Distinct role of polarity factors Boi1 and Boi2 in the control of exocytosis and abscission in budding yeast

Andrea Battola

TESI DOCTORAL UPF / 2017

DIRECTOR DE LA TESI

Manuel Mendoza

Departament de Biología Cel·lular y del Desenvolupament

upf. Universitat Pompeu Fabra Barcelona

Acknowledgements

This work would have not been possible without the help of many people:

First, I would like to show my gratitude to my PhD supervisor Manuel Mendoza. Thank you for giving me the opportunity to work in your lab and for the professional support you gave me throughout my doctoral studies.

I would like to thank my PhD committee composed by Vivek Malhotra, Snezhana Oliferenko and Maria Pia Cosma. Thank you for all the useful advice!

Thank you to my PhD panel members Isabelle Vernos, Ethel Queralt and Maribel Geli for accepting to evaluate my research work.

Thank you to the entire CRG Cell and Developmental department and to all the team of the Advanced Microscopy Unit.

Gracias Trini, he apreciado muchísimo toda la ayuda técnica que me has dado en estos 4 anos. Ha sido un placer colaborar contigo y estoy muy feliz que por fin conseguimos publicar nuestro querido paper.

Thank you to all the people from my lab: Arun, Nuno, Aina, Petra, Michael, Tsvetomira and Ping. Thank you for all the professional and personal support. A special Thank you to Nicola for listening to my crazy ideas about NoCut and for being supportive when I needed. Grazie Francesca per il tuo essere amica. Averti nel lab mi ha dato grande forza e sicurezza.

Gracias Tony, tus consejos y tu paciencia en estos dos anos me han ayudado muchísimo. Si he llegado hasta aquí lo debo a ti y por eso considera esta tesis un poco tuya también.

Thanks to all the PhD students I met during this fantastic experience. Hana, Reza, Miquel, Laura y Laia: it has been a very big pleasure to have spent my free time with you. I learned a lot from all of you!

Thank you to Elias, Alessandro, Titi, Jonas and HC for the nice chats, and the funny moments.

Un grazie speciale va alla mia famiglia. Siete la mia forza e vi ringrazio per tutto l'appoggio morale che mi avete dato sinora.

Abstract

Boi1 and Boi2 (Boi1/2) are budding yeast plasma membrane proteins that function in polarized growth, and in cytokinesis inhibition in response to chromosome bridges via the NoCut abscission checkpoint. How Boi1/2 act in these two distinct processes is not understood. We demonstrate that Boi1/2 are required for a late step in the fusion of secretory vesicles with the plasma membrane of the growing bud. Cells lacking Boi1/2 accumulate secretory vesicles and are defective in bud growth. In contrast, Boi2 is specifically required for abscission inhibition in cells with chromatin bridges. The SH3 domain of Boi2, which is dispensable for bud growth and targets Boi2 to the site of abscission, is necessary and sufficient for abscission inhibition. Gain of function of the exocyst, a conserved protein complex involved in tethering of exocytic vesicles to the plasma membrane, rescued secretion and bud growth defects in boi mutant cells, and abrogated NoCut checkpoint function. Thus, Boi2 functions redundantly with Boi1 to promote the fusion of secretory vesicles with the plasma membrane at sites of polarized growth, and acts as an abscission inhibitor during cytokinesis in response to chromatin bridges.

Resumen

Problemes en la replicació de l'ADN, condensació o de-catenació generen ponts anafàsics al punt de divisió (Baxter et al., 2015) (Chan et al., 2007), que en molts casos provoquen inestabilitat genòmica (Chan et al., 2009). La cèl·lula respon a aquest problema activant la via del NoCut, que retarda l'abscisió (la divisió física de la membrana plasmàtica) en cèl·lules de llevat i humanes (Norden et al., 2006). Estudis anteriors en *Saccharomyces cerevisiae*, mostren que el retràs de l'abscisió requereix el complex APC^{cdh1}, el qual estabilitza el fus anafàsic i permet que Aurora B quinasa detecti l'ADN extraviat al centre del fus (Amaral et al., 2016). Tanmateix, no es coneix massa sobre els mecanismes per mitjà dels quals el NoCut bloqueja la resolució de la membrana plasmàtica. La meva tesis i l'article adjunt mostren que la proteïna de membrana Boi2 is essencial en aquest procés. Durant el meu PhD he estudiat el rol de Boi2 en interfase i citocinesis. Als estadis inicials del cicle cel·lular, Boi2 i el seu paràlog Boi1 són essencials pel creixement de la superfície de la cèl·lula i el seu creixement polaritzat, durant aquests processos regulen la exocitosis de vesícules de secreció específiques. Boi1 i Boi2 no són essencials per citocinesis, però en presència de defectes de segregació cromosòmica, Bio2 actua com a inhibidor de la resolució de la membrana plasmàtica. He demostrat que el domini SH3 de Boi2, necessari pel reclutament de Boi2 al punt de gemmació, és necessari i suficient pel NoCut per retardar l'abscisió. En conjunt, els nostres resultats suggereixen que l'existència

d'una regulació precisa del tràfic de membranes és essencial per el retard de l'abscisió per part del NoCut.

Preface

Problems in DNA replication, condensation or decatenation generate chromosome bridges that span the site of division during cytokinesis (Baxter et al., 2015; Chan et al., 2007) and that lead to genomic instability (Chan et al., 2009). The cell responds to this issue by activating the NoCut pathway, which delays abscission (the physical division of the plasma membrane) in yeast and human cells (Norden et al., 2006; Steigemann et al., 2009). Previous findings in *Saccharomyces cerevisiae,* showed that the abscission delay requires the APC^{Cdh1} complex that stabilizes the anaphase spindle and allows Aurora B kinase to sense the lagging DNA in the midzone (Amaral et al., 2016). Little is known, however, about the mechanism behind the blockage of the plasma membrane resolution in NoCut. My thesis shows that the membrane protein Boi2 is essential in this process. In my PhD I investigate the role of Boi2 during both interphase and cytokinesis. At early stages of the cell cycle, Boi2 and its paralog Boi1 are essential for cell growth and polarized growth, in which they regulate exocytosis of specific secretory vesicles. Boi1 and Boi2 are not essential for cytokinesis, but in presence of chromosome segregation defects Boi2 acts as plasma membrane resolution inhibitor. I show that the SH3 domain of Boi2, which is required for the Boi2 protein localization to the bud-neck, is required and sufficient for the abscission delay in NoCut. Together this data suggests that a mechanism involving the careful regulation of membrane trafficking is essential for implementation of the NoCut abscission delay.

Table of Contents

1. Introduction

The introduction of my thesis attempts to give an overall knowledge of the eukaryotic cell cycle, chromosome segregation, cytokinesis and secretion, which are the cellular aspects I have been interested in during my research. I will discuss with more emphasis about these topics in *Saccharomyces cerevisiae*, as this is the model system I used for my experimental studies.

1.1. The Eukaryotic cell cycle

Cell reproduction is a fundamental feature of all living organisms. It occurs by an elaborate series of events called the cell cycle, governed by a complex network of proteins (Morgan, 2007).

Eukaryotic cells have a complex cell cycle that is divided in four main steps: G1 (growth or gap phase 1), S (synthesis phase), G2 (gap phase 2) and M (Mitosis) (Figure 1).

During G1 phase a massive mRNA and protein synthesis occurs with consequent increase of cell size and mass. Once the required proteins and growth are complete, the cell enters the next phase of the cell cycle. G_1 phase is particularly important in the cell cycle because it determines whether a cell commits to division or to leaving the cell cycle (Morgan, 2007). In fact, internal and external conditions can make the cell either delay G1, or enter a quiescent state known as G0 (Alberts, 2007). This cell regulation is called G1 checkpoint, known also as the restriction point in mammalian cells and the start point in yeast. The decision to commit to a new round of cell division occurs upon activation of the cyclin-CDK-dependent transcription that promotes entry into S phase (Bertoli et al., 2013).

S phase is the time window where DNA and the microtubule-organizing center (centrosomes in human cells or SPBs in yeast) duplicate. DNA replication by the DNA polymerases results in the generation of sister chromatids. In eukaryotic cells, genotoxic stress activates a surveillance mechanism, the S phase checkpoint, which detects and blocks cell cycle progression to protect DNA replication. This checkpoint is conserved from yeast to humans (Hartwell et al., 1989; Hartwell et al., 1994). An impaired checkpoint response results in genomic instability and promotes cancer in metazoan organisms (Bartkova et al., 2005).

In M (mitosis) phase, both nucleus and cell divide (cytokinesis) to

generate two cells with identical DNA. Nuclear division is the result of a multistep process called chromosome segregation where the sister chromatids, formed in S phase, are pulled apart to the opposite poles of the cell. Chromosome segregation is characterized by distinct phases. In prophase, sister chromatids condense, spindles form, centrioles in humans and spindle pole bodies in yeast move to opposite poles and microtubules polymerize. In metaphase microtubules coming from opposite poles attach to the kinetocores, protein structure found at the centromere of the sister chromatids, forming a bipolar structure. During anaphase, sister chromatid are pulled apart, due to the cleavage of cohesin, that holds them together. Then the mitotic splindle elongates resulting in the complete segregation of the chromatids into daughter cells.

The last step of mitosis is called telophase, when chromatin is packed into two identical nuclei and the spindle disassembles (Morgan, 2007). Following mitosis, cells physically separate by a process called cytokinesis.

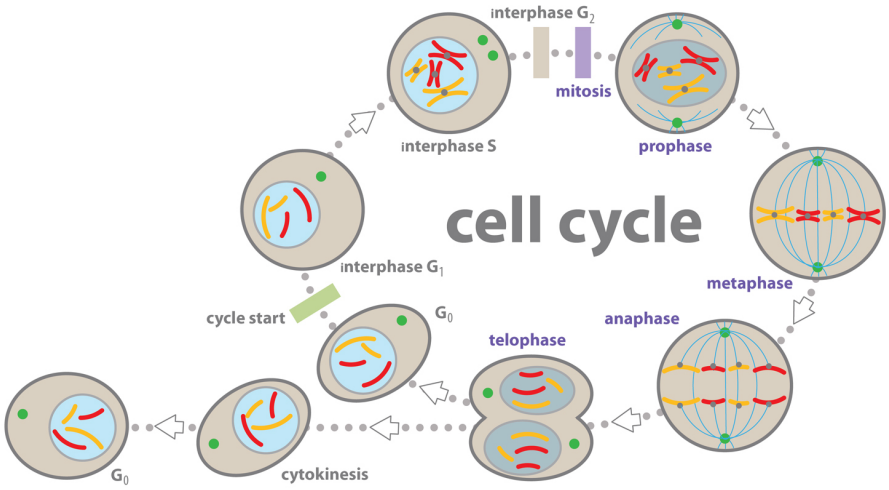


Figure 1. **The Eukaryotic cell cycle.** The phases G1, S and G2 constitute the interphase (I) while the M phase is mitosis and cytokinesis. Adapted from (http://www.scienceset.co.uk/portfolio-6.html)

1.2. The cell cycle in yeast

1.2.1. Polarized cell growth

Cell polarization is crucial for performing specific functions such as neuronal transmission (Witte et al., 2008) or ion transport across epithelia (Drubin et al., 1996) in higher eukaryotes. In *Saccharomices cerevisiae*,

polarized growth is cell cycle-regulated and it involves the conserved small GTPase Cdc42, the cytoskeletal polarization, and the exocytosis. During budding, the growth machinery (such as actin and exocytosis) is directed toward the bud cortex to promote bud growth. During budding, the growth is targeted to the bud tip from late G1 to G2, known as "apical growth" and then to the entire bud upon the entry into mitosis, known as "isotropic growth", driving uniform bud expansion (Lew et al., 1993)

Later in the cell cycle, the same growth machinery is redirected to the mother-bud neck to promote cytokinesis (Pringle et al., 1980) (Figure 1.1).

The actin cytoskeleton and the septins are also polarized during the cell cycle and are the major determinants of cellular morphogenesis in budding yeast. Filamentous (F) actin structures in yeast include actin cables, actin patches, and the cytokinetic actin ring (Pruyne et al., 2000). Polarized actin cables guide the transport of secretory vesicles toward the site of growth, actin patches regulate endocytosis and the actin ring is involved in cytokinesis (Prune et al., 1998; Kaksonen et al., 2003; Lippincott et al., 1998).

Septins are assembled into a cortical ring at the nascent bud site, which is turned into an hourglass structure upon bud emergence (Figure 1.1). At the onset of cytokinesis, the septin hourglass is split into two cortical rings that sandwich the cytokinesis machinery.

1.2.1.1 Cdc42

The conserved small GTPase Cdc42 plays a central role in cell polarity and in *S. cerevisiae* is polarized at a pre-established cortical site to drive bud growth (Park et al., 2007). Cdc42 belongs to the Ras superfamily and therefore cycles between its inactive (GDP)-bound and active (GTP)-bound states. Cdc42 activation is catalyzed by the guanine nucleotide-exchange factor (GEF) Cdc24 (Tcheperegine et al., 2005), and its inactivation depends on the GTP hydrolysis by the GTPase-activating proteins (GAPs), such as Bem2 and Bem3 (Zheng et al., 1993). Cdc42 is also regulated by the Rho GDP-dissociation inhibitor (GDI) (Tcheperegine et al., 2005), which acts by inhibiting either the dissociation of GDP or the GAP-stimulated GTPase activity (Chuang et al., 1993).

Cdc42 controls actin cable-mediated exocytosis by regulating the localization and/or activity of the formins (Ozaki-Kuroda et al., 2001) and it has been also shown to directly regulate exocytosis in an actin cable independent manner (Adamo et al., 2001).

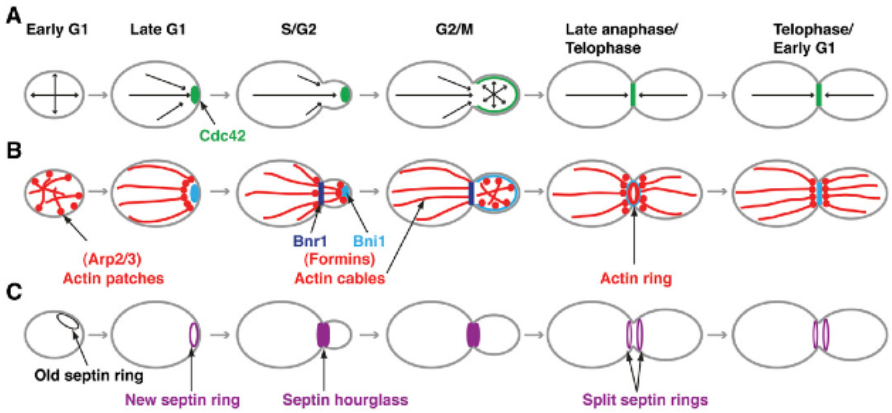


Figure 1.1. **Polarized growth factors during the cell cycle.** The figure depicts the localization of Cdc42 (A), actin (B) and septins (C) during the cell cycle (Bi et al., 2012).

1.2.2. The cell cycle regulation in yeast

In eukaryotic cells, the cell cycle is controlled by cyclin dependent kinases (CDKs). These kinases are dependent on cyclin subunits for catalytic activity, allowing a very tight regulation of their activation by production and destruction of the cyclins during the cell cycle. Cdk specificity is regulated by both an intrinsic selectivity in the active site and by substrate docking sites on the cyclin subunit. The oscillation of the different cyclin

during the cell cycle is an important switching mechanism that triggers the Start (G1/S), the mitotic entry, and the metaphase-anaphase transition events (Morgan, 2007) (Figure 1.2.). This general mechanism of sequential cyclin signals is conserved throughout the eukaryotes.

Six conserved CDKs exist in the budding yeast *S. cerevisiae* (Liu et al., 2000). Budding yeast Cdk1 was first identified in a landmark genetic screen for genes that control the cell cycle (Hartwell et al., 1970). It is a proline-directed kinase that preferentially phosphorylates the consensus sequence S/T-P-x-K/R (where × is any amino acid), although the minimal consensus sequence is S/T-P (Nigg et al., 1993). Cdk1 interacts with nine different cyclins throughout the cell cycle. The interaction with cyclins is important for activation of its kinase activity and also for recruitment and selection of substrates (Archambault et al., 2005). Cell cycle progression depends on the orderly expression of cyclins (Bloom et al., 2007) indicating that different cyclin-Cdk1 complexes are important for phosphorylation of the right proteins at the right time.

Cdk1 is inactive during G1 due to low concentrations of cyclins and the presence of the cyclin dependent kinase inhibitors (CKIs) Sic1 and Far1 (Schwob et al., 1994; Alberghina et al., 2004). Its activity increases at late G1, when cyclin concentrations rise and the CKIs are degraded (Mendenhall et al., 1998). Cdk1 activity stays high until anaphase, when it drops because cyclins are destroyed and CKIs are re-expressed (Amon et al., 1994). This drop in Cdk1 activity is paramount to exit from mitosis

and it resets the cell cycle to a basic G1 state of low Cdk1 activity.

As mentioned before, *S. cerevisiae* expresses nine cyclins that associate with Cdk1 throughout the cell cycle: three G1 cyclins and six B-type cyclins. The three G1 cyclins Cln1, Cln2 and Cln3 are involved in entry into S phase (Richardson et al., 1989). Cln3 stimulates the transcription of the CLN1 and CLN2 genes (Tyers et al., 1993) while Cln1 and Cln2 are important for spindle pole body duplication and initiation of bud morphogenesis.

Six B-type cyclins (Clb1-6) function after the G1 cyclins in the cell cycle. Expression of both Clb5 and Clb6 is induced during G1 phase. Clb5,6 are thought to be involved in timely initiation of S phase (Shwob et al., 1994). Furthermore, Clb5 is required for efficient DNA replication (Donaldson et al., 1998) while Clb6 inhibits transcription of G1 programs (Geymonat et al., 2004). Clb3 and Clb4 are expressed from S phase until anaphase and are involved in DNA replication, spindle assembly, and the G2/M-phase transition (Richardson et al., 1992). Clb1 and Clb2 are expressed during the G2-M phase of the cell cycle and destroyed at the end of M phase (Mendenhall et al., 1998; Seufert et al., 1995) and are involved in regulation of mitotic events such as spindle elongation, but also in bud morphogenesis by inducing the switch from polar to isotropic bud growth (Lew et al., 1993).

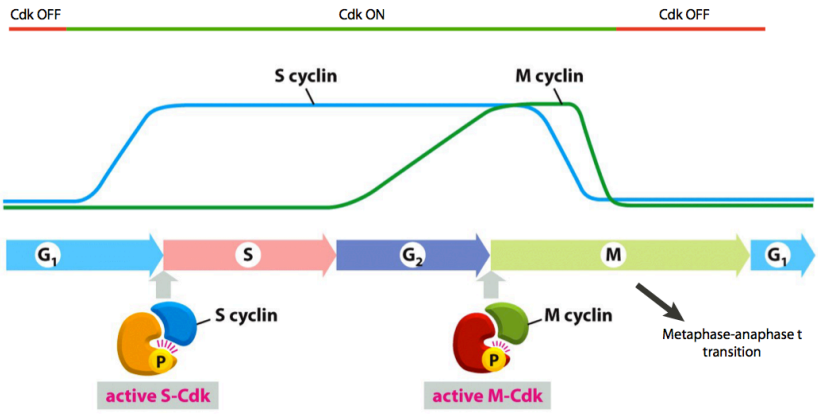


Figure 1.2. CDK1 and cyclin activity during the cell cycle. Adapted from Essential cell biology 3/e (Garland Science).

1.3. Membrane trafficking

Protein secretion is characterized by numerous steps that involve several hundred proteins and is essential for living organisms (Delic et al., 2013). In eukaryotic cells, membrane and soluble proteins are generally translocated to the endoplasmic reticulum (ER) during synthesis and then

transported to their target compartment. All newly synthesized proteins move from ER to the Golgi apparatus where they are either transported to the plasma membrane (PM) or the external medium through the SEC pathway (exocytosis) or are targeted to the vacuole either through endosomes (vacuolar protein sorting pathway). Plasma membrane proteins can be internalized through endocytosis and then transported to endosomes (Figure 1.3.). Here they are targeted for vacuolar degradation or they can be redirected to the Golgi (recycling pathway) where they enter the secretory pathway to be readdressed to the PM (Feyder et al., 2015).

1.3.1. The SEC pathway

At the TGN (Trans Golgi Network), cargo proteins are loaded into vesicles that are directed to the plasma membrane (PM) via the SEC pathway. These SEC vesicles are targeted to the polarized sites of growth by tropomyosin-actin cables and delivered to the PM with which they are tethered by the exocyst complex (composed of Sec3, Sec5, Sec10, Sec6, Sec8, Sec15, Exo70 and Exo84) prior to Snc1 SNARE complex dependent fusion in a Sec4 GTPase manner (Figure 1.4.) (TerBush et al., 1996). In my PhD studies I focused more on the last part of the SEC pathway, and therefore I will discuss about the exocyst and the Rab

GTPases functions as well as the endocytosis, and again giving more attention to these mechanisms in *S.Cerevisiae.*

1.3.2. The exocyst complex and late stages of exocytosis

The exocyst is a conserved octameric complex consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 subunits (Figure 1.5.). Six members of the exocyst complex have been identified by Novick and Schekman in the late 1970s using a genetic screen to isolate mutants involved in secretion in *Saccharomyces cerevisiae* (Novick et al., 1980). The inactivation of exocyst genes leads to post-Golgi secretory vesicles accumulation in yeast cells (Guo et al., 1999). The exocyst acts after the delivery of secretory vesicles to the plasma membrane but before the SNARE-mediated vesicle fusion (Grote et al., 2000). Thus, the exocyst complex functions as a tether that mediates contact between the secretory vesicles and the plasma membrane before docking and fusion. Exocyst usually localizes to sites of active exocytosis and cell surface expansion (TerBush et al., 1995; Finger et al., 1998; Guo et al., 1999).

In mammalian cells, although the exocyst mostly localizes to internal membrane compartments such as Golgi and recycling endosomes (Ang et al., 2004; Oztan et al., 2007), it is recruited to designated regions of the plasma membrane where active exocytosis and membrane expansion occur (Murthy et al., 2003).

It was proposed that two of the exocyst subunits, Exo70 and Sec3, function as landmarks at the plasma membrane for the remaining exocyst components, which arrive on post-Golgi secretory vesicles along the actin cables (Boyd et al., 2004). The yeast Exo70 (He et al., 2007) and Sec3 (Zhang et al., 2008) bind directly to PI(4,5)P2 on the plasma membrane.

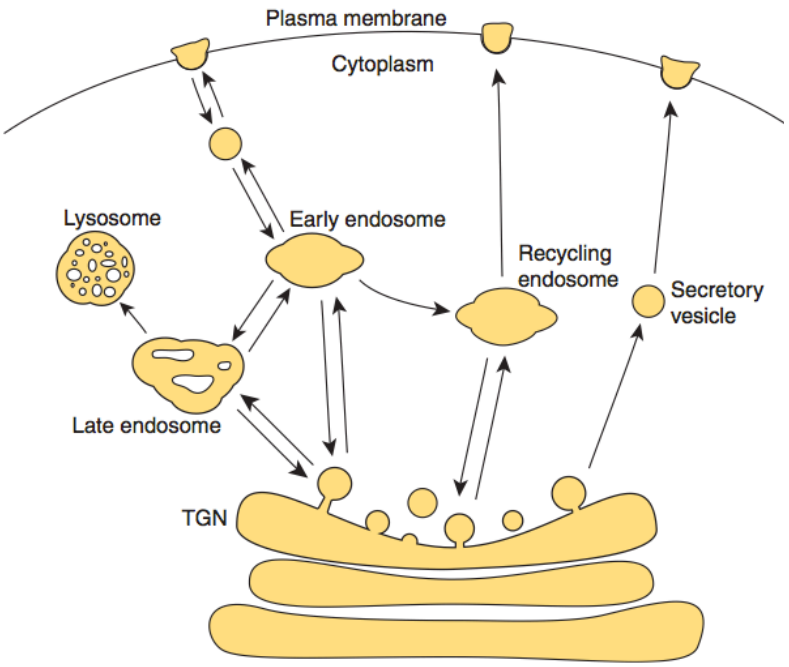


Figure 1.3. Membrane trafficking to the plasma membrane. During exocytosis, vesicles leave the TGN or the recycling endosomes in vesicular carriers to the PM. At the membrane, proteins are either internalized or transported to early endosomes. Here then either they travel to the lysosome passing by the late endosomes to be degraded or return to the plasma membrane through the recycling endosomes (Figure adapted from (Orlando et al., 2009).

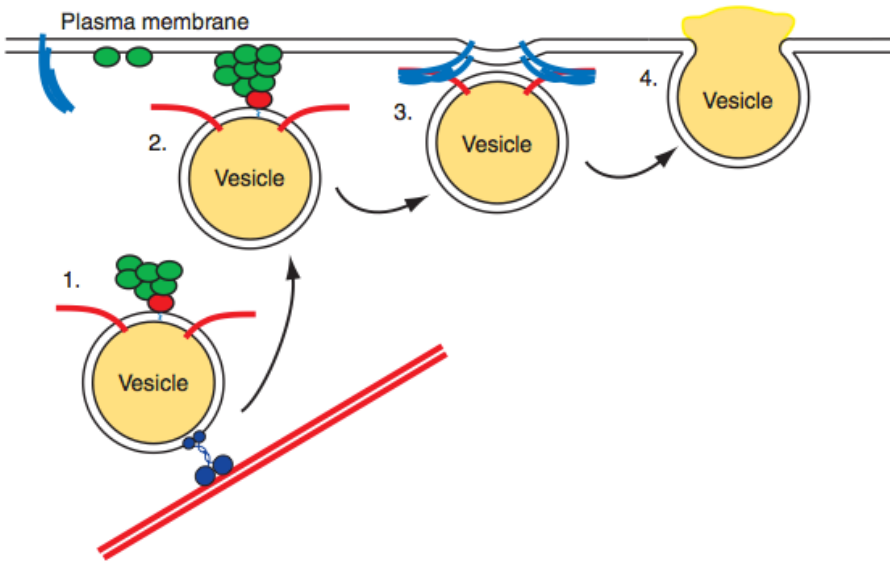


Figure 1.4. Late stages of exocytosis at the plasma membrane. 1. Secretory vesicles carrying the Sec4 Rab-GTPase (red), v-SNAREs (red curves), and part of the exocyst complex (green) are delivered to the plasma membrane along the cytoskeleton (red lines) via motors (blue). 2. Tethering of secretory vesicles to the plasma membrane through the exocyst. 3 and 4. The t-SNAREs (blue curves) and v-SNAREs bind on the plasma membrane with consequent membrane fusion (Park et al., 2012).

This protein-lipid interaction is conserved from yeast to mammals (Liu et al. 2007). These interactions play a critical role in recruiting Exo70, Sec3, and ultimately the other exocyst components to the plasma membrane for vesicle tethering (Figures 1.5. and 1.6.).

The assembly of the exocyst complex is cell cycle controlled in *S.cerevisiae*. It has been shown that the cyclin-dependent kinase, Cdk1, when in complex with Clb2, directly phosphorylates Exo84. Mitotic phosphorylation of Exo84 partially disrupts the exocyst assembly, leading to a blockage of exocytosis. This regulation causes a stop in cell growth before the metaphase-anaphase transition and it is believed to happen to prepare cells for division. Therefore there is a careful coordination between membrane trafficking and cell cycle in budding yeast (Luo et al., 2013).

1.3.3. Rab GTPases

Rab GTPases are molecular switches that cycle between active and inactive states and serve as scaffolds between membrane trafficking and intracellular signaling. Rab proteins are usually small as 20-25 kDa, but they consist of many interaction surfaces that associate with regulatory molecules and downstream effectors to exert their functions. They are involved in the regulation of many basic cellular functions but they are best known for their essential roles in exocytic and endocytic membrane trafficking (Schwartz et al., 2007), playing a key role in defining organelle identity and the direction of vesicular transport. A guanine nucleotide exchange factor (GEF) and a GTPase activating protein (GAP) are required for their activation and GTP hydrolysis, respectively. As the membrane flow change through the exocytic or endocytic pathways, the

Rabs associated with that membranes change too. The new Rabs decorating the membrane recruit a distinct set of effectors that, in turn, help to redefine the functional identity of the membrane (Novick et al., 2016).

The first Rab involved in the yeast secretory pathway is Ypt1, that promotes ER to Golgi transport as well as early stages of transport within the Golgi.

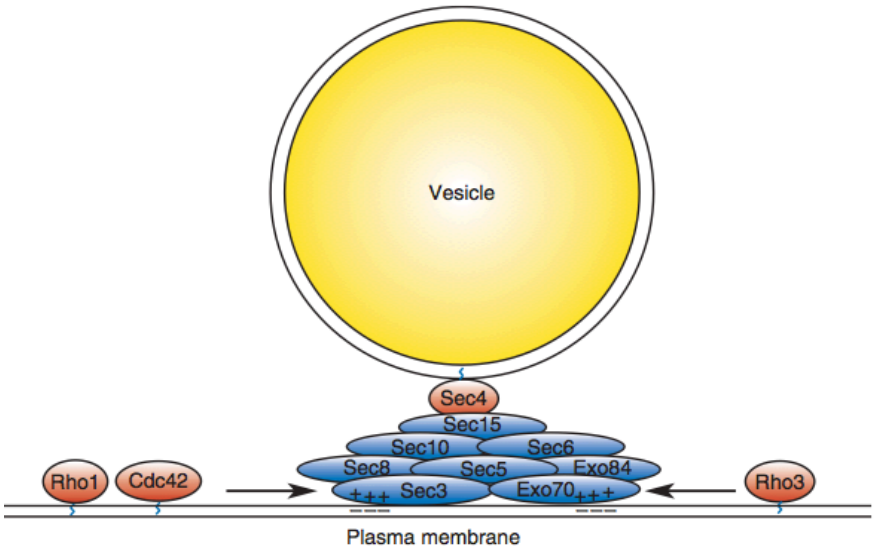


Figure 1.5. The exocyst complex. The exocyst subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84) are represented by blue rods. The GTPases are represented by red circles. Sec3 and Exo70 associate directly with the plasma membrane through their basic residues that bind to PI(4,5)P2. The Rab GTPase Sec4 binds to secretory vesicles, and interacts with the exocyst component Sec15. This interaction promotes the assembly of the exocyst complex at the plasma membrane. Sec3 is known to interact with Rho1 and Cdc42, whereas Exo70 interacts with Rho3 (Park et al., 2012). These small GTPases may regulate the polarization and activation of the exocyst at the plasma membrane.

The second Rab, Ypt32, activates the formation of secretory vesicles from the Golgi and drives the first stages of secretory vesicle maturation (Jedd et al., 1997). The final Rab, Sec4 (Rab8 in human cells) drives the vectorial delivery of those vesicles to the sites of polarized cell surface expansion as

well as the docking and fusion of the vesicles to the plasma membrane (Grosshans et al., 2006). Sec4 is activated by a GEF called Sec2, which is, like Sec4, highly concentrated on the surface of secretory vesicles (Novick et al., 2016). Sec2 is recruited to the Golgi membrane by a combination of RabGTPase Ypt32 and PI(4)P, thus recognizing only those membrane domains with both ligands. Here it rectruits Sec4 that in turns recruits its effector Sec15 (Ortiz et al., 2002; Mizuno-Yamasaki et al., 2010). When secretory vesicles are made and the PI(4)P concentration is high, Sec2 is phosphorylated by Yck1 and Yck2 (Stalder et al., 2015). Phosphorylated Sec2 binds to Ypt32 and its interaction with Sec15 is blocked. As secretory vesicles mature, the PI(4)P concentration is reduced and Sec2 is dephosphorilated, leading to Sec2-Sec15 interaction that enhances Sec4-Sec15 interaction (Medkova et al., 2006) and that prepares the vesicles for docking and fusion with the plasma membrane (Mizuno-Yamasaki et al., 2010). The reduction of PI(4)P levels is Osh4-dependent (Figure 1.6).

1.3.4. Endocytosis

Endocytosis is the process by which extracellular material and plasma membrane-bound proteins are collected and packaged into vesicles that enter the cytosol where they fuse with other internal organel. The material in the vesicles is then either recycled or degraded (Goode et al., 2015). Many features of this pathway are evolutionarily conserved from yeast to

human cells (Engqvist-Goldstein et al., 2003).

In budding yeast, sites for endocytosis are organized through the action of membrane proteins called eisosomes (Figure 1.7) (Walther et al., 2006). Initially, clathrin (that coats the budding vesicle) and Ede1p arrive at endocytic sites (Newpher et al., 2005). Clathrin is necessary for the proper recruitment of later endocytic proteins and Ede1p is involved in the proper initation of endocytic sites (Kaksonen et al., 2005). Then Las17p (the yeast ortholog of the human Wiskott-Aldrich syndrome protein, WASP), which activates the Arp2/3 complex to promote actin assembly, begins to be recruited along with its negative regulators (Sla2p and the Pan1 complex) (Kaksonen et al., 2003). Then Vrp1p stimulates the capacity of Myo5p to activate the Arp2/3 complex, and it promotes Arp2/3-mediated actin assembly for internalization (Sun et al., 2006) As the coat module internalizes, amphiphysin proteins are recruited to the endocytic site where they contribute to the release of the forming vesicle (Kaksonen et al., 2005). Then Abp1 starts recruiting coat-disassembly factors, and inhibits the nucleating activity of the Arp2/3 complex (D'Agostino et al., 2005). After the coat has moved inwards approximately 200 nm the protein kinases Ark1p and Prk1p, which are recruited in a Abp1-dependent way, regulate the endocytic site disassembly (Sekiya-Kawasaki et al., 2003; Zeng et al., 2001). The endocytic vesicles fuse with early endosomes in a process that is made more efficient by the association of the vesicles and early endosomes with actin cables (Toshima et al., 2006). The proteins that mediate association

of endocytic vesicles and early endosomes with actin cables, and the underlying mechanisms for facilitating efficient docking and fusion, remain to be identified.

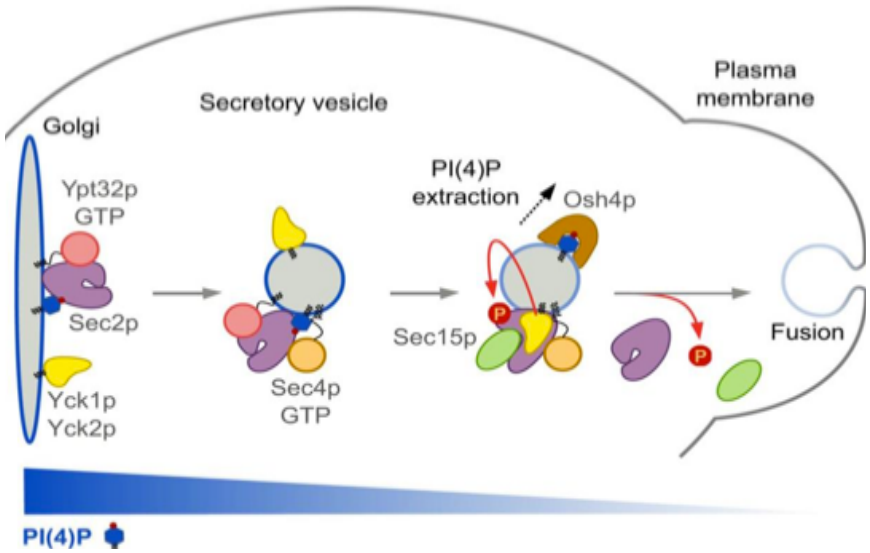


Figure 1.6. Rabs in exocytosis. (from left to right) Sec2 is recruited to membranes by the combination of Ypt32-GTP and PI(4)P. The secretory vesicle buds off and Sec2 activates Sec4, which then recruits its effector, Sec15. PI(4)P is removed by Osh4 and this allows Sec15 to interact with Sec2 which is now phosphorylated by Yck1/2, enhancing the Sec2–Sec15 interaction. This process generates a micro-domain of high Sec4-GTP and high Sec15, facilitating the delivery, tethering, and fusion of the vesicle with the plasma membrane. Sec2 is dephosphorylated and it dissociates from Sec15 and thus dissociate from the vesicle (Novick et al., 2016).

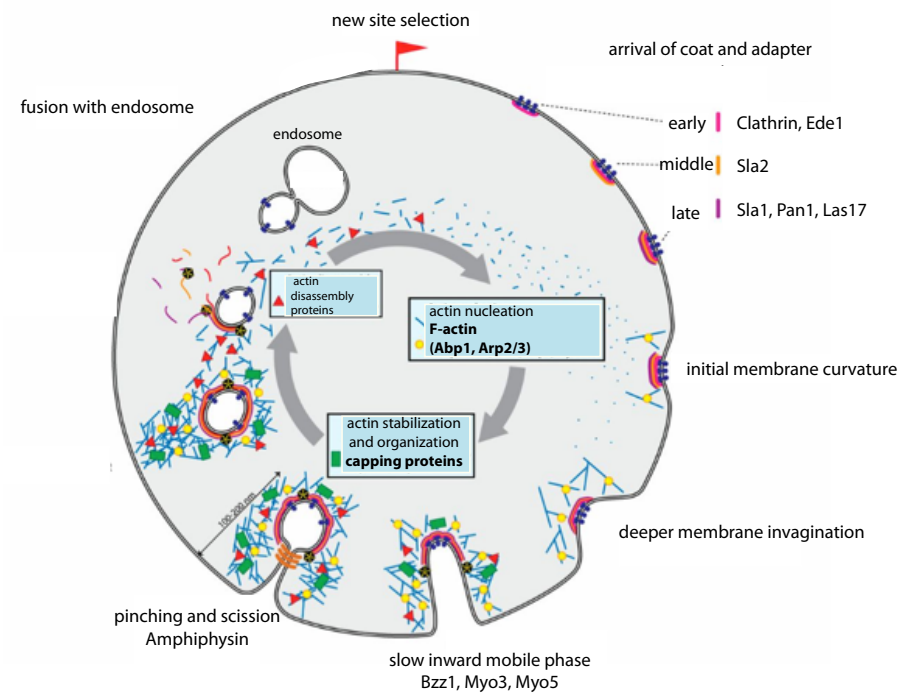


Figura 1.7. Schematic view of endocytosis in budding yeast After new site selection (red flag) coat and adapter protein arrive to the site of endocytosis. They can divided in early (i.e. Clathrin and Ede1), middle (i.e. Sla2) and late (i.e. Sla1, Pan1 and Las17) endocytosis protein. Las17 activates the Arp1/3 complex that promote actin assembly. Myo5, through the Arp2/3, promote the actin assembly for internalization. As the bud internalizes amphiphysin proteins act for the release of the forming endocytic vesicle. Once formed, the endocytic vesicle fuses with early endosomes. Adapted from (Goode et al., 2015)

1.4. Chromosome segregation

The transition from metaphase to anaphase is a crucial event, since it leads to the segregation of the sister chromatids, mitotic exit and cytokinesis. For this purpose, the spindle assembly checkpoint (SAC) ensures that all the chromosome kinetochores (protein structures at the centromeres) are properly linked to the metaphase spindle. The binding of SAC components to kinetochores that failed to bind with microtubules (Eytan et al, 2008) starts a cascade that inhibits the anaphase-promoting complex (APC) (Shwab et al., 2001) stopping therefore the onset of anaphase.

The APC polyubiquitinates proteins for their degradation by the proteasome and has two major substrates: Securin and the M-phase Cyclins. Securin (Yamamoto A) is an inhibitor of the Separase. This enzyme, after Securin degradation through the protesome, cleaves Scc1 (a protein of the cohesin complex), permitting chromosomes to segregate (Uhlmann et al., 1999). Degradation of M-phase cyclins inactivates Cdk1 activity and therefore allows exit from mitosis (Hornig et al., 2002) (Figure 1.8). For a proper exit from mitosis, Cdk-Clb2 substrate-phosphorylations are reversed by the Cdc14 phosphatase (Visintin et al., 1998). Net1 sequesters Cdc14 at the nucleolus until the onset of anaphase. The release of Cdc14, which coincides with its activation, occurs in two phases: initially Cdc14 is released from the nucleolus to the nucleus through the FEAR (fourteen early anaphase release) network and then from the

nucleus to the cytoplasm through the MEN (mitotic exit network). Cdc14 activity is essential to promote cytokinesis.

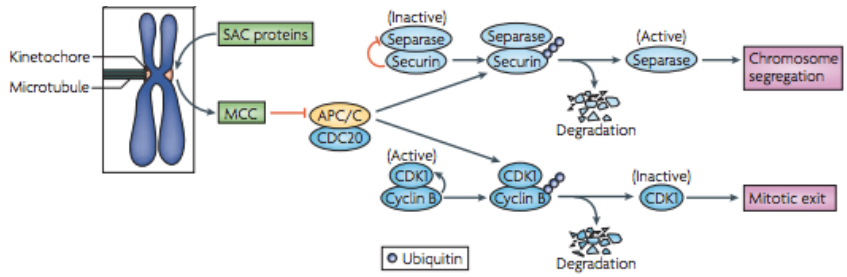


Figure 1.8. Chromosome segregation and the SAC checkpoint in yeast. Adapted from (Yanagida et al., 2009).

1.5. Cytokinesis

Cytokinesis is a crucial event in the distribution of cellular constituents between the two new-forming cells after DNA replication and chromosome segregation, and is essential for the survival of all organisms (Bhavsar-Jog Yogini et al., 2016). Cytokinesis involves the selection of a division site, membrane furrow ingression through a contractile

actomyosin ring, and membrane remodeling that ultimately leads to abscission (the physical division of the two membranes).

In *S. cerevisiae*, the site of budding is the future division plane and it is determined by Cdc42 in late G1 (Bi et al., 2012). Cdc42 is involved in the polarization of the actin cables and patches and in the nascent septin ring assembly at the bud site. The septin ring recruits Myo1, the sole myosin-II in budding yeast. After bud emergence, the septin ring expands into an hourglass and along with the myosin ring, marks the bud neck. To drive bud growth and morphogenesis in S/G2/M, actin cables and actin patches polarize towards the bud cortex and the bud neck where they mediate exocytosis and endocytosis, respectively (Moseley et al., 2006).

Upon the entry into anaphase, actin filaments and myosin-II interact to form an actin-myosin ring, which is sandwiched by the septin hourglass. The latter is converted into a double ring after activation of the mitotic exit network (MEN) (Lippincott et al., 2001). The actin-myosin ring begins to constrict centripetally and meanwhile, actin cables are polarized towards the bud neck to mediate the delivery of post-Golgi vesicles, which fuse with the plasma membrane (PM) to increase surface area as well as release cargoes such as the chitin synthase-II (Chs2) to drive primary septum (PS) formation (Chunag et al., 1996). Actin-myosin ring constriction is followed closely by PS formation. In the absence of PS formation, the actin-myosin ring undergoes asymmetric constriction

towards one side of the bud neck, which might reflect a partial detachment of actin-myosin ring from the PM (VerPlank et al., 2005). Thus, the PS is thought to stabilize the actin-myosin ring during its constriction. When PS formation is almost concluded, a secondary septum (SS) is synthesized at both sides of the PS by glucan synthases (EL). Following SS formation, a kinase cascade called RAM (Regulation of Ace2 and Morphogenesis) is activated and transcription factor Ace2 localizes exclusively to the daughter cell nucleus where promotes the transcription of cell wall hydrolases that digest the PS and part of the SS, leading to cell separation (Wloka et al., 2012).

The temporal coordination between cytokinesis and cell cycle progression is achieved by the Cdc14p early anaphase release (FEAR) and the mitotic exit network (MEN). The FEAR pathway acts at anaphase and controls the timing that connects chromosome separation to mitotic exit, setting the stage for mitosis to be completed. Once the FEAR has become activated, the MEN fires up the cytokinesis process (Seshan et al., 2004). Apart from its role in the mitotic exit, MEN induces the relocalization of several SPB-associated proteins from the bud to the neck, and this change is essential for the progression of cytokinesis.

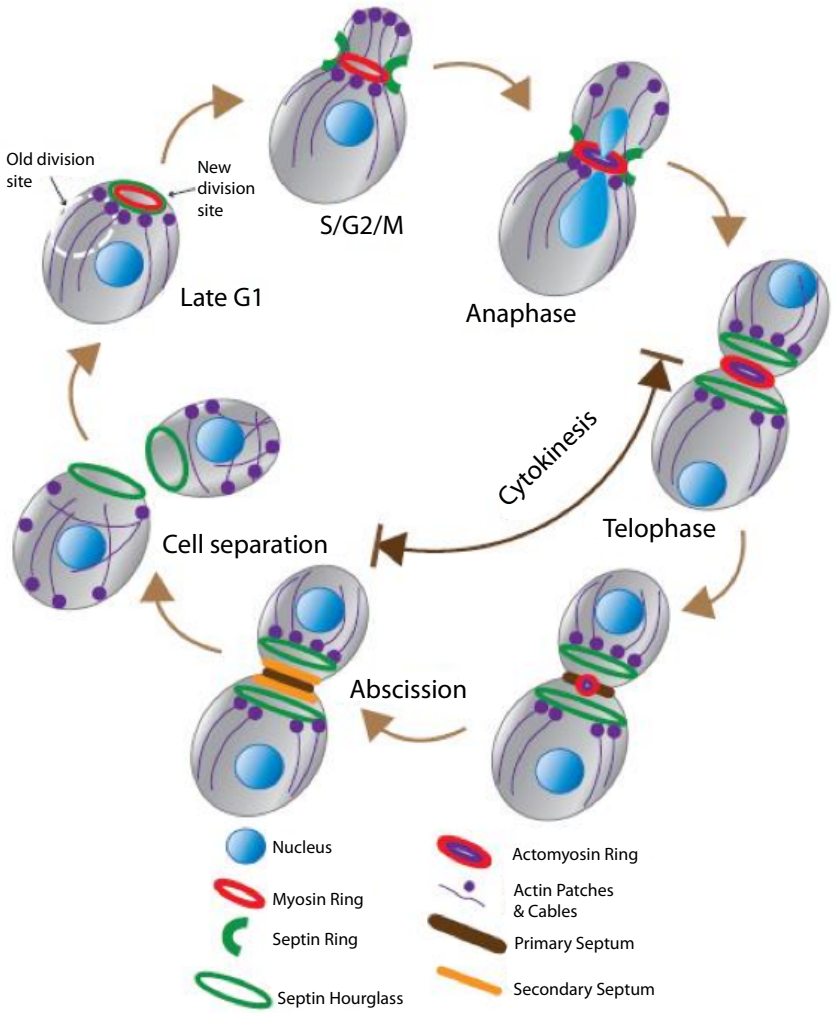


Figure 1.9. Schematic representation of cytokinesis proteins during the *Saccharomyces cerevisiae* cell cycle. Adapted from (Bhavsar-Jog Yogini et al., 2016)

1.6. Cell separation

Cell separation is directed by hydrolytic enzymes that partially degrade the septum wall between mother and daughter cells. In *Saccharomyces Cerevisiae*, this degradation occurs only at the neck of the daughter cell (Baladron et al., 2002; Colman-Lerner et al., 2001). RAM signalling network is behind this asymmetry since the hydrolases involved in the process are under its control. In fact, RAM activates a specific set of genes in the daughter nucleus through the Ace2p transcription factor, which is activated by Cbk1 through phosphoprylation. Ace2 triggers the late expression of the CTS1 and ENG1 genes, coding for the major chitinase and glucanase activities, respectively. Cts1p and Eng1p are then transported to the neck, where are secreted to the periplasmic space(Baladron et al., 2002; Colman-Lerner et al., 2001). After cell separation, Ace2 is translocated to the cytoplasm by CDK phosphorylation and remains there until the next division (Mazanka et al., 2010).

1.7. The NoCut checkpoint

After anaphase onset, sister chromatids are separated from each other and pulled to opposite poles of the cells. When the cleavage site is cleared from DNA the membrane furrows, contracts and eventually resolves. Resolution of the membranes is called abscission. In presence of

chromosome segregation defects, generated by problems in DNA damage, DNA condensation or decatenation, budding yeast and human cells activate a pathway called NoCut that delays abscission (Norden et al., 2006; Steigemann et al., 2009). The arrested cells have a specific defect in membrane abscission (in budding yeast, cells are kept in the contracted stage), since they are not able to separate even after treatment with the cell wall-degrading enzyme zymolyase (Norden et al., 2006).

Aurora B (Ipl1 in yeast) is central to this pathway. Inactivating Ipl1 in yeast or preventing its localization to the spindle midzone, abscsission is restored in mutants with chromosome segregation defects. This suggests that Aurora regulates this response (Norden et al., 2006) . Ipl1 is part of the chromosome passenger complex (CPC) and is thought to be the sensor of the lagging chromosomes. In line with this hypothesis, Ipl1 covalently bound to chromatin triggers a delay in cytokinesis (Mendoza et al., 2009).

Aurora B also mediates abscission delays in Hela cells caused by chromosome segregation defects (Steigemann et al., 2009) through the Aurora-B-mediated phosphorylation of CHMP4C, a component of the endosomal sorting complex required for transport (ESCRT) that mediates abscission (Carlton et al., 2012).

In budding yeast, APC-Cdh1 is impaired in anaphase of cells with lagging chromosomes, resulting in a stabilization of the anaphase spindle that is

required for the abscission delay. The stabilized spindle is thought to act as a platform for Ipl1 that can now detect chromatin in the spindle midzone (Figure 1.10) (Amaral et al., 2016).

Here I show that Boi2 is also a protein required for abscission inhibition in NoCut. I also show that the SH3 domain of Boi2 is sufficient trigger the abscission delay in NoCut. The mechanism(s) by which Boi2 acts in NoCut is still unknown, but it may rely on the regulation of the exocyst complex, since a gain of function mutation of Exo70 (subunit of the exocyst) restore the abscission delay in NoCut and since the SH3 domain of Boi2 interacts, *in vitro*, with some exocyst components (Masgrau, Battola et al., 2017).

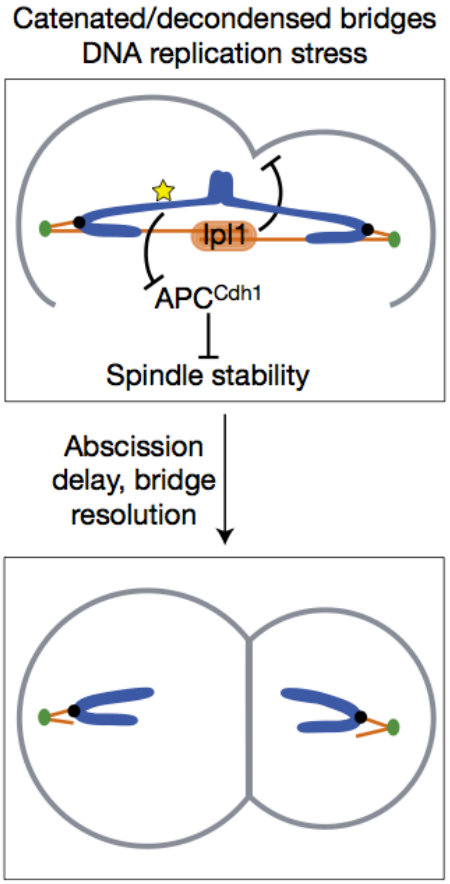


Figure 1.10. The NoCut Model. The defect in chromosome segregation stabilizes the APC^{Cdh1} complex, leading to a stabilization of the spindle. Ipl1 can now detect the lagging chromatin and promote the abscission delay (Amaral et al., 2016).

1.8. Boi1 and Boi2

Boi1 and Boi2 are two scaffold proteins involved in cell polarity. They contain an SRC homology 3 (SH3) domain essential for bud neck localization (Hallett et al., 2002) a sterile-alpha motif (SAM), a prolin-rich region, that usually interact with other SH3 domains of other proteins, and a Pleckstrin Homology (PH) domain at the C-terminal part (Figure 1.11) which is essential for the interaction with the plasma membrane and for the viability of the cell (Bender et al., 1996; Matsui et al., 1996).

Boi proteins localize at the bud cortex during interphase and later on, during cytokinesis they relocate to the bud neck. Deletion of either Boi1 or Boi2 does not affect cell viability or growth rate (Matsui et al., 1996). In contrast, cells lacking Boi1 and Boi2, are round and large and they die with a bud. This study has been confirmed recently in a study conducted in our lab (Masgrau, Battola et al., 2017). Lethality of *boil*Δ *boi2*Δ mutants is rescued by multicopy plasmids expressing RHO3, RHO4 suggesting that Boi1 and Boi2 promote the activation of Rho3/4 GTPases and they co-operate in the maintenance of cell polarity for bud formation (Bender et al., 1996). Overexpression of Boi1 inhibits bud emergence and this inhibition is rescued by a co-overexpression of Cdc42. Boi1 and Boi2 also control secretion of Bgl2 vesicles in interphase by regulating the exocyst complex and the SH3 domain of Boi2 is required for inhibiting abscission in NoCut (Masgrau, Battola et al., 2017).

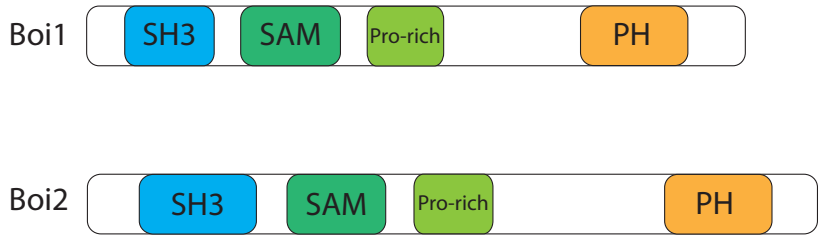


Figure 1.11. Schematic view of Boi1 and Boi2 protein structure. Both proteins contain a SH3 , SAM, Pro-rich and PH domain.

2. Aim of the work

Aina Masgrau (Aina Masgrau PhD thesis, 2014) previously showed that Boi1 and Boi2 depletion (through the construction of an auxin-inducible degron) is lethal. She also found that cells lacking the two proteins Boi1 and Boi2 accumulated secretory vesicles and this defect was rescued by a gain of function mutation of the exocyst complex. Additionally, her work provided evidence that Boi proteins might be involved in the NoCut checkpoint.

My aim was to characterize the mechanism through which Boi1 and Boi2 were acting in the regulation of the exocytosis and especially elucidate the role of them in the NoCut pathway. My data, complemented with the previous research of Aina Masgrau gave rise to the manuscript "Distinct roles of the polarity factors Boi1 and Boi2 in the control of exocytosis and abscission in budding yeast" (Masgrau, Battola et al., 2017).

3. Matherial and methods

3.1. Strains and media

S. cerevisiae strains are derivatives of S288c Yeast cells were grown in YPD/YPG/YPR (1% bacto-yeast extract; 2% bacto-peptone; 2% dextrose, galactose or raffinose; and 0.004% adenine). Gene tagging and deletions were generated by standard PCR-based methods.

3.2. Time-lapse and Fluorescence microscopy

Time-lapse microscopy was performed on cells in log phase, or after synchronization with alpha factor as indicated, plated in minimal synthetic medium in concanavalin A- coated Lab-Tek chambers (Nunc) and placed in a pre-equilibrated temperature- controlled chamber. Imaging was performed using a Cell Observer HS microscope with a 100x, 1.4 NA objective and an AxioCam MrX camera (Zeiss), an AF6000 wide-field microscope (Leica) with a 100x, 1.4 NA objective and an iXon 885 DU EM-CCD camera (Andor), or a Revolution XD spinning disc confocal microscope with a 100x, 1.45 NA objective and an iXon 897E Dual Mode EM-CCD camera (Andor). Bud volumes were calculated from DIC stacks using ImageJ and the BudJ plug-in (Ferrezuelo et al., 2012) customized for analysis of Z-stacks. To visualize F-actin, cells were fixed with 3.7%

formaldehyde for 1 h, washed in PBS, incubated with 0.2 U/ml Alexa 488-phalloidin for 1 h at 25 oC, washed in PBS and visualized immediately in 80% glycerol / 20% PBS. Abscission assays were performed as in (Amaral et al., 2016a). Briefly, the plasma membrane was visualized via GAL1,10 promoter driven GFP-CAAX integrated in the HIS3 locus. Expression of GFP-CAAX in glucose media was driven by the chimeric ADH1pr-Gal4-ER-VP16 transcription factor (Louvion et al., 1993) (URA3; gift of Francesc Posas, UPF) and addition of 90 nM β-estradiol (Sigma) 2 h before imaging. Only cells starting cytokinesis (membrane ingression) at least 40 min before the end of image acquisition were considered for the quantifications of abscission. Fluorescence intensities were measured from single sections of each cell to score membrane separation (abscission).

3.3. Electron microscopy

Cells were cryoimmobilized using a EM HPM 100 high-pressure freezer (Leica), freeze-substituted in anhydrous acetone containing 2% glutaraldehyde and 0.1% uranyl acetate (Fig. 3) or 2% OsO4 and 0.1% uranyl acetate and warmed to room temperature (EM AFS-2, Leica). After several acetone rinses, cells were incubated with 1% tannic acid, washed, incubated with OsO4 at 1% acetone, washed again, infiltrated with Epon resin and embedded and polymerised at 60 oC. Ultrathin sections were obtained using an Ultracut UC6 ultramicrotome (Leica) and observed in a

Tecnai Spirit electron microscope (FEI Company, The Netherlands) equipped with a Megaview III CCD camera.

3.4. Bgl2 assay

Bgl2 accumulation was determined as described (Curwin et al., 2012) with minor modifications. Cells were grown in YPR to log phase, transferred to YPG for 2 h, and cells were either shifted to 37 oC (sec14-1) or incubated with 0.5 mM NAA (boi1Δ boi2-aid). After 2 hours, equal cell numbers were harvested by centrifugation and washed in 10 mM NaN3/NaF solution. Total protein extracts were prepared by TCA extraction. For the internal fraction, cells were resuspended in pre-spheroplasting buffer (100 mM Tris- H2SO4, pH 9.4; 50 mM β-mercaptoethanol; 10 mM NaN3; 10 mM NaF) and washed with spheroplasting buffer without zymolyase (50 mM KH2PO4-KOH, pH 7.4; 1.4 M sorbitol; 10 mM NaN3). Then, cells were resuspended in spheroplasting buffer containing 167 μg/ml zymolyase 100T (Seikagaku Biobusiness), and incubated with gentle mixing. Spheroplasts were harvested and resuspended in sample buffer before separation by SDS–PAGE and western blotting to detect Bgl2 (specific antibody gift from Randy Schekman, University of California, Berkeley) and Glucose-6-phosphate dehydrogenase as a loading control (Sigma).

3.5. Immunoprecipitation assay

Strains expressing Boi2-myc and Exo70-HA were grown to mid-log phase in YPDA at 25°C. 200OD units of cells were pelleted and washed with cold lysis buffer (50mM Tris-HCl pH7.4; 200mM NaCl; 1mM EDTA; PIC). Pellets were resuspended in lysis buffer and were lysed by bead beating. Lysates were cleared by centrifugation at 3000rpm for 10min. The supernatant was collected and the membranes were pelleted for 45min at 45000rpm in an L100-XP Beckman ultracentrifuge. The cytosolic fraction was saved to perform the IP and the membranes were solubilised and incubated with 1% NP40; 50mM Tris-HCl pH7.4; 200mM NaCl; 1mM EDTA; PIC for 4h. Then samples were incubated with dynabeads (invitrogen) and 1:1000 of antiHA 12CA5 (Roche) over night. Samples were washed three times with 1% NP40; 50mM Tris-HCl pH7.4; 200mM NaCl; 1mM EDTA; PIC. Samples to analyze by MS were collected with digestion buffer (6M urea (Bio-Rad Laboratories S.A.), 200mM NH_4HCO_3). Samples to analyze by SDS- PAGE gels were collected with sample buffer and boiled at 95°C for 5min.

4. Results

4.1. Boi1 and Boi2 are essential for cell growth

As previously shown, single *boi1*Δ and *boi2*Δ mutants grow perfectly and with no defects in polarization. The double deletion mutants, instead, are lethal (Bender et al., 1996; Matsui et al., 1996). In order to understand the role of Boi1 and Boi2 during cell growth, a conditional mutant was generated by Aina Masgrau in which Boi2 is tagged with an auxin-inducible degron peptide (AID) at its C-terminal, in a strain where the plant E2 ligase Tir1 is under a Galactose promoter and Boi1 is deleted. Upon addition of 1-naphthaleneacetic acid (NAA), Tir1 ligase binds *boi2-aid* that then is poly-ubiquitinated and eventually degraded by the proteosome (Nishimura et al., 2009); Boi2-aid takes normally 2 hours upon NAA addition to be totally degraded (Masgrau, Battola et al., 2017).

The Δ*boi1 boi2-aid* strain do not grow in NAA containing plates (Aina Masgrau thesis, 2014), confirming that Boi2 is almost totally degraded, since, as I already discussed, the absence of the two proteins is not viable (Matsui et al., 1996).

My aim was to investigate the role of the two proteins in cell growth, and to understand why the Boi1/2 double deletion induces cell death. For this purpose, I performed a 6 hours DIC time-lapse microscopy of *GalTir1* and *GalTir1 boi1*Δ *boi2-aid* strains after 2 hours from addition of 0.25 mM

NAA, when Boi2-aid protein levels are almost disappeared and more generally, the two proteins are near to be completely depleted. *GalTir1* cells grew perfectly during the 6 hours movie, with or without NAA, indicating that neither the ligase Tir1 nor the drug NAA affected cell growth, and that the NAA-degron-strategy was perfectly suitable for this study. On the contrary, Boi1/2 depleted cells showed a severe defect in surface growth. Indeed, NAA-treated *boi1*Δ *boi2-aid* cells with small or medium buds, either grew slowly, or they became dark suggesting cell death or stopped in growing (no growth) (Figure 4.1). Interestingly, a 20% of cell death occurred in *boi1*Δ *boi2-aid* cells incubated with DMSO, indicating that the absence of Boi1 partially affects cell viability.

The *boi1*Δ *boi2-aid* cells were also observed 24 hours after NAA addition. Cells were rounded and not polarized, confirming the hypothesis that Boi1 and Boi2 are involved in cell polarization (Figure 4.2).

In conclusion, Boi1/2 are essential in cell growth and in cell polarization.

Surprisingly, the Δboi1 Δboi2 phenotype was different between the *Saccharomyces cerevisiae* strain we used in the lab (backround s288c) and the one used in a previous work (BF264-15D) (Norden et al., 2006) where, differently from our findings, the double deletion was not lethal. Genome sequencing analysis of this strain, performed by the laboratory of Tony Gabaldon, revealed the presence of a suppressor mutation in the EXO70 gene (EXO70-G388R, hereby called EXO70*) (Aina Masgrau thesis,

2014). The gene codes for the Exo70p exocyst subunit, involved in tethering secretory vesicles to the plasma membrane. That specific mutation was already shown to be a gain of function mutation, able to rescue lethality and growth defects in Cdc42 temperature sensitive mutants (Wu et al., 2010). We inserted this mutation in our Boi1/2 degron strain to check whether it rescued lethality and the growth defect due to absence of Boi1 and Boi2. The EXO70* rescued lethality of Boi1/2 depleted cells (Masgrau, Battola et al., 2017) and, as shown in the graph of Figure 4.1, also the bud growth.

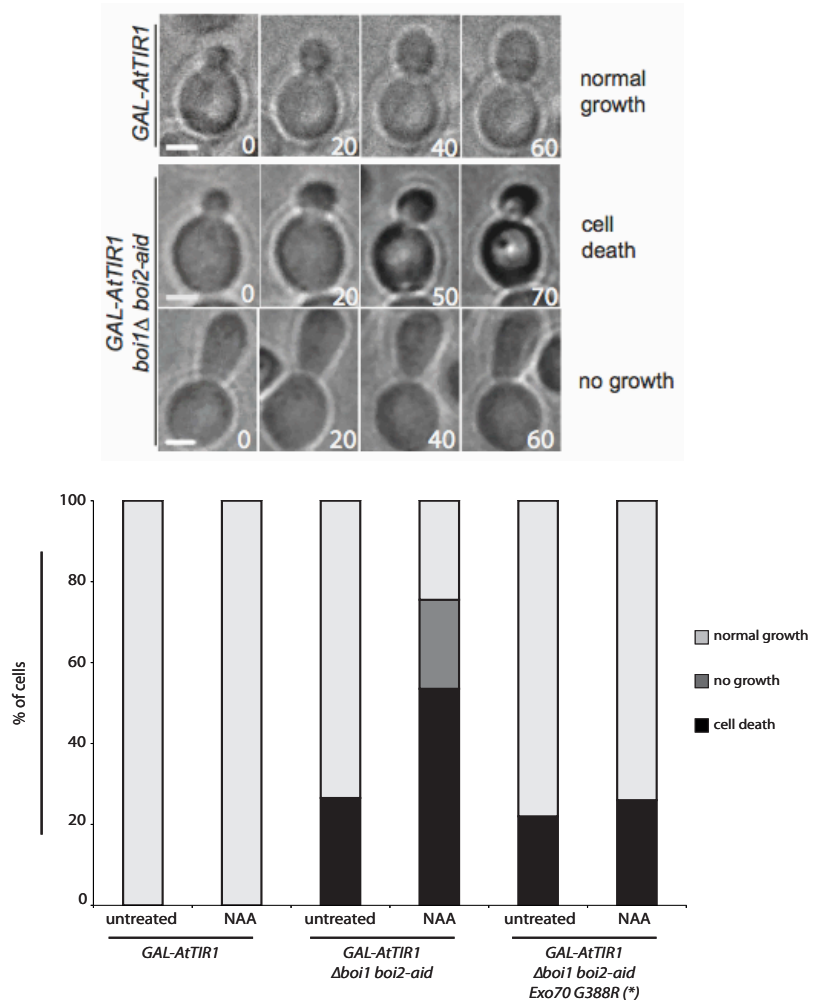


Figure 4.1. Boi1 and Boi2 are essential for growth. Top. Montages of *GalAtTir1* and *GalAtTir1* Δ*boi1 boi2-aid*. Bottom. Graph displaying the percentage of cells that grow (normal growth), die (cell death) and stop in growth (no growth) in *GalAtTir1, GalAtTir1* Δ*boi1 boi2-aid* and *GalAtTir1* Δ*boi1 boi2-aid EXO70**.

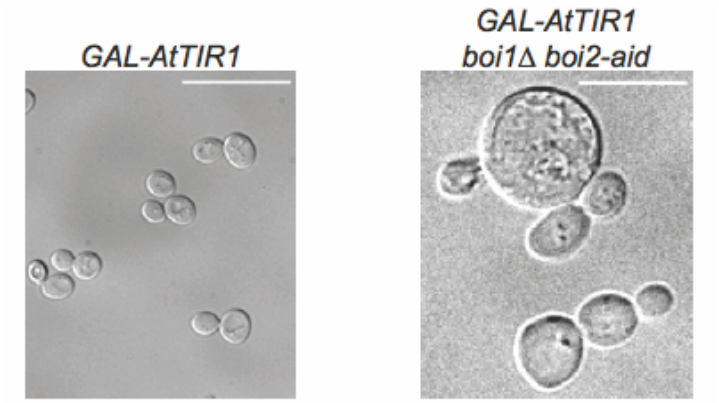


Figure 4.2. Depolarization defect in long-term Boi1/2 depletion. *Gal-AtTir1* cells show normal morphology after 24h of NAA treatment, whether Boi1/2 depleted cells (right) are bigger and rounded, suggesting a defect in polarization (Masgrau, Battola et al., 2017). Scale bar = 10 micron

4.2. Boi1 and Boi2 control secretion

The defect in growth observed by the depletion of Boi1 and Boi2 is rescued by a gain of function mutation of a component of the exocyst complex (EXO70*) (Masgrau-Battola et al., 2017). The exocyst complex, as discussed in the chapter "Membrane trafficking", is required for secretion and therefore we addressed the question if secretion might be affected in Boi1/2-depleted cells.

Budding yeast cells have two major types of exocytic vesicles, light-dense vesicles containing Bgl2, an endoglucanase that will be part of the cell wall, and more dense-vesicles that contain invertase, an enzyme that digests sucrose into monosaccharides (Bretscher et al., 1995).

Aina Masgrau showed that invertase secretory vesicles do not accumulate in Boi1/2 depleted cells but in her work it was not clear whether Bgl2 secretion was affected or not. For this purpose I performed immunoblotting of the cytoplasmic-internal Bgl2 fraction in WT, *boi1*Δ *boi2-aid*, Δ*boi1 boi2-aid* EXO70* and temperature sensitive mutants *sec14-1* spheroplasts (Figure 4.3). All the strains, but the *sec14-1* one, bear the GalAtTir1 ligase gene. Temperature sensitive *sec14-1* strain accumulates Bgl2 in the cytoplasm (Curwin et al., 2009), and therefore it has been used as a positive control for our Bgl2 assay. Boi-depleted cells as well as *sec14-1* cells (at non permissive temperature) accumulate large amounts of Bgl2 in the cytoplasm relative to the total cellular content. The EXO70*

mutation is capable of rescuing the strong secretion defect of Bgl2 vesicles in Boi-depleted cells, indicating that the exocyst complex might play a role, together with Boi1 and Boi2 in ensuring a correct functioning of the SEC pathway.

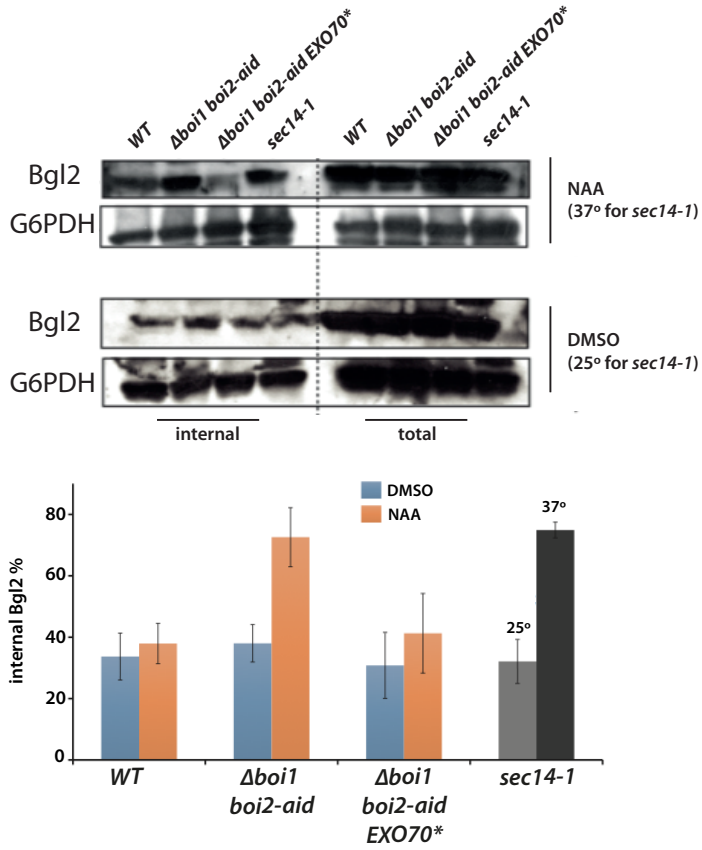


Figure 4.3. Boi1 and Boi2 are essential for secretion of Bgl2 vesicles. A. Western Blot of Bgl2 in spheroplasts (left lanes) and in total cell extracts (right lanes) of WT, Δ*boi1 boi2-aid*, Δ*boi1 boi2-aid* EXO70-G388R and *sec14-1* strains incubated with both NAA and at 37° (for *sec14-1*) (top rows) and DMSO and at 25° (for *sec14-1*) (bottom rows). B. Schematic representation of Bgl2 internal % in the aforementioned strains.

4.3. Boi2 is essential for NoCut

Boi1 and Boi2 were shown to be involved in the inhibition of abscission in presence of chromatin bridges spanning the site of division (Norden et al., 2006; Mendoza et al., 2009). The strains used for that study bear also a gain of function mutation of the exocyst complex component Exo70p (EXO70*), and thus it was not clear if the reverted NoCut-dependent abscission inhibition in the Δboi1 Δboi2 strain was due to the absence of Boi1 and Boi2 or to the hyperactivation of the exocytosis. For this reason I studied again the role of Boi1 and Boi2 in NoCut in the genetic backround we use in the lab (s288c) where no suppressors of the exocyst were found. To activate NoCut, chromatin bridges were induced by inactivating the temperature sensitive allele of topoisomerase II (*top2-4*) at non-permissive temperature (Holm et al., 1985). Wild-type, boi1Δ, boi2Δ, *top2-4*, *top2-4* boi1Δ and *top2-4* boi2Δ cells were released from a G1 arrest and shifted to 37º to induce chromatin bridges by the top2-4 mutation. In order to check abscission (when PM resolves) GFP was fused to the PM targeting CAAX motif of Ras2 (Amaral et al., 2016). WT cells as well as boi1Δ and Δboi2 completed abscission after about 10 minutes from membrane ingression, indicating that the temperature shift did not affect the normal abscission timing (Figure 4.4 and 4.5). In cells with chromatin bridges *top2-4* and *top2-4* boi1Δ abscission did not occur since NoCut is active. Intriguingly, *top2-4* boi2Δ cells resolve PM at the bud-neck as the same rate as WT cells These results indicate that Boi1 is dispensable and Boi2 is required for the NoCut-dependent abscission inhibition.

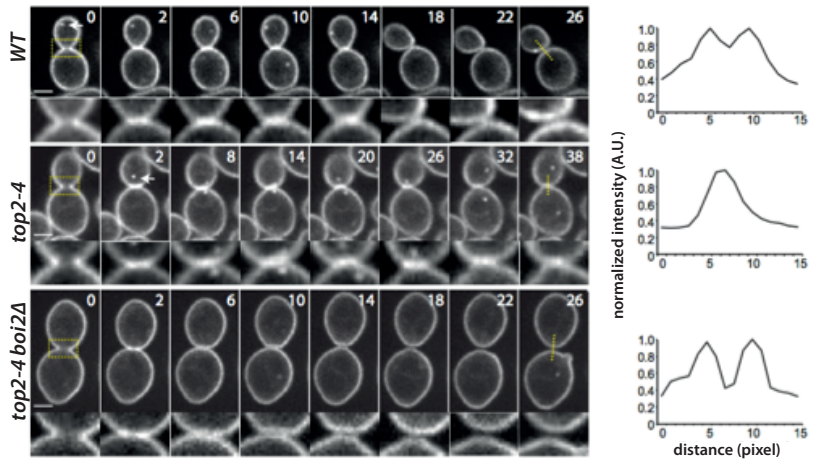


Fig. 4.4. Montage of cells during abscission. Time 0 represents the start of membrane closure that marks the beginning of cytokinesis. WT and *top2-4* boi2Δ resolve membrane as shown in the montage and in the graph at the bottom. Cells with the sole mutation *top2-4*, instead, are delayed in final abscission.

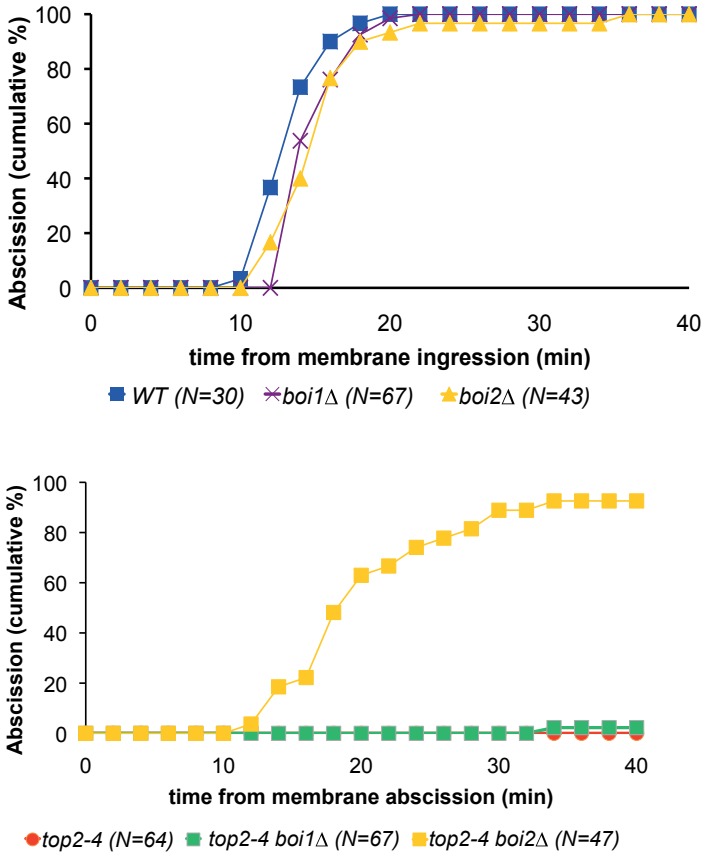


Figure 4.5. Boi2 deletion inhibits NoCut. Graph A shows membrane abscission timing is displayed for WT, Δboi1 and Δboi2 cells; all the strains complete abscission after around 12 minutes from membrane furrowing. Graph B shows *top2-4* and *top2-4* Δboi1 mutants divide because NoCut is active; deletion of Boi2 in *top2-4* cells, instead, inhibits abscission delay, since a 90% of cells divide after 40' from membrane furrowing.

4.4. The SH3 domain of Boi2 is required for NoCut

As already discussed in the introduction, the SH3 domain of Boi2 is responsible of its localization at the bud-neck during cytokinesis (Bender et al., 1996; Hallett et al., 2002). For this reason I checked whether the presence of Boi2 in the site of division was required for the correct abscission inhibition in NoCut. Boi2 was deprived of 102 amino acids at its N-terminal (including the SH3 domain) and the gene was under the GalS promoter. We did not use a stronger promoter because over-expression of Boi2 leads to an arrest in cell growth (Matsui et al., 1996).

The Boi2$^{\Delta SH3}$ protein under the GalS promoter does not affect cell growth and Boi2$^{\Delta SH3}$-GFP localizes correctly in the cortex at early phases of the cell cycle but fails to localize at the division site during cytokinesis. Differently, the localization of whole Boi2-GFP under the GalS promoter is not affected during the all cell cycle (Figure 4.6). This result shows that the GalS promoter does not change Boi2 dynamics in the cell and confirms that the SH3 domain of Boi2 is essential for its localization at the neck in cytokinesis.

I then looked at abscission of cells bearing the GalS-Boi2 or the GalS-Boi2$^{\Delta SH3}$ genes, in presence or absence of chromosome segregation defects. GalS-Boi2 cells do not encounter any issues in PM resolution during cytokinesis, but fail to divide after inactivation of Topoisomerase II and so when chromatin bridges form and NoCut is active. On the other

hand, cells expressing the Boi2$^{\Delta SH3}$ protein resolve PM in cytokinesis regardless of the presence of chromosome segregation defects (Figure 4.7) Thus, the SH3 domain of Boi2 and therefore its localization at the site of division, are required for delaying abscission in NoCut.

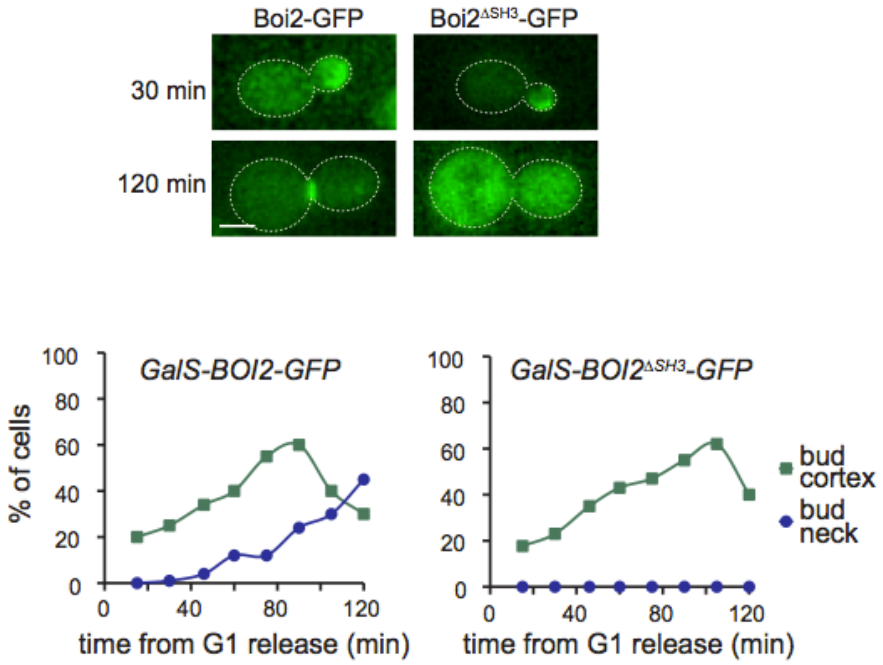


Figure 4.6. **Boi2-GFP and Boi2$^{\Delta SH3}$-GFP dynamics during the cell cycle.** Cell localization of Boi2-GFP and Boi2$^{\Delta SH3}$-GFP in interphase (30 minutes after G1 release) and cytokinsis (120 minutes after G1 release). Boi2$^{\Delta SH3}$-GFP concentrates at the cell cortex during interphase but fails to localize to the site of division during cytokinesis. Number of cells with Boi2-GFP and Boi2$^{\Delta SH3}$-GFP at the cortex and the bud-neck has been quantified and it is displayed in the graph.

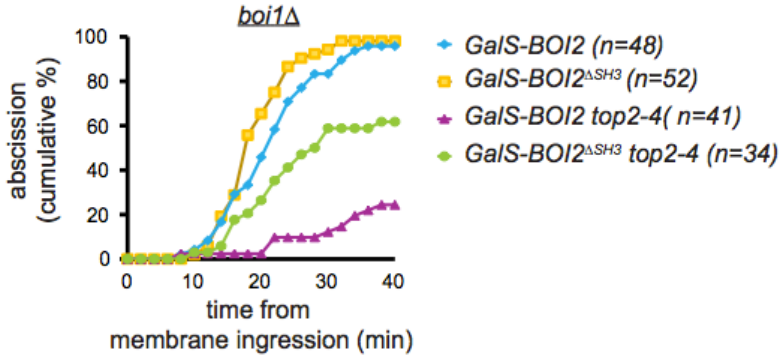


Figure 4.7. NoCut assay of WT and *top2-4* cells where Boi2 proteins is expressed as a whole (GalS-BOI2) or as in lack of its SH3 domain (GalS-BOI2$^{\Delta SH3}$). The graph shows the number of cells (expressed in cumulative percentage) that complete abscission in function of the time. Time zero (0) is the start of membrane ingression, which marks the onset of cytokinesis (X axes). Membrane of GalS-BOI2 and GalS-BOI2$^{\Delta SH3}$ cells resolve after around 25 minutes from membrane ingression. Different abscission dynamics arose in presence of catenated DNA (inactivating the gene of Topoisomerase II). A very low amount of cells expressing the whole Boi2 protein (GalS-BOI2 *top2-4*) undergo to membrane resolution. This defect is rescued in cells where Boi2 lacks of its SH3 domain (GalS-BOI2$^{\Delta SH3}$ *top2-4*).

4.5. Boi2-SH3 domain is sufficient to trigger NoCut

As shown in the introduction, Boi2, in addition to a SH3 domain, contains also a SAM, a Proline rich and a PH domain. To characterize the function of Boi2 in NoCut I investigated whether other domains of Boi2 were involved, and if the sole SH3 domain of Boi2 was sufficient to trigger the NoCut abscission delay. For this purpose, I generated a Boi2 mutant that lacks the protein part downstream of the SH3 domain and hereby referred as $SH3_{Boi2}$. The peptide was also tagged with GFP in order to track its localization during the cell cycle. $SH3_{Boi2}$-GFP exhibits a localization to the cortex (during interphase) and to the bud-neck (during cytokinesis) that is similar to the WT version of the protein (Boi2-GFP) (Figure 4.8). This result reveals that the peptide is transported somehow to the PM even in the absence of the other domains.

Cells expressing the $SH3_{Boi2}$ peptide grew perfectly in rich medium and undergo to PM resolution in cytokinesis in timings almost equal to WT cells. But, in presence of chromatin bridges, when NoCut is active, *top2-4* $SH3_{Boi2}$ fails to resolve PM (Figure 4.9). This result proves that the SH3 domain is sufficient to trigger NoCut.

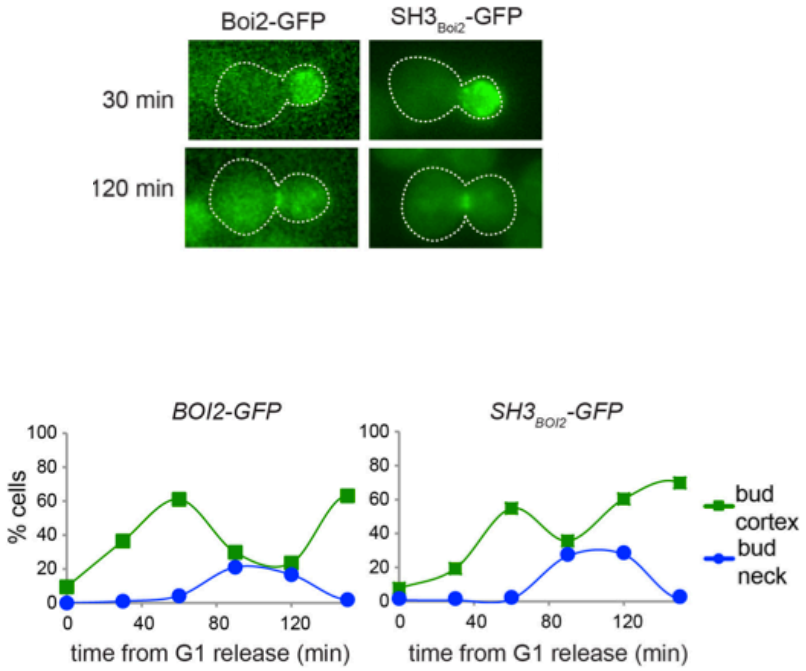


Figure 4.8. Boi2-GFP and $SH3_{Boi2}$-GFP dynamics during the cell cycle. Cell localization of Boi2-GFP and $SH3_{Boi2}$-GFP in interphase (30 minutes after G1 release) and cytokinsis (120 minutes after G1 release). Boi2-GFP and $SH3_{Boi2}$-GFP don't differ in terms of their localization at the cortex and the neck during the cell cycle.

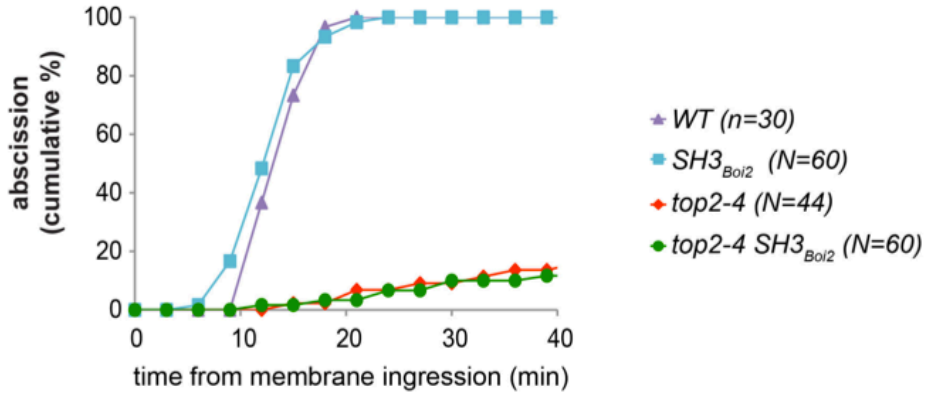


Figure 4.9. **NoCut assay of WT and *top2-4* cells in which Boi2 is expressed as a whole or as a peptide containing the sole SH3 domain ($SH3_{Boi2}$).** WT and $SH3_{Boi2}$ cells have a similar abscission timing, 20' after membrane resolution all the cells complete abscission. In the presence of catenated DNA (*top2-4*), not even 20% of cells undergo abscission in cytokinesis. These numbers are not reverted in the presence of $SH3_{Boi2}$.

4.6. Exocyst is downregulated in NoCut

Boi2 functions in interphase along with Boi1 by promoting the secretion of Bgl2 vesicles and the EXO70* mutation reverts the accumulation of these secretory vesicles in the bud of Boi1/2 depleted cells. (Masgrau, Battola et al., 2017). We then asked if exocytosis is impaired during cytokinesis in cells with chromosome segregation defects. For this purpose, along with Trinidad Sanmartin, we created a strain to check abscission in *top2-4* cells that bear the Exo70*, which is thought to hyperactivate exocytosis. WT, *top2-4*, *top2-4* Exo70* and Exo70* cells were arrested in G1 and then released at 37º. We observed that *top2-4* Exo70* cells resolve plasma membrane as the WT (Figure 4.10). These results suggest that exocyst, and maybe exocytosis, is downregulated in NoCut.

Interestingly the Exo70p subunit of the exocyst complex resides at the PM and it is thought to act as landmark for the other exocyst complex subunits that are attached to the secretory vesicles (Boyd et al., 2004). Provided that Boi2 and Exo70 are both membrane proteins and that Boi2 is involved in secretion in interphase, we can hypothesize that Boi2 may exert its function of abscission inhibitor in the NoCut pathway by negatively regulating secretion, in a pathway that involves the PM exocyst components (Exo70 and Sec3).

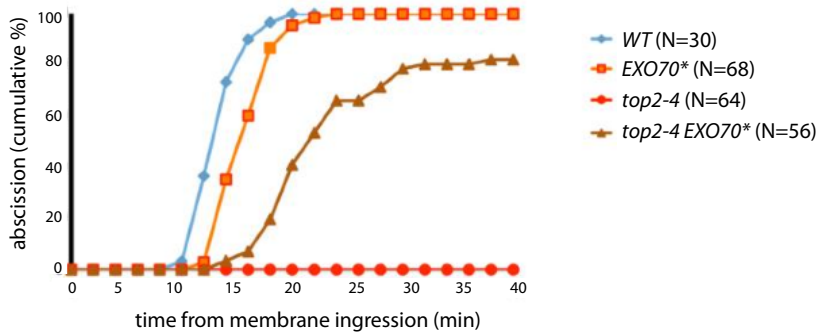


Figure 4.10. NoCut assay in cells bearing the EXO70*. WT and EXO70* cells resolve membranes in cytokinesis with the same dinamycs, after 15-20 minutes from membrane ingression. Cells with catenated chromosomes (*top2-4*) fail to divide unless they bear the EXO70* mutation (*top2-4 EXO70**) that partially revert the PM abscission delay.

A possible way to downregulate the exocyst complex could by promoting its disassembly. Recently it was shown that the phosphorylation of the exocyst subunit Exo84p by Cdk1 causes a partial disassembly of the complex that reflects a partial block in exocytosis (Luo et al., 2013). To test whether the exocyst complex was disassembled when chromosome segregation problems arise, I generated mutants where the EXO84 gene was deleted and replaced either by a phospho-mimic (5E) or a phospho-defective (5A) allele.

Asynchronous WT and *top2-4* cells bearing either the WT version of Exo84 (Exo84 WT) or the phospho-defective (Exo84-5A), or the phospho-mimick (Exo84-5E) allele, were incubated for 2 hours at 37° to

allow the formation of chromatin bridges in the strains with mutation in the Topisomerase II. During cytokinesis, membranes at the neck start furrowing, then contract and eventually resolve. In the presence of chromatin at the neck, membranes furrow, but they don't resolve, maintaining a contracted state (Norden et al., 2006). After two hours, cells were checked at the microscope and divided in three categories based on the plasma membrane state at the neck (open, contracted and resolved). As displayed in Figure 4.11, a high percentage of WT cells complete membrane resolution regardless the EXO84 allele they carry. The number of cells with resolved membrane is very similar among the three different WT strains carrying the three EXO84 alleles, suggesting that a constantly assembled or partially disassembled exocyst complex does not affect abscission timing. Differently, cells with inactive Topoisomerase II show differences in terms of abscission according to the different EXO84 allele they bear. Indeed, a 30% of *top2-4* EXO84 WT cells show contracted membranes at the site of division, due to the function of the NoCut pathway that delays abscission. Interestingly, this phenotype is reverted in cells with a tight assembled exocyst complex (*top2-4* Exo84-5A). Due to the higher numbers of resolved membranes compared to the WT strains with the different EXO84 alleles, we can suppose that a hyperactivation of the exocyst might accelerate the abscission process. A milder rescue of the abscission defect of *top2-4* EXO84 WT is observed also in cells where the exocyst complex is partial disassembly (Exo84-5E). This result might indicate that either the complex is still assembled in a way it does not perfectly mimic what happens in NoCut, or that the Exo84-5E is not

working correctly as a phosphomimic and thus is not promoting the disassembly of the complex.

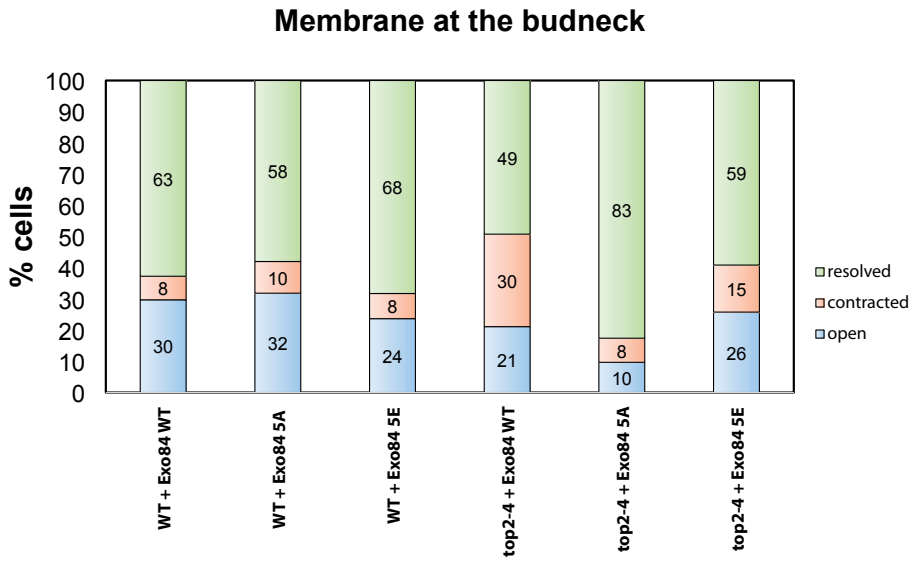


Figure 4.11. Quantification of cells with open, contracted and resolved membranes in WT and *top2-4* cells with the three variants of the exocyst subunit Exo84 (Exo84-WT, Exo84-5A and Exo84-5E). The graph shows that the number of cells that complete abscission (resolved membranes) is high in all the WT cells with the different EXO84 alleles. This number is lowered in the *top2-4* Exo84-WT strain, where NoCut is active and abscission is delayed. When the exocyst is constantly assembled, Nocut cannot delay abscission and the number of cells with resolved membranes at the neck strongly increases (*top2-4* Exo84-5A). The number of cells with contracted membranes decreases also in the *top2-4* Exo84-5E strain, where the exocyst is partially disassembled.

4.7. Boi1 or Boi2 interaction with Exo70 is not detectable *in vivo*

The Exo70* mutation rescues the growth and secretory defect in Boi1/2 depleted cells. Additionally, Boi2 is required for inhibit abscission in NoCut and Exo70* and Exo84-5A can revert this delay, suggesting a possible downregulation of the exocyst in NoCut. Given that Boi1 and Boi2 are known to interact with the exocyst complex *in vitro* (Tonikian et al., 2009) I aimed to study if Boi proteins interact with the exocyst *in vivo*.

Since Boi1 and Boi2 are membrane proteins, I firstly focused on checking their interaction with the exocyst subunit Exo70, which is a membrane protein too (Boyd et al., 2004). For this purpose I performed immunoprecipitation experiments in WT cells, pulling down the exocyst subunit Exo70 (tagged with 6-HA) to check if Boi2 (tagged with 9myc) co-precipates (Figure 4.12) and vice versa (data no shown). The same experiment has been performed to check interaction between Boi1 and Exo70 (data not shown). In none of the cases I could detect any interaction. Out of this result, we can assume that either Exo70 does not interact *in vivo* with Boi2, or that this interaction exists but is either weak or transient.

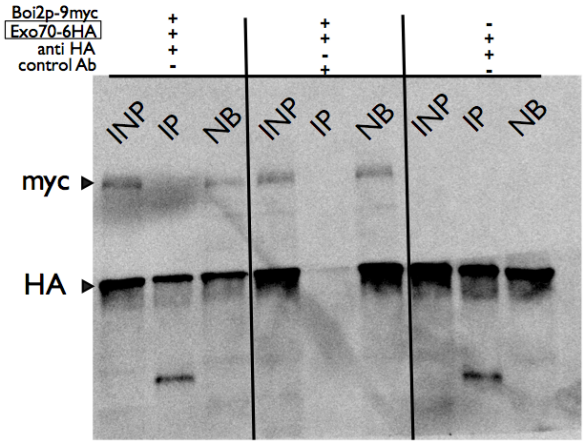


Figure 4.12. **Immunoprecipitation of Exo70-HA**. Protein extraction was made in cells in which Exo70 and Boi2 were tagged with 6-HA and 9-myc, respectively and in cells where only Exo70 was tagged with 6HA. Each of the three columns of the three lanes corresponds to a different solution: the input (INP) is the non-processed cell whole extract; part of the cell whole extract is then incubated with the complex beads-antibodies for the immunoprecipitation assay. After the immunoprecipitation, the beads are separated from the solution and resuspended in sample buffer (IP). The solution that has been separated from the beads is the non-bound fraction (NB). The first lane shows the blotting of Exo70-6HA and Boi2-9myc in the strain expressing both Exo70-6HA and Boi2-9myc. The immunoprecipitation was performed by using an anti-HA antibody. In the IP column Boi2 is not detectable. The second lane shows the blotting of the same proteins in the same strain. In this case, immunoprecipitation was performed by using an anti-IgG antibody to test the specificity of the anti-HA antibody. In the IP column no Exo70-6HA was detected. The third lane shows the blotting of the same protein in a strain that expresses only Exo70-HA. This to test the anti-myc antibody specificity. Boi2-myc, in fact, was not detected in none of the columns.

4.8. Exocyst is required for Boi2 localization at the bud-neck

The observation that the SH3 domain is sufficient for activating NoCut, also opened the question of how Boi2 is transported at the site of division. I then checked if exocyst is required for Boi2 localization at the bud-neck. Cells bearing mutation in either the SEC6 gene (*sec6-4*) or the SEC8 gene (*sec8-6*) (TerBush et al., 1995) were incubated at 37° for two hours and checked for Boi2-GFP localization. Cells were counted as small-medium and large budded cells (S/M, L) and then checked for Boi2-GFP localization at the cortex and the neck, respectively. As shown in Figure 4.13, cells did not show any mis-localization of Boi2-GFP at 25°, but after the switch to non-permissive temperatures, Boi2 fails to properly localize both at the cortex in S/M budded cells and the bud-neck in L budded cells already after 1 hour from the temperature switch in both *sec6-4* and *sec8-6* mutants. These results indicate that the exocyst complex is either involved in the transport of Boi2 to the cortex and the neck or that it stabilizes its localization at the PM.

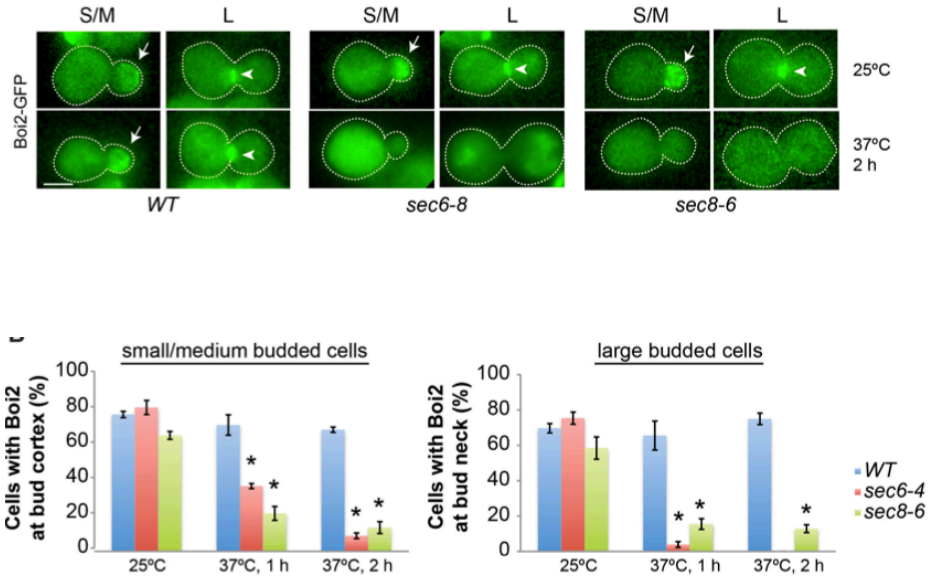
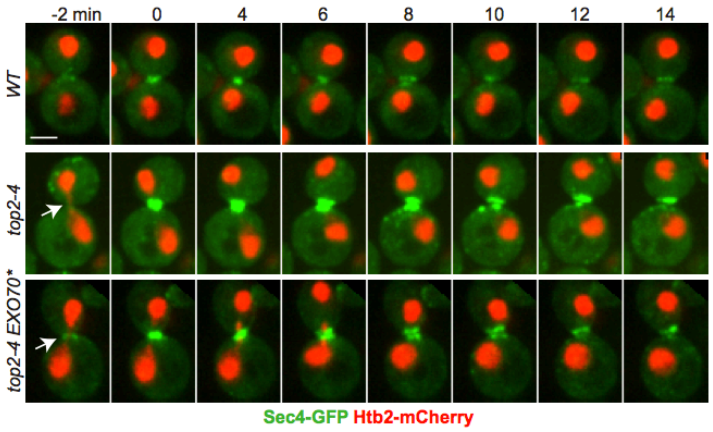


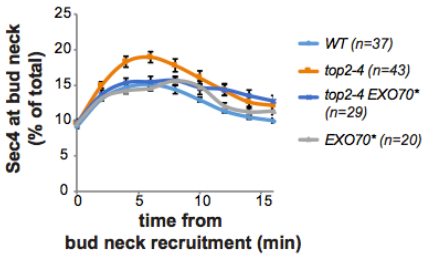
Figure 4.13. Boi2-GFP localization in WT and exocyst mutants. The panel A shows Boi2-GFP localization in the cortex and the neck in WT, *sec6-4* and *sec8-6* cells at 25º and 37º. Panel B displays the percentage of cells with Boi2-GFP at the cortex and the neck in the aforementioned strains incubated at 25º or at 37º for 1 and 2 hours. After 1h from switch to non-permissime temperature, Boi2-GFP fails to localize at the cortex or the neck of exocyst mutant strains.

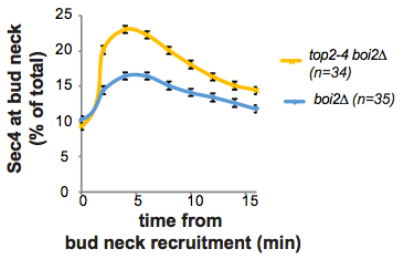
4.9. Sec4 Rab-GTPase accumulates in cells with chromatin bridges

To understand if exocytotic vesicles were accumulating at the neck of cells with cateneted DNA, we checked the localization and the dynamics of the vesicle marker Sec4 in cytokinesis. Sec4 is a RabGTPase that links the secretory vesicles with the exocyst complex (Guo et al., 1999; Lepore et al., 2016). After incubation at 37° for 1 hour, WT, *top2-4*, *top2-4* Exo70*, Exo70*, *top2-4* ΔBoi2 and ΔBoi2 cells expressing Sec4-GFP were exposed to 3 hours time lapse microscopy with a time resolution of 2 minutes. I then measured the Sec4-GFP intensity at the bud-neck relative to the total Sec4-GFP in the cell. The bud-neck intensity has been measured every 2 minutes from the first appearance of Sec4-GFP at the bud-neck (Figure 2.13). The bud-neck Sec4-GFP intensity is higher in *top2-4* cells compared to WT especially 6 minutes after the first appearance of Sec4-GFP at the neck. This high bud-neck intensity in *top2-4* cells is lowered by the presence of the Exo70* (Figure 2.13). This result indicates that when NoCut is active, Sec4-GFP accumulates more in the site of division. Furthermore, these levels are significantly reduced in *top2-4* Exo70*, suggesting that the exocyst regulates Sec4-GFP dynamics at the neck. Surprisingly the high Sec4-GFP levels at the neck of *top2-4* cells are even higher if Boi2 is absent (*top2-4* Δboi2).

In conclusion, Sec4-GFP and maybe exocytic vesicles are accumulating in the neck of NoCut activated cells and therefore the NoCut-dependent abscission inhibition may be due to a lack of vesicle fusion at the site of division.


-2 min
0
4
6
8
10
12
14
WT
top2-4
top2-4 EXO70*
Sec4-GFP Htb2-mCherry


Sec4 at bud neck (% of total)
time from bud neck recruitment (min)
WT (n=37)
top2-4 (n=43)
top2-4 EXO70* (n=29)
EXO70* (n=20)


Sec4 at bud neck (% of total)
time from bud neck recruitment (min)
top2-4 boi2Δ (n=34)
boi2Δ (n=35)

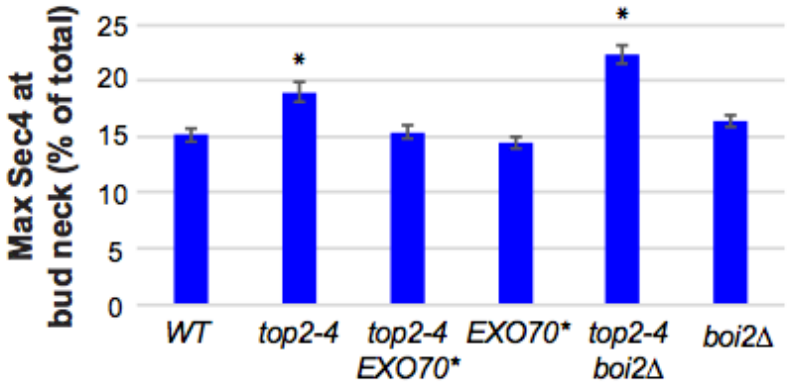

Max Sec4 at bud neck (% of total)
WT
top2-4
top2-4 EXO70*
EXO70*
top2-4 boi2Δ
boi2Δ

Figure 4.14. Sec4-GFP localization during **cytokinesis**. Sec4-GFP intensity was observed in the following strains: WT, *top2-4*, *top2-4* Exo70*, Exo70*, *top2-4* ΔBoi2 and ΔBoi2 . The cell cycle progression was tracked by looking at nucleus dynamics (Htb-mcherry). The top panel shows that Sec4-GFP signal in WT, *top2-4*, *top2-4* Exo70*. The graph below shows the Sec4-GFP bud-neck intensity pattern relative to the total Sec4-GFP in the cell. And the last graph shows the Sec4-GFP intensity at the neck relative to the total at the time point where the bud-neck Sec4-GFP signal intensity is visibly the highest.

5. Discussion

5.1. Role of Boi1 and Boi2 in exocytosis during polarized growth

In my thesis I demonstrated that Boi1 and Boi2 are essential for bud growth during early stages of the cell cycle where they positively regulate exocytosis of Bgl2 secretory vesicles. Indeed, depletion of both Boi1 and Boi2 causes a block in growth and leads to cell death, and this phenotype is restored by the insertion into the genome of a gain of function mutation of the exocyst complex (EXO70-G388R) (Wu et al., 2010). This is also valid for Electron Microscopy results that Aina Masgrau observed and described in her thesis (Aina Masgrau thesis, 2014) where secretory vesicles accumulated in in the bud of Boi1 and Boi2 depleted cells but were absent in Δ*boi1 Boi2-aid Exo70** cells.

The Exo70* gain of function mutation was also found to rescue exocytic defects in *cdc42* temperature sensitive mutants, indicating that Exo70 is a direct effector of Cdc42, and that Cdc42 might control exocytosis through the regulation of the exocyst assembly (Hu et al., 2010). So we hypothesize that Boi1 and Boi2 might function in the regulation of the exocytosis through the activation of the Cdc42-pathway (Bi et al., 2012). In confirmation of that a bem2 temperature sensitive mutation, which

causes a hyperactivation of Cdc42 (Arkins et al., 2013), rescues the Boi1/2 depletion lethality (Masgrau, Battola et al., 2017).

But how do Boi1 and Boi2 activate exocytosis? They might regulate exocyst assembly by interacting with proteins known to interact with the exocyst complex such as Cdc42, Cdc24 and Bem1 (McCusker et al., 2007).

A recently work (Kustermann et al., 2017) shows that Boi1 and Boi2 are involved in exocytosis, since, as we also observed, their loss leads to an accumulation of secretory vesicles at the bud tip. The defect in Boi1/2 depleted cells is rescued by the overexpression of both Sec1p, which is required for SNARE assembly, and Sso1p (a t-SNARE). Interestingly, the interactions between Boi proteins and the exocyst complex could have been detected in this paper. Boi1/2p interact directly with Sec3p which in turn interact with Sso1 (Yue et al., 2017). Sec3 and Sso1 bind with their PH domains phospholipids and active Cdc42p (Yamashita et al., 2010). In conclusion our paper (Masgrau, Battola et al., 2017) complements with the Kustermann paper, providing a model for the Boi1-Boi2 dependent exocytosis that includes their interaction with the exocyst complex, Rho-GTPase and SNAREs. This elaborated complex might be regulating exocytosis by controlling SNARE assembly.

As I already discussed, Boi1 and Boi2 regulate Cdc42 function in the regulation of polarized growth. Kustermann and colleagues were able to

better characterize this interaction, proving that Boi1 and Boi2 binding to Cdc42 through their PH domain, is not required for their function in exocytosis.

The exocyst complex assembly is cell cycle regulated (Luo et al., 2013). It will be interesting to understand if the absence of Boi1 and Boi2 can affect the assembly of the exocyst complex both in interphase and cytokinesis. As future plans it would be useful to test this assembly through immunoprecipitation of the exocyst complex subunits with or without Boi1 and Boi2 and with hyperactivated Cdc42 (*bem2-84*). Hypothetically, Boi1 and Boi2 positively regulate exocyst assembly and their depletion leads to a disassembly that block exocytosis and therefore cell growth, and then a hyperactivation of Cdc42 may revert this phenotype. In line with that hypothesis, Boi1 and Boi2 interact with many exocyst components in vitro and by two-hybrid assays (Tonikian et al., 2009) and Boi2 localization at the cortex in interphase and at the neck in cytokinesis depends on exocyst function (Masgrau, Battola et al., 2017).

5.2. The role of Boi2 in NoCut

The presence of chromatin bridges spanning the site of division during cytokinesis, activates the NoCut pathway in both yeast and human cells

(Norden et al., 2006; Steigemann et al., 2009). NoCut blocks abscission, the last step of cytokinesis, through regulation of the ESCRTIII complex and Boi2 in human cells and budding yeast, respectively (Carlton et al., 2012; Masgrau, Battola et al., 2017). My data demonstrated that in *Saccharomyces cerevisiae,* the SH3 domain itself of Boi2 is required and sufficient for the abscission delay in NoCut. This domain is responsible for Boi2 localization at the bud-neck during cytokinesis, indicating that the presence of Boi2 in the neck is required to inhibit abscission. As discussed before, Boi2, along with Boi1, is also responsible of controlling exocytosis during interphase (Kustermann et al., 2017; Hallett et al., 2002). This function relies on its PH domain, suggesting that Boi2 may act in NoCut through the regulation of exocytosis (but in a different way from interphase) or other mechanisms. In line with this last hypothesis, electron microscopy analysis revealed the absence of secretory vesicles in the neck of cells with chromatin bridges. However, we cannot completely rely on these results given that secretory vesicles are present for a very short time at the bud-neck during cytokinesis (see Sec-4 GFP-results). In agreement with our idea of Boi2 as regulator of exocytosis in NoCut, the SH3 domains of Boi1 and Boi2 can interact with exocyst components (Tonikian et al., 2009), and the exocyst is required for Boi2 recruitment to the bud neck (Masgrau, Battola et al., 2017).

It has been also shown that exocyst assembly could be regulated during the cell cycle (Luo et al., 2013) and we show that a constitutive assembly of the exocyst complex (Figure 4.11) generated by the phospho-defective

Exo84p mutant permits membrane resolution in cells with chromatin bridges during cytokinesis. In a hypothetical *scenario* Boi2 may act as regulator of exocytosis in NoCut through the control of the exocyst assembly. Specifically, Boi2 might act by disrupting interaction of the exocyst subunits in presence of chromosome segregation defects. This disassembly would then stop exocytosis causing an abscission delay. Exocytosis could promote abscission by either delivering important cytokinetic activators at the site of division or by adding membranes coming from the vesicles to the PM. Indeed, inactivation of some exocyst subunits blocks cytokinesis in budding yeast (Zhang et al., 2008; Dobbelaere et al., 2004) and in animal cells (Gromley et al., 2005).

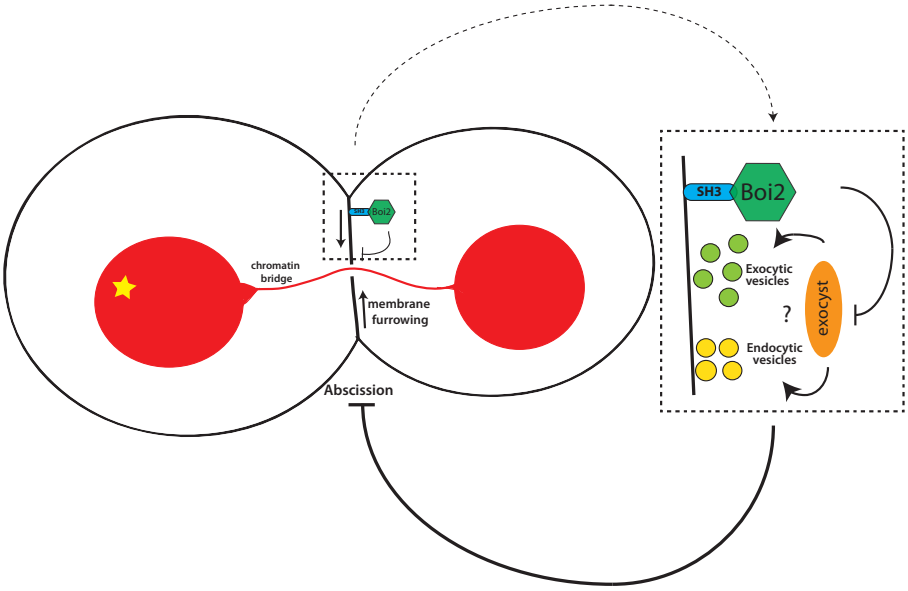
Consistenly, Rho-like GTPases mediate exocyst activation through the induction of conformational changes in Exo70 (Wu et al., 2008). This allosteric regulation model was further supported by the identification of point mutations in EXO70, including EXO70*, that support growth of cdc42 and rho3 mutants defective in exocytosis (Wu et al., 2010). Our finding that EXO70* mutants are defective in the NoCut checkpoint suggests that allosteric regulation of the exocyst may play a key role in the regulation of abscission timing in yeast, and perhaps also in human cells. Exo70* complexes might be refractory to modulation by Boi1/2, thereby enabling multiple exocytic pathways independently of Boi1/2 or topoisomerase II defects, and explaining proper growth of boi1Δ Boi2-aid EXO70* and normal cytokinesis in top2-ts EXO70* mutants.

Another possible way of how Boi2 can inhibit abscission is through the regulation of the endocytosis pathway. In fact, studies of the interactome of the Boi2-SH3 domain (Tonikian et al., 2009), revealed the presence of many proteins that are involved in the endocytosis, such as Sla1 and Pan1. SLA1 gene is not essential and codes for Sla1p that is required for assembly of the cortical actin cytoskeleton and interacts with proteins regulating actin dynamics in endocytosis (Holtzman et al., 1993; Warren et al., 2002). Deletion of the gene in cells with chromatin bridges would help us in understanding if this protein is involved in NoCut. Pan1p instead is an essential protein and is part of the actin cytoskeleton-regulatory complex Pan1p-Sla1p-End3p and promotes protein-protein interactions essential for endocytosis (Zeng et al., 2001; Duncan et al., 2001). To study if Boi2 interaction with Pan1 is required for NoCut, I aimed to modify the Prolin-rich domain of Pan1 in such a way its interaction with Boi2 is impeded. This mutation will be made through CRISPR strategies (DiCarlo et al., 2013) In line with the hypothesis that endocytosis is downregulated in NoCut, studies demonstrated that endocytosis is essential for a proper cytokinesis in HeLa cells (Schweitzer et al., 2005). In this study, they showed that endocytosis occurred at the ingressed cleavage furrow, and this allows termination of cytokinesis through either internalizing cytokinesis components that are no longer needed, or helping to physically seal off daughter cell membranes, facilitating the final completion of cytokinesis.

5.4. Sec4 accumulates in the neck of NoCut cells

Sec4 is a Rab-GTPase involved in the transport of secretory vesicles coming from the Golgi to the PM (Salminen et al., 1987; Novick et al., 1993). Our findings of Sec4-GFP accumulation in the neck of cells with chromatin bridges during cytokinesis, can suggest that in NoCut exocytosis is blocked. However, no secretory vesicles were found in the neck of NoCut activated cells through EM. The intense Sec4-GFP signal in *top2-4* cells is lowered by the presence of the Exo70* and increased in absence of Boi2. Sec4-GFP signal has used to track single secretory vesicles in budding yeast (Donovan et al., 2015). In this study they showed that single Sec4-GFP signal (corresponding to a single vesicle), disappears once arrived at the PM of a growing bud, and this marks the fusion of the secretory vesicle to the PM. My next experiments will be focused on checking the single vesicle through Sec4-GFP and verifying its disappearance in the neck of cells with or without chromatin bridges. This experiment will allow us to understand if the abscission delay in NoCut is due to a block in exocytosis. Absence of Boi2 in cells with or without chromosome bridges increases the levels of Sec4-GFP in the neck, suggesting that Boi2 is involved in its turnover in the neck. All these data suggest that Sec4 accumulation might mirror an accumulation of secretory vesicles at the neck in NoCut.

Sec4 is also involved in endocytosis: mutations in Sec4 disrupt actin patches and inhibited endocytosis (Johansen et al., 2016). Another scenario would be that Sec4 arrives at the neck and promotes exocytosis in NoCut. The accumulation of Sec4-GFP in NoCut could be then due to the failing of Sec4-GFP to bind the endocytotic vesicles since endocytosis is blocked.



4.15. The model. Problems in DNA catenation, replication or condensation (yellow star) lead to the formation of chromatin bridges. Boi2, after membrane furrowing, delays abscission through its SH3 domain. Boi2 might exert its function by either inhibiting exocytosis or endocytosis and through the control of the exocyst assembly.

6. Conclusions

- Boi1 and Boi2 regulate polarized growth in interphase through the control of the Bgl2 vesicles secretion

- Boi2 is essential for NoCut: its SH3 domain is responsible for the delay in abscission in cells with chromatin bridges

- The exocyst is downregulated in NoCut

Bibliography

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C., Mirabelle, S., Guha M., Sillibourne J,. "Centriolin Anchoring of Exocyst and SNARE Complexes at the Midbody Is Required for Secretory-Vesicle-Mediated Abscission." Cell 123 (1)75–87 (2005).

Adamo, JE., Moskow, JJ., Gladfelter ,AS., Viterbo, D., Lew, DJ., Brennwald, PJ. "Yeast Cdc42 functions at a late step in exocytosis, specifically during polarized growth of the emerging bud." J Cell Biol 155(4) 581-92 (2001).

Curwin, AJ., Fairn, G., McMaster, C. " Phospholipid transfer protein Sec14 Is required for trafficking from endosomes and regulates distinct trans-Golgi export pathways ." Journal of Biological Chemistry 284 7364–7375 (2009).

Alberghina, L., Rossi, RL, Querin, L., Wanke, V., Vanoni, M. "A cell sizer network involving Cln3 and Far1 controls entrance into S phase in the mitotic cycle of budding yeast." J Cell Biol 167.433–443 (2004).

Amon, A., Irniger, S., Nasmyth, K. "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 77.1037–1050 (1994).

Ang, AL., Taguch,i T., Francis, S., Folsch, H., Murrells, LJ., Pypaert, M. "Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells." J Cell Biol 167.531–543 (2004).

Bretscher, A., Harsay, E. "Parallel Secretory Pathways to the Cell Surface in Yeast." The Journal of Cell Biology 131.2.297-310 (1995).

Seshan, A., Amon, A. "Linked for life: temporal and spatial coordination of late mitotic events." Current Opinion in Cell Biology 16.41-48 (2004).

Archambault, V., Buchler, N., Wilmes, GM., Jacobson, MD., Cross, FR. "Two-faced cyclins with eyes on the targets." Cell Cycle 4.125-130 (2005).

Atkins, BD., Yoshida, S., Saito, K., Wu, C-F., Lew, DJ., Pellman, D. "Inhibition of Cdc42 during mitotic exit is required for cytokinesis." The Journal of Cell Biology 202.2.231-240 (2013).

Azvolinsky, A., Dunaway, S., Torres, JZ., Bessler, JB., Zakian, VA. "The S. cerevisiae Rrm3p DNA helicase moves with the replication fork and affects replication of all yeast chromosomes." Genes Dev. 20(22).3104-16 (2006).

Baladron, V., Ufano, S., Duenas, E., Martin-Cuadrado,A.B., del Rey, F., Vazquez de Aldana C.R. "Eng1p, an endo-1,3-beta-glucanase localized at the daughter side of the septum, is involved in cell separation in Saccharomyces cerevisiae." Eukaryot. Cell 1.774–786 (2002).

Bartek, J., Bartkova, J., and Lukas, J. "DNA damage signalling guards against activated oncogenes and tumour progression." Oncogene 26.7773–7779 (2007).

Baxter, J. "Breaking up is hard to do" The formation and resolution of sister chromatid intertwines." J Mol Biol. 427(3).590–607 (2015).

Bermejo, R., and Rodrigo. "The DNA damage checkpoint response to replication stress: A Game of Forks ." Front. Genet (2013).

Bertoli, C., Skotheim, M and de Bruin, R. "Control of cell cycle transcription during G1 and S phases." Nat Rev Mol Cell Biol. 14(8).518–528 (2013).

Bhavsar-Jog Yogini, P., Bi, E. "Mechanics and regulation of cytokinesis in budding yeast." Seminars in Cell and Developmental Biology 16.30499-2 (2016).

Bi, E., Park, HO. "Cell polarization and cytokinesis in budding yeast." Genetics 191.347-87 (2012).

Bing, H., Fengong, X., Zhang, X., Zhang, J., and Wei Guo. "Exo70 interacts with phospholipids and mediates the targeting of the exocyst to the plasma membrane." EMBO J 26(18).4053–4065 (2007).

Bloom J., Cross, FR. "Multiple levels of cyclin specificity in cell-cycle control." Nat Rev Mol Cell Biol 8.149–160 (2007).

Boyd, C., Hughes T., Pypaert M., Novick P. "Vesicles carry most exocyst subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p." J Cell Biol. 167(5).889-901 (2004).

Branze,i D., Foiani, M. "Maintaining genome stability at the replication fork." Nat Rev Mol Cell Biol. 11(3).208-19 (2010).

Alberts, B., Johnson, A., Lewis J., Raff, M., Roberts, R., Walter, P. "Molecular Biology of the Cell, 5th edition." (2007).

Goode, B., Eskin, J. and Wendland, B. "Actin and Endocytosis in Budding Yeast." Genetics 199.315–358 (2015).

Norden, C., Mendoza, M., Dobbelaere, J., Kotwaliwale, C., Biggins, S., Barral, Y. "The NoCut Pathway Links Completion of Cytokinesis to Spindle Midzone Function to Prevent Chromosome Breakage." Cell 125.1.85-98 (2006).

Caviston, JP., Longtine, M., Pringle, JR., Bi, E. "The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast." Mol Biol Cell 14.4051-66 (2003).

Chan, K.L., North, P.S., Hickson, I.D. "BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges." EMBO J. 26.3397–3409 (2007).

Chan, K.L., Palmai-Pallag, T., Ying, S., Hickson, I.D. "Replication stress induces sister-chromatid bridging at fragile site loci in mitosis." Nat. Cell Biol. 11.753–760 (2009).

Chin, BL., Ryan, O., Lewitter, F., Boone, C, Fink, GR. "Genetic variation in Saccharomyces cerevisiae: circuit diversification in a signal transduction network." Genetics 192(4).1523-32 (2012).

Chuang, JS., Schekman. RW. "Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p." J Cell Biol. 135(3).597-610 (1996).

Chuang, TH., Xu, X., Knaus, UG., Hart, MJ., Bokoch, GM. "GDP dissociation inhibitor prevents intrinsic and GTPase activating protein-stimulated GTP hydrolysis by the Rac GTP-binding protein." J Biol Chem 268(2).775-8 (1993).

Colman-Lerner, A., Chin, R., and Brent, R. "Yeast Cbk1 and Mob2 activate daughter specific genetic programs to induce asymmetric cell fates." Cell 107.739–750 (2001).

Holm, C., Goto, T., Wang, J. and David Botstein. "DNA topoisomerase II is required at the time of mitosis in yeast." Cell 41.2.553–563 (1985).

TerBush, D, Novick, P. "Sec6, Sec8, and Sec15 are components of a multisubunit complex which localizes to small bud tips in Saccharomyces cerevisiae." The Journal of Cell Biology 130.2.299-312 (1995).

D'Agostino, JL., Goode, BL. "Dissection of Arp2/3 complex actin nucleation mechanism and distinct roles for its nucleation-promoting factors in Saccharomyces cerevisiae." Genetics 171(1).35-47 (2005).

Delic, M., Valli, M., Graf, A. B., Pfeffer, M., Mattanovich, D. and Gasser, B. "The secretory pathway: exploring yeast diversity." FEMS Microbiol Rev 37.872–914 (2013).

McCusker, D., Denison, C., Anderson, S., Egelhofer, T., J Yates, J., Gygi, J. & Kellogg D. "Cdk1 coordinates cell-surface growth with the cell cycle." Nature Cell Biology 9.506-515 (2007).

DiCarlo, J. et al. "Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems." Nucleic Acids Research 41.4336-4343 (2013).

Donaldson, AD., Raghuraman, MK., Friedman, KL., Cross, FR., Brewer, BJ., Fangman, WL. "CLB5-dependent activation of late replication origins in S. cerevisiae." Mol Cell 2.173–182 (1998).

Donovan, KW., Bretscher, A. "Tracking individual secretory vesicles during exocytosis reveals an ordered and regulated process." J Cell Biol 210.2.181-9 (2015).

Drubin, DG., Nelson, WJ. "Origins of cell polarity." Cell 84(3).335-44 (1996).

Duncan, MC., Cope, MJ., Goode, BL., Wendland, B., Drubin, DG. "Yeast Eps15-like endocytic protein, Pan1p, activates the Arp2/3 complex. ." Nat Cell Biol. 3(7).687-90 (2001).

Weiss, E. "Mitotic exit and separation of mother and daughter cells." Genetics 192.165-202 (2012).

Mazanka, E., Alexander, J., Yeh, B., Charoenpong, P., Lowery, D., Yaffe, M., Weiss, E. "The NDR/LATS Family Kinase Cbk1 Directly Controls Transcriptional Asymmetry." PLoS Biol (2008).

Engqvist-Goldstein, AE., Drubin, DG. "Actin assembly and endocytosis: from yeast to mammals." Annu Rev Cell Dev Biol. 19.287-332 (2003).

Grote, E., Carr, C., Novick, P. "Ordering the Final Events in Yeast Exocytosis." The Journal of Cell Biology 151.2.439 (2000).

Evans, T ., Rosenthal, E.T ., Y oungblom, J., Distel, D., and Hunt, T . "yclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division." Cell 33.389–396 (1983).

Eytan, E., Braunstein, I., Ganoth, D., Teichner, A., Hittle, JC., Yen, TJ., Hershko, A. "Two different mitotic checkpoint inhibitors of the anaphase-promoting complex/cyclosome antagonize the action of the activator Cdc20." Proc Natl Acad Sci U S A. 105(27).9181-5 (2008).

Feyder, S., De Craene, J.-O., Bär, S., Bertazzi, D. L., & Friant, S. "Membrane Trafficking in the Yeast Saccharomyces cerevisiae Model." Int. J. Mol. Sci. 16.1509-1525 (2015).

Finger, F.P., T.E. Hughes, and P. Novick. "Sec3p is a spatial landmark for polarized secretion in budding yeast." Cell 92.559–571 (1998).

Finger, P., and Peter Novick. "Spatial Regulation of Exocytosis: Lessons from Yeast ." The Journal of Cell Biology 142.609-612 (1998).

Futcher, B. "Cyclins and the wiring of the yeast cell cycle." Yeast 12.1635–1646 (1996).

Geymonat, M., Spanos, A., Wells, GP., Smerdon, SJ., Sedgwick, SG. "Clb6/Cdc28 and Cdc14 regulate phosphorylation status and cellular localization of Swi6." Mol Cell Biol 24.2277–2285 (2004).

Grosshans, B.L., D. Ortiz, and P. Novick. "Rabs and their effectors: achieving specificity in membrane traffic." Proc Natl Acad Sci U S A 103.32.11821-7 (2006).

Luo, G., Zhang, J., Luca, F., Guo, W. "Mitotic phosphorylation of Exo84 disrupts exocyst assembly and arrests cell growth." The Journal of Cell Biology 202 (1).97 (2013).

Hallett, MA., Lo, HS., Bender, A. "Probing the importance and potential roles of the binding of the PH-domain protein Boi1 to acidic phospholipids. ." BMC Cell Biology 3.16 (2002).

Hartwell, LH., Culotti, J., Reid, B. "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).352-359 (1970).

Hartwell, L. H., Culotti, J., Pringle, J. R., and Reid, B. J. "Genetic control of the cell division cycle in yeast." Science 183.46–51 (1974).

He, B., Xi, F., Zhang, J., TerBush, D., Zhang, X, Guo, W. "Exo70p mediates the secretion of specific exocytic vesicles at early stages of the cell cycle for polarized cell growt." J Cell Biol. 176.771–777 (2007).

Holtzman, DA., Yang, S., Drubin, DG. "Synthetic-lethal interactions identify two novel genes, SLA1 and SLA2, that control membrane cytoskeleton assembly in Saccharomyces cerevisiae." J Cell Biol. 122(3).635-44 (1993).

Hornig, NC., Knowles, PP., McDonald, NQ., Uhlmann F. "The dual mechanism of separase regulation by securin." Curr Biol. 12(12).973-82 (2002).

Jansen, J., Barry, M., Yoo, C. and Eric L. Weiss. "Phosphoregulation of Cbk1 is critical for RAM network control of transcription and morphogenesis ." J Cell Biol. 175(5).755–766 (2006).

Jedd, G., J. Mulholland, and N. Segev. "Two new Ypt GTPases are required for exit from the yeast trans-Golgi compartment." The Journal of Cell Biology 137.3.563-80 (1997).

Carlton, J., Caballe, A., Agromayor, M., Kloc, M., Martin-Serrano.,J "ESCRT-III Governs the Aurora B–Mediated Abscission Checkpoint Through CHMP4C." Science 336.220-225 (2012).

Dobbelaere, Yves Barral. "Spatial Coordination of Cytokinetic Events by Compartmentalization of the Cell Cortex." Science 305.393-396 (2004).

Johansen, J., Alfaro, G., Beh, C."Polarized Exocytosis Induces Compensatory Endocytosis by Sec4p-Regulated Cortical Actin Polymerization." PLoS Biol 14(8) (2016).

Bartkova, J., Hořejší, Z., Koed, K., Krämer, A., X Tort, F., Zieger, Per Guldberg, K., Sehested, M., Nesland, K., Lukas, C., Ørntoft, T., Lukas, J. & Jiri Bartek. "DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis." Nature 434.864-870 (2005).

Kustermann, J., Wu, Y., Rieger, L., Dedden, D., Phan, T., Walther, P., Dünkler, A.and Nils Johnsson. "The cell polarity proteins Boi1p and

Boi2p stimulate vesicle fusion at the plasma membrane of yeast cells." J Cell Sci 9(5).506-15 (2017).

Pringle, J., Hartwell, L. "The Saccharomyces cerevisiae Cell Cycle." The Molecular Biology of the Yeast 97-142 (1980).

Kaksonen, M., Sun., Y, Drubin., DG. "A pathway for association of receptors, adaptors, and actin during endocytic internalization." Cell 115(4).475-87 (2003).

Kaksonen, M., Toret, CP., Drubin, DG. "A modular design for the clathrin- and actin-mediated endocytosis machinery." Cell 123(2).305-20 (2005).

Kaksonen, M., Sun, Y,. Drubin, DG. "A pathway for association of receptors, adaptors, and actin during endocytic internalization." Cell 115(4).475-87 (2003).

Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, H & Kanemaki, M. "An auxin-based degron system for the rapid depletion of proteins in nonplant cells." Nature Methods 6.917-922 (2009).

Kuranda, MJ., Robbins, PW. "Chitinase is required for cell separation during growth of Saccharomyces cerevisiae." J Biol Chem. 266(29).19758-67 (1991).

Bender, L., Lo, H S., Lee, H., Kokojan, V ., Peterson, V ., A Bender. "Associations among PH and SH3 domain-containing proteins and Rho-type GTPases in Yeast." The Journal of Cell Biology 133.4.879-894 (1996).

Lepore, D., Spassibojko, O., Pinto, G., Collins, RN. "Cell cycle–dependent phosphorylation of Sec4p controls membrane deposition during cytokinesis." he Journal of Cell Biology 214.6.691-703 (2016).

Lew, DJ., Reed, SI. "Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins." J Cell Biol 120.1305–1320 (1993).

Lew DJ, Reed SI. "Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins." J Cell Biol 120(6).1305-20 (1993).

Lew, D. J., Weinert, T., and Pringle, J. R. "Cell cycle control in Saccharomyces cerevisiae." Molecular and Cellular Biology of the Yeast Saccharomyces 607–695 (1997). Hartwell, MB Kastan. "Cell cycle control and cancer." Science 266.1821-1828 (1994).

Hartwell, L., Weinert, T. "Checkpoints: controls that ensure the order of cell cycle events." Science 246.629-634 (1989).

Lippincott J, Li R. "Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis." J Cell Biol 140(2).355-66 (1998).

Lippincott, J., Shannon, KB., Shou, W., Deshaies, RJ., Li, R. "The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis." J Cell Sci 114.1379-86 (2001).

Liu, J., Kipreos, ET. "Evolution of cyclin-dependent kinases (CDKs) and CDK-activating kinases (CAKs): differential conservation of CAKs in yeast and metazoa." Mol Biol Evol 17.1061–1074 (2000).

Luo, G., Zhang, J., Luca, FC., Guo W. "Mitotic phosphorylation of Exo84 disrupts exocyst assembly and arrests cell growth." The Journal of Cell Biology 202(1).97-111 (2013).

M. Murthy, D., Garza, R.H., Scheller, T.L. Schwarz. "Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists." Neuron 37.433-447 (2003).

Mendoza, M., Norden, C., Durrer, K., Rauter, H., Uhlmann, F & Barral, Y. "A mechanism for chromosome segregation sensing by the NoCut checkpoint." 11.477-483 (2009).

Masgrau, A., Battola, A., Sanmartin, T., Pryszcz, L., Gabaldón, T. and Mendoza, M. "Distinct roles of the polarity factors Boi1 and Boi2 in the control of exocytosis and abscission in budding yeast." Mol. Biol. Cell (2017).

Mazanka, E., Weiss, EL. "Sequential counteracting kinases restrict an asymmetric gene expression program to early G1." Mol Biol Cell. 21(16).2809-20 (2010).

McMurray, MA., Thorner, J. "Septins: molecular partitioning and the generation of cellular asymmetry." Cell Div 4.18 (2009).

Medkova, M., et al. "The rab exchange factor Sec2p reversibly associates with the exocyst." Mol Biol Cell 17.6.2757-69 (2006).

Mendenhall, MD,. Hodge, AE. "Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast Saccharomyces cerevisiae." Microbiol Mol Biol Rev 62.1191–1243 (1998).

Mizuno-Yamasaki, E., et al. "Phosphatidylinositol 4-phosphate controls both membrane recruitment and a regulatory switch of the Rab GEF Sec2p." Dev Cell 18.5.828-40 (2010).

Morgan, D., "The Cell Cycle." New Science Press (2007).

Moseley, JB., Goode, BL. "The yeast actin cytoskeleton: from cellular function to biochemical mechanism." Microbiol Mol Biol Rev 70.605-45 (2006).

Newpher, TM., Smith, RP., Lemmon, V., Lemmon, SK. "In vivo dynamics of clathrin and its adaptor-dependent recruitment to the actin-based endocytic machinery in yeast." Dev Cell. 9(1).87-98 (2005).

Nigg, EA. "Cellular substrates of p34(cdc2) and its companion cyclin-dependent kinases." Trends Cell Biol 3.296–301 (1993).

Novick, P., Brennwald, P., Walworth, NC., Kabcenell, AK., Garrett, M., Moya, M., Roberts, D., Müller, H., Govindan, B., Bowser, R. "The cycle of SEC4 function in vesicular transport." Ciba Found Symp. 176.218-28 (1993).

Novick, P., Field, C., Schekman, R. "Identification of 23 Complementation Groups Required for Post- translational Events in the Yeast Secretory Pathway." Cell 21.205-215 (1980.).

Novick, Peter. "Regulation of Membrane Traffic by Rab GEF and GAP Cascades." Small GTPases 4.252-256 (2016).

Amaral, N., Vendrell, A., Funaya, C., Idrissi, FZ., Maier, M., Kumar, A., Neurohr, G., Colomina, N., Torres-Rosell, J., Geli, MI., Mendoza, M "The Aurora-B-dependent NoCut checkpoint prevents damage of anaphase bridges after DNA replication stress." Nature Cell Biology 18.516–526 (2016).

O'Conallain, C., Doolin, MT., Taggart, C., Thornton, F., Butler, G. "Regulated nuclear localisation of the yeast transcription factor Ace2p controls expression of chitinase (CTS1) in Saccharomyces cerevisiae." Mol Gen Genet. 262(2).275-82 (1999).

Orlando, K., & Guo, W. "Membrane Organization and Dynamics in Cell Polarity." Cold Spring Harbor Perspectives in Biology 1.5 (2009).

Ortiz, D., et al. "Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles; evidence for a Rab cascade in yeast." The Journal of Cell Biology 157.6.1005-15 (2002).

Osborn, AJ., Elledge, SJ., Zou, L. "Checking on the fork: the DNA-replication stress-response pathway." Trends Cell Biol. 12(11).509-16 (2002).

Ozaki-Kuroda, K., Yamamoto, Y., Nohara, H., Kinoshita, M., Fujiwara, T., Irie, K., Takai, Y. "Dynamic localization and function of Bni1p at the sites of directed growth in Saccharomyces cerevisiae." Mol Cell Biol 21(3).827-39 (2001).

Oztan, A., Silvis, M., Weisz, OA., Bradbury, NA., Hsu, SC., Goldenring, JR. "Exocyst requirement for endocytic traffic directed toward the apical and basolateral poles of polarized MDCK cells." Mol Biol Cell 18.3978–3992 (2007).

Park, HO., Bi, E. "Central roles of small GTPases in the development of cell polarity in yeast and beyond." Microbiol Mol Biol Rev 71(1).48-96 (2007).

Park, H., Bi, E and Hay-Oak. "Cell Polarization and Cytokinesis in Budding Yeast." Genetics 191.2.347–387 (2012).

Pruyne, D., Bretscher, A. "Polarization of cell growth in yeast. I. Establishment and maintenance of polarity states." J Cell Sci. 113.365-75 (2000).

Pruyne, DW., Schott, DH., Bretscher, A. "Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast." J Cell Biol 143(7).1931-45 (1998).

Richardson, H,. Lew, DJ., Henze, M., Sugimoto, K., Reed. SI. "Cyclin-B homologs in Saccharomyces cerevisiae function in S phase and in G2." Genes Dev 6.2021–2034 (1992).

Richardson, HE., Wittenberg, C., Cross, F., Reed, SI. "An essential G1 function for cyclin-like proteins in yeast." Cell 59.1127–1133 (1989).

Chuang, JS., and Schekman, R. "Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p." J Cell Biol 135.597-610 (1996).

Salminen, A., Novick, PJ. "A ras-like protein is required for a post-Golgi event in yeast secretion." Cell 49.4.527-38 (1987).

Schwartz, S., Cao, C., Pylypenko, O., Rak, A., Wandinger-Ness. A "Rab GTPases at a glance." Journal of Cell Science 120.3905-3910 (2007).

Sburlati, A., Cabib, E. "Chitin synthetase 2, a presumptive participant in septum formation in Saccharomyces cerevisiae." J Biol Chem. 261(32).15147-52 (1986).

Schwab, M., Neutzner, M., Möcker, D., Seufert, W. "Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC." EMBO J. 20(18).5165-75 (2001).

Schweitzer, JK., Burke, EE., Goodson, HV., D'Souza-Schorey, C. "Endocytosis resumes during late mitosis and is required for cytokinesis." J Biol Chem. 280(50).41628-35 (2005).

Schwob, E., Bohm, T., Mendenhall, MD., Nasmyth, K. "The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in S. cerevisiae." Cell 79.233–244 (1994).

Seufert, W., Futcher, B., Jentsch, S. "Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins." Nature 373.78–81 (1995).

Stalder, D. and P.J. Novick. "The casein kinases Yck1p and Yck2p act in the secretory pathway, in part, by regulating the Rab exchange factor Sec2p." Molecular Biology of the Cell (2015).

Steigemann, P., Gerlich, DW. "Cytokinetic abscission: cellular dynamics at the midbody." Trends Cell Biol. 19(11).606-16 (2009).

Tcheperegine, SE., Gao, XD., Bi, E. "Regulation of cell polarity by interactions of Msb3 and Msb4 with Cdc42 and polarisome components." Mol Cell Biol. 25(19).8567-80 (2005).

Te, EM., Chai. CC., Yeong, FM. "Retention of Chs2p in the ER requires N-terminal CDK1-phosphorylation sites. ." Cell Cycle 8(18).2964-74 (2009).

TerBush, D.R., and P. Novick. "Sec6, Sec8, and Sec15 are components of a multisubunit complex which localizes to small bud tips in Saccharomyces cerevisiae." J. Cell Biol. 130.299–312 (1995).

TerBush, D.R., et al. "The exocyst is a multiprotein complex required for exocytosis in saccharomyces cerevisiae." EMBO J 15.6483–6494 (1996).

Tonikian, R., Xin, X., Toret, CP., Gfeller, D., Landgraf, C., Panni, S., Paoluzi, S., Castagnoli, L., Currell, B., Seshagiri, S., Yu, H., Winsor, B., Vidal M, Gerstein MB, Bader GD, Volkmer R, Cesareni G, Drubin DG, Kim, PM., Sidhu, SS., Boone, C. "Bayesian modeling of the yeast SH3 domain interactome predicts spatiotemporal dynamics of endocytosis proteins." PLoS Biol (2009).

Tyers, M., Tokiwa., G., Futcher, B. "Comparison of the Saccharomyces cerevisiae G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins." EMBO J 12.1955–1968 (1993).

Uhlmann, F., Lottspeich, F., Nasmyth, K. "Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1." 400(6739).37-42 (1999).

VerPlank, L., Li, R. "Cell cycle-regulated trafficking of Chs2 controls actomyosin ring stability during cytokinesis." Mol Biol Cell 16.2529-43 (2005).

Visintin, R., Craig, K., Hwang, ES., Prinz, S., Tyers, M., Amon, A. "The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation." Mol Cell. 2(6).709-18 (1998).

Walther, TC., Brickner, JH., Aguilar, PS., Bernales, S., Pantoj, C., Walter, P. "Eisosomes mark static sites of endocytosis." Nature 439 (7079).998-1003 (2006).

Warren, DT., Andrews, PD., Gourlay, CW., Ayscough, KR. "Sla1p couples the yeast endocytic machinery to proteins regulating actin dynamics." J Cell Sci. 115(Pt 8).1703-15 (2002).

Wei Guo, Dagmar Roth, Christiane Walch-Solimena, Peter Novick. "The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis." The EMBO Journal 18.1071-1080 (1999).

Guo, W., Roth, D., Walch-Solimena C, Novick P. —. "The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis." The EMBO Journal 18 (1999): 1071-1080.

Witte, H., Bradke, F. "The role of the cytoskeleton during neuronal polarization." Curr Opin Neurobiol. 18(5).479-87 (2008).

Wloka, C., Bi, E. "Mechanisms of cytokinesis in budding yeast." Cytoskeleton 69.710-26 (2012).

Wu, H., Turner, C., Gardner, J., Temple, B., Brennwald, P. "The Exo70 Subunit of the Exocyst Is an Effector for Both Cdc42 and Rho3 Function in Polarized Exocytosis." Molecular Biology of the Cell 21.3.430-442 (2010).

Zhang, X., Orlando, K., He, B., Xi, F., Zhang, J., Zajac, A., Guo, W. "Membrane association and functional regulation of Sec3 by phospholipids and Cdc42." The Journal of Cell Biology 180 (1).145 (2008).

Matsui, Y ., Matsui, R ., Akada, R ., Toh-e, A. "Yeast src homology region 3 domain-binding proteins involved in bud formation." The Journal of Cell Biology May 133.4.865-878 (1996).

Yamamoto, A., Guacci, V., Koshland, D. "Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae." J Cell Biol. 133(1).85-97 (1996).

Yanagida, M. "Clearing the way for mitosis: is cohesin a target?" Nature Reviews Molecular Cell Biology 10.489-496 (2009).

Zeng, G., Yu, X., Cai, M. "Regulation of yeast actin cytoskeleton-regulatory complex Pan1p/Sla1p/End3p by serine/threonine kinase Prk1p." Mol Biol Cell. 12(12).3759-72 (2001).

Zhang, X., Orlando, K., He, B., Xi, F., Zhang, J., Zajac, A., Guo, W. "Membrane association and functional regulation of Sec3 by phospholipids and Cdc4." J Cell Biol. 180.145–158 (2008).

Zheng, Y., Hart, MJ., Shinjo, K., Evans, T., Bender, A., Cerione, RA. "Biochemical comparisons of the Saccharomyces cerevisiae Bem2 and Bem3 proteins. Delineation of a limit Cdc42 GTPase-activating protein domain." J Biol Chem 268(33).24629-34 (1993).