid Fwd	ACGAAATTTGGAGTTTGAAACTGAACATAACGTAACAAATCGT ACGCTGCAGGTCGAC
699	NDC80-GFPRev	TTGCTGTAGATTGCTCGGGTATTATATATCATTTATTTTATCGAT GAATTCGAGCTCG
Primers used for plasmid construction:		
1025	Ndc80 US with Xho1	gcc ctcgag acc ATGCAAAGCTCAACAAGTAC
1037	Ndc80 DS with Not1	AGAAT GCggccgc TTAATTTGTTACGTTATGTT
1028	Spc24 US with Xho1	gcc ctcgag acc ATGTCACAAAAGGATAACCT
1029	Spc24 DS with Not1	AGAAT GCggccgc TCACTTTCCTAATCTTTCCC
1031	Spc25 US with Xho1	gcc ctcgag acc ATGGCCAGCATAGACGCATT
1032	Spc25 DS with Not1	AGAAT GCggccgc TTATAAAGATGCCAGAAGCA
1163	Nuf2 US with Sal1	gcc acgcgtcgac acc ATGAGTAGGAATCAAGAT
1164	Nuf2 DS with BamH1	CGCGGATCC CTATTGCATATATTCGAG

Table 6. Mass spectrometry raw data

Purification of: Mad1-TAP protein	total peptides	unique peptides	total peptides in control
sp|P40958|MAD2_YEAST	17	16	
sp|P40457|MLP2_YEAST	9	9	
sp|Q02455|MLP1_YEAST	8	8	
sp|P02557|TBB_YEAST	5	5	
sp|P38198|STU1_YEAST	4	4	
sp|P34077|NIC96_YEAST	3	3	
sp|P48837|NUP57_YEAST	3	3	
sp|P41695|BUB1_YEAST	3	3	
sp|P35719|MRP8_YEAST	3	3	
sp|P50095|IMDH3_YEAST	3	3	
sp|P36008|EF1G2_YEAST	2	2	
sp|Q06678|RM35_YEAST	2	2	
sp|P06704|CDC31_YEAST	2	2	
sp|Q04491|SEC13_YEAST	2	2	
sp|P38809|YHP7_YEAST	2	2	
sp|Q99257|MEX67_YEAST	2	2	
sp|P40513|MAM33_YEAST	1	1	
sp|P53622|COPA_YEAST	1	1	
sp|P45978|SCD6_YEAST	1	1	
sp|Q12087|RS30_YEAST	1	1	
sp|P38712|RRP3_YEAST	1	1	
sp|P46655|SYEC_YEAST	1	1	
sp|P53011|SEH1_YEAST	1	1	
sp|Q02821|IMA1_YEAST	1	1	
sp|P20484|MAK11_YEAST	1	1	
sp|P47035|NET1_YEAST	1	1	
sp|Q02931|UTP17_YEAST	1	1	
sp|P38833|YHS7_YEAST	1	1	
sp|Q12213|RL7B_YEAST	1	1	
sp|P25443|RS2_YEAST	1	1	
sp|P40010|NUG1_YEAST	1	1	
sp|P38879|NACA_YEAST	1	1	
sp|Q03640|TCB3_YEAST	1	1	
sp|P27614|CBPS_YEAST	1	1	
sp|P35191|MDJ1_YEAST	1	1	
sp|Q06287|EMG1_YEAST	1	1	
sp|P16862|K6PF2_YEAST	1	1	
sp|P38249|EIF3A_YEAST	1	1	
sp|P07283|PMM_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P26449|BUB3_YEAST	1	1	
sp|P14742|GFA1_YEAST	1	1	
sp|P41940|MPG1_YEAST	1	1	
sp|P50094|IMDH4_YEAST	1	1	
##sp|P32660|ATC5_YEAST	1	1	
sp|P32861|UGPA1_YEAST	1	1	
sp|P16861|K6PF1_YEAST	5	5	1
sp|P40957|MAD1_YEAST	69	56	19
sp|P04911|H2A1_YEAST	3	3	1
sp|P53297|PBP1_YEAST	3	3	1
sp|P10081|IF4A_YEAST	3	3	1
sp|P14907|NSP1_YEAST	3	3	1
sp|P22138|RPA2_YEAST	5	5	2
sp|P25567|SRO9_YEAST	5	4	2
sp|P53914|KRE33_YEAST	4	4	2
sp|P40991|NOP2_YEAST	4	4	2
sp|Q01080|RPA49_YEAST	4	4	2
sp|P38828|LSM12_YEAST	2	2	1
sp|P32495|NHP2_YEAST	2	2	1
sp|P22147|XRN1_YEAST	2	2	1
sp|P38922|HRB1_YEAST	2	2	1
sp|Q03940|RUVB1_YEAST	2	2	1
sp|P28007|GAR1_YEAST	2	1	1
sp|P04147|PABP_YEAST	16	14	9
sp|P02309|H4_YEAST	5	5	3
sp|P15108|HSC82_YEAST	5	5	3
sp|P20447|DBP3_YEAST	8	8	5
sp|P21576|VPS1_YEAST	8	8	5
sp|P15424|MS116_YEAST	9	9	6
sp|P40850|MKT1_YEAST	3	3	2

sp|P16140|VATB_YEAST	3	3	2
sp|P26785|RL16B_YEAST	3	3	2
sp|P00360|G3P1_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|Q01560|NOP3_YEAST	3	3	2
sp|P04449|RL24A_YEAST	3	3	2
sp|P07259|PYR1_YEAST	10	10	7
sp|P0C2I0|RL20_YEAST	8	8	6
sp|P24784|DBP1_YEAST	4	4	3
sp|P21304|PWP1_YEAST	4	4	3
sp|P26786|RS7A_YEAST	4	4	3
sp|P0C2H6|RL27A_YEAST	5	5	4
sp|P32481|IF2G_YEAST	6	6	5
sp|P10592|HSP72_YEAST	6	6	5
sp|P38631|FKS1_YEAST	24	23	23
sp|P11484|HSP75_YEAST	19	16	19
sp|P05750|RS3_YEAST	9	9	9
sp|P15646|FBRL_YEAST	7	6	7
sp|P02365|RS6_YEAST	6	6	6
sp|Q12159|YRA1_YEAST	6	6	6
sp|P00549|KPYK1_YEAST	6	6	6
sp|P05745|RL36A_YEAST	5	5	5
sp|P05317|RLA0_YEAST	5	5	5
sp|P02407|RS17A_YEAST	5	5	5
sp|P0C0W1|RS22A_YEAST	5	4	5
sp|P26782|RS24_YEAST	4	4	4
sp|P60010|ACT_YEAST	4	4	4
sp|P16474|GRP78_YEAST	4	4	4
sp|P05740|RL17A_YEAST	4	3	4
sp|P02400|RLA4_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P07245|C1TC_YEAST	3	3	3
sp|P00330|ADH1_YEAST	3	3	3
sp|P53252|PIL1_YEAST	3	3	3
sp|P05754|RS8_YEAST	3	3	3
sp|Q12230|LSP1_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|P17076|RL8A_YEAST	3	3	3
sp|P04451|RL23_YEAST	3	3	3
sp|Q02486|ABF2_YEAST	2	2	2
sp|P39730|IF2P_YEAST	2	2	2
sp|P50109|PSP2_YEAST	2	2	2
sp|Q04373|PUF6_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2
sp|O14467|MBF1_YEAST	2	2	2
sp|P0C0W9|RL11A_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|P05319|RLA2_YEAST	2	2	2
sp|P0C2H8|RL31A_YEAST	2	2	2
sp|Q03973|HMO1_YEAST	2	2	2
sp|Q06506|RRP9_YEAST	2	2	2
sp|P0C0V8|RS21A_YEAST	2	1	2
sp|P26755|RFA3_YEAST	2	1	2
sp|Q06511|RRP15_YEAST	1	1	1
sp|P19454|CSK22_YEAST	1	1	1
sp|P38711|RS27B_YEAST	1	1	1
sp|P32468|CDC12_YEAST	1	1	1
sp|P33201|MRT4_YEAST	1	1	1
sp|Q3E792|RS25A_YEAST	1	1	1
sp|P36160|RPF2_YEAST	1	1	1
sp|P46669|RPA43_YEAST	1	1	1
sp|P25342|CDC10_YEAST	1	1	1
sp|P33322|CBF5_YEAST	1	1	1
sp|P39938|RS26A_YEAST	1	1	1
sp|P37263|YC16_YEAST	1	1	1
sp|P53163|MNP1_YEAST	1	1	1
sp|Q04867|YM91_YEAST	1	1	1
sp|Q07623|NOP6_YEAST	1	1	1
sp|P05735|RL19_YEAST	1	1	1
sp|P41056|RL33B_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P07260|IF4E_YEAST	1	1	1
sp|P32529|RPA12_YEAST	1	1	1

sp|Q05946|UTP13_YEAST	1	1	1
sp|P41058|RS29B_YEAST	1	1	1
sp|P32583|SRP40_YEAST	1	1	1
sp|P38910|CH10_YEAST	1	1	1
sp|P0CH08|RL401_YEAST	1	1	1
sp|P35189|TAF14_YEAST	1	1	1
sp|P40070|LSM4_YEAST	1	1	1
sp|P53276|UTP8_YEAST	1	1	1
sp|P32589|HSP7F_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P10080|SSBP1_YEAST	1	1	1
sp|P40089|LSM5_YEAST	1	1	1
sp|Q08962|NIP7_YEAST	1	1	1
sp|P49631|RL43_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|Q06344|ESF1_YEAST	1	1	1
sp|P32827|RS23_YEAST	1	1	1
sp|Q04013|YHM2_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P09624|DLDH_YEAST	1	1	1
sp|P39567|IMDH1_YEAST	1	1	1
sp|P22336|RFA1_YEAST	11	11	12
sp|P02994|EF1A_YEAST	11	9	12
sp|P33442|RS3A1_YEAST	5	5	6
sp|P00924|ENO1_YEAST	5	5	6
sp|P05756|RS13_YEAST	5	4	6
sp|P40213|RS16_YEAST	5	4	6
sp|P12398|HSP77_YEAST	4	4	5
sp|P41805|RL10_YEAST	4	4	5
sp|P10664|RL4A_YEAST	11	10	14
sp|Q03690|TIF31_YEAST	7	7	9
sp|P06634|DED1_YEAST	9	7	12
sp|O13528|YA11A_YEAST	6	6	8
sp|P32445|RIM1_YEAST	6	5	8
sp|P39741|RL35_YEAST	3	3	4
sp|P09064|IF2B_YEAST	3	3	4
sp|Q12690|RL13A_YEAST	3	3	4
sp|P05744|RL33A_YEAST	3	3	4
sp|Q12499|NOP58_YEAST	8	7	11
sp|Q12460|NOP56_YEAST	10	10	14
sp|P32324|EF2_YEAST	5	5	7
sp|P17079|RL12_YEAST	5	4	7
sp|P16387|ODPA_YEAST	6	6	9
sp|P26783|RS5_YEAST	4	4	6
sp|P32787|MG101_YEAST	4	4	6
sp|P35271|RS18_YEAST	4	4	6

sp|P06367|RS14A_YEAST	4	4	6
sp|P24276|SSD1_YEAST	2	2	3
sp|P53883|NOP13_YEAST	2	2	3
sp|P05748|RL15A_YEAST	2	2	3
sp|Q06679|UTP4_YEAST	2	2	3
sp|P06778|RAD52_YEAST	2	2	3
sp|Q07457|BRE1_YEAST	2	2	3
sp|P25367|RNQ1_YEAST	2	2	3
sp|P12695|ODP2_YEAST	2	2	3
sp|P25491|MAS5_YEAST	2	2	3
sp|P53254|UTP22_YEAST	2	2	3
sp|P07280|RS19A_YEAST	2	2	3
sp|P26321|RL5_YEAST	2	2	3
sp|Q08745|RS10A_YEAST	2	1	3
sp|P06105|SC160_YEAST	14	14	22
sp|P10591|HSP71_YEAST	12	11	20
sp|P53030|RL1_YEAST	3	3	5
sp|P05736|RL2_YEAST	3	3	5
sp|P05737|RL7A_YEAST	3	3	5
sp|P05738|RL9A_YEAST	4	4	7
sp|P05753|RS4_YEAST	5	5	9
sp|P16521|EF3A_YEAST	5	5	9
sp|P42945|UTP10_YEAST	5	5	9
sp|P38011|GBLP_YEAST	4	4	8
sp|P00560|PGK_YEAST	4	4	8
sp|P14120|RL30_YEAST	3	3	6
sp|P27476|NSR1_YEAST	3	3	6
sp|Q02326|RL6A_YEAST	3	3	6
sp|P26781|RS11_YEAST	2	2	4
sp|P32905|RSSA1_YEAST	2	2	4
sp|P36105|RL14A_YEAST	2	2	4
sp|Q02753|RL21A_YEAST	2	2	4
sp|P10964|RPA1_YEAST	2	2	4
sp|P48164|RS7B_YEAST	2	2	4
sp|P29453|RL8B_YEAST	2	2	4
sp|P05743|RL26A_YEAST	2	2	4
sp|P26784|RL16A_YEAST	1	1	2
sp|P53927|NOP15_YEAST	1	1	2
sp|P39990|SNU13_YEAST	1	1	2
sp|P37838|NOP4_YEAST	1	1	2
sp|Q12220|UTP12_YEAST	1	1	2
sp|P34241|URB1_YEAST	1	1	2
sp|P05755|RS9B_YEAST	1	1	2
sp|P38112|MAK5_YEAST	1	1	2
sp|Q08235|BRX1_YEAST	1	1	2
sp|Q06078|UTP21_YEAST	1	1	2
sp|P14540|ALF_YEAST	1	1	2
sp|Q12000|TMA46_YEAST	1	1	2
sp|P23248|RS3A2_YEAST	1	1	2
sp|P39015|STM1_YEAST	1	1	2
sp|Q12024|YTM1_YEAST	1	1	2
sp|Q04305|UTP15_YEAST	1	1	2
sp|Q03532|HAS1_YEAST	4	4	9
sp|P05030|PMA1_YEAST	7	7	16
sp|P32473|ODPB_YEAST	3	3	7
sp|Q04660|ERB1_YEAST	3	3	7
sp|P48589|RS12_YEAST	3	3	7
sp|Q05022|RRP5_YEAST	9	9	22
sp|P20448|DBP4_YEAST	2	2	5
sp|P04456|RL25_YEAST	2	2	5
sp|P40024|ARB1_YEAST	2	2	6
sp|P38701|RS20_YEAST	2	2	6
sp|O13516|RS9A_YEAST	2	2	6
sp|P00950|PMG1_YEAST	1	1	3
sp|P06169|PDC1_YEAST	1	1	3
sp|P53551|H1_YEAST	1	1	3
sp|Q12176|MAK21_YEAST	3	3	10
sp|P14126|RL3_YEAST	2	2	8
sp|Q12136|SAS10_YEAST	1	1	4
sp|P00044|CYC1_YEAST	1	1	4
sp|P23301|IF5A2_YEAST	1	1	4
sp|P38934|BFR1_YEAST	3	3	17
sp|Q08208|NOP12_YEAST	1	1	6

Purification of: Mad1-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P41695|BUB1_YEAST	34	32	
sp|Q08581|SLK19_YEAST	16	16	
sp|P38198|STU1_YEAST	16	16	
sp|P40958|MAD2_YEAST	14	12	
sp|P26449|BUB3_YEAST	12	11	
sp|P40457|MLP2_YEAST	10	10	
sp|Q02931|UTP17_YEAST	8	8	
sp|Q03640|TCB3_YEAST	7	7	
sp|P40460|NDC80_YEAST	6	6	
sp|P32380|NUF1_YEAST	6	6	
sp|P47035|NET1_YEAST	5	5	
sp|P53148|SP105_YEAST	5	5	
sp|P34077|NIC96_YEAST	5	5	
sp|P48837|NUP57_YEAST	4	4	
sp|Q00684|CDC14_YEAST	4	4	
sp|P25623|SYP1_YEAST	4	4	
sp|P08153|SWI5_YEAST	3	3	
sp|Q02455|MLP1_YEAST	3	3	
sp|P25443|RS2_YEAST	3	3	
sp|P06704|CDC31_YEAST	3	3	
sp|P35719|MRP8_YEAST	3	3	
sp|P09440|C1TM_YEAST	3	3	
sp|P33419|NIP29_YEAST	3	3	
sp|Q02821|IMA1_YEAST	3	3	
sp|Q99207|NOP14_YEAST	3	3	
sp|P12385|ERF1_YEAST	2	2	
sp|Q99383|HRP1_YEAST	2	2	
sp|P02557|TBB_YEAST	2	2	
sp|P48361|ASK10_YEAST	2	2	
sp|P38809|YHP7_YEAST	2	2	
sp|P22276|RPC2_YEAST	2	2	
sp|Q08965|BMS1_YEAST	2	2	
sp|P38879|NACA_YEAST	2	2	
sp|Q02629|NU100_YEAST	2	2	
sp|P39731|MTW1_YEAST	2	2	
sp|P12945|NAT1_YEAST	2	2	
sp|Q04491|SEC13_YEAST	2	2	
sp|P36008|EF1G2_YEAST	2	2	
sp|P32562|CDC5_YEAST	2	2	
sp|Q3E705|EFG1P_YEAST	2	2	
sp|P09733|TBA1_YEAST	2	2	
sp|P42846|KRI1_YEAST	2	2	
sp|P16862|K6PF2_YEAST	2	2	
sp|P32597|STH1_YEAST	2	2	
sp|Q01477|UBP3_YEAST	2	2	
sp|P25644|PAT1_YEAST	2	2	
sp|P32598|PP12_YEAST	2	2	
sp|Q08237|REXO4_YEAST	2	2	
sp|P38712|RRP3_YEAST	2	2	
sp|P43609|RSC8_YEAST	2	2	
sp|P53221|RL26B_YEAST	2	2	
sp|Q02959|HOS3_YEAST	2	2	
sp|P50095|IMDH3_YEAST	2	2	
sp|P48231|TCB2_YEAST	1	1	
sp|Q04347|BUD22_YEAST	1	1	
sp|P40961|PHB1_YEAST	1	1	
sp|P36528|RM17_YEAST	1	1	
sp|P46673|NUP85_YEAST	1	1	
sp|P38199|YBD2_YEAST	1	1	
sp|P00817|IPYR_YEAST	1	1	
sp|P53011|SEH1_YEAST	1	1	
sp|P39685|PO152_YEAST	1	1	
##sp|P32660|ATC5_YEAST	1	1	
sp|P46956|PHO86_YEAST	1	1	
sp|P19524|MYO2_YEAST	1	1	
sp|P38249|EIF3A_YEAST	1	1	
sp|P39998|EDC3_YEAST	1	1	
sp|P53978|EF3B_YEAST	1	1	
sp|P12683|HMDH1_YEAST	1	1	

sp|Q04431|YD532_YEAST	1	1	
sp|P40215|NDH1_YEAST	1	1	
sp|P32499|NUP2_YEAST	1	1	
sp|P37297|STT4_YEAST	1	1	
sp|P38333|ENP1_YEAST	1	1	
sp|Q04477|SPC24_YEAST	1	1	
sp|P31383|2AAA_YEAST	1	1	
sp|P40217|EIF3I_YEAST	1	1	
sp|P41318|LST8_YEAST	1	1	
sp|P53040|TAF6_YEAST	1	1	
sp|Q06132|SGD1_YEAST	1	1	
sp|Q03735|NAB6_YEAST	1	1	
sp|P20434|RPAB1_YEAST	1	1	
sp|Q07657|SHS1_YEAST	1	1	
sp|Q12754|RRP12_YEAST	1	1	
sp|P38630|RFC1_YEAST	1	1	
sp|P40008|FMP52_YEAST	1	1	
sp|P04046|PUR1_YEAST	1	1	
sp|Q08972|NEW1_YEAST	1	1	
sp|Q03856|YD11A_YEAST	1	1	
sp|Q07915|RLP24_YEAST	1	1	
sp|P40010|NUG1_YEAST	1	1	
sp|Q04500|UTP14_YEAST	1	1	
sp|P32336|NUD1_YEAST	1	1	
sp|Q12389|DBP10_YEAST	1	1	
sp|P47037|SMC3_YEAST	1	1	
sp|P40568|DSN1_YEAST	1	1	
sp|P24000|RL24B_YEAST	1	1	
sp|P36094|SPC42_YEAST	1	1	
sp|Q02892|NOG1_YEAST	1	1	
sp|Q06412|TUS1_YEAST	1	1	
sp|P38041|BOB1_YEAST	1	1	
sp|P53732|RT12_YEAST	1	1	
sp|P38702|LEU5_YEAST	1	1	
sp|P50085|PHB2_YEAST	1	1	
sp|P38961|RRP8_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P39936|IF4F2_YEAST	1	1	
sp|P06103|EIF3B_YEAST	1	1	
sp|P40059|DOT6_YEAST	1	1	
sp|P50094|IMDH4_YEAST	1	1	
sp|P07271|PIF1_YEAST	1	1	
sp|Q12087|RS30_YEAST	1	1	
sp|P32457|CDC3_YEAST	1	1	
sp|P39007|STT3_YEAST	1	1	
sp|Q12466|TCB1_YEAST	1	1	
sp|P47149|NNF1_YEAST	1	1	
sp|P11325|SYLM_YEAST	1	1	
sp|P40066|GLE2_YEAST	1	1	
sp|P04840|VDAC1_YEAST	1	1	
sp|Q05123|ARP9_YEAST	1	1	
sp|P25299|RNA15_YEAST	1	1	
sp|Q12117|MRH1_YEAST	1	1	
sp|P36161|NU133_YEAST	1	1	
sp|P45818|ROK1_YEAST	1	1	
sp|Q03195|RLI1_YEAST	1	1	
sp|P18239|ADT2_YEAST	1	1	
sp|Q06631|BFR2_YEAST	1	1	
sp|Q12490|YB11B_YEAST	1	1	
sp|P38144|ISW1_YEAST	1	1	
sp|P39960|BEM2_YEAST	1	1	
sp|P28000|RPAC2_YEAST	1	1	
sp|Q99257|MEX67_YEAST	1	1	
sp|P50105|TAF4_YEAST	1	1	
sp|Q05518|PAL1_YEAST	1	1	
sp|P36516|RM03_YEAST	1	1	
sp|Q03430|RSM28_YEAST	1	1	
sp|P19262|ODO2_YEAST	1	1	
sp|Q06678|RM35_YEAST	1	1	
sp|P32832|RSC7_YEAST	1	1	
sp|P22147|XRN1_YEAST	7	7	1

sp|P07149|FAS1_YEAST	6	6	1
sp|P39935|IF4F1_YEAST	6	6	1
sp|P10080|SSBP1_YEAST	5	5	1
sp|P53914|KRE33_YEAST	8	8	2
sp|P53276|UTP8_YEAST	4	4	1
sp|P38882|UTP9_YEAST	4	4	1
sp|Q06218|DBP9_YEAST	4	4	1
sp|P53550|DCP2_YEAST	4	4	1
sp|P14907|NSP1_YEAST	4	4	1
sp|P39985|DPO5_YEAST	4	4	1
sp|P05735|RL19_YEAST	4	3	1
sp|P40957|MAD1_YEAST	67	50	19
sp|Q04373|PUF6_YEAST	6	6	2
sp|P25567|SRO9_YEAST	6	5	2
sp|Q05946|UTP13_YEAST	3	3	1
sp|P53261|PESC_YEAST	3	3	1
sp|P38828|LSM12_YEAST	3	3	1
sp|Q06106|MRD1_YEAST	3	3	1
sp|P53297|PBP1_YEAST	3	3	1
sp|P33322|CBF5_YEAST	3	3	1
sp|P09624|DLDH_YEAST	3	3	1
sp|P39744|NOC2_YEAST	3	3	1
sp|P20459|IF2A_YEAST	3	3	1
sp|P38061|RL32_YEAST	3	3	1
sp|P53734|DBP6_YEAST	3	3	1
sp|P04911|H2A1_YEAST	3	3	1
sp|Q3E792|RS25A_YEAST	3	2	1
sp|P40991|NOP2_YEAST	5	5	2
sp|P40055|UTP7_YEAST	5	5	2
sp|Q01080|RPA49_YEAST	5	4	2
sp|P04451|RL23_YEAST	7	5	3
sp|P32324|EF2_YEAST	15	15	7
sp|O13527|YA11B_YEAST	8	8	4
sp|P38788|SSZ1_YEAST	6	6	3
sp|P16140|VATB_YEAST	4	4	2
sp|P37838|NOP4_YEAST	4	4	2
sp|P26785|RL16B_YEAST	4	4	2
sp|P40850|MKT1_YEAST	4	4	2
sp|P0C0W9|RL11A_YEAST	4	4	2
sp|P04449|RL24A_YEAST	4	3	2
sp|P0C2H8|RL31A_YEAST	4	3	2
sp|P07703|RPAC1_YEAST	2	2	1
sp|P39567|IMDH1_YEAST	2	2	1
sp|Q12522|IF6_YEAST	2	2	1
sp|P32899|IMP3_YEAST	2	2	1
sp|P37263|YC16_YEAST	2	2	1
sp|P25617|YCQ6_YEAST	2	2	1
sp|Q12266|YB11A_YEAST	2	2	1
sp|P49626|RL4B_YEAST	2	2	1
sp|P38922|HRB1_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|Q03940|RUVB1_YEAST	2	2	1
sp|P33750|DCA13_YEAST	2	2	1
sp|P07260|IF4E_YEAST	2	2	1
sp|P00359|G3P3_YEAST	2	2	1
sp|P36160|RPF2_YEAST	2	2	1
sp|Q06511|RRP15_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|Q04177|UTP5_YEAST	2	2	1
sp|P32827|RS23_YEAST	2	2	1
sp|P40070|LSM4_YEAST	2	2	1
sp|P46669|RPA43_YEAST	2	2	1
sp|P49631|RL43_YEAST	2	1	1
sp|P04147|PABP_YEAST	17	15	9
sp|P33442|RS3A1_YEAST	11	11	6
sp|P15424|MS116_YEAST	11	10	6
sp|P21576|VPS1_YEAST	9	9	5
sp|P24276|SSD1_YEAST	5	5	3
sp|P26786|RS7A_YEAST	5	5	3
sp|P17076|RL8A_YEAST	5	4	3
sp|Q03690|TIF31_YEAST	14	14	9

sp|P40024|ARB1_YEAST	9	9	6
sp|P0C2I0|RL20_YEAST	9	8	6
sp|P26782|RS24_YEAST	6	5	4
sp|P53927|NOP15_YEAST	3	3	2
sp|P22138|RPA2_YEAST	3	3	2
sp|P05319|RLA2_YEAST	3	3	2
sp|Q12220|UTP12_YEAST	3	3	2
sp|P36049|EBP2_YEAST	3	3	2
sp|P26784|RL16A_YEAST	3	3	2
sp|P47077|YJB0_YEAST	3	3	2
sp|Q06506|RRP9_YEAST	3	3	2
sp|Q01560|NOP3_YEAST	3	3	2
sp|P38112|MAK5_YEAST	3	3	2
sp|P00360|G3P1_YEAST	3	3	2
sp|P34241|URB1_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|P05317|RLA0_YEAST	7	7	5
sp|P10592|HSP72_YEAST	7	7	5
sp|P20447|DBP3_YEAST	7	7	5
sp|P02407|RS17A_YEAST	7	6	5
sp|P41805|RL10_YEAST	7	6	5
sp|P38011|GBLP_YEAST	11	10	8
sp|P06105|SC160_YEAST	30	30	22
sp|P06634|DED1_YEAST	16	12	12
sp|P00549|KPYK1_YEAST	8	8	6
sp|P26783|RS5_YEAST	8	7	6
sp|P27476|NSR1_YEAST	8	7	6
sp|P25367|RNQ1_YEAST	4	4	3
sp|Q06679|UTP4_YEAST	4	4	3
sp|P05748|RL15A_YEAST	4	4	3
sp|Q12230|LSP1_YEAST	4	4	3
sp|P05754|RS8_YEAST	4	4	3
sp|P07245|C1TC_YEAST	4	4	3
sp|P02309|H4_YEAST	4	4	3
sp|P38779|CIC1_YEAST	4	4	3
sp|P15108|HSC82_YEAST	4	4	3
sp|P26321|RL5_YEAST	4	4	3
sp|P07280|RS19A_YEAST	4	4	3
sp|P53254|UTP22_YEAST	4	4	3
sp|P11484|HSP75_YEAST	25	19	19
sp|P14126|RL3_YEAST	10	9	8
sp|Q12690|RL13A_YEAST	5	5	4
sp|P10964|RPA1_YEAST	5	5	4
sp|P05743|RL26A_YEAST	5	5	4
sp|P0C2H6|RL27A_YEAST	5	5	4
sp|P32905|RSSA1_YEAST	5	4	4
sp|P05740|RL17A_YEAST	5	4	4
sp|Q12460|NOP56_YEAST	17	15	14
sp|P32481|IF2G_YEAST	6	6	5
sp|P05737|RL7A_YEAST	6	6	5
sp|P05736|RL2_YEAST	6	5	5
sp|P04456|RL25_YEAST	6	5	5
sp|P0C0W1|RS22A_YEAST	6	4	5
sp|Q12159|YRA1_YEAST	7	7	6
sp|P35271|RS18_YEAST	7	7	6
sp|O13516|RS9A_YEAST	7	7	6
sp|P02365|RS6_YEAST	7	6	6
sp|P05738|RL9A_YEAST	8	6	7
sp|O13528|YA11A_YEAST	9	7	8
sp|Q05022|RRP5_YEAST	24	23	22
sp|P10664|RL4A_YEAST	15	11	14
sp|P10591|HSP71_YEAST	21	18	20
sp|Q12176|MAK21_YEAST	10	10	10
sp|P05750|RS3_YEAST	9	9	9
sp|P16521|EF3A_YEAST	9	9	9
sp|P42945|UTP10_YEAST	9	9	9
sp|P32445|RIM1_YEAST	8	7	8
sp|Q04660|ERB1_YEAST	7	7	7
sp|P15646|FBRL_YEAST	7	6	7
sp|P32473|ODPB_YEAST	7	6	7

sp|P06367|RS14A_YEAST	6	6	6
sp|P17079|RL12_YEAST	7	5	7
sp|P40213|RS16_YEAST	6	5	6
sp|P14120|RL30_YEAST	6	5	6
sp|P05756|RS13_YEAST	6	5	6
sp|P05745|RL36A_YEAST	5	5	5
sp|P25635|PWP2_YEAST	5	5	5
sp|P47083|MPP10_YEAST	5	5	5
sp|Q02753|RL21A_YEAST	4	4	4
sp|P48164|RS7B_YEAST	4	4	4
sp|Q12136|SAS10_YEAST	4	4	4
sp|P26754|RFA2_YEAST	3	3	3
sp|P00925|ENO2_YEAST	3	3	3
sp|P12695|ODP2_YEAST	3	3	3
sp|P53551|H1_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|P24784|DBP1_YEAST	3	3	3
sp|P21304|PWP1_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|Q08745|RS10A_YEAST	3	2	3
sp|P35178|RRP1_YEAST	2	2	2
sp|P50109|PSP2_YEAST	2	2	2
sp|P39990|SNU13_YEAST	2	2	2
sp|Q06078|UTP21_YEAST	2	2	2
sp|Q02486|ABF2_YEAST	2	2	2
sp|P39730|IF2P_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2
sp|P40007|NOP16_YEAST	2	2	2
sp|P23248|RS3A2_YEAST	2	2	2
sp|O14467|MBF1_YEAST	2	2	2
sp|P05755|RS9B_YEAST	2	2	2
sp|Q03973|HMO1_YEAST	2	2	2
sp|P40693|RLP7_YEAST	2	2	2
sp|P05739|RL6B_YEAST	2	2	2
sp|P39015|STM1_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|Q04305|UTP15_YEAST	2	2	2
sp|P26755|RFA3_YEAST	2	1	2
sp|P28007|GAR1_YEAST	1	1	1
sp|P38789|SSF1_YEAST	1	1	1
sp|P40089|LSM5_YEAST	1	1	1
sp|P19454|CSK22_YEAST	1	1	1
sp|P53941|IMP4_YEAST	1	1	1
sp|P29311|BMH1_YEAST	1	1	1
sp|P35189|TAF14_YEAST	1	1	1
sp|P32495|NHP2_YEAST	1	1	1
sp|P0CH08|RL401_YEAST	1	1	1
sp|Q07362|PBP4_YEAST	1	1	1
sp|P02829|HSP82_YEAST	1	1	1
sp|Q08962|NIP7_YEAST	1	1	1
sp|P41056|RL33B_YEAST	1	1	1

sp|P46990|RL17B_YEAST	1	1	1
sp|Q07623|NOP6_YEAST	1	1	1
sp|P33201|MRT4_YEAST	1	1	1
sp|P32529|RPA12_YEAST	1	1	1
sp|P37304|PAM1_YEAST	1	1	1
sp|P47006|RPA34_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P53141|MLC1_YEAST	1	1	1
sp|P16861|K6PF1_YEAST	1	1	1
sp|Q06344|ESF1_YEAST	1	1	1
sp|P10962|MAK16_YEAST	1	1	1
sp|Q04867|YM91_YEAST	1	1	1
sp|P53163|MNP1_YEAST	1	1	1
sp|Q04013|YHM2_YEAST	1	1	1
sp|P20967|ODO1_YEAST	1	1	1
sp|P25694|CDC48_YEAST	1	1	1
sp|P02405|RL44_YEAST	1	1	1
sp|Q99216|PNO1_YEAST	1	1	1
sp|P32468|CDC12_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P25342|CDC10_YEAST	1	1	1
sp|P39523|YM11_YEAST	1	1	1
sp|P05030|PMA1_YEAST	15	15	16
sp|Q12499|NOP58_YEAST	10	10	11
sp|P05753|RS4_YEAST	8	7	9
sp|P02994|EF1A_YEAST	10	9	12
sp|P00924|ENO1_YEAST	5	5	6
sp|Q08208|NOP12_YEAST	5	5	6
sp|P32787|MG101_YEAST	5	5	6
sp|P38631|FKS1_YEAST	19	18	23
sp|P38934|BFR1_YEAST	14	13	17
sp|P12398|HSP77_YEAST	4	4	5
sp|Q03532|HAS1_YEAST	7	7	9
sp|P16387|ODPA_YEAST	7	7	9
sp|P22336|RFA1_YEAST	9	9	12
sp|P09064|IF2B_YEAST	3	3	4
sp|P60010|ACT_YEAST	3	3	4
sp|P16474|GRP78_YEAST	3	3	4
sp|P53131|PRP43_YEAST	3	3	4
sp|P36105|RL14A_YEAST	3	3	4
sp|P05744|RL33A_YEAST	3	3	4
sp|P29453|RL8B_YEAST	3	2	4
sp|P23301|IF5A2_YEAST	3	2	4
sp|P48589|RS12_YEAST	5	4	7
sp|P38701|RS20_YEAST	4	4	6
sp|P06778|RAD52_YEAST	2	2	3
sp|P00950|PMG1_YEAST	2	2	3
sp|P00330|ADH1_YEAST	2	2	3
sp|P53883|NOP13_YEAST	2	2	3
sp|P53252|PIL1_YEAST	2	2	3
sp|Q08492|BUD21_YEAST	2	2	3
sp|P00560|PGK_YEAST	5	5	8
sp|P53030|RL1_YEAST	3	3	5
sp|P20448|DBP4_YEAST	3	3	5
sp|Q02326|RL6A_YEAST	3	3	6
sp|P26781|RS11_YEAST	2	2	4
sp|P39741|RL35_YEAST	2	2	4
sp|Q02354|UTP6_YEAST	1	1	2
sp|Q12000|TMA46_YEAST	1	1	2
sp|Q12024|YTM1_YEAST	1	1	2
sp|Q08235|BRX1_YEAST	1	1	2
sp|P14540|ALF_YEAST	1	1	2
sp|Q12035|FCF2_YEAST	1	1	2
sp|Q08287|NOP8_YEAST	1	1	2
sp|P07259|PYR1_YEAST	3	3	7
sp|P25491|MAS5_YEAST	1	1	3
sp|P06169|PDC1_YEAST	1	1	3
sp|P19882|HSP60_YEAST	1	1	4

Purification of: Mad2-TAP protein	total peptides	unique peptides	total peptides in control
sp|P40958|MAD2_YEAST	13	12	
sp|P09440|C1TM_YEAST	6	6	
sp|P38249|EIF3A_YEAST	6	6	
sp|Q12754|RRP12_YEAST	4	4	
sp|P16862|K6PF2_YEAST	4	4	
sp|P25443|RS2_YEAST	4	4	
sp|P38879|NACA_YEAST	4	4	
sp|P19097|FAS2_YEAST	4	4	
sp|P40217|EIF3I_YEAST	4	4	
sp|P12385|ERF1_YEAST	4	4	
sp|Q08972|NEW1_YEAST	3	3	
sp|P24783|DBP2_YEAST	3	3	
sp|Q03640|TCB3_YEAST	3	3	
sp|Q00684|CDC14_YEAST	3	3	
sp|Q04491|SEC13_YEAST	3	3	
sp|Q00955|ACAC_YEAST	3	3	
sp|P40961|PHB1_YEAST	2	2	
sp|P53235|EIF2A_YEAST	2	2	
sp|P06103|EIF3B_YEAST	2	2	
sp|Q12490|YB11B_YEAST	2	2	
sp|Q02931|UTP17_YEAST	2	2	
sp|P05749|RL22A_YEAST	2	2	
sp|Q03195|RLI1_YEAST	2	2	
sp|P06704|CDC31_YEAST	2	2	
sp|P53221|RL26B_YEAST	2	2	
sp|P26309|CDC20_YEAST	2	2	
sp|P24000|RL24B_YEAST	2	1	
sp|P12945|NAT1_YEAST	1	1	
sp|P32497|EIF3C_YEAST	1	1	
sp|Q05518|PAL1_YEAST	1	1	
sp|Q12260|YB21A_YEAST	1	1	
sp|P39007|STT3_YEAST	1	1	
sp|P47035|NET1_YEAST	1	1	
##sp|Q12071|VPS54_YEAST	1	1	
sp|P36516|RM03_YEAST	1	1	
sp|P50085|PHB2_YEAST	1	1	
sp|P09435|HSP73_YEAST	1	1	
sp|P26449|BUB3_YEAST	1	1	
##sp|Q12510|YD156_YEAST	1	1	
sp|Q3E754|RS21B_YEAST	1	1	
sp|P50095|IMDH3_YEAST	1	1	
sp|Q12339|UTP23_YEAST	1	1	
sp|P33767|OSTB_YEAST	1	1	
sp|P47079|TCPQ_YEAST	1	1	
sp|P10622|RLA3_YEAST	1	1	
sp|P37292|GLYM_YEAST	1	1	
sp|Q02642|NACB1_YEAST	1	1	

sp|P06102|NOT3_YEAST	1	1	
sp|P51401|RL9B_YEAST	1	1	
sp|Q12117|MRH1_YEAST	1	1	
sp|P42846|KRI1_YEAST	1	1	
sp|P53978|EF3B_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|Q08237|REXO4_YEAST	1	1	
sp|P53011|SEH1_YEAST	1	1	
sp|P48439|OST3_YEAST	1	1	
sp|Q02796|LGE1_YEAST	1	1	
sp|P32457|CDC3_YEAST	1	1	
sp|P07271|PIF1_YEAST	1	1	
sp|P45818|ROK1_YEAST	1	1	
sp|P35732|YKF4_YEAST	1	1	
sp|Q02892|NOG1_YEAST	1	1	
sp|P38911|FKBP3_YEAST	1	1	
sp|Q06631|BFR2_YEAST	1	1	
sp|Q12514|NOT5_YEAST	1	1	
sp|P20081|FKBP_YEAST	1	1	
sp|P36009|DHR2_YEAST	1	1	
sp|P48837|NUP57_YEAST	1	1	
sp|P38248|ECM33_YEAST	1	1	
sp|Q12213|RL7B_YEAST	1	1	
sp|P46956|PHO86_YEAST	1	1	
sp|P20434|RPAB1_YEAST	1	1	
sp|Q02948|VPS30_YEAST	1	1	
sp|P07347|ARD1_YEAST	1	1	
sp|P38930|CSK2C_YEAST	1	1	
sp|Q12672|RL21B_YEAST	1	1	
sp|P02557|TBB_YEAST	1	1	
sp|Q08965|BMS1_YEAST	1	1	
sp|P16861|K6PF1_YEAST	9	9	1
sp|P07149|FAS1_YEAST	6	6	1
sp|P32527|ZUO1_YEAST	6	6	1
sp|P39935|IF4F1_YEAST	6	6	1
sp|P05735|RL19_YEAST	5	4	1
sp|Q3E792|RS25A_YEAST	4	3	1
sp|P04451|RL23_YEAST	11	8	3
sp|P0C2H8|RL31A_YEAST	7	6	2
sp|P07245|C1TC_YEAST	10	10	3
sp|P17076|RL8A_YEAST	10	8	3
sp|P32324|EF2_YEAST	22	22	7
sp|P40957|MAD1_YEAST	58	47	19
sp|P20459|IF2A_YEAST	3	3	1
sp|Q06218|DBP9_YEAST	3	3	1
sp|P22147|XRN1_YEAST	3	3	1
sp|P07260|IF4E_YEAST	3	3	1
sp|P46669|RPA43_YEAST	3	3	1
sp|P33322|CBF5_YEAST	3	2	1

sp|P53914|KRE33_YEAST	6	6	2
sp|P04449|RL24A_YEAST	6	5	2
sp|P26784|RL16A_YEAST	6	5	2
sp|P25567|SRO9_YEAST	5	4	2
sp|P38788|SSZ1_YEAST	7	7	3
sp|P10964|RPA1_YEAST	9	9	4
sp|P25342|CDC10_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|P53261|PESC_YEAST	2	2	1
sp|P39729|RBG1_YEAST	2	2	1
sp|P53276|UTP8_YEAST	2	2	1
sp|Q06344|ESF1_YEAST	2	2	1
sp|P40070|LSM4_YEAST	2	2	1
sp|P04911|H2A1_YEAST	2	2	1
sp|P38061|RL32_YEAST	2	2	1
sp|P38882|UTP9_YEAST	2	2	1
sp|Q06511|RRP15_YEAST	2	2	1
sp|P39744|NOC2_YEAST	2	2	1
sp|P02405|RL44_YEAST	2	2	1
sp|P53550|DCP2_YEAST	2	2	1
sp|P38922|HRB1_YEAST	2	2	1
sp|Q12522|IF6_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|P33201|MRT4_YEAST	2	2	1
sp|P49626|RL4B_YEAST	2	2	1
sp|P32495|NHP2_YEAST	2	2	1
sp|P49631|RL43_YEAST	2	1	1
sp|P28007|GAR1_YEAST	2	1	1
sp|P22138|RPA2_YEAST	4	4	2
sp|Q06506|RRP9_YEAST	4	4	2
sp|P0C0W9|RL11A_YEAST	4	4	2
sp|P16140|VATB_YEAST	4	4	2
sp|P39015|STM1_YEAST	4	3	2
sp|P0C0V8|RS21A_YEAST	4	3	2
sp|P26785|RL16B_YEAST	4	3	2
sp|P26321|RL5_YEAST	6	6	3
sp|O13527|YA11B_YEAST	8	8	4
sp|P05737|RL7A_YEAST	10	10	5
sp|P02407|RS17A_YEAST	10	8	5
sp|P05736|RL2_YEAST	10	8	5
sp|P06367|RS14A_YEAST	12	11	6
sp|P14126|RL3_YEAST	15	13	8
sp|P33442|RS3A1_YEAST	11	11	6
sp|P40024|ARB1_YEAST	11	11	6
sp|P20447|DBP3_YEAST	9	9	5
sp|Q03690|TIF31_YEAST	16	15	9
sp|P26782|RS24_YEAST	7	6	4
sp|Q01855|RS15_YEAST	5	5	3
sp|P15108|HSC82_YEAST	5	5	3

sp|P05748|RL15A_YEAST	5	4	3
sp|P04147|PABP_YEAST	15	13	9
sp|P04456|RL25_YEAST	8	7	5
sp|P05745|RL36A_YEAST	8	7	5
sp|P05317|RLA0_YEAST	8	7	5
sp|P16521|EF3A_YEAST	14	13	9
sp|Q01560|NOP3_YEAST	3	3	2
sp|P40991|NOP2_YEAST	3	3	2
sp|P47077|YJB0_YEAST	3	3	2
sp|O14467|MBF1_YEAST	3	3	2
sp|Q04305|UTP15_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P02406|RL28_YEAST	3	3	2
sp|P05319|RLA2_YEAST	3	3	2
sp|Q01080|RPA49_YEAST	3	3	2
sp|P53131|PRP43_YEAST	6	6	4
sp|P05740|RL17A_YEAST	6	5	4
sp|O13516|RS9A_YEAST	9	9	6
sp|P35271|RS18_YEAST	9	9	6
sp|P00549|KPYK1_YEAST	9	9	6
sp|P0C2I0|RL20_YEAST	9	7	6
sp|P06634|DED1_YEAST	18	15	12
sp|P06105|SC160_YEAST	32	32	22
sp|P05753|RS4_YEAST	13	10	9
sp|P41805|RL10_YEAST	7	7	5
sp|P38011|GBLP_YEAST	11	11	8
sp|P11484|HSP75_YEAST	26	23	19
sp|P05754|RS8_YEAST	4	4	3
sp|P02309|H4_YEAST	4	4	3
sp|P25491|MAS5_YEAST	4	4	3
sp|P02400|RLA4_YEAST	4	4	3
sp|P07280|RS19A_YEAST	4	4	3
sp|P26786|RS7A_YEAST	4	4	3
sp|P27476|NSR1_YEAST	8	8	6
sp|P38701|RS20_YEAST	8	7	6
sp|P05756|RS13_YEAST	8	7	6
sp|P15424|MS116_YEAST	8	7	6
sp|P26783|RS5_YEAST	8	7	6
sp|P05738|RL9A_YEAST	9	8	7
sp|P15646|FBRL_YEAST	9	8	7
sp|Q12690|RL13A_YEAST	5	5	4
sp|P05743|RL26A_YEAST	5	5	4
sp|Q12136|SAS10_YEAST	5	5	4
sp|P05744|RL33A_YEAST	5	5	4
sp|P29453|RL8B_YEAST	5	4	4
sp|P32905|RSSA1_YEAST	5	4	4
sp|P10592|HSP72_YEAST	6	6	5
sp|P53030|RL1_YEAST	6	5	5
sp|P02365|RS6_YEAST	7	7	6

sp|P14120|RL30_YEAST	7	6	6
sp|P32473|ODPB_YEAST	8	7	7
sp|P10664|RL4A_YEAST	16	11	14
sp|Q05022|RRP5_YEAST	25	25	22
sp|P38631|FKS1_YEAST	26	24	23
sp|O13528|YA11A_YEAST	9	7	8
sp|P42945|UTP10_YEAST	10	10	9
sp|P05750|RS3_YEAST	10	10	9
sp|Q12460|NOP56_YEAST	15	14	14
sp|Q07623|NOP6_YEAST	1	1	1
sp|P10081|IF4A_YEAST	1	1	1
sp|P36160|RPF2_YEAST	1	1	1
sp|P39985|DPO5_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P38711|RS27B_YEAST	1	1	1
sp|Q05946|UTP13_YEAST	1	1	1
sp|Q12266|YB11A_YEAST	1	1	1
sp|P33750|DCA13_YEAST	1	1	1
sp|Q08096|RCL1_YEAST	1	1	1
sp|P29311|BMH1_YEAST	1	1	1
sp|P37263|YC16_YEAST	1	1	1
sp|P32529|RPA12_YEAST	1	1	1
sp|P41056|RL33B_YEAST	1	1	1
sp|P39567|IMDH1_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|Q03940|RUVB1_YEAST	1	1	1
sp|P07251|ATPA_YEAST	1	1	1
sp|P53941|IMP4_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P14907|NSP1_YEAST	1	1	1
sp|P32589|HSP7F_YEAST	1	1	1
sp|P38828|LSM12_YEAST	1	1	1
sp|P35189|TAF14_YEAST	1	1	1
sp|P10962|MAK16_YEAST	1	1	1
sp|P53297|PBP1_YEAST	1	1	1
sp|Q04177|UTP5_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|P32827|RS23_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P19454|CSK22_YEAST	1	1	1
sp|P32468|CDC12_YEAST	1	1	1
sp|P25617|YCQ6_YEAST	1	1	1
sp|P32899|IMP3_YEAST	1	1	1
sp|P53141|MLC1_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|Q08962|NIP7_YEAST	1	1	1
sp|P53163|MNP1_YEAST	1	1	1
sp|P53734|DBP6_YEAST	1	1	1
sp|P25694|CDC48_YEAST	1	1	1

sp|P46990|RL17B_YEAST	1	1	1
sp|P0CH08|RL401_YEAST	1	1	1
sp|P39954|SAHH_YEAST	1	1	1
sp|P53742|NOG2_YEAST	1	1	1
sp|P07703|RPAC1_YEAST	1	1	1
sp|P47006|RPA34_YEAST	1	1	1
sp|Q12000|TMA46_YEAST	2	2	2
sp|P53927|NOP15_YEAST	2	2	2
sp|P05739|RL6B_YEAST	2	2	2
sp|Q02486|ABF2_YEAST	2	2	2
sp|Q08235|BRX1_YEAST	2	2	2
sp|P37838|NOP4_YEAST	2	2	2
sp|P40055|UTP7_YEAST	2	2	2
sp|Q03973|HMO1_YEAST	2	2	2
sp|P39730|IF2P_YEAST	2	2	2
sp|P23248|RS3A2_YEAST	2	2	2
sp|P38112|MAK5_YEAST	2	2	2
sp|Q04373|PUF6_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|P40693|RLP7_YEAST	2	2	2
sp|P36049|EBP2_YEAST	2	2	2
sp|P26755|RFA3_YEAST	2	1	2
sp|P53883|NOP13_YEAST	3	3	3
sp|P24784|DBP1_YEAST	3	3	3
sp|P21304|PWP1_YEAST	3	3	3
sp|P26754|RFA2_YEAST	3	3	3
sp|P25367|RNQ1_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P06169|PDC1_YEAST	3	3	3
sp|P53551|H1_YEAST	3	3	3
sp|Q07457|BRE1_YEAST	3	3	3
sp|P48164|RS7B_YEAST	4	4	4
sp|P0C2H6|RL27A_YEAST	4	4	4
sp|P23301|IF5A2_YEAST	4	4	4
sp|P16474|GRP78_YEAST	4	4	4
sp|P39741|RL35_YEAST	4	3	4
sp|P20448|DBP4_YEAST	5	5	5
sp|P32481|IF2G_YEAST	5	5	5
sp|P0C0W1|RS22A_YEAST	5	3	5
sp|P40213|RS16_YEAST	6	6	6
sp|Q12159|YRA1_YEAST	6	6	6
sp|P16387|ODPA_YEAST	9	9	9
sp|P22336|RFA1_YEAST	12	12	12

sp|P02994|EF1A_YEAST	12	10	12
sp|P10591|HSP71_YEAST	19	15	20
sp|P38934|BFR1_YEAST	15	14	17
sp|P48589|RS12_YEAST	6	6	7
sp|P17079|RL12_YEAST	6	5	7
sp|Q08208|NOP12_YEAST	5	5	6
sp|P32787|MG101_YEAST	5	5	6
sp|P05030|PMA1_YEAST	13	13	16
sp|P25635|PWP2_YEAST	4	4	5
sp|Q12176|MAK21_YEAST	8	8	10
sp|P09064|IF2B_YEAST	3	3	4
sp|Q02753|RL21A_YEAST	3	3	4
sp|P36105|RL14A_YEAST	3	2	4
sp|P32445|RIM1_YEAST	6	5	8
sp|P06778|RAD52_YEAST	2	2	3
sp|P00925|ENO2_YEAST	2	2	3
sp|P53252|PIL1_YEAST	2	2	3
sp|P53254|UTP22_YEAST	2	2	3
sp|P00330|ADH1_YEAST	2	2	3
sp|Q06679|UTP4_YEAST	2	2	3
sp|P12695|ODP2_YEAST	2	2	3
sp|Q08745|RS10A_YEAST	2	1	3
sp|Q02326|RL6A_YEAST	4	4	6
sp|P21576|VPS1_YEAST	3	3	5
sp|P47083|MPP10_YEAST	3	3	5
sp|P12398|HSP77_YEAST	3	3	5
sp|Q04660|ERB1_YEAST	4	4	7
sp|Q03532|HAS1_YEAST	5	5	9
sp|Q12499|NOP58_YEAST	6	6	11
sp|P39990|SNU13_YEAST	1	1	2
sp|P05755|RS9B_YEAST	1	1	2
sp|P40007|NOP16_YEAST	1	1	2
sp|Q12024|YTM1_YEAST	1	1	2
sp|Q06078|UTP21_YEAST	1	1	2
sp|P50109|PSP2_YEAST	1	1	2
sp|Q12035|FCF2_YEAST	1	1	2
sp|P14540|ALF_YEAST	1	1	2
sp|Q08287|NOP8_YEAST	1	1	2
sp|Q12220|UTP12_YEAST	1	1	2
sp|P26781|RS11_YEAST	2	2	4
sp|P60010|ACT_YEAST	2	2	4
sp|P00924|ENO1_YEAST	3	3	6
sp|P07259|PYR1_YEAST	3	3	7
sp|P00560|PGK_YEAST	3	3	8
sp|P00950|PMG1_YEAST	1	1	3
sp|P38779|CIC1_YEAST	1	1	3
sp|Q08492|BUD21_YEAST	1	1	3
sp|P24276|SSD1_YEAST	1	1	3
sp|Q12230|LSP1_YEAST	1	1	3

Purification of: Mad2-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P26309|CDC20_YEAST	19	16	
sp|P41695|BUB1_YEAST	15	15	
sp|P40958|MAD2_YEAST	13	11	
sp|P26449|BUB3_YEAST	9	9	
sp|P47074|MAD3_YEAST	9	9	
sp|P16862|K6PF2_YEAST	6	6	
sp|P09798|CDC16_YEAST	6	6	
sp|P09440|C1TM_YEAST	5	5	
sp|Q02931|UTP17_YEAST	5	5	
sp|Q03640|TCB3_YEAST	4	4	
sp|P38879|NACA_YEAST	4	4	
sp|P38249|EIF3A_YEAST	4	4	
sp|P24783|DBP2_YEAST	4	4	
sp|Q12754|RRP12_YEAST	3	3	
sp|P53886|APC1_YEAST	3	3	
sp|P25443|RS2_YEAST	3	3	
sp|P39076|TCPB_YEAST	3	3	
sp|P39077|TCPG_YEAST	3	3	
sp|Q04491|SEC13_YEAST	3	3	
sp|P40217|EIF3I_YEAST	3	3	
sp|Q08972|NEW1_YEAST	3	3	
sp|P39078|TCPD_YEAST	3	3	
sp|P47079|TCPQ_YEAST	3	3	
sp|P39079|TCPZ_YEAST	3	3	
sp|P24000|RL24B_YEAST	3	2	
sp|P16522|CDC23_YEAST	2	2	
sp|P42846|KRI1_YEAST	2	2	
sp|P32497|EIF3C_YEAST	2	2	
sp|P06103|EIF3B_YEAST	2	2	
sp|P53221|RL26B_YEAST	2	2	
sp|Q3E705|EFG1P_YEAST	2	2	
sp|P12385|ERF1_YEAST	2	2	
sp|Q12490|YB11B_YEAST	2	2	
sp|P32598|PP12_YEAST	2	2	
sp|P50095|IMDH3_YEAST	2	2	
sp|Q08965|BMS1_YEAST	2	2	
sp|P25586|KRR1_YEAST	2	2	
sp|P12945|NAT1_YEAST	2	2	
sp|P40413|TCPE_YEAST	2	2	
sp|P40513|MAM33_YEAST	1	1	
sp|P40010|NUG1_YEAST	1	1	
sp|P25644|PAT1_YEAST	1	1	
sp|P35718|RPC8_YEAST	1	1	
sp|P05759|RS27A_YEAST	1	1	
sp|Q12339|UTP23_YEAST	1	1	
sp|Q12087|RS30_YEAST	1	1	
sp|P35732|YKF4_YEAST	1	1	
sp|Q12379|SWM1_YEAST	1	1	
sp|P48837|NUP57_YEAST	1	1	
sp|Q08237|REXO4_YEAST	1	1	
sp|P38930|CSK2C_YEAST	1	1	
sp|Q12117|MRH1_YEAST	1	1	
sp|P38353|SSH1_YEAST	1	1	
sp|P33767|OSTB_YEAST	1	1	
sp|P46956|PHO86_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P34077|NIC96_YEAST	1	1	
sp|P39007|STT3_YEAST	1	1	
sp|P38697|IMDH2_YEAST	1	1	
sp|P40150|HSP76_YEAST	1	1	

sp|Q02642|NACB1_YEAST	1	1	
sp|P15705|STI1_YEAST	1	1	
sp|P25555|GBP2_YEAST	1	1	
sp|P05749|RL22A_YEAST	1	1	
sp|P37292|GLYM_YEAST	1	1	
sp|Q06631|BFR2_YEAST	1	1	
sp|P12612|TCPA_YEAST	1	1	
sp|Q12491|YB21B_YEAST	1	1	
sp|P50094|IMDH4_YEAST	1	1	
sp|Q99207|NOP14_YEAST	1	1	
sp|P41057|RS29A_YEAST	1	1	
sp|Q12213|RL7B_YEAST	1	1	
sp|P20434|RPAB1_YEAST	1	1	
sp|P06704|CDC31_YEAST	1	1	
sp|P40215|NDH1_YEAST	1	1	
sp|Q02892|NOG1_YEAST	1	1	
sp|Q99257|MEX67_YEAST	1	1	
sp|P41543|OST1_YEAST	1	1	
sp|P20436|RPAB3_YEAST	1	1	
sp|Q12517|DCP1_YEAST	1	1	
sp|P39998|EDC3_YEAST	1	1	
sp|Q00684|CDC14_YEAST	1	1	
sp|P25623|SYP1_YEAST	1	1	
sp|P35191|MDJ1_YEAST	1	1	
sp|P10622|RLA3_YEAST	1	1	
sp|Q12260|YB21A_YEAST	1	1	
sp|P38042|CDC27_YEAST	1	1	
sp|Q12672|RL21B_YEAST	1	1	
sp|P16861|K6PF1_YEAST	12	12	1
sp|P05735|RL19_YEAST	6	5	1
sp|P39935|IF4F1_YEAST	5	5	1
sp|P17076|RL8A_YEAST	13	11	3
sp|P38061|RL32_YEAST	4	4	1
sp|P53550|DCP2_YEAST	4	4	1
sp|P07149|FAS1_YEAST	4	4	1
sp|P39567|IMDH1_YEAST	4	3	1
sp|P07245|C1TC_YEAST	11	11	3
sp|P32324|EF2_YEAST	22	22	7
sp|P38788|SSZ1_YEAST	9	8	3
sp|P0C0W9|RL11A_YEAST	6	6	2
sp|P22138|RPA2_YEAST	6	6	2
sp|P39015|STM1_YEAST	6	5	2
sp|P39729|RBG1_YEAST	3	3	1
sp|P32527|ZUO1_YEAST	3	3	1
sp|Q03940|RUVB1_YEAST	3	3	1
sp|Q06106|MRD1_YEAST	3	3	1
sp|P04911|H2A1_YEAST	3	3	1
sp|Q06218|DBP9_YEAST	3	3	1
sp|P39744|NOC2_YEAST	3	3	1
sp|P07703|RPAC1_YEAST	3	3	1
sp|P39985|DPO5_YEAST	3	3	1
sp|P32827|RS23_YEAST	3	3	1
sp|P09624|DLDH_YEAST	3	3	1
sp|Q3E792|RS25A_YEAST	3	2	1
sp|P40957|MAD1_YEAST	52	46	19
sp|P26784|RL16A_YEAST	5	5	2
sp|P02406|RL28_YEAST	5	5	2
sp|P0C2H8|RL31A_YEAST	5	4	2
sp|P04449|RL24A_YEAST	5	4	2
sp|P04451|RL23_YEAST	7	6	3
sp|P10964|RPA1_YEAST	9	9	4
sp|P05740|RL17A_YEAST	9	8	4

sp|P33442|RS3A1_YEAST	12	11	6
sp|P02407|RS17A_YEAST	10	8	5
sp|P26782|RS24_YEAST	8	7	4
sp|P36049|EBP2_YEAST	4	4	2
sp|P07279|RL18_YEAST	4	4	2
sp|P53914|KRE33_YEAST	4	4	2
sp|P40991|NOP2_YEAST	4	4	2
sp|P05319|RLA2_YEAST	4	4	2
sp|P0C0V8|RS21A_YEAST	4	3	2
sp|P26785|RL16B_YEAST	4	3	2
sp|O14467|MBF1_YEAST	4	3	2
sp|P33201|MRT4_YEAST	2	2	1
sp|P38828|LSM12_YEAST	2	2	1
sp|Q05946|UTP13_YEAST	2	2	1
sp|P20459|IF2A_YEAST	2	2	1
sp|P33322|CBF5_YEAST	2	2	1
sp|P53141|MLC1_YEAST	2	2	1
sp|P53742|NOG2_YEAST	2	2	1
sp|P25694|CDC48_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|P40070|LSM4_YEAST	2	2	1
sp|P49167|RL38_YEAST	2	2	1
sp|Q06344|ESF1_YEAST	2	2	1
sp|P22147|XRN1_YEAST	2	2	1
sp|P32589|HSP7F_YEAST	2	2	1
sp|P49626|RL4B_YEAST	2	2	1
sp|P02405|RL44_YEAST	2	2	1
sp|P53261|PESC_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|Q08962|NIP7_YEAST	2	2	1
sp|P53276|UTP8_YEAST	2	2	1
sp|P53297|PBP1_YEAST	2	2	1
sp|P32899|IMP3_YEAST	2	2	1
sp|P06367|RS14A_YEAST	11	11	6
sp|P0C2I0|RL20_YEAST	11	9	6
sp|P05736|RL2_YEAST	9	8	5
sp|P05317|RLA0_YEAST	9	7	5
sp|P04147|PABP_YEAST	16	14	9
sp|P14126|RL3_YEAST	14	12	8
sp|P40024|ARB1_YEAST	10	10	6
sp|O13516|RS9A_YEAST	10	10	6
sp|P26786|RS7A_YEAST	5	5	3
sp|P07280|RS19A_YEAST	5	5	3
sp|P12695|ODP2_YEAST	5	5	3
sp|P05748|RL15A_YEAST	5	4	3
sp|P02400|RLA4_YEAST	5	3	3
sp|P41805|RL10_YEAST	8	8	5
sp|P04456|RL25_YEAST	8	7	5
sp|P38011|GBLP_YEAST	12	11	8
sp|P26783|RS5_YEAST	9	7	6
sp|O13527|YA11B_YEAST	6	6	4
sp|P32905|RSSA1_YEAST	6	5	4
sp|P39730|IF2P_YEAST	3	3	2
sp|Q02486|ABF2_YEAST	3	3	2
sp|P23248|RS3A2_YEAST	3	3	2
sp|P37838|NOP4_YEAST	3	3	2
sp|P47077|YJB0_YEAST	3	3	2
sp|P05739|RL6B_YEAST	3	2	2
sp|Q03690|TIF31_YEAST	13	13	9
sp|P16521|EF3A_YEAST	13	13	9
sp|P05738|RL9A_YEAST	10	8	7
sp|P21576|VPS1_YEAST	7	7	5

sp|P05745|RL36A_YEAST	7	7	5
sp|P05737|RL7A_YEAST	7	7	5
sp|P05750|RS3_YEAST	12	12	9
sp|P05753|RS4_YEAST	12	9	9
sp|P35271|RS18_YEAST	8	8	6
sp|P14120|RL30_YEAST	8	6	6
sp|P24784|DBP1_YEAST	4	4	3
sp|P21304|PWP1_YEAST	4	4	3
sp|Q01855|RS15_YEAST	4	4	3
sp|P02309|H4_YEAST	4	4	3
sp|P15108|HSC82_YEAST	4	4	3
sp|P25491|MAS5_YEAST	4	4	3
sp|P05754|RS8_YEAST	4	4	3
sp|P06105|SC160_YEAST	29	29	22
sp|P05744|RL33A_YEAST	5	5	4
sp|P39741|RL35_YEAST	5	5	4
sp|P0C2H6|RL27A_YEAST	5	4	4
sp|P23301|IF5A2_YEAST	5	4	4
sp|P11484|HSP75_YEAST	23	18	19
sp|P10592|HSP72_YEAST	6	6	5
sp|P20447|DBP3_YEAST	6	6	5
sp|P32481|IF2G_YEAST	6	6	5
sp|P53030|RL1_YEAST	6	5	5
sp|P02365|RS6_YEAST	7	7	6
sp|P05756|RS13_YEAST	7	6	6
sp|P38701|RS20_YEAST	7	6	6
sp|Q12460|NOP56_YEAST	16	14	14
sp|P10664|RL4A_YEAST	16	11	14
sp|P15646|FBRL_YEAST	8	7	7
sp|P38631|FKS1_YEAST	26	25	23
sp|Q05022|RRP5_YEAST	24	24	22
sp|P06634|DED1_YEAST	13	13	12
sp|P38934|BFR1_YEAST	18	17	17
sp|P10591|HSP71_YEAST	20	17	20
sp|P02994|EF1A_YEAST	12	10	12
sp|P42945|UTP10_YEAST	9	9	9
sp|O13528|YA11A_YEAST	8	7	8
sp|P32473|ODPB_YEAST	7	6	7
sp|Q12159|YRA1_YEAST	6	6	6
sp|P40213|RS16_YEAST	6	5	6
sp|P20448|DBP4_YEAST	5	5	5
sp|P0C0W1|RS22A_YEAST	5	4	5
sp|P48164|RS7B_YEAST	4	4	4
sp|Q12690|RL13A_YEAST	4	4	4
sp|P05743|RL26A_YEAST	4	4	4
sp|Q12136|SAS10_YEAST	4	4	4
sp|P36105|RL14A_YEAST	4	4	4
sp|Q02753|RL21A_YEAST	4	4	4
sp|P26781|RS11_YEAST	4	4	4
sp|P29453|RL8B_YEAST	4	3	4
sp|P25367|RNQ1_YEAST	3	3	3
sp|P53254|UTP22_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P00330|ADH1_YEAST	3	3	3
sp|Q06679|UTP4_YEAST	3	3	3
sp|Q08745|RS10A_YEAST	3	2	3
sp|Q01560|NOP3_YEAST	2	2	2
sp|Q12035|FCF2_YEAST	2	2	2
sp|P38112|MAK5_YEAST	2	2	2
sp|Q08235|BRX1_YEAST	2	2	2
sp|P40850|MKT1_YEAST	2	2	2
sp|P40055|UTP7_YEAST	2	2	2

sp|Q03973|HMO1_YEAST	2	2	2
sp|Q08287|NOP8_YEAST	2	2	2
sp|P53927|NOP15_YEAST	2	2	2
sp|P34241|URB1_YEAST	2	2	2
sp|P16140|VATB_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|Q04373|PUF6_YEAST	2	2	2
sp|P35178|RRP1_YEAST	2	2	2
sp|Q12000|TMA46_YEAST	2	2	2
sp|P05755|RS9B_YEAST	2	2	2
sp|P40007|NOP16_YEAST	2	2	2
sp|Q01080|RPA49_YEAST	2	2	2
sp|P53734|DBP6_YEAST	1	1	1
sp|Q12266|YB11A_YEAST	1	1	1
sp|P07251|ATPA_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P10962|MAK16_YEAST	1	1	1
sp|P14907|NSP1_YEAST	1	1	1
sp|P25342|CDC10_YEAST	1	1	1
sp|P38882|UTP9_YEAST	1	1	1
sp|P53163|MNP1_YEAST	1	1	1
sp|P43586|LOC1_YEAST	1	1	1
sp|P32529|RPA12_YEAST	1	1	1
sp|P07260|IF4E_YEAST	1	1	1
sp|P33750|DCA13_YEAST	1	1	1
sp|P46669|RPA43_YEAST	1	1	1
sp|Q04013|YHM2_YEAST	1	1	1
sp|P28007|GAR1_YEAST	1	1	1
sp|P40089|LSM5_YEAST	1	1	1
sp|Q04177|UTP5_YEAST	1	1	1
sp|P19454|CSK22_YEAST	1	1	1
sp|P37263|YC16_YEAST	1	1	1
sp|P46990|RL17B_YEAST	1	1	1
sp|P49631|RL43_YEAST	1	1	1
sp|P41056|RL33B_YEAST	1	1	1
sp|P0CH08|RL401_YEAST	1	1	1
sp|P47006|RPA34_YEAST	1	1	1
sp|P53941|IMP4_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P10080|SSBP1_YEAST	1	1	1

sp|P37304|PAM1_YEAST	1	1	1
sp|P38711|RS27B_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|Q07362|PBP4_YEAST	1	1	1
sp|P25617|YCQ6_YEAST	1	1	1
sp|P35189|TAF14_YEAST	1	1	1
sp|Q07623|NOP6_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P32495|NHP2_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P16387|ODPA_YEAST	8	8	9
sp|P17079|RL12_YEAST	6	5	7
sp|Q08208|NOP12_YEAST	5	5	6
sp|P27476|NSR1_YEAST	5	5	6
sp|P15424|MS116_YEAST	5	5	6
sp|P32787|MG101_YEAST	5	5	6
sp|P00549|KPYK1_YEAST	5	5	6
sp|P25635|PWP2_YEAST	4	4	5
sp|P16474|GRP78_YEAST	3	3	4
sp|P48589|RS12_YEAST	5	5	7
sp|Q12176|MAK21_YEAST	7	7	10
sp|P05030|PMA1_YEAST	11	11	16
sp|Q03532|HAS1_YEAST	6	6	9
sp|Q02326|RL6A_YEAST	4	4	6
sp|P00950|PMG1_YEAST	2	2	3
sp|P53883|NOP13_YEAST	2	2	3
sp|P53252|PIL1_YEAST	2	2	3
sp|P26321|RL5_YEAST	2	2	3
sp|P06169|PDC1_YEAST	2	2	3
sp|P38779|CIC1_YEAST	2	2	3
sp|Q12230|LSP1_YEAST	2	2	3
sp|Q12499|NOP58_YEAST	7	7	11
sp|P12398|HSP77_YEAST	3	3	5
sp|P22336|RFA1_YEAST	6	6	12
sp|P32445|RIM1_YEAST	4	4	8
sp|P00924|ENO1_YEAST	3	3	6
sp|P19882|HSP60_YEAST	2	2	4
sp|P09064|IF2B_YEAST	2	2	4
sp|Q12024|YTM1_YEAST	1	1	2
sp|P14540|ALF_YEAST	1	1	2
sp|P40693|RLP7_YEAST	1	1	2
sp|Q06078|UTP21_YEAST	1	1	2
sp|Q12692|H2AZ_YEAST	1	1	2
sp|Q02354|UTP6_YEAST	1	1	2
sp|Q06506|RRP9_YEAST	1	1	2
sp|Q04305|UTP15_YEAST	1	1	2
sp|P25567|SRO9_YEAST	1	1	2
sp|P26755|RFA3_YEAST	1	1	2
sp|Q12220|UTP12_YEAST	1	1	2
sp|P39990|SNU13_YEAST	1	1	2
sp|P50109|PSP2_YEAST	1	1	2
sp|Q04660|ERB1_YEAST	3	3	7
sp|P47083|MPP10_YEAST	2	2	5
sp|Q08492|BUD21_YEAST	1	1	3
sp|P26754|RFA2_YEAST	1	1	3
sp|P06778|RAD52_YEAST	1	1	3
sp|P24276|SSD1_YEAST	1	1	3
sp|P53551|H1_YEAST	1	1	3
sp|P00560|PGK_YEAST	2	2	8
sp|P53131|PRP43_YEAST	1	1	4
sp|P60010|ACT_YEAST	1	1	4
sp|P07259|PYR1_YEAST	1	1	7

Purification of: Mad3-TAP			
protein	total peptides	unique peptides	total peptides in control
sp|P47074|MAD3_YEAST	19	17	
sp|P40957|MAD1_YEAST	14	13	
sp|P26449|BUB3_YEAST	10	10	
sp|P12398|HSP77_YEAST	6	6	
sp|Q06142|IMB1_YEAST	6	6	
sp|Q03690|TIF31_YEAST	5	5	
sp|P41811|COPB2_YEAST	5	5	
sp|P07259|PYR1_YEAST	5	5	
sp|P32074|COPG_YEAST	5	5	
sp|P32324|EF2_YEAST	5	5	
sp|P25367|RNQ1_YEAST	4	4	
sp|P41810|COPB_YEAST	4	4	
sp|P50095|IMDH3_YEAST	4	4	
sp|Q02821|IMA1_YEAST	3	3	
sp|P15424|MS116_YEAST	3	3	
sp|P00330|ADH1_YEAST	3	3	
sp|P06778|RAD52_YEAST	3	3	
sp|Q12230|LSP1_YEAST	3	3	
sp|Q01080|RPA49_YEAST	3	3	
sp|P21576|VPS1_YEAST	3	3	
sp|P00560|PGK_YEAST	3	3	
sp|P53622|COPA_YEAST	3	3	
sp|P07260|IF4E_YEAST	3	3	
sp|P40958|MAD2_YEAST	3	3	
sp|P38922|HRB1_YEAST	2	2	
sp|Q06344|ESF1_YEAST	2	2	
sp|Q12117|MRH1_YEAST	2	2	
sp|P32589|HSP7F_YEAST	2	2	
sp|P53551|H1_YEAST	2	2	
sp|P02557|TBB_YEAST	2	2	
sp|P16521|EF3A_YEAST	2	2	
sp|P39730|IF2P_YEAST	2	2	
sp|P40850|MKT1_YEAST	2	2	
sp|P47006|RPA34_YEAST	2	2	
sp|P18239|ADT2_YEAST	2	2	
sp|P15108|HSC82_YEAST	2	2	
sp|P37263|YC16_YEAST	2	2	
sp|P19882|HSP60_YEAST	1	1	
sp|P38911|FKBP3_YEAST	1	1	
sp|Q06205|FKBP4_YEAST	1	1	
sp|O14455|RL36B_YEAST	1	1	
sp|P53163|MNP1_YEAST	1	1	
sp|P33338|SLA2_YEAST	1	1	
sp|P53221|RL26B_YEAST	1	1	
sp|P38061|RL32_YEAST	1	1	
sp|P25491|MAS5_YEAST	1	1	
sp|P40024|ARB1_YEAST	1	1	
sp|P06704|CDC31_YEAST	1	1	
sp|P16140|VATB_YEAST	1	1	
sp|P32529|RPA12_YEAST	1	1	
sp|Q07362|PBP4_YEAST	1	1	
sp|P11633|NHP6B_YEAST	1	1	
sp|P34077|NIC96_YEAST	1	1	
sp|P40070|LSM4_YEAST	1	1	
sp|P38691|KSP1_YEAST	1	1	
sp|P42846|KRI1_YEAST	1	1	
sp|P39003|HXT6_YEAST	1	1	
sp|P35189|TAF14_YEAST	1	1	
sp|P07251|ATPA_YEAST	1	1	
sp|P38697|IMDH2_YEAST	1	1	
sp|P39015|STM1_YEAST	1	1	
sp|P49631|RL43_YEAST	1	1	

sp|P40509|COPE_YEAST	1	1	
sp|Q12329|HSP42_YEAST	1	1	
sp|P46669|RPA43_YEAST	1	1	
sp|Q04867|YM91_YEAST	1	1	
##sp|P34241|URB1_YEAST	1	1	
sp|P22138|RPA2_YEAST	1	1	
sp|P32590|HSP79_YEAST	1	1	
sp|P50094|IMDH4_YEAST	1	1	
sp|Q08235|BRX1_YEAST	1	1	
sp|P00830|ATPB_YEAST	1	1	
sp|P36000|AP1B1_YEAST	1	1	
sp|P40513|MAM33_YEAST	1	1	
sp|P16861|K6PF1_YEAST	1	1	
sp|P31383|2AAA_YEAST	1	1	
sp|P38711|RS27B_YEAST	1	1	
sp|P46990|RL17B_YEAST	1	1	
##sp|Q03018|ESP1_YEAST	1	1	
sp|P10080|SSBP1_YEAST	1	1	
sp|P26755|RFA3_YEAST	1	1	
sp|P36049|EBP2_YEAST	1	1	
sp|P53734|DBP6_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P38712|RRP3_YEAST	1	1	
sp|Q05024|TRI1_YEAST	1	1	
##sp|Q08977|YP260_YEAST	1	1	
sp|P10081|IF4A_YEAST	1	1	
sp|P32796|CACP_YEAST	1	1	
sp|P00925|ENO2_YEAST	1	1	
sp|P53742|NOG2_YEAST	1	1	
sp|P38779|CIC1_YEAST	1	1	
sp|P47037|SMC3_YEAST	1	1	
sp|P23301|IF5A2_YEAST	1	1	
sp|P32827|RS23_YEAST	1	1	
sp|P39935|IF4F1_YEAST	1	1	
sp|P00950|PMG1_YEAST	1	1	
sp|P05759|RS27A_YEAST	1	1	
sp|P14907|NSP1_YEAST	1	1	
sp|P38809|YHP7_YEAST	1	1	
sp|P53141|MLC1_YEAST	1	1	
sp|P16387|ODPA_YEAST	6	6	1
sp|P24276|SSD1_YEAST	11	11	2
sp|P32473|ODPB_YEAST	5	5	1
sp|P10964|RPA1_YEAST	5	5	1
sp|P53252|PIL1_YEAST	5	5	1
sp|Q02486|ABF2_YEAST	5	5	1
sp|P05740|RL17A_YEAST	5	4	1
sp|P00549|KPYK1_YEAST	4	4	1
sp|P32787|MG101_YEAST	4	4	1
sp|P07280|RS19A_YEAST	4	4	1
sp|P10592|HSP72_YEAST	4	4	1
sp|Q03973|HMO1_YEAST	4	3	1
sp|P39567|IMDH1_YEAST	4	3	1
sp|P10591|HSP71_YEAST	15	14	4
sp|P04911|H2A1_YEAST	3	3	1
sp|P16474|GRP78_YEAST	3	3	1
sp|P04451|RL23_YEAST	3	3	1
sp|P04449|RL24A_YEAST	3	2	1
sp|P0C2H6|RL27A_YEAST	5	5	2
sp|P05738|RL9A_YEAST	5	5	2
sp|P06105|SC160_YEAST	11	11	5
sp|P05030|PMA1_YEAST	17	17	8
sp|P38631|FKS1_YEAST	10	10	5
sp|P14126|RL3_YEAST	6	6	3
sp|P26783|RS5_YEAST	6	6	3

sp|P38011|GBLP_YEAST	4	4	2
sp|P35271|RS18_YEAST	4	4	2
sp|P47083|MPP10_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|P60010|ACT_YEAST	2	2	1
sp|P53254|UTP22_YEAST	2	2	1
sp|P26321|RL5_YEAST	2	2	1
sp|P53336|YG5X_YEAST	2	2	1
sp|P05735|RL19_YEAST	2	2	1
sp|P05736|RL2_YEAST	2	2	1
sp|P00924|ENO1_YEAST	2	2	1
sp|P05755|RS9B_YEAST	2	2	1
sp|P23248|RS3A2_YEAST	2	2	1
sp|P26781|RS11_YEAST	2	2	1
sp|P53030|RL1_YEAST	2	2	1
sp|P53914|KRE33_YEAST	2	2	1
sp|P20447|DBP3_YEAST	14	14	8
sp|P02994|EF1A_YEAST	12	10	7
sp|P05753|RS4_YEAST	5	5	3
sp|P0C0W1|RS22A_YEAST	5	4	3
sp|P32445|RIM1_YEAST	5	4	3
sp|P11484|HSP75_YEAST	16	15	10
sp|P04147|PABP_YEAST	11	11	7
sp|P38934|BFR1_YEAST	6	6	4
sp|Q12159|YRA1_YEAST	6	6	4
sp|P04456|RL25_YEAST	3	3	2
sp|P09064|IF2B_YEAST	3	3	2
sp|P05743|RL26A_YEAST	3	3	2
sp|P02309|H4_YEAST	3	3	2
sp|P41805|RL10_YEAST	3	3	2
sp|P53927|NOP15_YEAST	3	3	2
sp|P26785|RL16B_YEAST	3	3	2
sp|P0C0W9|RL11A_YEAST	3	3	2
sp|P25567|SRO9_YEAST	3	3	2
sp|P21304|PWP1_YEAST	3	3	2
sp|P40213|RS16_YEAST	3	3	2
sp|P14120|RL30_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P39741|RL35_YEAST	3	3	2
sp|P32905|RSSA1_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|P22336|RFA1_YEAST	10	10	7
sp|P05750|RS3_YEAST	8	8	6
sp|P06367|RS14A_YEAST	4	4	3
sp|Q02753|RL21A_YEAST	4	4	3
sp|O13528|YA11A_YEAST	4	4	3
sp|Q06506|RRP9_YEAST	4	3	3
sp|P02365|RS6_YEAST	5	5	4
sp|P33442|RS3A1_YEAST	5	5	4
sp|P05317|RLA0_YEAST	6	6	5
sp|P27476|NSR1_YEAST	6	6	5
sp|Q12460|NOP56_YEAST	12	11	11
sp|Q07457|BRE1_YEAST	7	7	7
sp|P02407|RS17A_YEAST	6	6	6
sp|P15646|FBRL_YEAST	6	5	6
sp|P10664|RL4A_YEAST	5	5	5
sp|P32481|IF2G_YEAST	4	4	4
sp|P05745|RL36A_YEAST	4	4	4
sp|P0C2I0|RL20_YEAST	4	4	4
sp|P05737|RL7A_YEAST	4	4	4
sp|Q12690|RL13A_YEAST	3	3	3
sp|P39990|SNU13_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3

sp|Q01560|NOP3_YEAST	3	3	3
sp|P05754|RS8_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|P17076|RL8A_YEAST	3	3	3
sp|P17079|RL12_YEAST	3	3	3
sp|P26782|RS24_YEAST	3	3	3
sp|Q12692|H2AZ_YEAST	2	2	2
sp|P05744|RL33A_YEAST	2	2	2
sp|Q3E792|RS25A_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|Q04373|PUF6_YEAST	2	2	2
sp|P12695|ODP2_YEAST	2	2	2
sp|Q02326|RL6A_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|P47077|YJB0_YEAST	1	1	1
sp|Q12087|RS30_YEAST	1	1	1
sp|P26784|RL16A_YEAST	1	1	1
sp|P40007|NOP16_YEAST	1	1	1
sp|P20448|DBP4_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P20459|IF2A_YEAST	1	1	1
sp|P38985|SRP14_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P05319|RLA2_YEAST	1	1	1
sp|P32583|SRP40_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P28007|GAR1_YEAST	1	1	1
sp|P29453|RL8B_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|P50109|PSP2_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|Q06078|UTP21_YEAST	1	1	1
sp|P05739|RL6B_YEAST	1	1	1
sp|P36160|RPF2_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P07703|RPAC1_YEAST	1	1	1
sp|P39938|RS26A_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P06634|DED1_YEAST	12	9	13
sp|Q12499|NOP58_YEAST	9	8	10
sp|Q03532|HAS1_YEAST	6	6	7
sp|P33322|CBF5_YEAST	3	3	4
sp|P26786|RS7A_YEAST	3	3	4
sp|P38701|RS20_YEAST	2	2	3
sp|Q08492|BUD21_YEAST	2	2	3
sp|P24784|DBP1_YEAST	3	3	5
sp|Q04660|ERB1_YEAST	2	2	4
sp|P32495|NHP2_YEAST	1	1	2
sp|P48164|RS7B_YEAST	1	1	2
sp|P48589|RS12_YEAST	1	1	2
sp|P05748|RL15A_YEAST	1	1	2
sp|P02406|RL28_YEAST	1	1	2
sp|P0C2H8|RL31A_YEAST	1	1	2
sp|Q08287|NOP8_YEAST	1	1	2
sp|P36105|RL14A_YEAST	1	1	2
sp|P53883|NOP13_YEAST	1	1	2
sp|Q12024|YTM1_YEAST	1	1	2
sp|P40991|NOP2_YEAST	1	1	2
sp|Q05022|RRP5_YEAST	7	7	15
sp|Q12176|MAK21_YEAST	2	2	5
sp|P05756|RS13_YEAST	2	2	5
sp|O13516|RS9A_YEAST	2	2	5
sp|Q08208|NOP12_YEAST	1	1	3
sp|P42945|UTP10_YEAST	1	1	8

purification of: Mad3-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P47074|MAD3_YEAST	17	16	
sp|P26309|CDC20_YEAST	17	15	
sp|Q03690|TIF31_YEAST	10	10	
sp|P26449|BUB3_YEAST	10	9	
sp|P16521|EF3A_YEAST	9	9	
sp|P09798|CDC16_YEAST	7	7	
sp|P21576|VPS1_YEAST	7	7	
sp|P12398|HSP77_YEAST	7	7	
sp|Q06142|IMB1_YEAST	6	6	
sp|P41810|COPB_YEAST	5	5	
sp|Q12230|LSP1_YEAST	5	5	
sp|P32324|EF2_YEAST	5	5	
sp|P40958|MAD2_YEAST	5	5	
sp|P07245|C1TC_YEAST	5	5	
sp|Q01080|RPA49_YEAST	5	5	
sp|P07260|IF4E_YEAST	5	5	
sp|P41811|COPB2_YEAST	4	4	
sp|P34241|URB1_YEAST	4	4	
sp|P22138|RPA2_YEAST	4	4	
sp|Q02821|IMA1_YEAST	4	4	
sp|P22147|XRN1_YEAST	4	4	
sp|P40024|ARB1_YEAST	4	4	
sp|P15424|MS116_YEAST	4	4	
sp|P36049|EBP2_YEAST	4	4	
sp|P07259|PYR1_YEAST	4	4	
sp|P37838|NOP4_YEAST	3	3	
sp|Q02354|UTP6_YEAST	3	3	
sp|P25491|MAS5_YEAST	3	3	
sp|P16522|CDC23_YEAST	3	3	
sp|P38779|CIC1_YEAST	3	3	
sp|Q06344|ESF1_YEAST	3	3	
sp|P32899|IMP3_YEAST	3	3	
sp|P53141|MLC1_YEAST	3	3	
sp|P25694|CDC48_YEAST	3	3	
sp|P39730|IF2P_YEAST	3	3	
sp|P23301|IF5A2_YEAST	3	3	
sp|Q03640|TCB3_YEAST	3	3	
sp|P53886|APC1_YEAST	3	3	
sp|P00044|CYC1_YEAST	2	2	
sp|P40215|NDH1_YEAST	2	2	
sp|Q12754|RRP12_YEAST	2	2	
sp|P25367|RNQ1_YEAST	2	2	
sp|Q08965|BMS1_YEAST	2	2	
sp|P00330|ADH1_YEAST	2	2	
sp|P50094|IMDH4_YEAST	2	2	
sp|P53261|PESC_YEAST	2	2	
sp|P31383|2AAA_YEAST	2	2	
sp|P42846|KRI1_YEAST	2	2	
sp|P32074|COPG_YEAST	2	2	
sp|P39985|DPO5_YEAST	2	2	
sp|P06778|RAD52_YEAST	2	2	
sp|P53297|PBP1_YEAST	2	2	
sp|Q06218|DBP9_YEAST	2	2	

sp|P32796|CACP_YEAST	2	2
sp|P00560|PGK_YEAST	2	2
sp|Q06287|EMG1_YEAST	2	2
sp|P38061|RL32_YEAST	2	2
sp|P40850|MKT1_YEAST	2	2
sp|P02557|TBB_YEAST	2	2
sp|P07149|FAS1_YEAST	2	2
sp|P15108|HSC82_YEAST	2	2
sp|P53622|COPA_YEAST	2	2
sp|Q12117|MRH1_YEAST	2	2
sp|P53131|PRP43_YEAST	2	2
sp|Q08235|BRX1_YEAST	2	2
sp|P38788|SSZ1_YEAST	2	2
sp|P53734|DBP6_YEAST	2	2
sp|P38144|ISW1_YEAST	2	2
sp|Q06631|BFR2_YEAST	2	2
sp|P38249|EIF3A_YEAST	2	2
sp|P16140|VATB_YEAST	2	2
sp|P53221|RL26B_YEAST	2	2
sp|P38903|2A5D_YEAST	2	2
sp|Q03940|RUVB1_YEAST	2	2
sp|P38911|FKBP3_YEAST	2	2
sp|P26755|RFA3_YEAST	2	1
sp|Q06106|MRD1_YEAST	1	1
sp|P37263|YC16_YEAST	1	1
sp|P39935|IF4F1_YEAST	1	1
sp|P50085|PHB2_YEAST	1	1
sp|P12945|NAT1_YEAST	1	1
sp|P38691|KSP1_YEAST	1	1
sp|P38912|IF1A_YEAST	1	1
sp|Q06132|SGD1_YEAST	1	1
sp|P20967|ODO1_YEAST	1	1
sp|P30771|NAM7_YEAST	1	1
sp|P39936|IF4F2_YEAST	1	1
sp|P40485|SLM1_YEAST	1	1
sp|Q07623|NOP6_YEAST	1	1
sp|P25443|RS2_YEAST	1	1
sp|P32589|HSP7F_YEAST	1	1
sp|P38697|IMDH2_YEAST	1	1
sp|Q04013|YHM2_YEAST	1	1
sp|P09624|DLDH_YEAST	1	1
sp|Q12000|TMA46_YEAST	1	1
sp|P38922|HRB1_YEAST	1	1
sp|P10622|RLA3_YEAST	1	1
sp|Q12266|YB11A_YEAST	1	1
sp|P40509|COPE_YEAST	1	1
sp|P50095|IMDH3_YEAST	1	1
sp|P02405|RL44_YEAST	1	1
sp|P06704|CDC31_YEAST	1	1
sp|Q07896|NOC3_YEAST	1	1
sp|P32598|PP12_YEAST	1	1
sp|Q04305|UTP15_YEAST	1	1
sp|Q06205|FKBP4_YEAST	1	1
sp|Q12379|SWM1_YEAST	1	1
sp|P38041|BOB1_YEAST	1	1

sp|P40513|MAM33_YEAST	1	1
sp|P38711|RS27B_YEAST	1	1
sp|P39003|HXT6_YEAST	1	1
sp|P10962|MAK16_YEAST	1	1
sp|P38042|CDC27_YEAST	1	1
sp|P40693|RLP7_YEAST	1	1
sp|P06787|CALM_YEAST	1	1
sp|Q08096|RCL1_YEAST	1	1
sp|P14907|NSP1_YEAST	1	1
sp|P38712|RRP3_YEAST	1	1
sp|P43586|LOC1_YEAST	1	1
##sp|Q08977|YP260_YEAST	1	1
sp|P53551|H1_YEAST	1	1
sp|Q04500|UTP14_YEAST	1	1
sp|P35189|TAF14_YEAST	1	1
sp|P39015|STM1_YEAST	1	1
sp|P48231|TCB2_YEAST	1	1
sp|Q12464|RUVB2_YEAST	1	1
sp|P53040|TAF6_YEAST	1	1
sp|Q02892|NOG1_YEAST	1	1
sp|P25623|SYP1_YEAST	1	1
sp|P32827|RS23_YEAST	1	1
sp|P43609|RSC8_YEAST	1	1
sp|P18239|ADT2_YEAST	1	1
sp|P48361|ASK10_YEAST	1	1
sp|Q00684|CDC14_YEAST	1	1
sp|Q12490|YB11B_YEAST	1	1
sp|P32471|EF1B_YEAST	1	1
sp|P46669|RPA43_YEAST	1	1
sp|Q04867|YM91_YEAST	1	1
sp|P19454|CSK22_YEAST	1	1
sp|P39729|RBG1_YEAST	1	1
sp|P00925|ENO2_YEAST	1	1
sp|P11632|NHP6A_YEAST	1	1
sp|P38828|LSM12_YEAST	1	1
sp|P46990|RL17B_YEAST	1	1
sp|P53742|NOG2_YEAST	1	1
sp|P20434|RPAB1_YEAST	1	1
sp|P00950|PMG1_YEAST	1	1
sp|Q01477|UBP3_YEAST	1	1
sp|P11633|NHP6B_YEAST	1	1
sp|P40070|LSM4_YEAST	1	1
sp|P41056|RL33B_YEAST	1	1
sp|P53163|MNP1_YEAST	1	1
sp|Q08683|APC5_YEAST	1	1
sp|P47006|RPA34_YEAST	1	1
sp|O14455|RL36B_YEAST	1	1
sp|P49626|RL4B_YEAST	1	1
sp|P38333|ENP1_YEAST	1	1
sp|P40089|LSM5_YEAST	1	1
sp|P47037|SMC3_YEAST	1	1
sp|P10080|SSBP1_YEAST	1	1
sp|Q12213|RL7B_YEAST	1	1
sp|P36516|RM03_YEAST	1	1
sp|P49631|RL43_YEAST	1	1

sp|P06169|PDC1_YEAST	1	1	
sp|Q07362|PBP4_YEAST	1	1	
sp|P32529|RPA12_YEAST	1	1	
sp|P16451|ODPX_YEAST	1	1	
sp|P10964|RPA1_YEAST	10	10	1
sp|P25635|PWP2_YEAST	8	8	1
sp|P16387|ODPA_YEAST	8	8	1
sp|P10592|HSP72_YEAST	6	6	1
sp|P32473|ODPB_YEAST	6	6	1
sp|P53252|PIL1_YEAST	6	6	1
sp|P04449|RL24A_YEAST	6	4	1
sp|P53914|KRE33_YEAST	5	5	1
sp|P60010|ACT_YEAST	5	5	1
sp|O13527|YA11B_YEAST	5	5	1
sp|P07280|RS19A_YEAST	5	5	1
sp|P05736|RL2_YEAST	5	4	1
sp|P05740|RL17A_YEAST	5	4	1
sp|P10591|HSP71_YEAST	19	16	4
sp|P06105|SC160_YEAST	23	23	5
sp|P38934|BFR1_YEAST	18	17	4
sp|P38011|GBLP_YEAST	9	9	2
sp|P24276|SSD1_YEAST	8	8	2
sp|P05738|RL9A_YEAST	8	7	2
sp|P53254|UTP22_YEAST	4	4	1
sp|P00549|KPYK1_YEAST	4	4	1
sp|P05735|RL19_YEAST	4	4	1
sp|P29453|RL8B_YEAST	4	3	1
sp|P39567|IMDH1_YEAST	4	2	1
sp|P35271|RS18_YEAST	7	7	2
sp|P14120|RL30_YEAST	7	6	2
sp|P38631|FKS1_YEAST	17	16	5
sp|P14126|RL3_YEAST	9	9	3
sp|P41805|RL10_YEAST	6	6	2
sp|P32583|SRP40_YEAST	3	3	1
sp|P16474|GRP78_YEAST	3	3	1
sp|P47083|MPP10_YEAST	3	3	1
sp|P26781|RS11_YEAST	3	3	1
sp|P32787|MG101_YEAST	3	3	1
sp|P26784|RL16A_YEAST	3	3	1
sp|P00924|ENO1_YEAST	3	3	1
sp|P33201|MRT4_YEAST	3	3	1
sp|P53030|RL1_YEAST	3	2	1
sp|P05739|RL6B_YEAST	3	2	1
sp|P26783|RS5_YEAST	8	8	3
sp|P05753|RS4_YEAST	8	7	3
sp|P05743|RL26A_YEAST	5	5	2
sp|P0C2H6|RL27A_YEAST	5	5	2
sp|P40213|RS16_YEAST	5	5	2
sp|P05748|RL15A_YEAST	5	4	2
sp|P04456|RL25_YEAST	5	4	2
sp|O13528|YA11A_YEAST	7	6	3
sp|P04147|PABP_YEAST	16	13	7
sp|P05030|PMA1_YEAST	18	17	8
sp|Q05022|RRP5_YEAST	30	30	15
sp|O13516|RS9A_YEAST	10	10	5

sp|Q12176|MAK21_YEAST	10	10	5
sp|P10664|RL4A_YEAST	10	8	5
sp|Q04660|ERB1_YEAST	8	8	4
sp|P33442|RS3A1_YEAST	8	8	4
sp|P32445|RIM1_YEAST	6	5	3
sp|Q02326|RL6A_YEAST	4	4	2
sp|P09064|IF2B_YEAST	4	4	2
sp|P05744|RL33A_YEAST	4	4	2
sp|P48164|RS7B_YEAST	4	4	2
sp|P40991|NOP2_YEAST	4	4	2
sp|P39741|RL35_YEAST	4	4	2
sp|P02309|H4_YEAST	4	4	2
sp|P12695|ODP2_YEAST	4	4	2
sp|P20459|IF2A_YEAST	2	2	1
sp|Q03973|HMO1_YEAST	2	2	1
sp|P47077|YJB0_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|Q02486|ABF2_YEAST	2	2	1
sp|P53276|UTP8_YEAST	2	2	1
sp|P47108|URB2_YEAST	2	2	1
sp|P50109|PSP2_YEAST	2	2	1
sp|P05319|RLA2_YEAST	2	2	1
sp|P35178|RRP1_YEAST	2	2	1
sp|Q12087|RS30_YEAST	2	2	1
sp|P40007|NOP16_YEAST	2	2	1
sp|P38112|MAK5_YEAST	2	2	1
sp|P05755|RS9B_YEAST	2	2	1
sp|P40055|UTP7_YEAST	2	2	1
sp|P04451|RL23_YEAST	2	2	1
sp|P26321|RL5_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|Q06078|UTP21_YEAST	2	2	1
sp|P20448|DBP4_YEAST	2	2	1
sp|P11484|HSP75_YEAST	19	17	10
sp|P0C2I0|RL20_YEAST	7	7	4
sp|P22336|RFA1_YEAST	12	12	7
sp|P06367|RS14A_YEAST	5	5	3
sp|P26782|RS24_YEAST	5	5	3
sp|Q08208|NOP12_YEAST	5	5	3
sp|P39744|NOC2_YEAST	5	5	3
sp|P38701|RS20_YEAST	5	4	3
sp|P0C0W1|RS22A_YEAST	5	4	3
sp|P42945|UTP10_YEAST	13	13	8
sp|P05750|RS3_YEAST	9	9	6
sp|P15646|FBRL_YEAST	9	8	6
sp|P05745|RL36A_YEAST	6	6	4
sp|Q12159|YRA1_YEAST	6	6	4
sp|P53927|NOP15_YEAST	3	3	2
sp|P53941|IMP4_YEAST	3	3	2
sp|P21304|PWP1_YEAST	3	3	2
sp|Q12024|YTM1_YEAST	3	3	2
sp|Q04373|PUF6_YEAST	3	3	2
sp|P0C0W9|RL11A_YEAST	3	3	2
sp|Q02931|UTP17_YEAST	3	3	2
sp|P26785|RL16B_YEAST	3	3	2

sp|P48589|RS12_YEAST	3	3	2
sp|P32905|RSSA1_YEAST	3	3	2
sp|P53883|NOP13_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|Q08962|NIP7_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|P36105|RL14A_YEAST	3	2	2
sp|P02994|EF1A_YEAST	10	9	7
sp|P27476|NSR1_YEAST	7	7	5
sp|P05756|RS13_YEAST	7	6	5
sp|Q02753|RL21A_YEAST	4	4	3
sp|P17076|RL8A_YEAST	4	4	3
sp|Q08492|BUD21_YEAST	4	4	3

sp|Q05946|UTP13_YEAST	4	4	3
sp|Q12690|RL13A_YEAST	4	4	3
sp|P02400|RLA4_YEAST	4	3	3
sp|Q12460|NOP56_YEAST	14	13	11
sp|P02365|RS6_YEAST	5	5	4
sp|P05737|RL7A_YEAST	5	5	4
sp|P32481|IF2G_YEAST	5	5	4
sp|P05317|RLA0_YEAST	6	6	5
sp|P02407|RS17A_YEAST	7	6	6
sp|P06634|DED1_YEAST	15	12	13
sp|Q03532|HAS1_YEAST	8	8	7
sp|P20447|DBP3_YEAST	9	9	8
sp|Q12499|NOP58_YEAST	10	9	10
sp|P26786|RS7A_YEAST	4	4	4
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P17079|RL12_YEAST	3	3	3
sp|P05754|RS8_YEAST	3	3	3
sp|Q12136|SAS10_YEAST	3	3	3
sp|Q01560|NOP3_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|Q06506|RRP9_YEAST	3	2	3
sp|Q12220|UTP12_YEAST	2	2	2
sp|Q3E705|EFG1P_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|P0C2H8|RL31A_YEAST	2	2	2
sp|P38882|UTP9_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|P04911|H2A1_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|P33750|DCA13_YEAST	1	1	1
sp|P00358|G3P2_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|Q12035|FCF2_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P25617|YCQ6_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P23248|RS3A2_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P36160|RPF2_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P41058|RS29B_YEAST	1	1	1
sp|P28007|GAR1_YEAST	1	1	1
sp|P24784|DBP1_YEAST	4	4	5
sp|Q06679|UTP4_YEAST	3	3	4
sp|Q07457|BRE1_YEAST	5	5	7
sp|P39990|SNU13_YEAST	2	2	3
sp|P33322|CBF5_YEAST	2	2	4
sp|Q3E792|RS25A_YEAST	1	1	2
sp|P25567|SRO9_YEAST	1	1	2
sp|P32495|NHP2_YEAST	1	1	2

purification of: Bub1-TAP protein	total peptides	unique peptides	total peptides in control
sp|P41695|BUB1_YEAST	26	25	
sp|P26449|BUB3_YEAST	7	7	
sp|Q03690|TIF31_YEAST	6	6	
sp|P40957|MAD1_YEAST	5	5	
sp|P32324|EF2_YEAST	5	5	
sp|P07260|IF4E_YEAST	4	4	
sp|P15424|MS116_YEAST	4	4	
sp|P16140|VATB_YEAST	4	4	
sp|P39730|IF2P_YEAST	3	3	
sp|P00330|ADH1_YEAST	3	3	
sp|P15108|HSC82_YEAST	3	3	
sp|P38779|CIC1_YEAST	3	3	
sp|P38697|IMDH2_YEAST	3	3	
sp|Q01080|RPA49_YEAST	2	2	
sp|P36049|EBP2_YEAST	2	2	
sp|P40958|MAD2_YEAST	2	2	
sp|P53551|H1_YEAST	2	2	
sp|Q08965|BMS1_YEAST	2	2	
sp|P25443|RS2_YEAST	2	2	
sp|P12398|HSP77_YEAST	2	2	
sp|P47006|RPA34_YEAST	2	2	
sp|Q3E7Y3|RS22B_YEAST	1	1	
sp|P16862|K6PF2_YEAST	1	1	
sp|P40850|MKT1_YEAST	1	1	
sp|Q04305|UTP15_YEAST	1	1	
sp|O14455|RL36B_YEAST	1	1	
sp|P53297|PBP1_YEAST	1	1	
sp|Q06511|RRP15_YEAST	1	1	
sp|Q99207|NOP14_YEAST	1	1	
sp|Q08746|RRS1_YEAST	1	1	
sp|P38711|RS27B_YEAST	1	1	
sp|Q12230|LSP1_YEAST	1	1	
sp|P07259|PYR1_YEAST	1	1	
sp|P25367|RNQ1_YEAST	1	1	
sp|P38712|RRP3_YEAST	1	1	
sp|Q04500|UTP14_YEAST	1	1	
sp|P32827|RS23_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P39935|IF4F1_YEAST	1	1	
sp|P53742|NOG2_YEAST	1	1	
sp|Q07896|NOC3_YEAST	1	1	
sp|P02405|RL44_YEAST	1	1	
sp|P25491|MAS5_YEAST	1	1	
sp|P37263|YC16_YEAST	1	1	
sp|P38789|SSF1_YEAST	1	1	
sp|Q04867|YM91_YEAST	1	1	
sp|P49631|RL43_YEAST	1	1	
sp|P39985|DPO5_YEAST	1	1	

sp|Q08096|RCL1_YEAST	1	1	
sp|Q12117|MRH1_YEAST	1	1	
sp|P38061|RL32_YEAST	1	1	
sp|P38911|FKBP3_YEAST	1	1	
sp|P50095|IMDH3_YEAST	1	1	
sp|P00560|PGK_YEAST	1	1	
sp|P21576|VPS1_YEAST	1	1	
sp|P42846|KRI1_YEAST	1	1	
##sp|Q08977|YP260_YEAST	1	1	
sp|P11633|NHP6B_YEAST	1	1	
sp|Q08235|BRX1_YEAST	1	1	
sp|P34241|URB1_YEAST	1	1	
sp|Q06106|MRD1_YEAST	1	1	
sp|P00925|ENO2_YEAST	1	1	
sp|P16521|EF3A_YEAST	1	1	
sp|P46669|RPA43_YEAST	1	1	
sp|P38691|KSP1_YEAST	1	1	
sp|P39015|STM1_YEAST	1	1	
sp|Q06287|EMG1_YEAST	1	1	
sp|P00950|PMG1_YEAST	1	1	
sp|P06169|PDC1_YEAST	1	1	
sp|P16861|K6PF1_YEAST	1	1	
sp|P40693|RLP7_YEAST	1	1	
sp|P23301|IF5A2_YEAST	1	1	
sp|P35189|TAF14_YEAST	1	1	
sp|Q12213|RL7B_YEAST	1	1	
sp|P10964|RPA1_YEAST	6	6	1
sp|P10592|HSP72_YEAST	5	5	1
sp|Q02486|ABF2_YEAST	5	5	1
sp|P24276|SSD1_YEAST	9	8	2
sp|P00549|KPYK1_YEAST	4	4	1
sp|P16387|ODPA_YEAST	4	4	1
sp|P04449|RL24A_YEAST	4	3	1
sp|P32787|MG101_YEAST	3	3	1
sp|P07280|RS19A_YEAST	3	3	1
sp|P05736|RL2_YEAST	3	3	1
sp|P00924|ENO1_YEAST	3	3	1
sp|P53254|UTP22_YEAST	3	3	1
sp|Q03973|HMO1_YEAST	3	3	1
sp|P10591|HSP71_YEAST	11	11	4
sp|P38011|GBLP_YEAST	5	5	2
sp|P09064|IF2B_YEAST	5	5	2
sp|P05753|RS4_YEAST	7	6	3
sp|P26783|RS5_YEAST	6	6	3
sp|P04456|RL25_YEAST	4	4	2
sp|P35271|RS18_YEAST	4	4	2
sp|P41805|RL10_YEAST	4	4	2
sp|P40213|RS16_YEAST	4	4	2
sp|P39567|IMDH1_YEAST	2	2	1
sp|P02293|H2B1_YEAST	2	2	1

sp|P47077|YJB0_YEAST	2	2	1
sp|P26784|RL16A_YEAST	2	2	1
sp|P04911|H2A1_YEAST	2	2	1
sp|P47083|MPP10_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|P00358|G3P2_YEAST	2	2	1
sp|P20448|DBP4_YEAST	2	2	1
sp|P05319|RLA2_YEAST	2	2	1
sp|P05735|RL19_YEAST	2	2	1
sp|P25635|PWP2_YEAST	2	2	1
sp|P05755|RS9B_YEAST	2	2	1
sp|P26321|RL5_YEAST	2	2	1
sp|Q06078|UTP21_YEAST	2	2	1
sp|P16474|GRP78_YEAST	2	2	1
sp|P53252|PIL1_YEAST	2	2	1
sp|P60010|ACT_YEAST	2	2	1
sp|O13527|YA11B_YEAST	2	2	1
sp|P05740|RL17A_YEAST	2	2	1
sp|P04451|RL23_YEAST	2	2	1
sp|P53030|RL1_YEAST	2	1	1
sp|P06105|SC160_YEAST	9	9	5
sp|P0C2I0|RL20_YEAST	7	7	4
sp|P02365|RS6_YEAST	7	7	4
sp|P06367|RS14A_YEAST	5	5	3
sp|P17076|RL8A_YEAST	5	5	3
sp|Q12690|RL13A_YEAST	5	5	3
sp|P32445|RIM1_YEAST	5	5	3
sp|P05030|PMA1_YEAST	13	13	8
sp|P10664|RL4A_YEAST	8	8	5
sp|P33442|RS3A1_YEAST	6	6	4
sp|Q12159|YRA1_YEAST	6	6	4
sp|P02309|H4_YEAST	3	3	2
sp|P39741|RL35_YEAST	3	3	2
sp|P36105|RL14A_YEAST	3	3	2
sp|P05748|RL15A_YEAST	3	3	2
sp|P40991|NOP2_YEAST	3	3	2
sp|P32905|RSSA1_YEAST	3	3	2
sp|P25567|SRO9_YEAST	3	3	2
sp|P21304|PWP1_YEAST	3	3	2
sp|P05738|RL9A_YEAST	3	3	2
sp|P0C0W9|RL11A_YEAST	3	3	2
sp|P0C2H6|RL27A_YEAST	3	3	2
sp|P05743|RL26A_YEAST	3	3	2
sp|P14120|RL30_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|Q05022|RRP5_YEAST	21	21	15
sp|P38631|FKS1_YEAST	7	7	5
sp|Q12460|NOP56_YEAST	15	14	11
sp|P05750|RS3_YEAST	8	8	6
sp|P14126|RL3_YEAST	4	4	3

sp|Q02753|RL21A_YEAST	4	4	3
sp|P11484|HSP75_YEAST	13	12	10
sp|P04147|PABP_YEAST	9	9	7
sp|P26786|RS7A_YEAST	5	5	4
sp|P05737|RL7A_YEAST	5	5	4
sp|P38934|BFR1_YEAST	5	5	4
sp|P32481|IF2G_YEAST	5	5	4
sp|P02994|EF1A_YEAST	8	8	7
sp|Q03532|HAS1_YEAST	8	8	7
sp|P02407|RS17A_YEAST	6	6	6
sp|P15646|FBRL_YEAST	6	5	6
sp|O13516|RS9A_YEAST	5	5	5
sp|Q04660|ERB1_YEAST	4	4	4
sp|P33322|CBF5_YEAST	4	4	4
sp|Q06506|RRP9_YEAST	3	3	3
sp|P17079|RL12_YEAST	3	3	3
sp|Q01560|NOP3_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|P05754|RS8_YEAST	3	3	3
sp|Q12136|SAS10_YEAST	3	3	3
sp|P0C0W1|RS22A_YEAST	3	3	3
sp|P26782|RS24_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P05744|RL33A_YEAST	2	2	2
sp|P26785|RL16B_YEAST	2	2	2
sp|P48164|RS7B_YEAST	2	2	2
sp|P48589|RS12_YEAST	2	2	2
sp|P07279|RL18_YEAST	2	2	2
sp|Q12024|YTM1_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|P53927|NOP15_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2
sp|Q3E792|RS25A_YEAST	2	2	2
sp|P12695|ODP2_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|P53276|UTP8_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P32583|SRP40_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1

sp|P36160|RPF2_YEAST	1	1	1
sp|P41058|RS29B_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P28007|GAR1_YEAST	1	1	1
sp|P39938|RS26A_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P20459|IF2A_YEAST	1	1	1
sp|P33201|MRT4_YEAST	1	1	1
sp|Q12035|FCF2_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P29453|RL8B_YEAST	1	1	1
sp|P53914|KRE33_YEAST	1	1	1
sp|P38112|MAK5_YEAST	1	1	1
sp|P40007|NOP16_YEAST	1	1	1
sp|P38985|SRP14_YEAST	1	1	1
sp|P32473|ODPB_YEAST	1	1	1
sp|P23248|RS3A2_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P20447|DBP3_YEAST	7	7	8
sp|Q12499|NOP58_YEAST	8	8	10
sp|P05317|RLA0_YEAST	4	4	5
sp|P27476|NSR1_YEAST	4	4	5
sp|P05756|RS13_YEAST	4	4	5
sp|Q12176|MAK21_YEAST	4	4	5
sp|P06634|DED1_YEAST	10	10	13
sp|P42945|UTP10_YEAST	6	6	8
sp|P05745|RL36A_YEAST	3	3	4
sp|Q06679|UTP4_YEAST	3	3	4
sp|P38701|RS20_YEAST	2	2	3
sp|P39744|NOC2_YEAST	2	2	3
sp|Q05946|UTP13_YEAST	2	2	3
sp|P39990|SNU13_YEAST	2	2	3
sp|Q08208|NOP12_YEAST	2	2	3
sp|Q08492|BUD21_YEAST	2	2	3
sp|O13528|YA11A_YEAST	2	2	3
sp|P24784|DBP1_YEAST	3	3	5
sp|P22336|RFA1_YEAST	4	4	7
sp|P32495|NHP2_YEAST	1	1	2
sp|P0C2H8|RL31A_YEAST	1	1	2
sp|Q12220|UTP12_YEAST	1	1	2
sp|Q04373|PUF6_YEAST	1	1	2
sp|Q08962|NIP7_YEAST	1	1	2
sp|Q02326|RL6A_YEAST	1	1	2
sp|P53883|NOP13_YEAST	1	1	2
sp|P38882|UTP9_YEAST	1	1	2
sp|Q02931|UTP17_YEAST	1	1	2
sp|P53941|IMP4_YEAST	1	1	2
sp|Q08287|NOP8_YEAST	1	1	2
sp|Q07457|BRE1_YEAST	2	2	7

Purification of: Bub1-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P41695|BUB1_YEAST	46	42	
sp|P40957|MAD1_YEAST	31	31	
sp|P40460|NDC80_YEAST	13	13	
sp|P26449|BUB3_YEAST	12	12	
sp|Q03690|TIF31_YEAST	11	11	
sp|P32324|EF2_YEAST	10	10	
sp|P16521|EF3A_YEAST	8	8	
sp|P15424|MS116_YEAST	8	8	
sp|P40024|ARB1_YEAST	7	7	
sp|P22147|XRN1_YEAST	7	7	
sp|P16140|VATB_YEAST	7	7	
sp|P53148|SP105_YEAST	7	7	
sp|P06169|PDC1_YEAST	6	6	
sp|Q03640|TCB3_YEAST	6	6	
sp|P21576|VPS1_YEAST	6	6	
sp|P40850|MKT1_YEAST	6	6	
sp|P38779|CIC1_YEAST	6	6	
sp|P07149|FAS1_YEAST	5	5	
sp|Q08965|BMS1_YEAST	5	5	
sp|P25623|SYP1_YEAST	5	5	
sp|P38697|IMDH2_YEAST	5	5	
sp|P00560|PGK_YEAST	5	5	
sp|P12398|HSP77_YEAST	5	5	
sp|P39935|IF4F1_YEAST	5	5	
sp|P37838|NOP4_YEAST	5	5	
sp|P29311|BMH1_YEAST	4	4	
sp|P49631|RL43_YEAST	4	4	
sp|P00950|PMG1_YEAST	4	4	
sp|P00330|ADH1_YEAST	4	4	
sp|Q12754|RRP12_YEAST	4	4	
sp|P50095|IMDH3_YEAST	4	4	
sp|P47035|NET1_YEAST	4	4	
sp|P53297|PBP1_YEAST	4	4	
sp|Q04431|YD532_YEAST	4	4	
sp|P22138|RPA2_YEAST	4	4	
sp|Q03940|RUVB1_YEAST	3	3	
sp|P40958|MAD2_YEAST	3	3	
sp|P09440|C1TM_YEAST	3	3	
sp|P07245|C1TC_YEAST	3	3	
sp|P38041|BOB1_YEAST	3	3	
sp|Q04867|YM91_YEAST	3	3	
sp|P38828|LSM12_YEAST	3	3	
sp|P23301|IF5A2_YEAST	3	3	
sp|P07259|PYR1_YEAST	3	3	
sp|P39730|IF2P_YEAST	3	3	
sp|P16861|K6PF1_YEAST	3	3	

sp|P38691|KSP1_YEAST	3	3
sp|P34241|URB1_YEAST	3	3
sp|P53261|PESC_YEAST	3	3
sp|P15108|HSC82_YEAST	3	3
sp|P38249|EIF3A_YEAST	3	3
sp|P32899|IMP3_YEAST	3	3
sp|P08153|SWI5_YEAST	3	3
sp|P10080|SSBP1_YEAST	3	3
sp|Q06218|DBP9_YEAST	3	3
sp|P02557|TBB_YEAST	3	3
sp|P53550|DCP2_YEAST	3	3
sp|P10081|IF4A_YEAST	3	3
sp|Q04500|UTP14_YEAST	3	3
sp|P25491|MAS5_YEAST	3	3
sp|P39985|DPO5_YEAST	3	3
sp|P00925|ENO2_YEAST	3	3
sp|Q04177|UTP5_YEAST	3	2
sp|P00044|CYC1_YEAST	2	2
sp|Q00684|CDC14_YEAST	2	2
sp|Q12230|LSP1_YEAST	2	2
sp|P47149|NNF1_YEAST	2	2
sp|P53734|DBP6_YEAST	2	2
sp|Q07915|RLP24_YEAST	2	2
sp|P50085|PHB2_YEAST	2	2
sp|Q12000|TMA46_YEAST	2	2
sp|P53221|RL26B_YEAST	2	2
sp|Q08096|RCL1_YEAST	2	2
sp|P30771|NAM7_YEAST	2	2
sp|P38061|RL32_YEAST	2	2
sp|P39729|RBG1_YEAST	2	2
sp|Q01080|RPA49_YEAST	2	2
sp|P32589|HSP7F_YEAST	2	2
sp|P33895|NUF2_YEAST	2	2
sp|P39998|EDC3_YEAST	2	2
sp|P10962|MAK16_YEAST	2	2
sp|Q02892|NOG1_YEAST	2	2
sp|Q06631|BFR2_YEAST	2	2
sp|Q12339|UTP23_YEAST	2	2
sp|P36049|EBP2_YEAST	2	2
sp|P48231|TCB2_YEAST	2	2
sp|P07260|IF4E_YEAST	2	2
sp|Q08235|BRX1_YEAST	2	2
sp|P39731|MTW1_YEAST	2	2
sp|P16862|K6PF2_YEAST	2	2
sp|P38911|FKBP3_YEAST	2	2
sp|Q08237|REXO4_YEAST	2	2
sp|P42846|KRI1_YEAST	2	2
sp|P47006|RPA34_YEAST	2	2

sp|Q07362|PBP4_YEAST	2	2
sp|P38922|HRB1_YEAST	2	2
sp|Q06106|MRD1_YEAST	2	2
sp|P32892|DRS1_YEAST	2	2
sp|P40693|RLP7_YEAST	2	2
sp|P00817|IPYR_YEAST	2	2
sp|P43586|LOC1_YEAST	2	2
sp|P53131|PRP43_YEAST	2	2
sp|Q02354|UTP6_YEAST	2	2
sp|P15705|STI1_YEAST	2	2
sp|P25367|RNQ1_YEAST	2	2
sp|P00830|ATPB_YEAST	2	2
sp|P53136|NSA1_YEAST	2	2
sp|O14455|RL36B_YEAST	2	2
sp|Q04491|SEC13_YEAST	2	2
sp|P25443|RS2_YEAST	2	2
sp|Q06287|EMG1_YEAST	2	2
sp|P40070|LSM4_YEAST	2	2
sp|P53551|H1_YEAST	2	2
sp|P12945|NAT1_YEAST	2	2
sp|Q07896|NOC3_YEAST	2	2
sp|P39015|STM1_YEAST	2	2
sp|P49626|RL4B_YEAST	2	2
sp|P38333|ENP1_YEAST	2	2
sp|Q06344|ESF1_YEAST	2	2
sp|P24000|RL24B_YEAST	2	1
sp|P35194|UTP20_YEAST	1	1
sp|P38789|SSF1_YEAST	1	1
sp|P40089|LSM5_YEAST	1	1
sp|P47143|ADK_YEAST	1	1
sp|P53732|RT12_YEAST	1	1
sp|P39523|YM11_YEAST	1	1
sp|P16451|ODPX_YEAST	1	1
sp|P25555|GBP2_YEAST	1	1
sp|P32562|CDC5_YEAST	1	1
sp|P38629|RFC3_YEAST	1	1
sp|P39987|HSP7E_YEAST	1	1
sp|P45978|SCD6_YEAST	1	1
sp|P53163|MNP1_YEAST	1	1
sp|P20434|RPAB1_YEAST	1	1
sp|P35201|MIF2_YEAST	1	1
sp|P38809|YHP7_YEAST	1	1
sp|Q00955|ACAC_YEAST	1	1
sp|P38630|RFC1_YEAST	1	1
sp|P46675|STU2_YEAST	1	1
sp|P05759|RS27A_YEAST	1	1
sp|Q02821|IMA1_YEAST	1	1
sp|Q06511|RRP15_YEAST	1	1

sp|Q12266|YB11A_YEAST	1	1
sp|P40217|EIF3I_YEAST	1	1
sp|P47912|LCF4_YEAST	1	1
sp|P07251|ATPA_YEAST	1	1
sp|P53742|NOG2_YEAST	1	1
sp|Q04013|YHM2_YEAST	1	1
sp|P41056|RL33B_YEAST	1	1
sp|P50094|IMDH4_YEAST	1	1
sp|P25586|KRR1_YEAST	1	1
sp|P46951|YPP1_YEAST	1	1
sp|P06103|EIF3B_YEAST	1	1
sp|P35732|YKF4_YEAST	1	1
sp|P53865|CNM67_YEAST	1	1
sp|P14832|CYPH_YEAST	1	1
sp|P00359|G3P3_YEAST	1	1
sp|Q01477|UBP3_YEAST	1	1
sp|Q05123|ARP9_YEAST	1	1
sp|P32598|PP12_YEAST	1	1
sp|P34077|NIC96_YEAST	1	1
sp|P46956|PHO86_YEAST	1	1
sp|Q06678|RM35_YEAST	1	1
sp|Q12389|DBP10_YEAST	1	1
sp|P38904|SPP41_YEAST	1	1
##sp|P22516|CHL1_YEAST	1	1
sp|P40485|SLM1_YEAST	1	1
sp|P14907|NSP1_YEAST	1	1
sp|Q04225|RRB1_YEAST	1	1
sp|P32380|NUF1_YEAST	1	1
sp|P40010|NUG1_YEAST	1	1
sp|P46990|RL17B_YEAST	1	1
sp|P06168|ILV5_YEAST	1	1
sp|Q02959|HOS3_YEAST	1	1
sp|P20484|MAK11_YEAST	1	1
sp|Q12406|ARP7_YEAST	1	1
sp|P36084|MUD2_YEAST	1	1
##sp|P32660|ATC5_YEAST	1	1
sp|P40498|YIJ1_YEAST	1	1
sp|P48234|NOL10_YEAST	1	1
sp|Q04305|UTP15_YEAST	1	1
sp|P24783|DBP2_YEAST	1	1
sp|P38199|YBD2_YEAST	1	1
sp|Q12117|MRH1_YEAST	1	1
sp|P32827|RS23_YEAST	1	1
sp|P40014|SPC25_YEAST	1	1
sp|P02405|RL44_YEAST	1	1
sp|P11633|NHP6B_YEAST	1	1
sp|P40568|DSN1_YEAST	1	1
sp|P48361|ASK10_YEAST	1	1

sp|P32468|CDC12_YEAST	1	1
sp|P38203|LSM2_YEAST	1	1
sp|P53040|TAF6_YEAST	1	1
sp|P25644|PAT1_YEAST	1	1
sp|P34760|TSA1_YEAST	1	1
sp|P38711|RS27B_YEAST	1	1
sp|Q12464|RUVB2_YEAST	1	1
sp|Q99207|NOP14_YEAST	1	1
sp|P36521|RM11_YEAST	1	1
sp|P38930|CSK2C_YEAST	1	1
sp|P25342|CDC10_YEAST	1	1
sp|P0CH08|RL401_YEAST	1	1
sp|P18239|ADT2_YEAST	1	1
sp|Q06205|FKBP4_YEAST	1	1
sp|P38712|RRP3_YEAST	1	1
sp|P12683|HMDH1_YEAST	1	1
sp|Q03653|EFR3_YEAST	1	1
sp|Q07468|VAM6_YEAST	1	1
sp|Q12466|TCB1_YEAST	1	1
sp|P36528|RM17_YEAST	1	1
sp|P48837|NUP57_YEAST	1	1
sp|Q04477|SPC24_YEAST	1	1
sp|P38272|YBY0_YEAST	1	1
sp|P43603|LSB3_YEAST	1	1
sp|Q02457|TBF1_YEAST	1	1
sp|P19097|FAS2_YEAST	1	1
sp|P35179|SC61G_YEAST	1	1
sp|P40059|DOT6_YEAST	1	1
sp|P06704|CDC31_YEAST	1	1
sp|Q07623|NOP6_YEAST	1	1
sp|P28743|KIP2_YEAST	1	1
sp|Q12490|YB11B_YEAST	1	1
sp|P37263|YC16_YEAST	1	1
sp|P39003|HXT6_YEAST	1	1
sp|P40956|GTS1_YEAST	1	1
sp|Q08746|RRS1_YEAST	1	1
sp|P38330|RMD9L_YEAST	1	1
sp|P39960|BEM2_YEAST	1	1
sp|P43609|RSC8_YEAST	1	1
sp|P53141|MLC1_YEAST	1	1
sp|P19454|CSK22_YEAST	1	1
sp|Q12213|RL7B_YEAST	1	1
sp|P35189|TAF14_YEAST	1	1
sp|P38788|SSZ1_YEAST	1	1
sp|P06778|RAD52_YEAST	1	1
sp|Q03761|TAF12_YEAST	1	1
sp|P22276|RPC2_YEAST	1	1
sp|P29295|HRR25_YEAST	1	1

sp|P09435|HSP73_YEAST	1	1	
sp|P32529|RPA12_YEAST	1	1	
sp|P45818|ROK1_YEAST	1	1	
sp|P19524|MYO2_YEAST	1	1	
sp|P26755|RFA3_YEAST	1	1	
sp|O13527|YA11B_YEAST	14	14	1
sp|P10964|RPA1_YEAST	11	11	1
sp|P53914|KRE33_YEAST	9	9	1
sp|P00549|KPYK1_YEAST	8	8	1
sp|P10592|HSP72_YEAST	7	7	1
sp|P20448|DBP4_YEAST	7	7	1
sp|P53254|UTP22_YEAST	7	7	1
sp|P25635|PWP2_YEAST	7	7	1
sp|P47083|MPP10_YEAST	7	7	1
sp|P24276|SSD1_YEAST	13	13	2
sp|P53276|UTP8_YEAST	6	6	1
sp|P16387|ODPA_YEAST	6	6	1
sp|P32473|ODPB_YEAST	6	5	1
sp|P05735|RL19_YEAST	6	5	1
sp|P04449|RL24A_YEAST	6	4	1
sp|P39567|IMDH1_YEAST	6	4	1
sp|P10591|HSP71_YEAST	20	18	4
sp|P26784|RL16A_YEAST	5	5	1
sp|P07280|RS19A_YEAST	5	5	1
sp|P60010|ACT_YEAST	5	5	1
sp|P00924|ENO1_YEAST	5	5	1
sp|P47108|URB2_YEAST	5	5	1
sp|P05736|RL2_YEAST	5	4	1
sp|P38934|BFR1_YEAST	19	18	4
sp|P38011|GBLP_YEAST	9	9	2
sp|P35271|RS18_YEAST	9	8	2
sp|P06105|SC160_YEAST	22	22	5
sp|P14126|RL3_YEAST	12	10	3
sp|P26781|RS11_YEAST	4	4	1
sp|P33750|DCA13_YEAST	4	4	1
sp|P04451|RL23_YEAST	4	4	1
sp|P38112|MAK5_YEAST	4	4	1
sp|Q02486|ABF2_YEAST	4	4	1
sp|P05740|RL17A_YEAST	4	3	1
sp|P20459|IF2A_YEAST	4	3	1
sp|P53030|RL1_YEAST	4	3	1
sp|P38631|FKS1_YEAST	18	18	5
sp|P40213|RS16_YEAST	7	7	2
sp|Q02931|UTP17_YEAST	7	7	2
sp|P14120|RL30_YEAST	7	6	2
sp|P05738|RL9A_YEAST	7	6	2
sp|P0C2H6|RL27A_YEAST	7	6	2
sp|P05753|RS4_YEAST	10	9	3

sp|P26782|RS24_YEAST	9	8	3
sp|P25567|SRO9_YEAST	6	6	2
sp|P41805|RL10_YEAST	6	6	2
sp|P23248|RS3A2_YEAST	3	3	1
sp|P16474|GRP78_YEAST	3	3	1
sp|P00358|G3P2_YEAST	3	3	1
sp|P04911|H2A1_YEAST	3	3	1
sp|P32787|MG101_YEAST	3	3	1
sp|Q06078|UTP21_YEAST	3	3	1
sp|P26321|RL5_YEAST	3	3	1
sp|P35178|RRP1_YEAST	3	3	1
sp|P40055|UTP7_YEAST	3	3	1
sp|Q99216|PNO1_YEAST	3	3	1
sp|P33201|MRT4_YEAST	3	3	1
sp|Q03973|HMO1_YEAST	3	2	1
sp|P29453|RL8B_YEAST	3	2	1
sp|O14467|MBF1_YEAST	3	2	1
sp|P10664|RL4A_YEAST	13	10	5
sp|P0C2I0|RL20_YEAST	10	8	4
sp|P40991|NOP2_YEAST	5	5	2
sp|P02309|H4_YEAST	5	5	2
sp|P05743|RL26A_YEAST	5	5	2
sp|P12695|ODP2_YEAST	5	5	2
sp|P05748|RL15A_YEAST	5	4	2
sp|P32905|RSSA1_YEAST	5	4	2
sp|P04147|PABP_YEAST	17	14	7
sp|Q12176|MAK21_YEAST	12	12	5
sp|O13528|YA11A_YEAST	7	7	3
sp|P05737|RL7A_YEAST	9	8	4
sp|P11484|HSP75_YEAST	22	18	10
sp|P42945|UTP10_YEAST	17	17	8
sp|Q05022|RRP5_YEAST	31	30	15
sp|P05030|PMA1_YEAST	16	15	8
sp|P27476|NSR1_YEAST	10	9	5
sp|Q04660|ERB1_YEAST	8	8	4
sp|P32481|IF2G_YEAST	8	8	4
sp|P26783|RS5_YEAST	6	6	3
sp|P06367|RS14A_YEAST	6	6	3
sp|P0C0W1|RS22A_YEAST	6	5	3
sp|P17076|RL8A_YEAST	6	5	3
sp|P17079|RL12_YEAST	6	4	3
sp|Q12024|YTM1_YEAST	4	4	2
sp|P38882|UTP9_YEAST	4	4	2
sp|P04456|RL25_YEAST	4	4	2
sp|P0C0W9|RL11A_YEAST	4	4	2
sp|P05744|RL33A_YEAST	4	4	2
sp|P07279|RL18_YEAST	4	4	2
sp|P21304|PWP1_YEAST	4	4	2

sp|P36105|RL14A_YEAST	4	4	2
sp|P02406|RL28_YEAST	4	4	2
sp|P09064|IF2B_YEAST	4	4	2
sp|P26785|RL16B_YEAST	4	3	2
sp|P0C2H8|RL31A_YEAST	4	3	2
sp|Q07897|CMS1_YEAST	2	2	1
sp|Q12522|IF6_YEAST	2	2	1
sp|P05739|RL6B_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|P02293|H2B1_YEAST	2	2	1
sp|P50109|PSP2_YEAST	2	2	1
sp|Q12087|RS30_YEAST	2	2	1
sp|P34247|UTP11_YEAST	2	2	1
sp|P05319|RLA2_YEAST	2	2	1
sp|P07703|RPAC1_YEAST	2	2	1
sp|P53336|YG5X_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|P28007|GAR1_YEAST	2	1	1
sp|P33442|RS3A1_YEAST	7	7	4
sp|P33322|CBF5_YEAST	7	7	4
sp|Q03532|HAS1_YEAST	12	12	7
sp|P02994|EF1A_YEAST	12	11	7
sp|P05750|RS3_YEAST	10	10	6
sp|P32445|RIM1_YEAST	5	5	3
sp|Q01855|RS15_YEAST	5	5	3
sp|P38701|RS20_YEAST	5	5	3
sp|P15646|FBRL_YEAST	9	8	6
sp|P02365|RS6_YEAST	6	6	4
sp|P26786|RS7A_YEAST	6	6	4
sp|Q12159|YRA1_YEAST	6	6	4
sp|P48164|RS7B_YEAST	3	3	2
sp|P00360|G3P1_YEAST	3	3	2
sp|P53927|NOP15_YEAST	3	3	2
sp|Q04373|PUF6_YEAST	3	3	2
sp|Q02326|RL6A_YEAST	3	3	2
sp|P48589|RS12_YEAST	3	3	2
sp|P53941|IMP4_YEAST	3	3	2
sp|Q12220|UTP12_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|Q3E792|RS25A_YEAST	3	2	2
sp|P32495|NHP2_YEAST	3	2	2
sp|O13516|RS9A_YEAST	7	7	5
sp|P05756|RS13_YEAST	7	6	5
sp|P20447|DBP3_YEAST	11	11	8
sp|P02407|RS17A_YEAST	8	7	6
sp|Q12690|RL13A_YEAST	4	4	3
sp|Q08208|NOP12_YEAST	4	4	3
sp|P39744|NOC2_YEAST	4	4	3

sp|Q12136|SAS10_YEAST	4	4	3
sp|P05754|RS8_YEAST	4	4	3
sp|P02400|RLA4_YEAST	4	3	3
sp|Q12460|NOP56_YEAST	14	12	11
sp|Q06679|UTP4_YEAST	5	5	4
sp|P05745|RL36A_YEAST	5	5	4
sp|P05317|RLA0_YEAST	6	6	5
sp|P06634|DED1_YEAST	15	13	13
sp|P22336|RFA1_YEAST	8	8	7
sp|Q06506|RRP9_YEAST	3	3	3
sp|Q05946|UTP13_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|Q08492|BUD21_YEAST	3	3	3
sp|Q02753|RL21A_YEAST	3	3	3
sp|Q12692|H2AZ_YEAST	2	2	2
sp|Q3E705|EFG1P_YEAST	2	2	2
sp|P53883|NOP13_YEAST	2	2	2
sp|P39741|RL35_YEAST	2	2	2
sp|Q08287|NOP8_YEAST	2	2	2
sp|Q08962|NIP7_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|Q02796|LGE1_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|P53252|PIL1_YEAST	1	1	1
sp|P38080|AKL1_YEAST	1	1	1
sp|P25617|YCQ6_YEAST	1	1	1
sp|Q12035|FCF2_YEAST	1	1	1
sp|P40007|NOP16_YEAST	1	1	1
sp|P47077|YJB0_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P05755|RS9B_YEAST	1	1	1
sp|Q12499|NOP58_YEAST	9	9	10
sp|P24784|DBP1_YEAST	4	4	5
sp|P39990|SNU13_YEAST	1	1	3
sp|Q01560|NOP3_YEAST	1	1	3
sp|Q07457|BRE1_YEAST	2	2	7

Purification of: Bub3-TAP protein	total peptides	unique peptides	total peptides in control
sp|P41695|BUB1_YEAST	20	20	
sp|P47074|MAD3_YEAST	16	15	
sp|P26449|BUB3_YEAST	16	12	
sp|P21576|VPS1_YEAST	7	7	
sp|P40513|MAM33_YEAST	7	6	
sp|Q03690|TIF31_YEAST	6	6	
sp|P32324|EF2_YEAST	6	6	
sp|P15424|MS116_YEAST	6	6	
sp|P07259|PYR1_YEAST	4	4	
sp|P15108|HSC82_YEAST	4	4	
sp|P00330|ADH1_YEAST	3	3	
sp|P12398|HSP77_YEAST	3	3	
sp|Q12230|LSP1_YEAST	3	3	
sp|Q01080|RPA49_YEAST	3	3	
sp|P41810|COPB_YEAST	3	3	
sp|P06169|PDC1_YEAST	2	2	
sp|P16140|VATB_YEAST	2	2	
sp|P53551|H1_YEAST	2	2	
sp|P50095|IMDH3_YEAST	2	2	
sp|P39015|STM1_YEAST	2	2	
sp|P40850|MKT1_YEAST	2	2	
sp|P39730|IF2P_YEAST	2	2	
sp|P10081|IF4A_YEAST	2	2	
sp|P02557|TBB_YEAST	2	2	
sp|P25367|RNQ1_YEAST	2	2	
sp|P41811|COPB2_YEAST	2	2	
sp|P53297|PBP1_YEAST	2	2	
sp|P25443|RS2_YEAST	2	1	
sp|P50094|IMDH4_YEAST	1	1	
sp|P40217|EIF3I_YEAST	1	1	
sp|P22138|RPA2_YEAST	1	1	
sp|P32827|RS23_YEAST	1	1	
sp|P37263|YC16_YEAST	1	1	
sp|P43586|LOC1_YEAST	1	1	
sp|O14455|RL36B_YEAST	1	1	
sp|Q06511|RRP15_YEAST	1	1	
sp|Q12117|MRH1_YEAST	1	1	
sp|P22147|XRN1_YEAST	1	1	
sp|P46669|RPA43_YEAST	1	1	
sp|P11633|NHP6B_YEAST	1	1	
sp|P38061|RL32_YEAST	1	1	
sp|P47006|RPA34_YEAST	1	1	
sp|P53163|MNP1_YEAST	1	1	
sp|P06704|CDC31_YEAST	1	1	
sp|P16521|EF3A_YEAST	1	1	
sp|Q03940|RUVB1_YEAST	1	1	

sp|P26755|RFA3_YEAST	1	1	
sp|P53221|RL26B_YEAST	1	1	
sp|P02405|RL44_YEAST	1	1	
sp|P06778|RAD52_YEAST	1	1	
sp|P16862|K6PF2_YEAST	1	1	
sp|Q12213|RL7B_YEAST	1	1	
sp|P23301|IF5A2_YEAST	1	1	
sp|P38697|IMDH2_YEAST	1	1	
sp|Q08235|BRX1_YEAST	1	1	
sp|P10080|SSBP1_YEAST	1	1	
sp|P34077|NIC96_YEAST	1	1	
sp|P07260|IF4E_YEAST	1	1	
sp|Q12329|HSP42_YEAST	1	1	
sp|P35189|TAF14_YEAST	1	1	
sp|P38711|RS27B_YEAST	1	1	
sp|P00560|PGK_YEAST	1	1	
sp|P39985|DPO5_YEAST	1	1	
sp|P53261|PESC_YEAST	1	1	
##sp|Q08977|YP260_YEAST	1	1	
sp|P38712|RRP3_YEAST	1	1	
sp|Q12464|RUVB2_YEAST	1	1	
sp|P36049|EBP2_YEAST	1	1	
sp|P38828|LSM12_YEAST	1	1	
sp|P00925|ENO2_YEAST	1	1	
sp|P40024|ARB1_YEAST	1	1	
sp|Q12000|TMA46_YEAST	1	1	
sp|P42846|KRI1_YEAST	1	1	
sp|P38922|HRB1_YEAST	1	1	
sp|P49631|RL43_YEAST	1	1	
sp|Q06344|ESF1_YEAST	1	1	
sp|P00950|PMG1_YEAST	1	1	
sp|P32796|CACP_YEAST	1	1	
sp|P26309|CDC20_YEAST	1	1	
sp|Q02486|ABF2_YEAST	6	6	1
sp|P10964|RPA1_YEAST	6	6	1
sp|P16387|ODPA_YEAST	5	5	1
sp|P24276|SSD1_YEAST	9	8	2
sp|P00549|KPYK1_YEAST	4	4	1
sp|P07280|RS19A_YEAST	4	4	1
sp|P39567|IMDH1_YEAST	4	3	1
sp|P10591|HSP71_YEAST	14	14	4
sp|P05738|RL9A_YEAST	6	6	2
sp|P04451|RL23_YEAST	3	3	1
sp|P53030|RL1_YEAST	3	3	1
sp|P60010|ACT_YEAST	3	3	1
sp|Q03973|HMO1_YEAST	3	3	1
sp|P32473|ODPB_YEAST	3	3	1
sp|P00924|ENO1_YEAST	3	3	1

sp|P10592|HSP72_YEAST	3	3	1
sp|P32787|MG101_YEAST	3	3	1
sp|P05740|RL17A_YEAST	3	3	1
sp|P41805|RL10_YEAST	5	5	2
sp|P35271|RS18_YEAST	5	5	2
sp|P26782|RS24_YEAST	6	6	3
sp|P02309|H4_YEAST	4	4	2
sp|P04456|RL25_YEAST	4	4	2
sp|P38011|GBLP_YEAST	4	4	2
sp|P0C2H6|RL27A_YEAST	4	4	2
sp|P40213|RS16_YEAST	4	4	2
sp|P09064|IF2B_YEAST	4	4	2
sp|P02293|H2B1_YEAST	2	2	1
sp|P26321|RL5_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|P16474|GRP78_YEAST	2	2	1
sp|P53914|KRE33_YEAST	2	2	1
sp|P04911|H2A1_YEAST	2	2	1
sp|P53252|PIL1_YEAST	2	2	1
sp|P05735|RL19_YEAST	2	2	1
sp|P05736|RL2_YEAST	2	2	1
sp|P26784|RL16A_YEAST	2	2	1
sp|O13527|YA11B_YEAST	2	2	1
sp|P04449|RL24A_YEAST	2	1	1
sp|P04147|PABP_YEAST	13	13	7
sp|P38631|FKS1_YEAST	9	9	5
sp|P05030|PMA1_YEAST	14	14	8
sp|P17076|RL8A_YEAST	5	5	3
sp|P26783|RS5_YEAST	5	5	3
sp|P0C0W1|RS22A_YEAST	5	4	3
sp|P32445|RIM1_YEAST	5	4	3
sp|P05753|RS4_YEAST	5	4	3
sp|P10664|RL4A_YEAST	8	8	5
sp|P06105|SC160_YEAST	8	8	5
sp|P0C2I0|RL20_YEAST	6	6	4
sp|P05743|RL26A_YEAST	3	3	2
sp|P0C0W9|RL11A_YEAST	3	3	2
sp|P40991|NOP2_YEAST	3	3	2
sp|P53927|NOP15_YEAST	3	3	2
sp|P14120|RL30_YEAST	3	3	2
sp|P39741|RL35_YEAST	3	3	2
sp|P48164|RS7B_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P25567|SRO9_YEAST	3	3	2
sp|P36105|RL14A_YEAST	3	3	2
sp|P02994|EF1A_YEAST	10	8	7
sp|P05750|RS3_YEAST	8	8	6
sp|Q02753|RL21A_YEAST	4	4	3

sp|Q01560|NOP3_YEAST	4	4	3
sp|O13528|YA11A_YEAST	4	3	3
sp|P22336|RFA1_YEAST	9	9	7
sp|P38934|BFR1_YEAST	5	5	4
sp|P02365|RS6_YEAST	5	5	4
sp|Q12159|YRA1_YEAST	5	5	4
sp|P15646|FBRL_YEAST	7	6	6
sp|P11484|HSP75_YEAST	11	10	10
sp|Q12460|NOP56_YEAST	12	12	11
sp|P02407|RS17A_YEAST	6	6	6
sp|P24784|DBP1_YEAST	5	5	5
sp|P05756|RS13_YEAST	5	4	5
sp|P05745|RL36A_YEAST	4	4	4
sp|P32481|IF2G_YEAST	4	4	4
sp|P05737|RL7A_YEAST	4	4	4
sp|P26786|RS7A_YEAST	4	4	4
sp|Q12690|RL13A_YEAST	3	3	3
sp|P06367|RS14A_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|Q08208|NOP12_YEAST	3	3	3
sp|P38701|RS20_YEAST	3	3	3
sp|Q08492|BUD21_YEAST	3	3	3
sp|P05754|RS8_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|Q06506|RRP9_YEAST	3	2	3
sp|P0C0V8|RS21A_YEAST	2	2	2
sp|P32905|RSSA1_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2
sp|P12695|ODP2_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|Q04373|PUF6_YEAST	2	2	2
sp|P48589|RS12_YEAST	2	2	2
sp|Q08962|NIP7_YEAST	2	2	2
sp|P26785|RL16B_YEAST	2	2	2
sp|P21304|PWP1_YEAST	2	2	2
sp|Q02326|RL6A_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|Q12087|RS30_YEAST	1	1	1
sp|P38985|SRP14_YEAST	1	1	1
sp|P28007|GAR1_YEAST	1	1	1
sp|P29453|RL8B_YEAST	1	1	1
sp|P33201|MRT4_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|P23248|RS3A2_YEAST	1	1	1
sp|P00358|G3P2_YEAST	1	1	1
sp|P41058|RS29B_YEAST	1	1	1

sp|P47077|YJB0_YEAST	1	1	1
sp|P26781|RS11_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|P05319|RLA2_YEAST	1	1	1
sp|P47083|MPP10_YEAST	1	1	1
sp|P53254|UTP22_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|P05755|RS9B_YEAST	1	1	1
sp|P53276|UTP8_YEAST	1	1	1
sp|P20459|IF2A_YEAST	1	1	1
sp|P32583|SRP40_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|Q06078|UTP21_YEAST	1	1	1
sp|P07703|RPAC1_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P36160|RPF2_YEAST	1	1	1
sp|P20447|DBP3_YEAST	7	7	8
sp|P06634|DED1_YEAST	11	8	13
sp|P05317|RLA0_YEAST	4	4	5
sp|O13516|RS9A_YEAST	4	4	5
sp|P33442|RS3A1_YEAST	3	3	4
sp|Q05022|RRP5_YEAST	11	11	15
sp|Q03532|HAS1_YEAST	5	5	7
sp|P14126|RL3_YEAST	2	2	3
sp|P39990|SNU13_YEAST	2	2	3
sp|P42945|UTP10_YEAST	5	5	8
sp|Q12499|NOP58_YEAST	6	6	10
sp|P27476|NSR1_YEAST	3	3	5
sp|Q06679|UTP4_YEAST	2	2	4
sp|P33322|CBF5_YEAST	2	2	4
sp|Q04660|ERB1_YEAST	2	2	4
sp|P53883|NOP13_YEAST	1	1	2
sp|P05744|RL33A_YEAST	1	1	2
sp|Q3E792|RS25A_YEAST	1	1	2
sp|P05748|RL15A_YEAST	1	1	2
sp|P32495|NHP2_YEAST	1	1	2
sp|Q12024|YTM1_YEAST	1	1	2
sp|Q12176|MAK21_YEAST	2	2	5
sp|P39744|NOC2_YEAST	1	1	3
sp|P17079|RL12_YEAST	1	1	3
sp|Q07457|BRE1_YEAST	2	2	7

Purification of: Bub3-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P40957|MAD1_YEAST	30	29	
sp|P41695|BUB1_YEAST	26	25	
sp|P47074|MAD3_YEAST	16	15	
sp|P26309|CDC20_YEAST	16	14	
sp|P26449|BUB3_YEAST	16	12	
sp|Q03690|TIF31_YEAST	11	11	
sp|P21576|VPS1_YEAST	8	8	
sp|P22147|XRN1_YEAST	8	8	
sp|P40958|MAD2_YEAST	8	8	
sp|P16521|EF3A_YEAST	8	8	
sp|P40513|MAM33_YEAST	8	7	
sp|P32324|EF2_YEAST	7	7	
sp|P40850|MKT1_YEAST	6	6	
sp|P07245|C1TC_YEAST	6	6	
sp|P07259|PYR1_YEAST	6	6	
sp|P15424|MS116_YEAST	5	5	
sp|P16140|VATB_YEAST	5	5	
sp|P40024|ARB1_YEAST	5	5	
sp|P53297|PBP1_YEAST	5	5	
sp|P34241|URB1_YEAST	5	5	
sp|Q06218|DBP9_YEAST	5	5	
sp|Q12754|RRP12_YEAST	5	5	
sp|P41810|COPB_YEAST	5	5	
sp|P22138|RPA2_YEAST	4	4	
sp|P53261|PESC_YEAST	4	4	
sp|P12398|HSP77_YEAST	4	4	
sp|P25623|SYP1_YEAST	4	4	
sp|P32074|COPG_YEAST	4	4	
sp|P09798|CDC16_YEAST	4	4	
sp|Q02354|UTP6_YEAST	4	4	
sp|P32796|CACP_YEAST	4	4	
sp|P37838|NOP4_YEAST	4	4	
sp|P41811|COPB2_YEAST	4	4	
sp|Q00684|CDC14_YEAST	3	3	
sp|P38779|CIC1_YEAST	3	3	
sp|P25491|MAS5_YEAST	3	3	
sp|P50095|IMDH3_YEAST	3	3	
sp|Q03640|TCB3_YEAST	3	3	
sp|P38061|RL32_YEAST	3	3	
sp|P10080|SSBP1_YEAST	3	3	
sp|P00330|ADH1_YEAST	3	3	
sp|Q03940|RUVB1_YEAST	3	3	
sp|P39985|DPO5_YEAST	3	3	
sp|Q12230|LSP1_YEAST	3	3	
sp|P32899|IMP3_YEAST	3	3	
sp|P15108|HSC82_YEAST	3	3	

sp|P25367|RNQ1_YEAST	3	3
sp|P26755|RFA3_YEAST	3	2
sp|P23301|IF5A2_YEAST	3	2
sp|P19097|FAS2_YEAST	2	2
sp|P38711|RS27B_YEAST	2	2
sp|Q08096|RCL1_YEAST	2	2
sp|P42846|KRI1_YEAST	2	2
sp|P25443|RS2_YEAST	2	2
sp|P40693|RLP7_YEAST	2	2
sp|Q01080|RPA49_YEAST	2	2
sp|P40010|NUG1_YEAST	2	2
sp|Q06631|BFR2_YEAST	2	2
sp|Q12464|RUVB2_YEAST	2	2
sp|P38788|SSZ1_YEAST	2	2
sp|P43586|LOC1_YEAST	2	2
sp|P38809|YHP7_YEAST	2	2
sp|Q08237|REXO4_YEAST	2	2
sp|Q99207|NOP14_YEAST	2	2
sp|P30771|NAM7_YEAST	2	2
sp|P36049|EBP2_YEAST	2	2
sp|P39730|IF2P_YEAST	2	2
sp|P43609|RSC8_YEAST	2	2
sp|P06778|RAD52_YEAST	2	2
sp|Q12117|MRH1_YEAST	2	2
sp|Q07362|PBP4_YEAST	2	2
sp|P38828|LSM12_YEAST	2	2
sp|P53550|DCP2_YEAST	2	2
sp|P06787|CALM_YEAST	2	2
sp|P32598|PP12_YEAST	2	2
sp|P02557|TBB_YEAST	2	2
sp|P53551|H1_YEAST	2	2
sp|P07149|FAS1_YEAST	2	2
sp|P20484|MAK11_YEAST	2	2
sp|P00560|PGK_YEAST	2	2
sp|P53141|MLC1_YEAST	2	2
sp|P25694|CDC48_YEAST	2	2
sp|P40215|NDH1_YEAST	2	2
sp|P38697|IMDH2_YEAST	2	2
sp|P49626|RL4B_YEAST	2	2
sp|P53221|RL26B_YEAST	2	2
sp|P53734|DBP6_YEAST	2	2
sp|Q06287|EMG1_YEAST	2	2
sp|P24000|RL24B_YEAST	2	1
sp|Q06344|ESF1_YEAST	1	1
sp|Q12266|YB11A_YEAST	1	1
sp|P50085|PHB2_YEAST	1	1
sp|P11632|NHP6A_YEAST	1	1
sp|P35191|MDJ1_YEAST	1	1

sp|P39523|YM11_YEAST	1	1
sp|Q04491|SEC13_YEAST	1	1
sp|P38041|BOB1_YEAST	1	1
sp|P53742|NOG2_YEAST	1	1
sp|P19454|CSK22_YEAST	1	1
sp|P32908|SMC1_YEAST	1	1
sp|O14455|RL36B_YEAST	1	1
sp|P50094|IMDH4_YEAST	1	1
sp|P11633|NHP6B_YEAST	1	1
sp|P29311|BMH1_YEAST	1	1
sp|P32529|RPA12_YEAST	1	1
sp|P38042|CDC27_YEAST	1	1
sp|P0CH08|RL401_YEAST	1	1
sp|P20434|RPAB1_YEAST	1	1
sp|Q01477|UBP3_YEAST	1	1
sp|Q08235|BRX1_YEAST	1	1
sp|Q6Q547|NOP10_YEAST	1	1
sp|P36013|MAOM_YEAST	1	1
sp|P39729|RBG1_YEAST	1	1
sp|P06704|CDC31_YEAST	1	1
sp|Q04867|YM91_YEAST	1	1
sp|P53886|APC1_YEAST	1	1
sp|P20436|RPAB3_YEAST	1	1
sp|Q12466|TCB1_YEAST	1	1
sp|P12683|HMDH1_YEAST	1	1
sp|P22276|RPC2_YEAST	1	1
sp|P32589|HSP7F_YEAST	1	1
sp|P48231|TCB2_YEAST	1	1
sp|P09440|C1TM_YEAST	1	1
sp|Q12490|YB11B_YEAST	1	1
sp|Q03761|TAF12_YEAST	1	1
sp|P31383|2AAA_YEAST	1	1
sp|P46669|RPA43_YEAST	1	1
sp|P06786|TOP2_YEAST	1	1
sp|P32597|STH1_YEAST	1	1
sp|P40070|LSM4_YEAST	1	1
sp|P48361|ASK10_YEAST	1	1
sp|P09624|DLDH_YEAST	1	1
sp|P53040|TAF6_YEAST	1	1
sp|P12945|NAT1_YEAST	1	1
sp|P36516|RM03_YEAST	1	1
sp|P46990|RL17B_YEAST	1	1
sp|P38333|ENP1_YEAST	1	1
sp|P40089|LSM5_YEAST	1	1
sp|P10622|RLA3_YEAST	1	1
sp|Q07623|NOP6_YEAST	1	1
sp|P34077|NIC96_YEAST	1	1
sp|P38911|FKBP3_YEAST	1	1

sp|P41056|RL33B_YEAST	1	1
sp|P53131|PRP43_YEAST	1	1
sp|Q08683|APC5_YEAST	1	1
sp|P36521|RM11_YEAST	1	1
sp|P39935|IF4F1_YEAST	1	1
sp|P47006|RPA34_YEAST	1	1
sp|Q06106|MRD1_YEAST	1	1
sp|P16861|K6PF1_YEAST	1	1
sp|P25644|PAT1_YEAST	1	1
##sp|P32660|ATC5_YEAST	1	1
sp|P48837|NUP57_YEAST	1	1
sp|Q07807|PUF3_YEAST	1	1
sp|P38930|CSK2C_YEAST	1	1
sp|Q02457|TBF1_YEAST	1	1
sp|Q04013|YHM2_YEAST	1	1
sp|P32380|NUF1_YEAST	1	1
sp|P36528|RM17_YEAST	1	1
sp|P47035|NET1_YEAST	1	1
sp|P53622|COPA_YEAST	1	1
sp|Q06205|FKBP4_YEAST	1	1
sp|Q12213|RL7B_YEAST	1	1
sp|P38691|KSP1_YEAST	1	1
##sp|Q08977|YP260_YEAST	1	1
sp|P10962|MAK16_YEAST	1	1
sp|Q07896|NOC3_YEAST	1	1
sp|P20606|SAR1_YEAST	1	1
sp|P53163|MNP1_YEAST	1	1
sp|Q04177|UTP5_YEAST	1	1
sp|P37263|YC16_YEAST	1	1
sp|P47037|SMC3_YEAST	1	1
sp|P53732|RT12_YEAST	1	1
sp|P32827|RS23_YEAST	1	1
sp|P40217|EIF3I_YEAST	1	1
sp|P20967|ODO1_YEAST	1	1
sp|P39003|HXT6_YEAST	1	1
sp|P00925|ENO2_YEAST	1	1
sp|Q04305|UTP15_YEAST	1	1
sp|P14907|NSP1_YEAST	1	1
sp|Q08965|BMS1_YEAST	1	1
sp|P07260|IF4E_YEAST	1	1
sp|P18239|ADT2_YEAST	1	1
sp|P40509|COPE_YEAST	1	1
sp|P49631|RL43_YEAST	1	1
sp|Q00402|NUM1_YEAST	1	1
sp|Q07915|RLP24_YEAST	1	1
sp|P28000|RPAC2_YEAST	1	1
sp|P35189|TAF14_YEAST	1	1
sp|P39015|STM1_YEAST	1	1

sp|P00950|PMG1_YEAST	1	1	
sp|Q02892|NOG1_YEAST	1	1	
sp|Q12000|TMA46_YEAST	1	1	
sp|P39998|EDC3_YEAST	1	1	
sp|P53741|BRE5_YEAST	1	1	
sp|P10964|RPA1_YEAST	8	8	1
sp|O13527|YA11B_YEAST	7	7	1
sp|P04449|RL24A_YEAST	7	5	1
sp|P53254|UTP22_YEAST	6	6	1
sp|P05735|RL19_YEAST	6	6	1
sp|Q02486|ABF2_YEAST	6	6	1
sp|P10591|HSP71_YEAST	20	17	4
sp|P24276|SSD1_YEAST	10	10	2
sp|P53276|UTP8_YEAST	5	5	1
sp|P16387|ODPA_YEAST	5	5	1
sp|P10592|HSP72_YEAST	5	5	1
sp|P00549|KPYK1_YEAST	5	5	1
sp|P05736|RL2_YEAST	5	5	1
sp|P32473|ODPB_YEAST	5	5	1
sp|P05740|RL17A_YEAST	5	4	1
sp|P38011|GBLP_YEAST	9	9	2
sp|P06105|SC160_YEAST	22	22	5
sp|P38631|FKS1_YEAST	20	19	5
sp|P38934|BFR1_YEAST	16	16	4
sp|P14126|RL3_YEAST	12	10	3
sp|P14120|RL30_YEAST	8	6	2
sp|P07280|RS19A_YEAST	4	4	1
sp|P47108|URB2_YEAST	4	4	1
sp|P25635|PWP2_YEAST	4	4	1
sp|P32787|MG101_YEAST	4	4	1
sp|P00924|ENO1_YEAST	4	4	1
sp|P35271|RS18_YEAST	7	7	2
sp|P40213|RS16_YEAST	7	7	2
sp|P05753|RS4_YEAST	9	9	3
sp|P41805|RL10_YEAST	6	6	2
sp|P26781|RS11_YEAST	3	3	1
sp|P40055|UTP7_YEAST	3	3	1
sp|P53030|RL1_YEAST	3	3	1
sp|P16474|GRP78_YEAST	3	3	1
sp|P20448|DBP4_YEAST	3	3	1
sp|P33750|DCA13_YEAST	3	3	1
sp|P23248|RS3A2_YEAST	3	3	1
sp|P60010|ACT_YEAST	3	3	1
sp|P26321|RL5_YEAST	3	3	1
sp|P53252|PIL1_YEAST	3	3	1
sp|P29453|RL8B_YEAST	3	2	1
sp|P05739|RL6B_YEAST	3	2	1
sp|P26783|RS5_YEAST	8	8	3

sp|Q02931|UTP17_YEAST	5	5	2
sp|P05743|RL26A_YEAST	5	5	2
sp|P40991|NOP2_YEAST	5	5	2
sp|P48589|RS12_YEAST	5	5	2
sp|P05738|RL9A_YEAST	5	5	2
sp|P0C2H6|RL27A_YEAST	5	5	2
sp|P21304|PWP1_YEAST	5	5	2
sp|Q02326|RL6A_YEAST	5	4	2
sp|P26785|RL16B_YEAST	5	4	2
sp|P33442|RS3A1_YEAST	9	9	4
sp|P0C2I0|RL20_YEAST	8	8	4
sp|P10664|RL4A_YEAST	10	7	5
sp|O13528|YA11A_YEAST	6	6	3
sp|P06367|RS14A_YEAST	6	6	3
sp|Q08208|NOP12_YEAST	6	6	3
sp|P38701|RS20_YEAST	6	6	3
sp|P26782|RS24_YEAST	6	5	3
sp|P0C0W1|RS22A_YEAST	6	5	3
sp|P32905|RSSA1_YEAST	4	4	2
sp|P02309|H4_YEAST	4	4	2
sp|P05744|RL33A_YEAST	4	4	2
sp|P48164|RS7B_YEAST	4	4	2
sp|P09064|IF2B_YEAST	4	4	2
sp|P39741|RL35_YEAST	4	4	2
sp|P0C0W9|RL11A_YEAST	4	4	2
sp|P04456|RL25_YEAST	4	3	2
sp|P47083|MPP10_YEAST	2	2	1
sp|P39567|IMDH1_YEAST	2	2	1
sp|P04911|H2A1_YEAST	2	2	1
sp|P33201|MRT4_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|P50109|PSP2_YEAST	2	2	1
sp|P25617|YCQ6_YEAST	2	2	1
sp|P53914|KRE33_YEAST	2	2	1
sp|P38112|MAK5_YEAST	2	2	1
sp|P20459|IF2A_YEAST	2	2	1
sp|P26784|RL16A_YEAST	2	2	1
sp|Q03973|HMO1_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|P11484|HSP75_YEAST	19	16	10
sp|P05030|PMA1_YEAST	15	15	8
sp|Q05022|RRP5_YEAST	28	27	15
sp|P04147|PABP_YEAST	13	11	7
sp|Q12176|MAK21_YEAST	9	9	5
sp|Q04660|ERB1_YEAST	7	7	4
sp|P02365|RS6_YEAST	7	7	4
sp|Q12159|YRA1_YEAST	7	7	4

sp|P39744|NOC2_YEAST	5	5	3
sp|Q06506|RRP9_YEAST	5	4	3
sp|O13516|RS9A_YEAST	8	8	5
sp|Q03532|HAS1_YEAST	11	11	7
sp|P42945|UTP10_YEAST	12	12	8
sp|P05750|RS3_YEAST	9	9	6
sp|P05745|RL36A_YEAST	6	6	4
sp|P05748|RL15A_YEAST	3	3	2
sp|P12695|ODP2_YEAST	3	3	2
sp|P53927|NOP15_YEAST	3	3	2
sp|P53941|IMP4_YEAST	3	3	2
sp|Q12220|UTP12_YEAST	3	3	2
sp|Q04373|PUF6_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|P02994|EF1A_YEAST	10	8	7
sp|P15646|FBRL_YEAST	8	7	6
sp|Q12690|RL13A_YEAST	4	4	3
sp|P05754|RS8_YEAST	4	4	3
sp|P17076|RL8A_YEAST	4	4	3
sp|P32445|RIM1_YEAST	4	4	3
sp|P22336|RFA1_YEAST	9	9	7
sp|P05737|RL7A_YEAST	5	5	4
sp|P27476|NSR1_YEAST	6	6	5
sp|P05756|RS13_YEAST	6	5	5
sp|P20447|DBP3_YEAST	9	9	8
sp|Q12499|NOP58_YEAST	11	10	10
sp|Q12460|NOP56_YEAST	12	11	11
sp|P06634|DED1_YEAST	14	13	13
sp|P02407|RS17A_YEAST	6	5	6
sp|P05317|RLA0_YEAST	5	5	5
sp|Q06679|UTP4_YEAST	4	4	4
sp|P33322|CBF5_YEAST	4	4	4
sp|P26786|RS7A_YEAST	4	4	4
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|Q05946|UTP13_YEAST	3	3	3
sp|Q12136|SAS10_YEAST	3	3	3
sp|P17079|RL12_YEAST	3	3	3
sp|Q02753|RL21A_YEAST	3	3	3
sp|Q12024|YTM1_YEAST	2	2	2
sp|P53883|NOP13_YEAST	2	2	2
sp|Q08287|NOP8_YEAST	2	2	2
sp|P36105|RL14A_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2

sp|Q08962|NIP7_YEAST	2	2	2
sp|Q3E705|EFG1P_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|P28007|GAR1_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P40007|NOP16_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|Q12035|FCF2_YEAST	1	1	1
sp|P07703|RPAC1_YEAST	1	1	1
sp|Q12087|RS30_YEAST	1	1	1
sp|P38080|AKL1_YEAST	1	1	1
sp|P00358|G3P2_YEAST	1	1	1
sp|Q99216|PNO1_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|P05319|RLA2_YEAST	1	1	1
sp|Q06078|UTP21_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P05755|RS9B_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P35178|RRP1_YEAST	1	1	1
sp|P47077|YJB0_YEAST	1	1	1
sp|P04451|RL23_YEAST	1	1	1
sp|P24784|DBP1_YEAST	4	4	5
sp|P32481|IF2G_YEAST	3	3	4
sp|Q01560|NOP3_YEAST	2	2	3
sp|Q08492|BUD21_YEAST	2	2	3
sp|P39990|SNU13_YEAST	2	2	3
sp|P0C2H8|RL31A_YEAST	1	1	2
sp|Q3E792|RS25A_YEAST	1	1	2
sp|P32495|NHP2_YEAST	1	1	2
sp|P25567|SRO9_YEAST	1	1	2
sp|P38882|UTP9_YEAST	1	1	2
sp|Q07457|BRE1_YEAST	3	3	7

Purification of: Bub3-TAP benomyl arrested			
protein	total peptides	unique peptides	total peptides in control
sp|P40957|MAD1_YEAST	30	29	
sp|P41695|BUB1_YEAST	26	25	
sp|P47074|MAD3_YEAST	16	15	
sp|P26309|CDC20_YEAST	16	14	
sp|P26449|BUB3_YEAST	16	12	
sp|Q03690|TIF31_YEAST	11	11	
sp|P21576|VPS1_YEAST	8	8	
sp|P22147|XRN1_YEAST	8	8	
sp|P40958|MAD2_YEAST	8	8	
sp|P16521|EF3A_YEAST	8	8	
sp|P40513|MAM33_YEAST	8	7	
sp|P32324|EF2_YEAST	7	7	
sp|P40850|MKT1_YEAST	6	6	
sp|P07245|C1TC_YEAST	6	6	
sp|P07259|PYR1_YEAST	6	6	
sp|P15424|MS116_YEAST	5	5	
sp|P16140|VATB_YEAST	5	5	
sp|P40024|ARB1_YEAST	5	5	
sp|P53297|PBP1_YEAST	5	5	
sp|P34241|URB1_YEAST	5	5	
sp|Q06218|DBP9_YEAST	5	5	
sp|Q12754|RRP12_YEAST	5	5	
sp|P41810|COPB_YEAST	5	5	
sp|P22138|RPA2_YEAST	4	4	
sp|P53261|PESC_YEAST	4	4	
sp|P12398|HSP77_YEAST	4	4	
sp|P25623|SYP1_YEAST	4	4	
sp|P32074|COPG_YEAST	4	4	
sp|P09798|CDC16_YEAST	4	4	
sp|Q02354|UTP6_YEAST	4	4	
sp|P32796|CACP_YEAST	4	4	
sp|P37838|NOP4_YEAST	4	4	
sp|P41811|COPB2_YEAST	4	4	
sp|Q00684|CDC14_YEAST	3	3	
sp|P38779|CIC1_YEAST	3	3	
sp|P25491|MAS5_YEAST	3	3	
sp|P50095|IMDH3_YEAST	3	3	
sp|Q03640|TCB3_YEAST	3	3	
sp|P38061|RL32_YEAST	3	3	
sp|P10080|SSBP1_YEAST	3	3	
sp|P00330|ADH1_YEAST	3	3	
sp|Q03940|RUVB1_YEAST	3	3	
sp|P39985|DPO5_YEAST	3	3	
sp|Q12230|LSP1_YEAST	3	3	
sp|P32899|IMP3_YEAST	3	3	
sp|P15108|HSC82_YEAST	3	3	

sp|P25367|RNQ1_YEAST	3	3
sp|P26755|RFA3_YEAST	3	2
sp|P23301|IF5A2_YEAST	3	2
sp|P19097|FAS2_YEAST	2	2
sp|P38711|RS27B_YEAST	2	2
sp|Q08096|RCL1_YEAST	2	2
sp|P42846|KRI1_YEAST	2	2
sp|P25443|RS2_YEAST	2	2
sp|P40693|RLP7_YEAST	2	2
sp|Q01080|RPA49_YEAST	2	2
sp|P40010|NUG1_YEAST	2	2
sp|Q06631|BFR2_YEAST	2	2
sp|Q12464|RUVB2_YEAST	2	2
sp|P38788|SSZ1_YEAST	2	2
sp|P43586|LOC1_YEAST	2	2
sp|P38809|YHP7_YEAST	2	2
sp|Q08237|REXO4_YEAST	2	2
sp|Q99207|NOP14_YEAST	2	2
sp|P30771|NAM7_YEAST	2	2
sp|P36049|EBP2_YEAST	2	2
sp|P39730|IF2P_YEAST	2	2
sp|P43609|RSC8_YEAST	2	2
sp|P06778|RAD52_YEAST	2	2
sp|Q12117|MRH1_YEAST	2	2
sp|Q07362|PBP4_YEAST	2	2
sp|P38828|LSM12_YEAST	2	2
sp|P53550|DCP2_YEAST	2	2
sp|P06787|CALM_YEAST	2	2
sp|P32598|PP12_YEAST	2	2
sp|P02557|TBB_YEAST	2	2
sp|P53551|H1_YEAST	2	2
sp|P07149|FAS1_YEAST	2	2
sp|P20484|MAK11_YEAST	2	2
sp|P00560|PGK_YEAST	2	2
sp|P53141|MLC1_YEAST	2	2
sp|P25694|CDC48_YEAST	2	2
sp|P40215|NDH1_YEAST	2	2
sp|P38697|IMDH2_YEAST	2	2
sp|P49626|RL4B_YEAST	2	2
sp|P53221|RL26B_YEAST	2	2
sp|P53734|DBP6_YEAST	2	2
sp|Q06287|EMG1_YEAST	2	2
sp|P24000|RL24B_YEAST	2	1
sp|Q06344|ESF1_YEAST	1	1
sp|Q12266|YB11A_YEAST	1	1
sp|P50085|PHB2_YEAST	1	1
sp|P11632|NHP6A_YEAST	1	1
sp|P35191|MDJ1_YEAST	1	1

sp|P39523|YM11_YEAST	1	1
sp|Q04491|SEC13_YEAST	1	1
sp|P38041|BOB1_YEAST	1	1
sp|P53742|NOG2_YEAST	1	1
sp|P19454|CSK22_YEAST	1	1
sp|P32908|SMC1_YEAST	1	1
sp|O14455|RL36B_YEAST	1	1
sp|P50094|IMDH4_YEAST	1	1
sp|P11633|NHP6B_YEAST	1	1
sp|P29311|BMH1_YEAST	1	1
sp|P32529|RPA12_YEAST	1	1
sp|P38042|CDC27_YEAST	1	1
sp|P0CH08|RL401_YEAST	1	1
sp|P20434|RPAB1_YEAST	1	1
sp|Q01477|UBP3_YEAST	1	1
sp|Q08235|BRX1_YEAST	1	1
sp|Q6Q547|NOP10_YEAST	1	1
sp|P36013|MAOM_YEAST	1	1
sp|P39729|RBG1_YEAST	1	1
sp|P06704|CDC31_YEAST	1	1
sp|Q04867|YM91_YEAST	1	1
sp|P53886|APC1_YEAST	1	1
sp|P20436|RPAB3_YEAST	1	1
sp|Q12466|TCB1_YEAST	1	1
sp|P12683|HMDH1_YEAST	1	1
sp|P22276|RPC2_YEAST	1	1
sp|P32589|HSP7F_YEAST	1	1
sp|P48231|TCB2_YEAST	1	1
sp|P09440|C1TM_YEAST	1	1
sp|Q12490|YB11B_YEAST	1	1
sp|Q03761|TAF12_YEAST	1	1
sp|P31383|2AAA_YEAST	1	1
sp|P46669|RPA43_YEAST	1	1
sp|P06786|TOP2_YEAST	1	1
sp|P32597|STH1_YEAST	1	1
sp|P40070|LSM4_YEAST	1	1
sp|P48361|ASK10_YEAST	1	1
sp|P09624|DLDH_YEAST	1	1
sp|P53040|TAF6_YEAST	1	1
sp|P12945|NAT1_YEAST	1	1
sp|P36516|RM03_YEAST	1	1
sp|P46990|RL17B_YEAST	1	1
sp|P38333|ENP1_YEAST	1	1
sp|P40089|LSM5_YEAST	1	1
sp|P10622|RLA3_YEAST	1	1
sp|Q07623|NOP6_YEAST	1	1
sp|P34077|NIC96_YEAST	1	1
sp|P38911|FKBP3_YEAST	1	1

sp|P41056|RL33B_YEAST	1	1
sp|P53131|PRP43_YEAST	1	1
sp|Q08683|APC5_YEAST	1	1
sp|P36521|RM11_YEAST	1	1
sp|P39935|IF4F1_YEAST	1	1
sp|P47006|RPA34_YEAST	1	1
sp|Q06106|MRD1_YEAST	1	1
sp|P16861|K6PF1_YEAST	1	1
sp|P25644|PAT1_YEAST	1	1
##sp|P32660|ATC5_YEAST	1	1
sp|P48837|NUP57_YEAST	1	1
sp|Q07807|PUF3_YEAST	1	1
sp|P38930|CSK2C_YEAST	1	1
sp|Q02457|TBF1_YEAST	1	1
sp|Q04013|YHM2_YEAST	1	1
sp|P32380|NUF1_YEAST	1	1
sp|P36528|RM17_YEAST	1	1
sp|P47035|NET1_YEAST	1	1
sp|P53622|COPA_YEAST	1	1
sp|Q06205|FKBP4_YEAST	1	1
sp|Q12213|RL7B_YEAST	1	1
sp|P38691|KSP1_YEAST	1	1
##sp|Q08977|YP260_YEAST	1	1
sp|P10962|MAK16_YEAST	1	1
sp|Q07896|NOC3_YEAST	1	1
sp|P20606|SAR1_YEAST	1	1
sp|P53163|MNP1_YEAST	1	1
sp|Q04177|UTP5_YEAST	1	1
sp|P37263|YC16_YEAST	1	1
sp|P47037|SMC3_YEAST	1	1
sp|P53732|RT12_YEAST	1	1
sp|P32827|RS23_YEAST	1	1
sp|P40217|EIF3I_YEAST	1	1
sp|P20967|ODO1_YEAST	1	1
sp|P39003|HXT6_YEAST	1	1
sp|P00925|ENO2_YEAST	1	1
sp|Q04305|UTP15_YEAST	1	1
sp|P14907|NSP1_YEAST	1	1
sp|Q08965|BMS1_YEAST	1	1
sp|P07260|IF4E_YEAST	1	1
sp|P18239|ADT2_YEAST	1	1
sp|P40509|COPE_YEAST	1	1
sp|P49631|RL43_YEAST	1	1
sp|Q00402|NUM1_YEAST	1	1
sp|Q07915|RLP24_YEAST	1	1
sp|P28000|RPAC2_YEAST	1	1
sp|P35189|TAF14_YEAST	1	1
sp|P39015|STM1_YEAST	1	1

sp|P00950|PMG1_YEAST	1	1	
sp|Q02892|NOG1_YEAST	1	1	
sp|Q12000|TMA46_YEAST	1	1	
sp|P39998|EDC3_YEAST	1	1	
sp|P53741|BRE5_YEAST	1	1	
sp|P10964|RPA1_YEAST	8	8	1
sp|O13527|YA11B_YEAST	7	7	1
sp|P04449|RL24A_YEAST	7	5	1
sp|P53254|UTP22_YEAST	6	6	1
sp|P05735|RL19_YEAST	6	6	1
sp|Q02486|ABF2_YEAST	6	6	1
sp|P10591|HSP71_YEAST	20	17	4
sp|P24276|SSD1_YEAST	10	10	2
sp|P53276|UTP8_YEAST	5	5	1
sp|P16387|ODPA_YEAST	5	5	1
sp|P10592|HSP72_YEAST	5	5	1
sp|P00549|KPYK1_YEAST	5	5	1
sp|P05736|RL2_YEAST	5	5	1
sp|P32473|ODPB_YEAST	5	5	1
sp|P05740|RL17A_YEAST	5	4	1
sp|P38011|GBLP_YEAST	9	9	2
sp|P06105|SC160_YEAST	22	22	5
sp|P38631|FKS1_YEAST	20	19	5
sp|P38934|BFR1_YEAST	16	16	4
sp|P14126|RL3_YEAST	12	10	3
sp|P14120|RL30_YEAST	8	6	2
sp|P07280|RS19A_YEAST	4	4	1
sp|P47108|URB2_YEAST	4	4	1
sp|P25635|PWP2_YEAST	4	4	1
sp|P32787|MG101_YEAST	4	4	1
sp|P00924|ENO1_YEAST	4	4	1
sp|P35271|RS18_YEAST	7	7	2
sp|P40213|RS16_YEAST	7	7	2
sp|P05753|RS4_YEAST	9	9	3
sp|P41805|RL10_YEAST	6	6	2
sp|P26781|RS11_YEAST	3	3	1
sp|P40055|UTP7_YEAST	3	3	1
sp|P53030|RL1_YEAST	3	3	1
sp|P16474|GRP78_YEAST	3	3	1
sp|P20448|DBP4_YEAST	3	3	1
sp|P33750|DCA13_YEAST	3	3	1
sp|P23248|RS3A2_YEAST	3	3	1
sp|P60010|ACT_YEAST	3	3	1
sp|P26321|RL5_YEAST	3	3	1
sp|P53252|PIL1_YEAST	3	3	1
sp|P29453|RL8B_YEAST	3	2	1
sp|P05739|RL6B_YEAST	3	2	1
sp|P26783|RS5_YEAST	8	8	3

sp|Q02931|UTP17_YEAST	5	5	2
sp|P05743|RL26A_YEAST	5	5	2
sp|P40991|NOP2_YEAST	5	5	2
sp|P48589|RS12_YEAST	5	5	2
sp|P05738|RL9A_YEAST	5	5	2
sp|P0C2H6|RL27A_YEAST	5	5	2
sp|P21304|PWP1_YEAST	5	5	2
sp|Q02326|RL6A_YEAST	5	4	2
sp|P26785|RL16B_YEAST	5	4	2
sp|P33442|RS3A1_YEAST	9	9	4
sp|P0C2I0|RL20_YEAST	8	8	4
sp|P10664|RL4A_YEAST	10	7	5
sp|O13528|YA11A_YEAST	6	6	3
sp|P06367|RS14A_YEAST	6	6	3
sp|Q08208|NOP12_YEAST	6	6	3
sp|P38701|RS20_YEAST	6	6	3
sp|P26782|RS24_YEAST	6	5	3
sp|P0C0W1|RS22A_YEAST	6	5	3
sp|P32905|RSSA1_YEAST	4	4	2
sp|P02309|H4_YEAST	4	4	2
sp|P05744|RL33A_YEAST	4	4	2
sp|P48164|RS7B_YEAST	4	4	2
sp|P09064|IF2B_YEAST	4	4	2
sp|P39741|RL35_YEAST	4	4	2
sp|P0C0W9|RL11A_YEAST	4	4	2
sp|P04456|RL25_YEAST	4	3	2
sp|P47083|MPP10_YEAST	2	2	1
sp|P39567|IMDH1_YEAST	2	2	1
sp|P04911|H2A1_YEAST	2	2	1
sp|P33201|MRT4_YEAST	2	2	1
sp|O14467|MBF1_YEAST	2	2	1
sp|P32583|SRP40_YEAST	2	2	1
sp|P50109|PSP2_YEAST	2	2	1
sp|P25617|YCQ6_YEAST	2	2	1
sp|P53914|KRE33_YEAST	2	2	1
sp|P38112|MAK5_YEAST	2	2	1
sp|P20459|IF2A_YEAST	2	2	1
sp|P26784|RL16A_YEAST	2	2	1
sp|Q03973|HMO1_YEAST	2	2	1
sp|P39938|RS26A_YEAST	2	2	1
sp|P11484|HSP75_YEAST	19	16	10
sp|P05030|PMA1_YEAST	15	15	8
sp|Q05022|RRP5_YEAST	28	27	15
sp|P04147|PABP_YEAST	13	11	7
sp|Q12176|MAK21_YEAST	9	9	5
sp|Q04660|ERB1_YEAST	7	7	4
sp|P02365|RS6_YEAST	7	7	4
sp|Q12159|YRA1_YEAST	7	7	4

sp|P39744|NOC2_YEAST	5	5	3
sp|Q06506|RRP9_YEAST	5	4	3
sp|O13516|RS9A_YEAST	8	8	5
sp|Q03532|HAS1_YEAST	11	11	7
sp|P42945|UTP10_YEAST	12	12	8
sp|P05750|RS3_YEAST	9	9	6
sp|P05745|RL36A_YEAST	6	6	4
sp|P05748|RL15A_YEAST	3	3	2
sp|P12695|ODP2_YEAST	3	3	2
sp|P53927|NOP15_YEAST	3	3	2
sp|P53941|IMP4_YEAST	3	3	2
sp|Q12220|UTP12_YEAST	3	3	2
sp|Q04373|PUF6_YEAST	3	3	2
sp|P07279|RL18_YEAST	3	3	2
sp|P0C0V8|RS21A_YEAST	3	2	2
sp|P02994|EF1A_YEAST	10	8	7
sp|P15646|FBRL_YEAST	8	7	6
sp|Q12690|RL13A_YEAST	4	4	3
sp|P05754|RS8_YEAST	4	4	3
sp|P17076|RL8A_YEAST	4	4	3
sp|P32445|RIM1_YEAST	4	4	3
sp|P22336|RFA1_YEAST	9	9	7
sp|P05737|RL7A_YEAST	5	5	4
sp|P27476|NSR1_YEAST	6	6	5
sp|P05756|RS13_YEAST	6	5	5
sp|P20447|DBP3_YEAST	9	9	8
sp|Q12499|NOP58_YEAST	11	10	10
sp|Q12460|NOP56_YEAST	12	11	11
sp|P06634|DED1_YEAST	14	13	13
sp|P02407|RS17A_YEAST	6	5	6
sp|P05317|RLA0_YEAST	5	5	5
sp|Q06679|UTP4_YEAST	4	4	4
sp|P33322|CBF5_YEAST	4	4	4
sp|P26786|RS7A_YEAST	4	4	4
sp|Q3E7X9|RS28A_YEAST	3	3	3
sp|P02400|RLA4_YEAST	3	3	3
sp|Q01855|RS15_YEAST	3	3	3
sp|Q05946|UTP13_YEAST	3	3	3
sp|Q12136|SAS10_YEAST	3	3	3
sp|P17079|RL12_YEAST	3	3	3
sp|Q02753|RL21A_YEAST	3	3	3
sp|Q12024|YTM1_YEAST	2	2	2
sp|P53883|NOP13_YEAST	2	2	2
sp|Q08287|NOP8_YEAST	2	2	2
sp|P36105|RL14A_YEAST	2	2	2
sp|P02406|RL28_YEAST	2	2	2
sp|P00360|G3P1_YEAST	2	2	2
sp|Q12692|H2AZ_YEAST	2	2	2

sp|Q08962|NIP7_YEAST	2	2	2
sp|Q3E705|EFG1P_YEAST	2	2	2
sp|Q08745|RS10A_YEAST	2	1	2
sp|P28007|GAR1_YEAST	1	1	1
sp|P02293|H2B1_YEAST	1	1	1
sp|P40007|NOP16_YEAST	1	1	1
sp|P26754|RFA2_YEAST	1	1	1
sp|Q12035|FCF2_YEAST	1	1	1
sp|P07703|RPAC1_YEAST	1	1	1
sp|Q12087|RS30_YEAST	1	1	1
sp|P38080|AKL1_YEAST	1	1	1
sp|P00358|G3P2_YEAST	1	1	1
sp|Q99216|PNO1_YEAST	1	1	1
sp|P53336|YG5X_YEAST	1	1	1
sp|P05319|RLA2_YEAST	1	1	1
sp|Q06078|UTP21_YEAST	1	1	1
sp|Q12522|IF6_YEAST	1	1	1
sp|P49167|RL38_YEAST	1	1	1
sp|P61830|H3_YEAST	1	1	1
sp|P34247|UTP11_YEAST	1	1	1
sp|P05755|RS9B_YEAST	1	1	1
sp|P14540|ALF_YEAST	1	1	1
sp|P87262|RL34A_YEAST	1	1	1
sp|Q07897|CMS1_YEAST	1	1	1
sp|P35178|RRP1_YEAST	1	1	1
sp|P47077|YJB0_YEAST	1	1	1
sp|P04451|RL23_YEAST	1	1	1
sp|P24784|DBP1_YEAST	4	4	5
sp|P32481|IF2G_YEAST	3	3	4
sp|Q01560|NOP3_YEAST	2	2	3
sp|Q08492|BUD21_YEAST	2	2	3
sp|P39990|SNU13_YEAST	2	2	3
sp|P0C2H8|RL31A_YEAST	1	1	2
sp|Q3E792|RS25A_YEAST	1	1	2
sp|P32495|NHP2_YEAST	1	1	2
sp|P25567|SRO9_YEAST	1	1	2
sp|P38882|UTP9_YEAST	1	1	2
sp|Q07457|BRE1_YEAST	3	3	7

Alexandra Weirich CV

Address Ottawa Institute of Systems Biology
Department of Biochemistry, Microbiology and Immunology
University of Ottawa, 451 Smyth Rd., Ottawa, Ontario KlH 8M5

Lab: (613) 562-5800 ext. 8661
Fax: (613) 562-5452

Education

M.Sc.	2010-present	Biochemistry University of Ottawa, Ottawa, Ontario
B.Sc.	2009	Biochemistry University of Ottawa, Ottawa, Ontario

Research Experience

July2010-June2011	Technical Research Assistant Bio-Rad Medical Diagnostics Dreieich, Hessen, Germany
Summer 2010	Summer Student Ottawa Institute of Systems Biology University of Ottawa, Ottawa, Ontario
Summer 2008	Summer Student Ottawa Health Research Institute Ottawa, Ontario
Sept2007-May2008	Student Research Assistant Spartan Bioscience Inc. Ottawa, Ontario

Awards

2004-2005	University of Ottawa Entrance Scholarship
2007	National Sciences and Engineering Research Council (NSERC) Undergraduate Student Research Award
2009	Dean's Honours List
2009	Deutscher Akademischer Austausch Dienst RISEprofessional Scholarship

Published abstracts:

Alexandra Weirich and Adam Rudner. Purification of Spindle Assembly Checkpoint Complexes. 7th Salk Institute Cell Cycle Meeting, La Jolla CA, 2011

Elliott Faller, **Alexandra Weirich**, Paul MacPherson. Extracellular HIV-1 TAT Stimulates STAT3 phosphorylation in CD8 T cells through induction of a soluble factor. Ontario HIV Treatement Network Conference, Toronto ON, 2008

Posters**:**

Weirich, A. and Rudner, A. REGULATION OF THE ANAPHASE PROMOTING COMPLEX BY THE SPINDLE ASSEMBLY CHECKPOINT. Ottawa Institute of Systems Biology Retreat, Montebello QC, 2011

Weirich, A. and Rudner, A. REGULATION OF THE ANAPHASE PROMOTING COMPLEX BY THE SPINDLE ASSEMBLY CHECKPOINT. University of Ottawa, Faculty of Medicine Student Research Day, Ottawa ON, 2011

Elliott Faller, **Alexandra Weirich**, Paul MacPherson. Extracellular HIV-1 TAT Stimulates STAT3 phosphorylation in CD8 T cells through induction of a soluble factor. University of Ottawa Honours Project research Day, Ottawa ON, 2009

Presentations:

Mitotic Regulation of the Aanaphase Promoting Complex by the Spindle Assembly Checkpoint. Department of Biochemistry, Microbiology and Immunology, Work In Progress Seminar. Ottawa ON, 2011

Extracellular HIV-1 TAT Stimulates STAT3 phosphorylation in CD8 T cells through induction of a soluble factor. Ontario Health Research Institute Summer Student Research Seminars, Ottawa ON, 2008

Non-refereed publications:

S Prevost, **A Weirich**, NA Arbour, R Liu, E Vaillancourt, CJ Harder. Comparison of DNA purification kits for whole blood. Spartan Bioscience Inc. Application Notes.

N Arbour, **A Weirich**, D Cornejo-Palma, S Prevost, K Ramotar, CJ Harder. Real-time PCR detection of VRE. Spartan Bioscience Inc. Application Notes

Acknowledgements:

"**Real-time PCR detection of VRE**" presentation at the Canadian Association for Clinical Microbiology and Infectious Diseases (CACMID) conference, February 2008 Acknowledgement