THE DYNEIN ADAPTOR, LIS1/PAC1, IS A SUMOYLATION AND UBIQUITIN SUBSTRATE

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2015

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SUMOYLATION AND UBIQUITIN SUBSTRATE

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ACKNOWLEDGEMENTS

I would like to to thank a number of outstanding individuals who helped me through my graduate career at Oklahoma State University. First I would like to thank my parents, Rafael and Maria Alonzo, for their love, support, and encouragement to pursue my dreams for so many years. “Gracias por siempre creer en mi!” I would further like to thanks my grandmother, Ana Chavez, who didn’t live long enough to see me achieve my goal, but has been here with me always, and my grandparents Salvador Alonzo, Guillermina Perez, and Luis Camarena along with my siblings Janet, Zaray and Rafael Jr. Alonzo for their love and support over the years.

Additionally, I would like to thank my husband Enrique Alcala and daughter Hailey Anna Alcala for being my backbone these past years. Thank you for all the love, support and patience. With out neither of you this Ph.D. would of not been possible. I also will like to thank my OSU family, The Miller Lab; I will always cherish all the memories we have created.

Lastly I would like to thank my advisor Dr. Rita K. Miller. This thesis would have not been possible without your help, support and patience over the years. I would like to extend my deep gratitude and appreciation for your constant support and encouragement during my graduate career.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

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Date of Degree: May 2015

Title of Study: THE DYNEIN ADAPTOR, LIS1/PAC1, IS A SUMOYLATION AND UBIQUITIN SUBSTRATE

Major Field: Biochemistry and Molecular Biology

Abstract:

Microtubules are proteinaceous that contribute to cell motility and cell division. Microtubules are highly dynamic, continuously growing and shrinking. Because microtubules are fundamental for cell division, it is important to understand the molecular mechanisms that regulate them. Pac1p, the Lis1 homologue in yeast, is a microtubule plus-end-tracking protein. Mutations in the *LIS1* gene have been correlated with Miller-Dieker Lissencephaly syndrome due to defects in neuronal migration. Spindle positioning in yeast is an essential process during cell division that is regulated by the *KAR9* and dynein pathways. The *KAR9* pathway guides cytoplasmic microtubules into the bud. Dynein pulls the spindle across the bud neck via the forces it exerts on the cytoplasmic microtubule from the cortex. As part of the dynein pathway, Pac1p is important for recruiting dynein to the plus end of the microtubule and regulates its motility. Pac1p interacts with other plus-end microtubule binding proteins including Bik1p, the CLIP-170 homologue in yeast. Although Pac1p plays vital roles for microtubule function, little is known about its regulation.

Sumoylation is a post-translational modification that covalently attaches a Small Ubiquitin-like Modifier (SUMO) protein to the target substrate. Using a two-hybrid assay, Pac1p interacts with *SMT3*, the SUMO homologue in yeast, and other key players of the sumoylation pathway. Ubiquitin-like-specific protease-1 (Ulp1) is a protease that specifically cleaves Smt3p from its protein conjugates. Using a temperature sensitive strain that inactivates the Ulp1p protease, we observe an accumulation of higher molecular weight Pac1p bands. This suggests that the higher molecular weight forms of Pac1p are due to the accumulation of *SMT3* conjugates. In contrast to ubiquitination, sumoylation is not a modification that tags the target substrate for direct degradation. However, SUMO-targeted ubiquitin ligases (STUbLs) can recognize a sumoylated substrate and promote its degradation of the substrate via ubiquitination. Using a two-hybrid assay, we show that Pac1p interacts with the STUbL enzyme Nis1p/Ris1p. Pac1p also co-purified with ubiquitin. Strains deleted for *RIS1* and the SUMO isopeptidase *WSS1*, display an accumulation of higher molecular weight Pac1p conjugates, in comparison to WT. This work suggests a novel molecular mechanism of regulation for a microtubule-associated protein that regulates a critical microtubule motor.

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CHAPTER I

INTRODUCTION

Microtubules are proteinaceous polymers that are involved in cell motility, cell division, and serve as a scaffold for many microtubule-binding proteins (Desai and Mitchison, 1997; Conde and Caceres, 2009; Valiron et al., 2001). It is well known that microtubules are key components of the mitotic spindle and serve as tracks for the transport of essential cargo in the cell. Microtubule binding proteins regulate critical microtubule-dependent processes associated with various diseases such as Alzheimer's, Lissencephaly, tauopathies, ciliopathies, and Parkinson's. Errors in microtubule and chromosome attachment can also lead to chromosome instability, resulting in aneuploidy and/or polyploidy, which is common in many types of cancers.

Dynein is the major motor protein that walks toward the minus end of microtubules. It both captures chromosomes during mitosis and carries a variety of different cargoes to specific destinations within the cell. However, regulation of the attachment of cargo to dynein is poorly understood. Dynein is a complex motor comprised of several accessory proteins. Two of these are the dynein-activating complex called the dynactin complex, and Lis1/Pac1p. Lis1/Pac1p is a dynein adaptor that regulates dynein motor activity. Once Lis1 binds to dynein, dynein can switch to a "high load transport state" (Huang et al., 2012; McKenney et al., 2011). This allows dynein to transport a heavier load of cargo across the cell. Overall, Lis1 plays a major role in the regulation of dynein motor function and is

important for microtubule organization in the brain. Although much is known about Lis1 activity and function, little is known about the regulation of Lis1 or its homologues.

SUMO is a post-translational modification that regulates many cellular processes, but it has never been previously linked to dynein. A significant gap in our knowledge exists about the signal transduction system that regulates dynein function. Advancing our understanding of dynein regulation will help improve therapies that target human disease associated with dynein function, including Lissencephaly and cancer.

The specific goal of this dissertation is to identify a new regulatory mechanism that controls dynein through modification of its Lis1/Pac1p adaptor. My model is that dynein activity is controlled by sumoylation of Lis1/Pac1p, which switches dynein to a "high" gear motor. Later in the cell cycle, ubiquitination of Pac1p by SUMO-targeted ubiquitin ligases (STUbLs) targets Pac1p for proteasome degradation, thus returning dynein to its "normal" state. The work described in this thesis test several aspects of this model.

CHAPTER II

LITERATURE REVIEW

Microtubules

Microtubules are proteinaceous polymers that contribute to intracellular motility and cell division (reviewed in (Conde and Caceres, 2009; Desai and Mitchison, 1997; Etienne-Manneville, 2013; Howard and Hyman, 2003; Valiron et al., 2001)). They serve as tracks along which various motors move cargoes throughout the cell. As a major structural element of the mitotic spindle, they are often referred to as "ropes," owing to their ability to generate pulling forces on chromosomes.

Microtubules are highly dynamic, continuously growing and shrinking (Akhmanova and Steinmetz, 2008; Cassimeris et al., 1988; Chretien et al., 1995; Gardner et al., 2008; Sammak and Borisy, 1988; Schulze and Kirschner, 1988). The faster growing end is referred to as the plus end. The less dynamic end is referred to as the minus end (Allen and Borisy, 1974; Bergen and Borisy, 1980). In many cell types, the centrosome serves as a major microtubule-organizing center (MTOC), and stabilizes the minus ends of microtubules embedded within it (Mitchison and Kirschner, 1984b). In yeast, the spindle pole body serves as the MTOC (reviewed in (Jaspersen and Winey, 2004; Kahana et al., 1995; Rout and Kilmartin, 1990)).

Two types of tubulin protein, α and β tubulin, are used in the construction of the microtubule wall (Kirschner, 1978). α and β tubulin form heterodimers, which associate head to tail with β tubulin facing toward the plus end of the MT (Desai and Mitchison, 1997). This establishes the structural basis for microtubule polarity (Allen and Borisy, 1974; Bergen and Borisy, 1980; Amos and Schlieper, 2005). Tubulin heterodimers associate into linear protofilaments. In a classical microtubule structure, thirteen protofilaments associate laterally to form the hollow microtubule cylinder, which is about 25nm in diameter. Although both α and β tubulin are GTP-binding proteins, only β-tubulin has GTP hydrolyzing activity (Hyman et al., 1992). The hydrolysis of GTP affects the structure of the tubulin dimer within the polymer. The tubulin dimer containing the GDP nucleotide experiences mechanical strain that leads to a kink in the α-β tubulin interface, whereas the tubulin dimer containing GTP remains straight (Chretien et al., 1999; Mahadevan and Mitchison, 2005).

The plus-end of the microtubule alternates between growing and shrinking phases, a behavior known as dynamic instability (Mitchison and Kirschner, 1984a). Hydrolysis of the GTP cap at the end of the microtubules leads to microtubule depolymerization or "catastrophe" (Hyman et al., 1992). Microtubule depolymerization can be reversed by the addition of new GTP-bound tubulin dimers to the plus end of the microtubule, thus providing a new GTP cap. The switch from depolymerization to growth is called microtubule "rescue" (Hyman et al., 1992).

Due to their dynamic instability, microtubules can engage in "search-and-capture" phenomena (Lansbergen and Akhmanova, 2006; Vaughan, 2004). When a microtubule becomes attached to an organelle, the microtubule has the potential to perform mechanical work. Microtubules can generate a pushing force by polymerization and a pulling force by depolymerization (Inoue and Salmon, 1995). Both pulling and pushing forces can be observed during mitotic cell division. During the early stages of mitosis, microtubules continually "search" for the kinetochore of chromosomes. Once bound to the kinetochore, the microtubules can either push the sister chromatids away from the spindle pole or pull them toward the pole, by either polymerizing or depolymerizing.

Spindle positioning

Asymmetric cell division is a process used by many organisms as a mechanism to create cell-type diversity. In eukaryotes, correct spindle positioning is fundamental for proper chromosomal segregation to occur, and ensures that the offspring cells inherit a complete set of chromosomes. Errors in spindle positioning can lead to aneuploidy and/or polyploidy, which is common in various types of cancers. Spindle positioning in eukaryotes is highly regulated. The cell can either divide symmetrically, where both cells are identical in size, or asymmetrically, producing two different size cells (Figure 2-1). The orientation of the spindle can promote various differentiation outcomes. For instance, in vertebrates neuroepithelial cells can divide vertically to give rise to more neuroepithelial cells, or horizontally to produce the downstream neuron and progenitor cells (Huttner and Kosodo, 2005). Other examples of asymmetric cell division include cell division of animal oocytes. In oocytes the spindle is moved closer to the cell cortex making the cell divide asymmetrically in order to provide maturing oocytes more cytoplasm to aid in development. During brain development in *Drosophila*, progenitor cells use asymmetric cell division to create both the peripheral and central nervous system (Rhyu et al., 1994). Stem cell differentiation also depends on asymmetrical cell division. Stem cells must give rise to either daughter stem cells that will maintain stem cell properties, and daughter cells that will further differentiate (Bu et al., 2013).

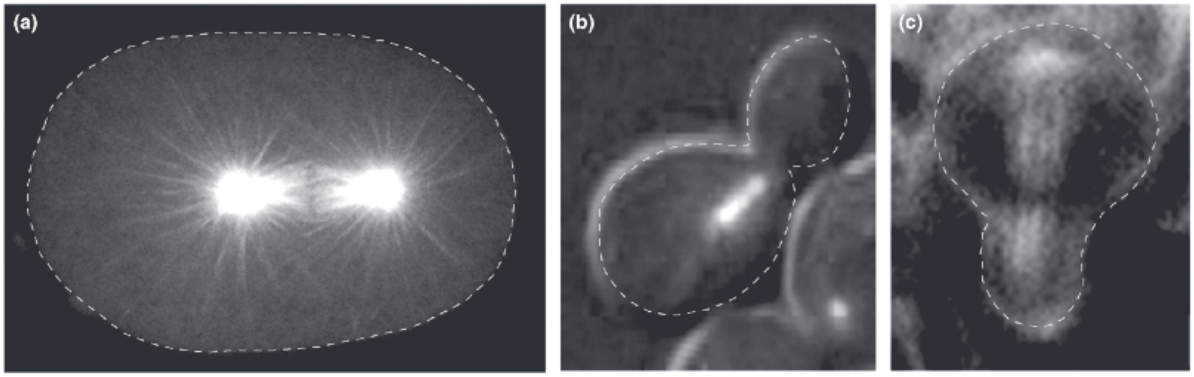

Figure 2-1. The mitotic spindle is asymmetrically localized. (A) In *C. elegans* embryo, (B) budding yeast (C) and *Drosophila* (Figure adapted from (McCarthy and Goldstein, 2006)).

Asymmetrically dividing cells use either established landmarks that are inherited from their parent cell, or form random symmetry-breaking events in response to environment. These signals are referred as polarity signals. In *Saccharomyces cerevisiae*, asymmetric cell division is the main form of cell division. Spatial landmarks are passed on to daughter cells, pre-establishing the site of cell division (Pringle et al., 1995). Various proteins, Bud8p, Bud5p, Bud2p, Cdc42, and Cdc24, are targeted to the bud neck, which will be the site of cell division (Irazoqui and Lew, 2004). Cdc42 activity at the bud neck is crucial. Cdc42 signals the organization of the actin network at the site where the daughter bud emerges. In yeast, two partially redundant pathways, the *KAR9* and dynein pathway, regulate spindle positioning in response to cell polarity (Miller and Rose, 1998) (Figure 2-2). Disruption of either pathway causes a spindle position defect, while disruption of both the dynein and *KAR9* pathways is lethal for the cell (Miller and Rose, 1998).

***KAR9* Pathway**

The *KAR9* pathway functions to position the nucleus and orient the mitotic spindle (Miller and Rose, 1998). *KAR9* is thought to be analogous to the mammalian adenomatous polyposis coli protein (APC). Mutations in the APC protein are strongly correlated to a number of human colorectal cancers (Bienz, 2001; Groden et al., 1991; Markowitz and Bertagnolli, 2009). The Kar9 protein links the microtubules that extend from the spindle pole body (SPB) to the actin network. This link is achieved via a bridging complex comprised of Myo2p-Kar9p-Bim1p (Beach et al., 2000; Hwang et al., 2003). Bim1p is the yeast homologue of the plus-end tracking protein EB1, while Myo2p is a type V myosin. Kar9p binds the older SPB, which will be inherited by the daughter cell, whereas the new SPB will remain in the mother cell (Ferreira et al., 2014; Liakopoulos et al., 2003; Moore et al., 2006;

Moore and Miller, 2007). Therefore it is important that Kar9p only localize on the microtubules that will be directed towards the bud, otherwise both poles would be pulled into the bud.

When the Myo2p-Kar9p-Bim1p complex is formed, Myo2p walks up the actin cable. As Myo2p walks, it directs the cytoplasmic microtubule into the bud and the attached spindle to the mother-bud neck (Beach et al., 2000; Korinek et al., 2000; Miller et al., 2000; Yin et al., 2000). Once the spindle is oriented at the bud neck, the dynein pathway can continue the process**.**

Dynein pathway

The dynein pathway is responsible for moving the spindle across the mother-bud neck prior to cytokinesis. The motor protein dynein is a major player in this pathway. Dynein pulls the spindle across the bud neck via the forces it exerts on the cytoplasmic microtubule from the cell cortex (Adames and Cooper, 2000; Li et al., 2005; Yeh et al., 1995). Cells lacking the motor dynein fail to move the spindle across the bud neck resulting in a binucleated mother cell (Eshel et al., 1993). A similar phenotype is observed when proteins of the dynactin complex, such as *JNM1*, are disrupted (McMillan and Tatchell, 1994). *JNM1,* Just Nuclear Migration protein, is a component of the dynactin complex that also includes Arp1p, and Nip100p.

Dynein participates in a wide range of cellular functions. Dynein plays several roles in the mitotic spindle and at the kinetochore (Kardon and Vale, 2009). Dynein is important in chromosome capture and alignment, as well in silencing the spindle assembly checkpoint (Bader and Vaughan, 2010; Howell et al., 2001; Mao et al., 2010). Together with NuMa, dynein plays a critical role in focusing the poles of the mitotic spindle, helping to generate its cone-shaped geometry (Gaglio et al., 1997). Dynein also carries a variety of different cargoes to specific destinations within the cell. Cargoes include endocytic vesicles, viral particles, organelles in retrograde axonal transport, melanosomes, and ER to Golgi transport vesicles (Holzbaur and Vallee, 1994; Johansson et al., 2007; LaMonte et

al., 2002; Moughamian and Holzbaur, 2012; Rocha et al., 2009; Scherer and Vallee, 2011; Tan et al., 2011; Watson et al., 2005). However, regulation of the attachment of cargo to dynein is still poorly understood. Errors in any of these processes can lead to increases in abnormally segregated chromosomes, a condition known as aneuploidy.

Dynein can be recruited to the plus end of microtubules either by the Kip2p-Bik1p complex or dynein's adaptor protein, Pac1p (Caudron et al., 2008; Markus and Lee, 2011; Sheeman et al., 2003). Once dynein is at the plus end of the microtubule, it can bind to the cortical protein Num1p. Num1p serves as a cortical anchor for dynein. Once bound, dynein can start walking towards the minus end of the microtubule. As it walks towards the minus end, it pulls the spindle across the bud neck. Disruption of either the dynactin complex or the cortical anchoring protein Num1p causes anaphase to occur earlier in the mother cell, leading to bi-nucleated cells (Clark et al., 1994; Heil-Chapdelaine et al., 2000; Kahana et al., 1998; McMillan and Tatchell, 1994; Muhua et al., 1994).

During these processes of spindle alignment it is also crucial to be able to control the dynamicity of the microtubules. Microtubule binding proteins have been shown to regulate microtubule dynamics by binding to their plus-end. Plus-end tracking proteins or +TIPs are a group of protein families that can alter microtubule dynamics (Akhmanova and Hoogenraad, 2005; Schuyler and Pellman, 2001). These protein families include the Cytoplasmic Linker Protein, CLIP-170/Bik1p (Diamantopoulos et al., 1999; Rickard and Kreis, 1991), the end-binding protein EB1/Bim1p (Schwartz et al., 1997), the Xenapus Microtubule Associated Protein XMAP215/Stu2p (Belmont and Mitchison, 1996; McNally and Vale, 1993), CLIP-associated protein (CLASP), Adenomatous Polyposis Coli (APC) (Kita et al., 2006), dynactin subunit $p150^{Glued}$ (Waterman-Storer et al., 1995a), and the type 1 Lissencephaly disease gene Lis1/Pac1p (Sapir et al., 1999). (Figure 2-3).

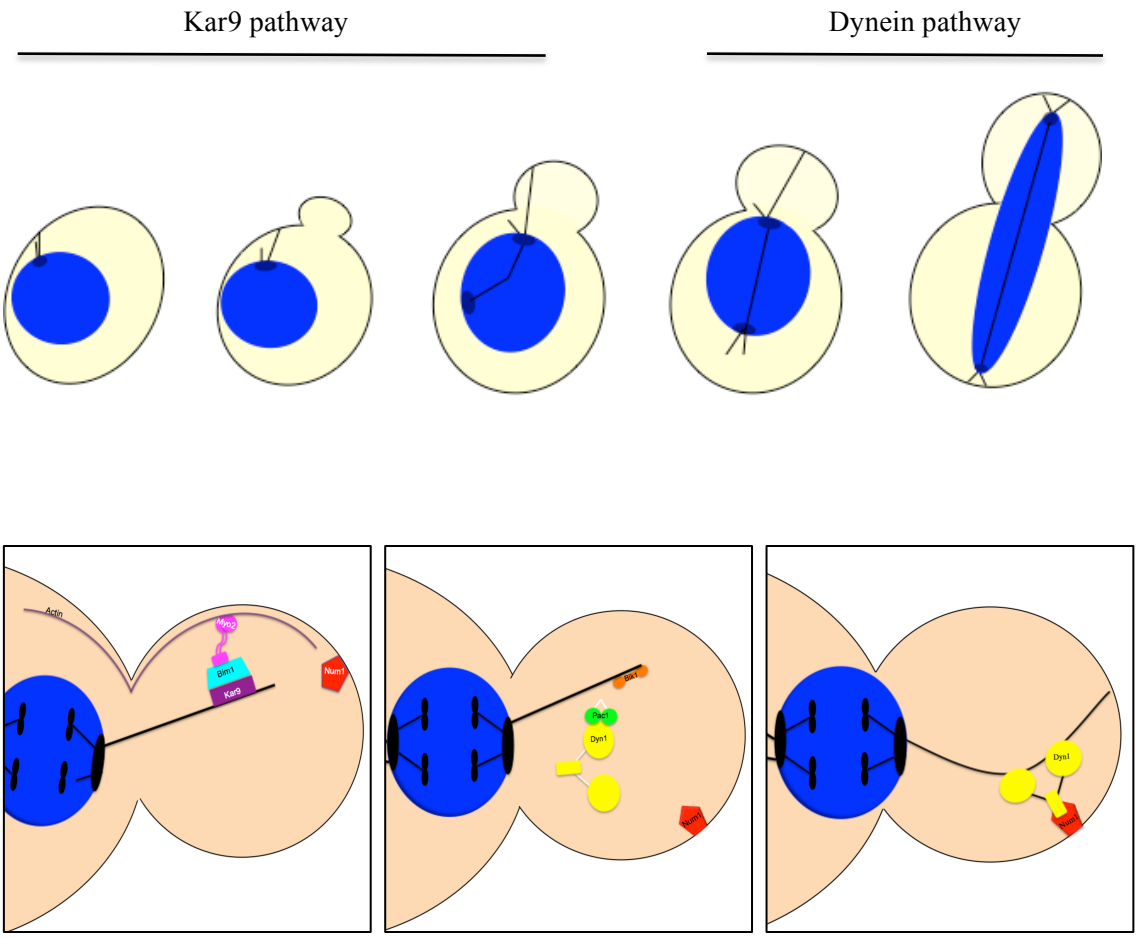

Figure 2-2. Spindle positioning in budding yeast. The Kar9 pathway links the actin and microtubule cytoskeleton via the Kar9p-Bim1p-Myo2p complex. The Kar9p pathway positions the mitotic spindle at the mother-bud neck. Dynein then is targeted to the plus-end of the microtubule via Bik1p and Pac1p. Once Dynein is at the plus end of the microtubule, dynein can be off loaded to the cell cortex via Num1p. After dynein is anchored to the cell cortex, it pulls on microtubules that are attached to the microtubule-organizing center, thus pulling the spindle across the mother-bud neck at the onset of anaphase.

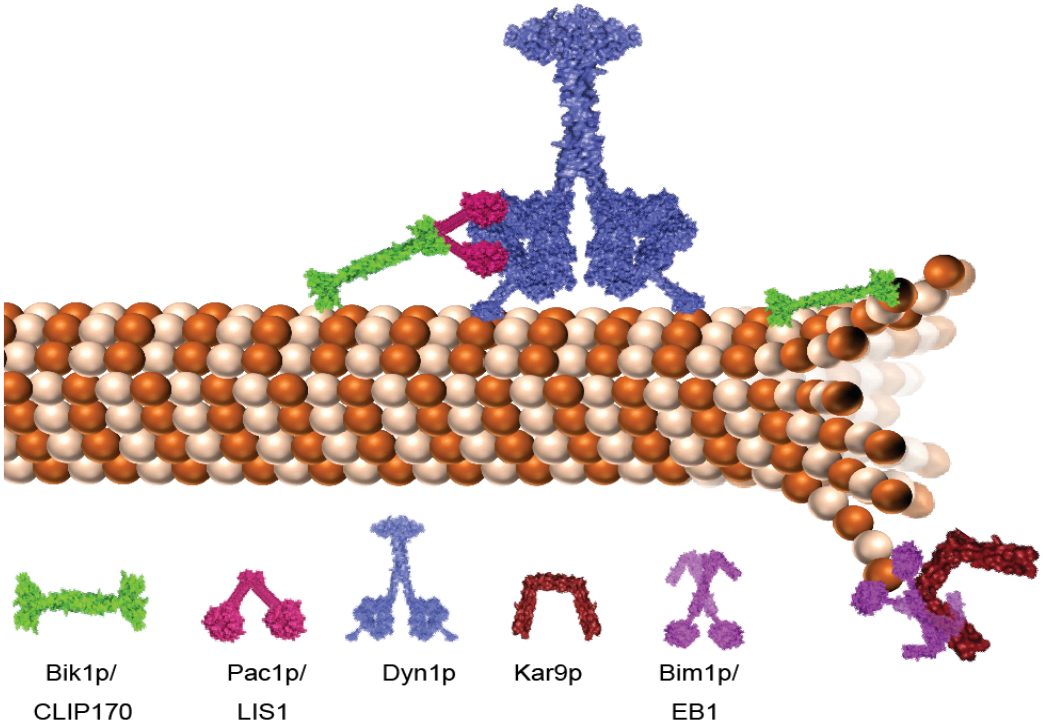

Figure 2-3: Microtubule Associated Proteins (MAPs). An artistic representation is shown of the following MAPs: Bik1p/Clip170, Pac1p/Lis1, Stu2p/XMAP215, Bim1p/EB1, Kar9p, and Dyn1/dynein. These drawings were developed by combining various crystalized domains of each protein to create a stylized structure. The following Protein Data Bank accession numbers were used: Dyn1 (4AKG), Bik1p/CLIP170 (2HQH), Stu2p/XMAP215 (4U3J, 2QK1), and Bim1p (4E61). No crystal structure is available for Kar9p, and thus it is a hypothetical structure. The microtubule portion of the figure was in collaboration with Matt Greenlee.

SUMOYLATION PATHWAY

The SUMO gene

SUMO is ~100 amino acids in size (Johnson, 2004). Although SUMO and ubiquitin share only ~18% sequence identity, they are structurally quite similar (Bayer et al., 1998; Vijay-Kumar et al., 1987) (Figure 2-4). Like ubiquitin, the tertiary structure of SUMO contains a ββαββαβ fold, known as the β-grasp fold, which is a common characteristic of the ubiquitin protein family (Bayer et al., 1998).

However, there are some differences between the two molecules. SUMO has an amino-terminal extension approximately twenty amino acids long that is absent in ubiquitin. Both are processed after translation, but by slightly different mechanisms. Ubiquitin can be translated as either a monomer or as a multimer that is subsequently cleaved into single ubiquitin units by deubiquitinating enzymes to expose the terminal glycine used in conjugation (Fang and Weissman, 2004; Larsen et al., 1998; Li and Ye, 2008; Ozkaynak et al., 1987; Wilkinson, 1997). In contrast, SUMO is translated as a single unit, but with a carboxy-terminal extension that is proteolytically processed by Ulp1p to expose the terminal glycine residue (Li and Hochstrasser, 1999).

Consensus sites

Classically, SUMO conjugates to a lysine residue lying within the consensus sequence ΨKxE/D, where Ψ is a large hydrophobic residue and x is any amino acid (Johnson, 2004; Melchior, 2000). However, about half of known conjugation events occur within non-consensus or incomplete consensus sites (Blomster et al., 2009; Matic et al., 2010; Teng et al., 2012). Computational algorithms designed to accurately predict sumoylation sites are continuously being improved (Blomster et al., 2009; Chen et al., 2012; Teng et al., 2012; Xu et al., 2008).

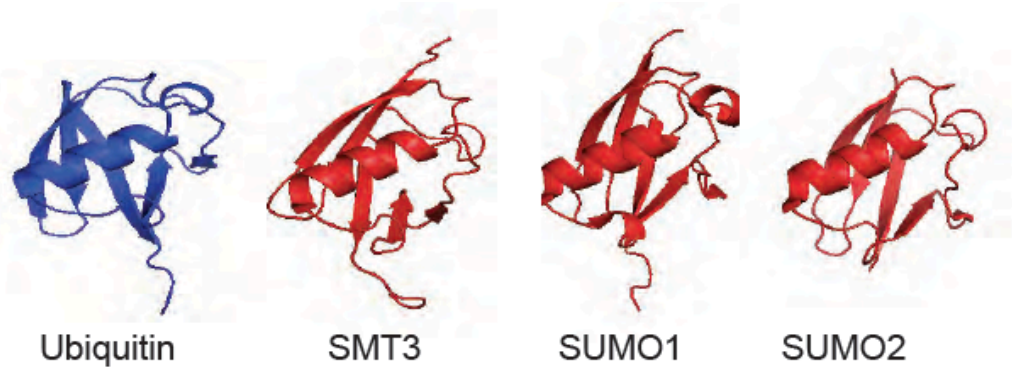

Figure 2-4: Structure of ubiquitin and SUMO proteins. Ribbon drawings of ubiquitin, SMT3, SUMO1, and SUMO2. These molecules share a common secondary structure that assembles into a ubiquitin-like fold. Renderings were developed using the crystallography coordinates available from the Protein Data Bank with the following accession numbers: ubiquitin (1UBQ), SMT3 (3V60), SUMO1 (2UYZ), and SUMO2 (1WM3). The structures for the above molecules were analyzed using the PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC. Figure and figure legend from Alonso et al. 2015.

SUMO paralogs

There are four SUMO genes in humans: SUMO1, SUMO2, SUMO3 and SUMO4. In *Saccharomyces cerevisiae* there is only one *SMT3,* and one in the *Schizosaccharomyces pombe,* Pmt3 (Meluh and Koshland, 1995; Tanaka et al., 1999). In humans, SUMO1, SUMO2, and SUMO3 can be found in multiple tissues, whereas SUMO4 mRNA expression is most pronounced in lymph nodes and kidney (Citro and Chiocca, 2013). Notably, the SUMO4 variant M55V has been linked to type I and type II diabetes (Guo et al., 2004). SUMO2 and SUMO3 are 97% identical in sequence and are considered redundant with each other. Thus, they are often referred to as SUMO 2/3. SUMO1 shares ~50% sequence identity with SUMO2/3 (Saitoh and Hinchey, 2000). SUMO1 is most similar to the yeast Smt3p, sharing fifty percent amino acid sequence identity and a longer N-terminal extension (Schwarz et al., 1998; Sheng and Liao, 2002).

The enzyme cascade

The enzyme cascade of the sumoylation pathway is analogous to the ubiquitination pathway, but the enzymes are distinct for each (reviewed in (Ulrich, 2009). Three different classes of enzymes are required for SUMO conjugation to the target protein: an activating enzyme (E1), a conjugating enzyme (E2), and a ligating enzyme (E3), which enhances the efficiency of conjugation and specificity for SUMO targets (Hochstrasser, 2001; Johnson, 2004) (Figure 2-5).

SUMO conjugation consists of an isopeptide bond formation between the carboxyl group of the terminal glycine of SUMO to the epsilon amino group of a lysine residue within the target protein. SUMO can either be attached to one lysine residue (mono-sumoylation), multiple lysine residues (multi-sumoylation), or form SUMO chains on the target lysine residue (poly-sumoylation) (Bencsath et al., 2002; Hickey et al., 2012).

SUMO-activating enzyme

The conjugation of SUMO to its target substrate requires ATP. The activation of SUMO is initiated with the adenylation of the C-terminal carboxyl group of SUMO in an ATP-dependent reaction. The process continues with the SUMO-activating enzyme, an E1. These enzymes consist of a heterodimer of Aos1p and Uba2p, which conserved from yeast to human (Desterro et al., 1999; Dohmen et al., 1995; Johnson et al., 1997). The thiol group of cysteine within the active site of Aos1p-Uba2p attacks the adenylated SUMO, forming a high-energy thioester bond between the Aos1p-Uba2p heterodimer and the C-terminus of SUMO (Olsen et al., 2010). Next, the activated SUMO is transferred to a cysteine within the active site of the E2 SUMO-conjugating enzyme, Ubc9p, forming a new thioester bond.

SUMO-conjugating enzyme

The E2 SUMO-conjugating enzyme Ubc9p is conserved from yeast to humans, sharing ~56% sequence similarity (Johnson and Blobel, 1997; Schwarz et al., 1998). In *Saccharomyces cerevisiae,* the *UBC9* gene is essential. In a clear demonstration of its conservation, the budding yeast Ubc9p can be replaced by the human version (Desterro et al., 1997; Gong et al., 1999; Yasugi and Howley, 1996). Ubc9p is the sole SUMO-conjugating enzyme, in contrast to multiple E2's in the ubiquitin system (Desterro et al., 1997; Johnson and Blobel, 1997; Jones et al., 2002; Lee et al., 1998).

Ubc9, is regulated by multiple posttranslational modifications. Ubc9p is sumoylated on two lysines (Ho et al., 2011). Preventing sumoylation at one site enhances its activity, while sumoylation at the other site diminishes it (Ho et al., 2011). Sumoylation of Ubc9p also reported to promote discrimination between substrates (Knipscheer et al., 2008). Acetylation also promotes targeting of Ubc9p to distinct substrates (Hsieh et al., 2013). Ubc9p phosphorylation

by CDK1 implies that sumoylation is coordinated with the cell cycle (Su et al., 2012). This has significant ramifications for control of the cytoskeleton with its myriad layers of cell cycle input.

SUMO ligating enzyme

SUMO conjugation can take place in the absence of a SUMO E3. However, the E3 is thought to bring the Ubc9p in close proximity with the target substrate to enhance SUMO conjugation and its specificity (Desterro et al., 1999; Okuma et al., 1999; Takahashi et al., 2001). SUMO E3 enzymes share similar features with the RING-domain found in the ubiquitin E3s (Hochstrasser, 2001; Johnson and Gupta, 2001) (Figure 2-6). There are several classes of E3s in humans; the protein inhibitor of activated STAT, known as the PIAS family (Shuai, 2000), polycomb group protein Pc2 (Kagey et al., 2003), and the nuclear pore protein complex RanBP2/Nup358 (Pichler et al., 2002). In budding yeast, there are four SUMO E3 ligases Siz1p, Siz2p/Nfi1p, and Mms21p/Nse2p and Cst9p/Zip3p (Duan et al., 2011; Heideker et al., 2011; Johnson and Gupta, 2001; Reindle et al., 2006; Stephan et al., 2011). Siz1p and Siz2p are responsible for the majority of SUMO conjugation *in vivo*, with Siz1p having the larger effect on global sumoylation levels (Johnson and Gupta, 2001; Takahashi et al., 2001). Mms21p/Nse2p was found in a screen for mutants that are sensitive to the drug methyl methanesulfonate (MMS), an agent that stalls DNA replication forks (Prakash and Prakash, 1977). Mms21p is part of the Smc5/6 complex important for chromosomal integrity, and functions in suppressing gross chromosomal rearrangements (Albuquerque et al., 2013; Rai et al., 2011; Stephan et al., 2011; Yong-Gonzales et al., 2012). Mms21p targets include telomeric and kinetochore proteins (Wan et al., 2013; Yong-Gonzales et al., 2012). Cst9p/Zip3p localizes at double-strand breaks associated with cross-over sites in meiosis (Serrentino et al., 2013).

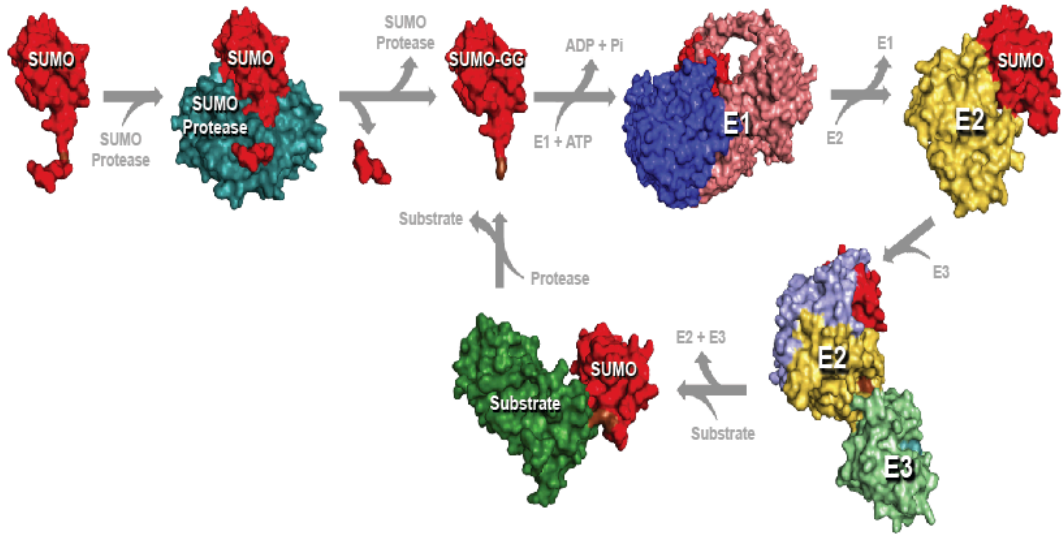

Figure 2-5: SUMOylation Pathway: To portray each state in the sumoylation pathway, surface maps were developed using crystallography coordinates available from PDB with the following accession numbers: SUMO1 and Senp1 (2IY1), E1 complex (3KYC), E2 complex (2UYZ), E3 complex (3UIP), and sumoylated PCNA (3V60). SUMO is shown in the same orientation throughout the sumoylation process depicted above. Figure and figure legend from (Alonso et al., 2015, artwork by Matt Greenlee)

SUMO chains

Like ubiquitin, SUMO can form poly-SUMO chains, known as polysumoylation (Johnson and Gupta, 2001). SUMO chains occur mainly through SUMO's flexible N-terminal extension containing a ψKxE sequence (Tatham et al., 2001). SUMO2/3 uses mainly lysine 11 for SUMO chain formation (Tatham et al., 2001). In contrast, SUMO1 lacks the lysines needed for chain formation. Therefore it is less likely to form chains (Matic et al., 2008), although a few examples of SUMO1 chains have been observed (Blomster et al., 2010). SUMO1 can also cap the end of a SUMO 2/3 chain, limiting its length (Matic et al., 2008). In contrast, the budding yeast SUMO, Smt3p, has three attachment sites for chain formation, K11, K15, and K19 (Bylebyl et al., 2003). SUMO chain formation is controlled and "edited" via the ubiquitin like specific protease Ulp2p in budding yeast (Bylebyl et al., 2003). Although SUMO chains are not essential for vegetative growth in yeast (Bylebyl et al., 2003), they are required for synaptonemal complex formation during meiosis (Cheng et al., 2006; Klug et al., 2013). Interestingly, the sumoylated form of Ubc9p promotes chain formation (Klug et al., 2013). Chains also play a role in targeting STUbL enzymes to a sumoylated protein (Tatham et al., 2008; Uzunova et al., 2007).

Conjugation and non-covalent binding

SUMOylation can regulate cellular processes via two major mechanisms. SUMO can become covalently cross-linked to a target protein or it can interact non-covalently with a binding partner (reviewed in (Kerscher, 2007)). This latter type of interaction typically occurs through SUMO interaction motifs (SIMs) on the interacting protein (Kroetz and Hochstrasser, 2009; Minty et al., 2000; Song et al., 2004). These are short stretches of the branched hydrophobic amino acids, (isoleucine, leucine, valine), in the pattern (I/L/V) X (I/L/V) (I/L/V) with x being any amino acid (Kroetz and Hochstrasser, 2009; Yang and Sharrocks, 2010). This motif is sometimes flanked on

one side by acidic residues, and this enhances binding to SUMO (Hannich et al., 2005; Hecker et al., 2006; Kerscher, 2007; Uzunova et al., 2007). Some proteins, like the kinetochore kinesin CENP-E, can interact both covalently and non-covalently (Zhang et al., 2008).

Proteases make sumoylation a reversible process

Regulation of cellular processes by SUMO can be quite dynamic. SUMO is removed from its targets by cleavage of the isopeptide bond between the glycine of SUMO and the target lysine. The deconjugating enzymes responsible for this specialized clipping are termed ULPs in yeast for ubiquitin-like specific protease (Li and Hochstrasser, 1999) and SENPs in plants and metazoans for SUMO/sentrin-specific proteases. Sentrin was an early name for SUMO (Kamitani et al., 1997). Several insightful reviews have been written recently on SENPs and Ulps (Drag and Salvesen, 2008; Gillies and Hochstrasser, 2012; Hickey et al., 2012; Mukhopadhyay and Dasso, 2007; Su and Hochstrasser, 2010).

SUMO deconjugating enzymes are grouped into four classes

In mammals, there are six SUMO-cleaving enzymes: SENP1, SENP2, SENP3, SENP5, SENP6, and SENP7 (Mukhopadhyay and Dasso, 2007). Phylogenetically, SENPs 1, 2, 3, 5 are classed with the yeast Ulp1p. SENP 6 and 7 are homologues of the yeast Ulp2 protease (Lima and Reverter, 2008; Mukhopadhyay and Dasso, 2007). SENP 8 is not actually a SUMO-SENP, as it removes another ubiquitin-like protein, Nedd8, from its conjugates (Gan-Erdene et al., 2003; Shin et al., 2011; Wu et al., 2003). Whereas SENPs are members of the large cysteine protease family, Wss1p is predicted to be a zinc-metalloprotease (Iyer et al., 2004; Mullen et al., 2010). In *Saccharomyces cerevisiae,* there are only three SUMO proteases (Ulp1p, Ulp2p, and Wss1p),

each belonging to a distinct class (Gillies and Hochstrasser, 2012). In mammals, a fourth class has recently been described the de-sumoylating isopeptidase (DESI) (Shin et al., 2012; Suh et al., 2012).

SENPs

Unlike traditional proteases, SENPs/Ulps do not degrade either SUMO or their targets. Instead, these enzymes possess two related cleavage activities, endopeptidase and isopeptidase activity. Whereas both the Ulp1p and Ulp2p families of SENPs desumoylate substrates by cleaving the isopeptide bond located between SUMO and the target, the Ulp1p class (but not the Ulp2 group) can also act as an endopeptidase (Drag and Salvesen, 2008; Li and Hochstrasser, 1999; Lima and Reverter, 2008; Mikolajczyk et al., 2007). This activity processes the full-length pro-SUMO to a conjugatable form by cleaving several amino acids from the carboxy-terminus to expose the terminal-glycine used in conjugation (Drag and Salvesen, 2008). In *Saccharomyces cerevisiae*, this removes three amino acids, ATY. For mammalian SUMOs, two to eleven amino acids are removed depending on the SUMO paralog (Hickey et al., 2012).

The Ulp1 and Ulp2 classes display distinct substrate specificities (Li and Hochstrasser, 2000), as evidenced by the fact that when either of the two proteases is absent, different sets of sumoylated products accumulate (Johnson and Blobel, 1999; Li and Hochstrasser, 1999; Li and Hochstrasser, 2000; Schwienhorst et al., 2000). These two proteases also display different subcellular localizations and virtually non-overlapping interactomes (Cubenas-Potts et al., 2013; Panse et al., 2003; Srikumar et al., 2013).

Although few substrates are known for the Ulp2p family of enzymes, localization and protein-interactome data suggest that it plays a role in the nucleus. Recent evidence also suggests it may function within the nucleolus, an organelle important for ribosome biogenesis and ribosomal

DNA transcription (Srikumar et al., 2013). We are not aware of any functional evidence that physically links Ulp2p to the major cytoskeletal polymers.

In addition to cleaving SUMO from substrates, the Ulp2p class of proteases modify SUMO chains in a process known as "chain editing" (Bylebyl et al., 2003; Mikolajczyk et al., 2007). Chain editing reduces the length and/or complexity of poly-SUMO chains, preferring to act on longer chains over mono-sumoylated entities (Bylebyl et al., 2003; Li and Hochstrasser, 2000; Lima and Reverter, 2008; Mukhopadhyay and Dasso, 2007; Mullen et al., 2010).

Structurally, SENPs/Ulps contain distinct amino and carboxyl domains (Lima and Reverter, 2008). The carboxy-terminal domain contains the catalytic domain, which is conserved across species. Functional studies using the amino–terminal domain suggests that it is important for subcellular localization and substrate targeting (Cubenas-Potts et al., 2013; Di Bacco et al., 2006; Goeres et al., 2011; Hang and Dasso, 2002; Klein et al., 2009; Kolli et al., 2010; Lima and Reverter, 2008; Yun et al., 2008).

Wss1p

Wss1p is the original member of a distinct class of SUMO proteases termed the WLM family of proteases (Wss1-Like Metalloproteases) (Iyer et al., 2004; Mullen et al., 2010). WSS1 was originally identified as a weak suppressor of *smt3-1*, a temperature sensitive allele of SUMO (Biggins et al., 2001), clearly implicating it in SUMO-related functions. Wss1 is predicted to be a zinc-dependent metalloprotease. Several observations make this a particularly fascinating new protease. Wss1p contains two SIMs (SUMO interacting motifs) within its extreme carboxyl-terminal domain, and has significant conservation with deubiquitinating enzymes (DUBs) (Mullen et al., 2010; Uzunova et al., 2007)

These observations prompted the Brill lab to ask which type of protease Wss1p might actually be, a SUMO protease or a DUB? Their work suggests that while Wss1p may have both types of activity, it is more efficient as a SUMO-cleaving enzyme than a ubiquitin-cleaving one. Several lines of evidence support this contention. A pull down assay showed that Wss1p binds GST-SUMO but not GST-ubiquitin (Mullen et al., 2010). Testing for SUMO peptidase activity directly, purified Wss1p was much more effective in cleaving synthesized poly-SUMO chains than poly-ubiquitin chains *in vitro*. It also appeared more effective at cleaving longer SUMO chains rather than shorter chains, similar to Ulp2p.

Wss1p was also tested for its ability to cleave a terminal ubiquitin from a SUMO chain. It could, but preferred to cut the single ubiquitin off, rather than a SUMO-ubiquitin double residue (Mullen et al., 2010). As Wss1p has sequence conservation with deubiquitingating enzymes (DUBs) (Mullen et al., 2010), the question has been raised as to whether Wss1 could also act as a SUMO-directed deubiquitinating enzyme. In this capacity, it might have a more generalized function (Su and Hochstrasser, 2010). Wss1p's ability to cleave ubiquitin correlates well with its physical interaction with proteins of the proteasome (Mullen et al., 2010).

Wss1p plays an important role in sister chromatid recombination, a type of double-strand break repair (Munoz-Galvan et al., 2013). Consistent with this, a portion of GFP-Wss1p localizes diffusely through the nucleus (van Heusden and Steensma, 2008). Its physical interaction with Psy2p and Tof2p is consistent with it being involved in stabilizing or processing stalled replication forks (van Heusden and Steensma, 2008).

In addition to its importance in DNA repair, Wss1p has recently been linked to another SUMO-utilizing process, microtubule biology. Two-hybrid analysis showed that Wss1p interacts with four distinct classes of microtubule-binding proteins, Kar9p, Bim1p, Bik1p and Pac1p (Alonso et al., 2012; Meednu et al., 2008). What makes this finding remarkable is that these different

classes of MAPs carry out diverse functions for microtubules (Berlin et al., 1990; Blake-Hodek et al., 2010; Gundersen and Bretscher, 2003; Huang et al., 2012; Hwang et al., 2003; Lee et al., 2003; Miller et al., 2000; Miller et al., 1999; Moore et al., 2006; Schwartz et al., 1997; Sheeman et al., 2003).

A portion of Wss1p also localizes to foci in the cytoplasm (van Heusden and Steensma, 2008). Curiously, this localization is dependent upon the actin-related component of the dynactin complex, Arp1p, but not on another dynactin component Jnm1p (van Heusden and Steensma, 2008). Wss1p is reported to localize only in the mother cell (van Heusden and Steensma, 2008). While little is known about how this protease affects any component of the cytoskeleton, we speculate that the mother-specific localization of Wss1p could play a role in inactivating dynein or dynactin components by regulating the levels of their ubiquitin-like modifications, and thus their delivery to the proteasome in mother cells but not the bud.

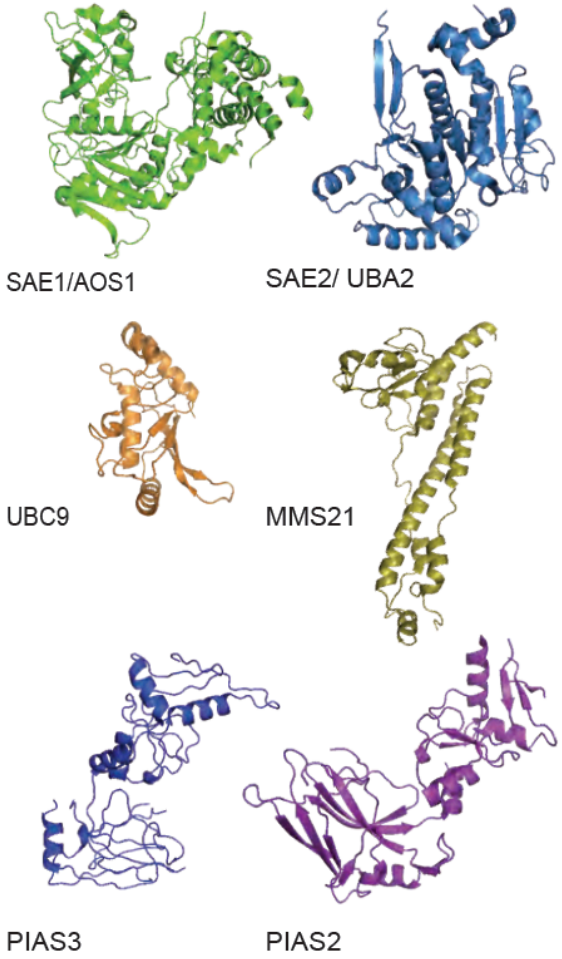

Figure 2-6: Structure of SUMO E1, E2, E3 enzymes. Tertiary ribbon structure of the SUMO-activating enzyme dimers SAE1, SAE2, SUMO conjugating enzyme UBC9, and SUMO ligating enzymes MMS21, PIAS3, PIAS2, and SIZ1. These renderings were developed from crystallography coordinates available from the following PDB accession numbers: SAE1 (1Y8Q), SAE2 (1Y8Q), UBC9 (2GRR), MMS2 (3HTK), PIAS3 (4MVT), PIAS2 (4FO9), and SIZ1 (3I2D). Using the PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC. Figure and figure legend from (Alonso et al., 2015).

SUMO-Targeted Ubiquitin Ligases (STUbLs)

Prior to 2007, sumoylation and ubiquitination were viewed as distinct modification systems with limited cross talk (Ulrich, 2005). In one example, ubiquitin and SUMO modify the same lysine at different times, in a competitive relationship (Desterro et al., 1998; Hoege et al., 2002; Steffan et al., 2004). In this model, SUMO protects the protein from ubiquitin-mediated degradation. Another type of cross talk employs cooperation between the two modifications, in which a serial modification of a target occurs first by SUMO and then by ubiquitin (Huang et al., 2003).

In 2007, a new class of enzyme was described, the SUMO-targeted ubiquitin ligase (STUbL) (Prudden et al., 2007; Sun et al., 2007; Uzunova et al., 2007; Xie et al., 2007). A STUbL is an enzyme with ubiquitin ligase activity that recognizes a sumoylated protein and poly-ubiquitinates it (reviewed in (Perry et al., 2008; Praefcke et al., 2012)). Poly-ubiquitination then targets that protein for degradation via the proteasome. Thus, SUMOylation can be an indirect, upstream signal for protein degradation (Figure 2-7).

Three STUbL families have been characterized, the Uls1p-Nis1p complex, and the Slx5p-Slx8p/RNF4 complex, and the Rad18 enzyme. Both Uls1p-Nis1p and Slx5p-Slx8p function as heterodimers (Yang et al., 2006). While both Slx5p and Slx8p contain RING domains, Slx5p is the subunit that targets the complex to substrates via its two SIMs (Cook et al., 2009; Szymanski and Kerscher, 2013; Xie et al., 2007). Slx8p has the more robust catalytic activity and can auto-ubiquitinate *in vitro* (Xie et al., 2007). Slx5p-Slx8p is the yeast homologue of the human RNF4, which functions as a single molecule (Sun et al., 2007; Uzunova et al., 2007; Xie et al., 2007). The human RNF4 can functionally replace the double deletion of slx5Δ slx8Δ (Uzunova et al., 2007). Both the yeast and mammalian versions function in DNA repair and genome integrity (Galanty et al., 2012; Kerscher, 2007; Mullen et al., 2001; Prudden et al., 2007; Xie et al., 2007).

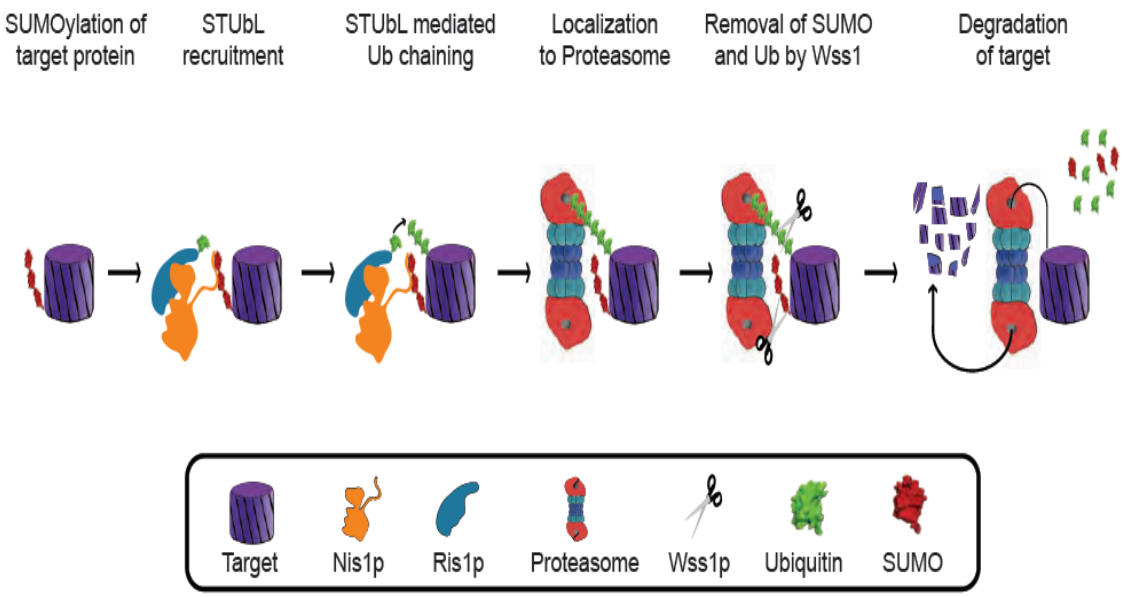

Figure 2-7: STUbL Pathway Model. The figure above outlines the targeting of a protein to the proteasome via the RIS1-NIS1 STUbL complex. (Adapted from Alonso et al., 2012; artwork by Jake Kline)

Uls1p/Nis1p contains a RING domain and binds to SUMO conjugates via SIMs. Little information is presently known about Uls1p and its targets, with only a few targets of Uls1p currently identified. These include the microtubule associated protein Pac1p and the DNA binding protein Rap1p (Alonso et al., 2012; Grunstein, 1997; Jain and Cooper, 2010; Zhang et al., 2012).

STUbLs also play an important role in cancer. In one of the best characterized examples, RNF4 functions in the degradation of PML in nuclear bodies (reviewed in (Hay, 2013)). In acute promyelocytic leukemia, the PML protein forms an in-frame fusion with the retinoic acid receptor alpha (RAR α), forming an oncoprotein that initiates this blood cancer (Tatham et al., 2008). Arsenic, a treatment for acute promyelocytic leukemia, causes the sumoylation of PML-RARa by SUMO2. RNF4 then polyubiquitinates these SUMO chains, resulting in degradation of the aberrant PML by the proteasome (Liu et al., 2012; Maroui et al., 2012; Rojas-Fernandez et al., 2014; Tatham et al., 2008). Recently, the novel STUbL Arkadia was found to function similarly in PML degradation (Erker et al., 2013). The elegant work describing PML cell biology and its relationship to effective therapeutic interventions for this disease gives hope to the idea that cytoskeletal accumulation diseases might one day be treated by targeting the SUMO system.

Rad18p, a RING finger protein, is the most recently reported protein to exhibit the hallmarks of a STUbL. Early studies showed that Rad18p complexes with Rad6p and mono-ubiquitinates the proliferating-cell nuclear antigen (PCNA). PCNA is the sliding clamp processivity factor for DNA polymerases (Hoege et al., 2002; Parker and Ulrich, 2012). Subsequently, Rad18p was shown to contain a SIM, which is used to bind sumoylated PCNA. Rad18p then polyubiquitinates PCNA and targets it for proteasome degradation (Parker and Ulrich, 2012). Rad18p has also been shown to serve as a SUMOylation enhancer of PCNA *in vitro* (Parker and Ulrich, 2014). In contrast to the yeast Rad18p, the human Rad18 does not bind SUMO and is not activated by the sumoylation of PCNA (Parker and Ulrich, 2012).

In just a few short years, the number of targets for STUbL enzymes and processes governed by STUbLs has exploded, with STUbLs playing critical roles in almost as many cellular processes as SUMO itself. These include governing genome stability and proper cellular responses to several types of DNA damage (Luo et al., 2012), particularly double strand break repair (Cook et al., 2009; Galanty et al., 2012; Guzzo et al., 2012; Nagai et al., 2011), non-homologous end-joining at telomeres (Lescasse et al., 2013), nucleotide excision repair (Poulsen et al., 2013), and homologous recombination repair (Prudden et al., 2007; Yin et al., 2012). STUbLs are also involved in the process of transcription (Xie et al., 2010; Yang et al., 2013), and regulate replication stress, such as those seen with hydroxyurea and MMS treatment (Cal-Bakowska et al., 2011). STUbLs have also been found to influence kinetochore stability, which in turn influences chromosome stability (Dasso, 2008; Mukhopadhyay and Dasso, 2010; van de Pasch et al., 2013). STUbLs are also central to the maintenance of SUMO levels themselves by modulating the levels of SUMO E3 ligases (Westerbeck et al., 2014). Methods for STUbL research continue to be improved (Szymanski and Kerscher, 2013). With these new roles for STUbLs, it remains to be determined the extent to which STUbL enzymes ubiquitinate all or some of their targets on the end of a polysumo chain or on a distinct lysine.

It is perhaps not surprising that STUbLs have now been linked to the cytoskeleton, including interactions with several microtubule-associated proteins. However, the amount of information concerning STUbLs and the cytoskeleton is still limited.

Microtubules and SUMO

Many classes of microtubule-associate proteins (MAPs) modify and regulate a multitude of microtubule behaviors. Some of these functions include directing microtubules towards distinct subcellular locations, cross-linking microtubules, mediating protein-protein interactions, and

either stabilizing or destabilizing microtubules. Some classes of MAPs bind directly to the tubulin dimers to help regulate their addition to the microtubule polymer (Cheerambathur and Desai, 2014; Etienne-Manneville, 2010; Ferreira et al., 2014; Gupta et al., 2013). Other MAPs, like tau, bind along the sides of microtubules (Al-Bassam et al., 2002), whereas other classes of MAPs bind at the plus-end (+TIPs) (Akhmanova and Steinmetz, 2008).

Recently several classes of MAPs have been identified as SUMO substrates, and several others that interact with SUMO either physically or by two-hybrid analysis. The MAPs that can be covalently modified include the spindle positioning protein Kar9p, heavy chain of the dynein motor, La, the Alzheimer's MAP Tau, and the kinetochore attachment protein Ndc80p (Alonso et al., 2012; Dorval and Fraser, 2006; Dorval and Fraser, 2007; Leisner et al., 2008; Meednu et al., 2008; Montpetit et al., 2006). The kinetochore kinesin CENP-E is both modified by SUMO and interacts non-covalently with SUMO (Zhang et al., 2008). Interaction with the SUMO machinery has also been seen with the Kar9 interacting protein, Bim1p. Bim1p is the EB1 homologue in yeast, but it is not known whether this interaction occurs through conjugation or non-covalent interactions (Meednu et al., 2008).

Kar9p

Kar9 is a cortical protein identified in a screen for bilateral karyogamy mutants (Kurihara et al., 1994). Kar9p is thought to be analogous to the adenomatous polyposis coli protein (APC), as they share similar functions (Gundersen, 2002). Kar9p is required for correct mitotic spindle orientation, and is important for nuclear migration as previously described above (Miller et al., 1999; Miller and Rose, 1998). Kar9 asymmetric localization is regulated by its post-translational modifications (Leisner et al., 2008; Moore et al., 2006; Moore and Miller, 2007). Kar9p can be phosphorylated and sumoylated. Cdc28p phosphorylates Kar9p at two serine sites, S197 and

S496 (Liakopoulos et al., 2003). Mutation of these phosphorylation sites causes Kar9p to localize to both SPB (Liakopoulos et al., 2003; Moore et al., 2006; Moore and Miller, 2007). Kar9p is also sumoylated *in vitro* and *in vivo* (Leisner et al., 2008; Meednu et al., 2008). Kar9p is sumoylated at lysine 304 (Meednu et al., 2008) and lysine 301, 333, 381 and 529 (Leisner et al., 2008). Mutation of lysine 304 to proline results in defects in positioning of the mitotic spindle, and Kar9p mislocalization to both spindle pole bodies (Meednu et al., 2008). The lysine 304 to proline mutant also results in a short-bipolar spindle, where the spindle is located away from mother-bud neck (Meednu et al., 2008). Similar results were observed when lysine 301, 333, 281, and 529 were mutated to arginine (Leisner et al., 2008). Inhibiting global SUMOylation levels also causes a mislocalization of the spindle apparatus (Leisner et al., 2008; Meednu et al., 2008). Because the global sumoylation defects is worse than the defect of the non-sumoylatable Kar9p, this suggests that other proteins involved in proper position of the mitotic spindle are also regulated via sumoylation. Preview reports demonstrate that phosphorylation regulates sumoylation (Hietakangas et al., 2006; Yang et al., 2003). This appears to be how Kar9p is regulated given that Kar9p phosphorylation at at serine 197 decreases its interaction with SUMO (Meednu et al., 2008). Meednu et. al., 2008 showed by two-hybrid analysis that S197E mutation does not interact with SUMO. Kar9p is also modified by ubiquitination, and has been shown to interact with the STUbL, Ris1p-Nis1p, and Wss1p by two-hybrid analysis (Meednu et al., 2008; Kammerer et al., 2010).

Dynein

There are two forms of cytoplasmic dynein DHC1a (dynein 1) and DHC1b (dynein 2), both of which are distinct from flageller dynein (Gibbons, 1995; Paschal et al., 1987). Dynein 1 is the major form of cytoplasmic dynein and is found in all eukaryotes, from fungi to human (King et

al., 2002). Dynein 2 is a less well characterized form of cytoplasmic dynein that is found in most ciliated eukaryotic cells, where it functions in intraflagellar transport and golgi organization (Grissom et al., 2002; Helfand et al., 2002; Mikami et al., 2002; Pazour et al., 1999; Signor et al., 1999). Mutations in the dynein 2 complex result in a number of ciliopathies (Schmidts et al., 2013a; Schmidts et al., 2013b). Dynein 2 associates with different intermediate and light chains than those associated with dynein 1. Dynein 2 also does not interact with other known regulators of dynein, including dynactin, Lis1, and BICD2 (Asante et al., 2014).

X-ray crystallography work from the Vale lab provides detailed insight into the structure of the dynein 1 motor (Carter et al., 2011; Carter et al., 2008). Globular microtubule-binding sites reside at the end of a 15nm long coiled-coil, termed the stalk (Carter et al., 2008; Gee et al., 1997; Mazumdar et al., 1996). The power for the movement of dynein is derived from the hydrolysis of ATP within a ring of six AAA ATPase motifs (Gee et al., 1997; Mocz and Gibbons, 2001; Neuwald et al., 1999). However, it is noteworthy that the six AAA domains are not created equal. Only four of the six AAA ATPase domains bind ATP (Kardon and Vale, 2009). ATP hydrolysis by one of them, AAA1, provides the primary energy required for allosteric conformational changes (Kon et al., 2004). It is believed that these allosteric changes within the AAA ATPase domains are transmitted through the "linker" to the "buttress," which generates a conformational change in the stalk, which is connected to the microtubule-binding domain. These changes produce the movement along the microtubule (Bhabha et al., 2014; Carter et al., 2011).

The dynein heavy chain is complexed with several accessory proteins. These are the intermediate chains, the light chains, and the light intermediate chains (Lo et al., 2001; Ma et al., 1999; Mok et al., 2001; Vaughan and Vallee, 1995; Waterman-Storer et al., 1995b). Two adaptors for dynein are Lis1/Pac1p and the dynein-activating complex, better known as the dynactin complex (Faulkner et al., 2000; Levy and Holzbaur, 2006; Schroer, 2004; Tai et al., 2002; Vaughan et al.,

1999). The dynactin complex consists of two sub-domains, a short actin-like filament connected to a shoulder-sidearm projection (Eckley et al., 1999; Quintyne et al., 1999). The short actin-like filament consists of Arp1, CapZ, p62, Arp11, p27, and p25. The shoulder-sidearm projection consists of 150^{Glued}, dynamitin, and p24 (Eckley et al., 1999; Garces et al., 1999). Both dynactin and Lis1/Pac1p are involved in attaching cargo to the dynein motor, but the mechanisms that regulate cargo attachment to dynein are unclear (Kardon and Vale, 2009; McKenney et al., 2011). A SUMOylation proteome screen suggests dynein heavy chain as a possible SUMO1 target (Matafora et al., 2009). This is the first identification of the dynein motor itself being a SUMO substrate.

La

La is a sumoylated RNA-binding protein that is transported by dynein (van Niekerk et al., 2007). La is also an antigen found in the autoimmune diseases systemic lupus erythematosus and Sjorgren's syndrome (Kumar et al., 2013), and La protein can enhance mRNA translation as well as viral replication (Kumar et al., 2013; Trotta et al., 2003).

The Twiss lab demonstrated that La is sumoylated at lysine 41 (van Niekerk et al., 2007). A non-sumoylatable form of La fails to immunoprecipitate with dynein. The non-sumoylatable La also moves down the axon in the anterograde direction, but not toward the cell body in the retrograde direction. Together these observations suggest that sumoylation of La promotes its interaction with dynein, and is required for La's retrograde transport in neurons by dynein. However, several questions remain. Does La transport involve the interaction with other dynein adaptors? Where in the neuron is La sumoylated, and does desumoylation regulate the un-loading of La cargo?

Tau

Tau, tubulin-associated unit, is a microtubule-associated protein that helps stabilize microtubules. Tau is highly conserved in higher eukaryotes (Goedert et al., 1996; Goedert et al., 1989a; Goedert et al., 1989; Maccioni et al., 1995). Tau is found mainly in neurons, where it stabilizes microtubules and promotes their polymerization (Binder et al., 1985; Cleveland et al., 1977; Drechsel et al., 1992; Drubin and Kirschner, 1986). Tau also has the ability to bundle microtubules (Kanai et al., 1992). Tau is a hydrophilic protein that consists of four regions; an acidic region, a proline-rich region, a microtubule-binding region, and a basic C-terminal region. The extreme difference in charge between the N-terminus and the C-terminus region of tau can be modulated by various post-translational modifications. Tau shares homology to other microtubule-associated proteins (MAPs) including MAP2 and MAP3/4 (Chapin and Bulinski, 1991). Mutations in tau are associated with several neurodegenerative disorders, including Alzheimer's, Pick's disease and several tauopathies (reviewed in (Goedert, 2001)). Alzheimer's is a neurodegenerative disease characterized by neurofibrillary tangles and senile plaques. The neurofibrillary tangles are intracellular aggregates containing abnormally phosphorylated tau, whereas senile plaques are extracellular deposits of amyloid β-peptides (Delacourte et al., 1999; Grundke-Iqbal et al., 1986; Ihara et al., 1986). In models for tau's role in Alzheimer's, tau first dissociates from microtubules in a phosphorylation-dependent manner, leading to their destabilization. Subsequently, unbound Tau forms dimers that oligomerize to form the paired helical filaments found in neurofibrillary tangles (reviewed in (Meraz-Rios et al., 2010)). As various forms of tau are found in cerebrospinal fluid, it is now being developed as biomarker for Alzheimer's disease to speed early diagnosis (reviewed in (Blennow et al., 2012; Kopeikina et al., 2012)).

Tau can be modified by numerous post-translational modifications, including phosphorylation, glycosylation, glycation, prolyl-isomerization, nitration, polyamination, ubiquitination, oxidation, and sumoylation (Arnaud et al., 2009; Bulbarelli et al., 2009; David et al., 2002; Dorval and Fraser, 2006; Dorval and Fraser, 2007; Grundke-Iqbal et al., 1986; Horiguchi et al., 2003; Kuhla et al., 2007; Landino et al., 2004; Liu et al., 2009; Murthy et al., 1998; Nacharaju et al., 1997; Necula and Kuret, 2004; Schweers et al., 1995; Takahashi et al., 2008; Takahashi et al., 1999; Wang et al., 2008; Wang et al., 1996; Zhang et al., 2005; Zhou et al., 2000). Tau has as many as thirty phosphorylation sites that can alter its structure, function, and localization (Fischer et al., 2009; Grundke-Iqbal et al., 1986; Litersky et al., 1996). In general, an increase in tau phosphorylation reduces its affinity for microtubules, and thus its ability to stabilize them (Drewes et al., 1995).

The relationship between SUMO and ubiquitin on tau is a noteworthy example of one type of crosstalk between two ubiquitin family members. Tau is ubiquitinated both *in vitro* and *in vivo* (Arnaud et al., 2009; David et al., 2002; Liu et al., 2009; Petrucelli et al., 2004; Zhang et al., 2005). Tau is sumoylated mainly by SUMO-1, but in some cases by SUMO-2 and SUMO-3 (Dorval and Fraser, 2006; Dorval and Fraser, 2007; Takahashi et al., 2008). Mutational analysis showed that the primary attachment site for SUMO is lysine 340, which is located within a microtubule-binding repeat. Tau has been seen shown to be heavily ubiquitinated in mature tangles of Alzheimer's patients, whereas the sumoylation levels in the mature tangles are low (Bancher et al., 1991; Dorval and Fraser, 2006). It is speculated that ubiquitin and SUMO compete for the same lysine residue. In this case, if one modification is up-regulated, the other would be down-regulated (Dorval and Fraser, 2006). Consistent with this model, inhibition of the proteasome causes a decrease on tau sumoylation, while increasing tau ubiquitination (Dorval and Fraser, 2006). Therefore, the sumoylation of tau could be one mechanism to modulate its turnover rate by blocking the ubiquitination that sends it to the proteasome (Dorval and Fraser,

2006). The diminished sumoylation of tau observed in Alzheimer's patients are consistent with the diminished proteasome function that is commonly found in many neurodegenerative diseases (Dorval and Fraser, 2006; Pountney et al., 2003).

Tau sumoylation is also partly dependent on phosphorylation. Treatment of cells with the phosphatase inhibitor, okadaic acid, promotes tau sumoylation (Dorval and Fraser, 2006; Dorval and Fraser, 2007). Sumoylation of tau is also increased by treatment of cells with the microtubule-depolymerizing drug, colchicine, which also releases tau from the microtubule. This finding is consistent with the sumoylation site being located inside the microtubule-binding region (Dorval and Fraser, 2006). These findings raise questions about the extent to which sumoylation may control tau solubility. Since tau is implicated in various human diseases, the levels of tau sumoylation should also be examined in other tauopathies. This information could provide insight into our understanding of the role of sumoylation in human disease pathogenesis.

Kinetochore MAPs

Numerous proteins of the kinetochore are sumoylated (Cubenas-Potts et al., 2013; Mukhopadhyay and Dasso, 2010). Indeed, SUMO/Smt3p in yeast was identified as the third Suppressor of Mif Two, which is a protein located at the centromere-kinetochore interface (Lampert and Westermann, 2011). While the sumoylation of centromere and kinetochore proteins is itself a field of rising interest, this section focuses on the kinetochore proteins that are also bona fide microtubule binding proteins.

Ndc80

Ndc80p is a conserved part of the kinetochore-associated Ndc80 complex, also referred to as Hec1p. Ndc80p is also a microtubule-associated protein. Ndc80p consists of a N-terminal microtubule-binding domain, which is negatively regulated by the kinase Aurora B, and a C-terminal coiled-coiled domain, which interacts with other components of the kinetochore-associated Ndc80 complex (Cheeseman et al., 2006; Guimaraes et al., 2008; Miller et al., 2008). The kinetochore consists of a collection of proteins that assembles on centromere DNA, to which the microtubules then attach. Ndc80p helps organize and stabilize kinetochore-microtubule interaction in order to facilitate proper chromosome segregation (Wei et al., 2011). Ndc80p forms a "dumbbell-like" heterotetramer with Nuf2p, Spc24p, and Spc25p to form the Ndc80 complex (Cheeseman et al., 2006; Tien et al., 2013). The Ndc80 complex also helps localize spindle assembly checkpoint proteins to the kinetochore (Gillett et al., 2004; Maiato et al., 2004).

In budding yeast, Ndc80p was identified as a sumoylated protein in several SUMO proteomes by mass spectrometry (Panse et al., 2004; Wykoff and O'Shea, 2005; Zhou et al., 2004). Later, it was confirmed that Ndc80p is sumoylated *in vivo* at a lysine residing at position 231 (Montpetit et al., 2006). Mutation of lysine 231 to arginine completely abolished the higher molecular forms of Ndc80p. It is unlikely that lysine 231 contributes to SUMO chain formation since the laddering effect remains the same in a strain in which SUMO chain formation is blocked (Montpetit et al., 2006). Instead, the abrogation of the multiple higher molecular weight forms of Ndc80p in the K231R mutant suggests that this amino acid is required for the sumoylation of other lysines. Ndc80p sumoylation levels remain relatively constant over the cell cycle. Its sumoylation is also not affected by the depolymerization of microtubules by nocodazole treatment or by activation of the spindle assembly checkpoint. This is unlike other sumoylated kinetochore proteins, Ndc10p, Bir1p, and Cep3p, in which SUMO does respond to these perturbations. This suggests that Ndc80p is regulated differently than other kinetochore proteins, like Ndc10p (Montpetit et al.,

2006). Although the evidence shows that Ndc80p is sumoylated *in vivo*, there are no phenotypes described as yet for the K231R mutant.

CENP-E

CENP-E is both a centromere-associated protein located in the outer plate of the kinetochore and a plus end-directed microtubule motor from the kinesin family (Yen et al., 1991). CENP-E is required for cell cycle progression from metaphase to anaphase by helping align chromosomes at the metaphase plate (Liu et al., 2007; Yen et al., 1991). CENP-E localization at the kinetochore is crucial for spindle checkpoint activation, which prevents defects in chromosome segregation (Liu et al., 2007). CENP-E has been shown to promote plus-end microtubule elongation *in vitro,* by stabilizing the microtubule as it walks towards the plus-end (Sardar et al., 2010).

CENP-E is both a SUMO substrate and a SUMO-binding protein (Zhang et al., 2008). The important role that SUMO plays in CENP-E function was demonstrated by inhibition of sumoylation using overexpression of SENP2, a SUMO-specific protease. This resulted in cell-cycle arrest at prometaphase, and the mislocalization of CENP-E from the kinetochore (Zhang et al., 2008). Overexpression of SENP2 also caused a decrease in sumoylation of other kinetochore-associated proteins that are needed for proper CENP-E localization to the kinetochore, since they bind CENP-E non-covalently (Zhang et al., 2008). CENP-E has also been shown to be a SUMO2/3 binding protein. Disruption of the SIMs in CENP-E also causes its mislocalization from the kinetochore (Zhang et al., 2008).

CHAPTER III

THE YEAST HOMOLOG OF THE MICROTUBULE-ASSOCIATED PROTEIN LIS1 INTERACTS WITH THE SUMOYLATION MACHINERY AND A SUMO-TARGETED UBIQUITIN LIGASE

As described above, a large number of microtubule-associated proteins have been shown to be SUMO substrates. However, an important MAP that had not been investigated until the work presented in this thesis was the dynein adaptor Pac1p/Lis1, although various sets of data suggested a connection. Pac1p interacts with Bim1p/EB1, which interacts by two-hybrid with SUMO (Meednu et al., 2008). Pac1p interacts with dynein, which was identified in a SUMOylation proteomic screen as a possible SUMO substrate (Matafora et al., 2009).

A number of recent studies have investigated the mechanisms that regulate microtubule plus-end binding proteins and spindle position. As described above, the phosphorylation of Kar9p has been shown to be important for proper spindle position. Recently, ubiquitin-related modifications have been shown to be involved in regulating kinetochore attachment to microtubules (Dasso, 2008; Joseph et al., 2004; Joseph et al., 2002; Montpetit et al., 2006; Tanaka et al., 1999). Previous work from the Miller lab and work described above, suggest the possibility that similar regulatory mechanisms could regulate cytoplasmic microtubule function.

Lis1/Pac1p

Lis1 is a microtubule-associated protein part of the plus-end tracking protein group, occasionally referred to as PAFAH1B1 (Hattori et al., 1994). Mutations in the *LIS1* gene are responsible for the severe brain disease, Type 1 lissencephaly, or "smooth brain." Lissencephaly is a rare brain formation disorder caused by dysfunction in neuronal migration, leading to severe mental disorders and early death (Kato and Dobyns, 2003; Liu, 2011; Reiner et al., 2006; Sapir et al., 1999). The hallmark of the disease is a drastic decrease in convolutions of the cerebral cortex (Reiner and Sapir, 2013). The human cerebral cortex consists of distinct cortical layers. Neuronal migration creates an inside-out arrangement of these layers. Neuronal migration dysfunction is thought to be the cause of cortical disorganizations found in patients diagnosed with lissencephaly. Therefore, any factors affecting the process of neuronal migration will physically affect the convolutions in the cerebral cortex (Aumais et al., 2001; Gupta et al., 2002; Lambert de Rouvroit and Goffinet, 2001).

Lis1 is a 46kDa dimeric protein, containing a novel LisH motif at the N-terminus, followed by a coiled-coil region and a WD-40 repeat region forming a seven β-propeller structure (Mateja et al., 2006). The two domains of the protein are equally important for function, since mutations causing lissencephaly are found to occur throughout the protein (Leventer et al., 2001; Leventer et al., 2001; Matsumoto et al., 2001). Lis1 binds directly to microtubules through its N-terminal region coil-coil domain (Sapir et al., 1997), while it binds dynein via the WD-40 repeats (Huang et al., 2012).

Pac1p, Lis1 homologue, is composed of three regions: a LisH domain, a small coiled-coil domain and a highly conserved WD40 repeat domain. None of these domains alone are sufficient for microtubule binding or plus end tracking *in vivo* (Markus et al., 2011); All three domains are necessary for proper activity (Markus et al., 2011). The WD40 repeats of Pac1p are thought to

bind across the intersection of the AAA3 and AAA4 ATPase motifs of dynein (Faulkner et al., 2000; Huang et al., 2012; McKenney et al., 2011; Toropova et al., 2014; Vallee et al., 2001; Wang et al., 2013).

While Lis1 is perhaps best known for its role in neurons, it is also important in desmosome stability and cortical microtubule organization in the epidermis. Loss of Lis1 results in fragile desmosomes, where it also localizes (Sumigray et al., 2011; Sumigray and Lechler, 2011). Lis1 is also critical in the development of hematopoietic stem cells, where it controls the positioning of the mitotic spindle during cell division and the inheritance of cell fate determinants (Zimdahl et al., 2014).

Earlier studies found that many homologues of Lis1 assist in the function of dynein (Geiser et al., 1997; Swan et al., 1999; Xiang et al., 1995b). In *S. cerevisiae*, mutants of dynein, dynactin and the Lis1 homologue Pac1p display defects in nuclear positioning during mitosis (Geiser et al., 1997; Xiang et al., 1995b). In *A. nidulans*, allele-specific suppression was observed between Lis1 homologue, nudF, and dynein heavy chains, nudA, implying that Lis1 might interact with dynein heavy chain directly (Xiang et al., 1995). Later studies found that Lis1 co-localizes with dynein and dynactin at numerous subcellular structures including the kinetochore, cell cortex, spindle, and spindle poles (Faulkner et al., 2000). Overexpression of Lis1 in mammalian cells disrupts cortical dynein localization causing mis-oriented spindles. While, inhibition of Lis1 caused failure in chromosome alignment at the metaphase plate, which is a phenotype observed when dynein activity is inhibited (Faulkner et al., 2000). This suggests that Lis1 is involved in spindle orientation during mitosis. It has been shown that Lis1 interacts directly with dynein at multiple sites including the tail and the motor domain (Tai et al., 2002). Lis1 also interacts with dynactin, a dynein accessory complex that mediates dynein attachment to various subcellular structures (Efimov and Morris, 2000). Overexpression of Lis1 causes dynactin to be displaced from the

microtubule plus end (Faulkner et al., 2000). Lis1 was found to participate in mitotic dynein functions, such as spindle orientation and metaphase chromosome capturing (Vallee et al., 2001).

Lis1 also promotes dynein's interaction with certain cargo (Sitaram et al., 2012; Splinter et al., 2012). The direct binding of Lis1/Pac1p to dynein can regulate several properties of the motor itself. These include its velocity, the load carried, and "processivity." By inducing a conformational change in the motor, Lis1/Pac1p also increases the "heaviness" of the load that the motor can carry (McKenney et al., 2010). Dynein bound to Lis1 walks at a slower speed than unbound dynein (Huang et al., 2012; Markus et al., 2011; McKenney et al., 2010; Torisawa et al., 2011; Toropova et al., 2014). The binding of Lis1 also increases its "processivity," which is the distance that a motor travels before stepping off the track. All of these parameters can be influenced by the time of attachment of dynein for the microtubule (Huang et al., 2012; Toropova et al., 2014). Thus, the binding of Lis1 to dynein can be thought of as transforming it into a more powerful diesel engine, one in low gear.

CLIP170/Bik1p

Bik1p is the yeast homologue of CLIP-170, a family of CAP-Gly proteins that track microtubule plus-ends (reviewed in (Gupta et al., 2014; Miller et al., 2006)). These are often referred to as a member of the a "+TIP" family of proteins (Akhmanova and Steinmetz, 2008). CLIP-170 binds the growing ends of microtubules, whereas Bik1p binds microtubules that are both growing and shrinking (Carvalho et al., 2004). Bik1p also stabilizes microtubules against catastrophe. When Bik1p is absent from the cell, microtubules are very short (Berlin 1990).

Structurally, Bik1p/ CLIP-170 is comprised of an amino-terminal head domain, a central coiled-coil domain, and a carboxy-terminal domain that contains metal-binding "zinc knuckle" motif. This domain is sometimes referred to as the "cargo-binding domain" (Gupta et al., 2010; Miller et

al., 2006). In contrast to the yeast Bik1p, the head domain of the mammalian CLIP-170 contains two CAP-Gly domain and several serine rich domains (Miller et al., 2006). Early work suggested that microtubule binding occurred through the CAP-Gly domains, but recent work demonstrates that the serine rich regions also make substantial contributions to microtubule binding (Gupta et al., 2010). In addition to binding the microtubule polymer, CLIP-170 also possesses a significant affinity for tubulin dimers (Folker et al., 2005). This interaction may play a role in a "co-polymerization" mechanism by which CLIP-170 tracks the plus-end of the growing microtubule (Folker et al., 2005). The interaction of Pac1p with Bik1p occurs though the carboxy-terminal domain of Bik1p (Sheeman et al., 2003) .

The functions of both CLIP-170 and Bik1p are closely connected to those of dynein (Caudron et al., 2008; Goodson et al., 2003; Sheeman et al., 2003; Tai et al., 2002; Vaughan et al., 1999). In yeast Bik1p together with Pac1p and Ndl1p, recruit dynein to the plus end of the microtubule, prior to dynein's off-loading to the cortex (Lee et al., 2005; Markus et al., 2011; Sheeman et al., 2003). In the absence of Pac1p or Bik1p, dynein fails to be recruited to the plus-end of microtubules, resulting in spindle positioning defects (Sheeman et al., 2003). Bik1p also interacts with Kar9p, providing a link between the Kar9p and dynein spindle positioning pathways (Moore et al., 2006).

The finding that Pac1p/Lis1 and Bik1p/CLIP-170 interact with SUMO leads to a new area of study of how sumoylation of these proteins affect microtubule dynamics. Various MAPs interact with each other to form a web of interactions along the microtubules (Akhmanova and Steinmetz, 2008). As described above, the dynamic nature of microtubules is important for many processes in higher eukaryotes including organization of the apical-basal polarity of the epithelial cells. Considering the expanding list of MAPS interacting with SUMO, there is a possibility that sumoylation may help promote or regulate interactions between the MAPs along the microtubules. This phenomenon is significant not only for spindle positioning but also for proper

sister chromatid division. The work described in Alonso et al. 2012 adds Pac1p and Bik1p, to the growing list of microtubule-associated proteins that interact with SUMO.

Understanding the mechanisms that regulate spindle positioning will contribute towards understanding various forms types of cancer. Pac1p and Bik1p are also part of the dynein pathway, which is important for correct spindle positioning. Mutations affecting spindle positioning also lead to a variety of developmental diseases. Pac1p homologues in other organisms have also been shown to be important for spindle orientation during neuroblast development (Siller and Doe, 2008). Therefore understanding how these proteins are modified could further our knowledge on the diseases and neuron development.

SUMMARY OF MANUSCRIPT

Pac1p and SUMO

Several approaches were employed to show that SUMO is linked to Lis1/Pac1p. First, two-hybrid analysis was used to show that Pac1p interacts with SUMO and several other members of the sumoylation pathway, including the E2 enzyme, Ubc9p, and the E3, Nfi1p (D'Silva, 2008). Second, inhibition of the SUMO protease Ulp1p resulted in multiple higher molecular weight forms of Pac1p, suggesting that Ulp1 removes SUMO from Pac1p. Third, the co-immunoprecipitation of Pac1p with SUMO strongly suggested that Pac1p is a SUMO substrate. Fourth, Pac1p interacted with both components of the STUbL enzyme Uls1p-Nis1p by two-hybrid analysis. Pac1p shift was increased in strains deleted for the STUbL Uls1p, and in strains where the proteasome was inhibited with the drug MG132. These data support a model in which the Uls1p-Nis1p STUbL recognizes a sumoylated Pac1p and thus targets it to the proteasome. Depending on the localization of the STUbL, this could represent a mechanism to degrade a subcellular pool of Pac1p, perhaps on the set of microtubules directed into the bud.

After the manuscript was published the Pac1p homologue PAFAH1B1/Lis1 was identified in a SUMO-1 and SUMO-2 SUMOylation proteomic screens (Bonacci et al., 2014; Wen et al., 2014). This finding suggests that the sumoylation of Pac1p might be conserved across species.

Bik1p and SUMO

Bik1p displays several interactions with the sumoylation machinery. Bik1p interacts with SUMO, the SUMO E2 conjugating enzyme Ubc9p, and the E3 Nfi1p by two-hybrid analysis. Interestingly, the carboxy-terminal domain of Bik1p, the domain that interacts with Pac1p, is also required for Pac1p's interaction with SUMO. In the reciprocal direction, Pac1p is required for Bik1p's interaction with SUMO in the two-hybrid assay. These findings suggest the possibility that a mutual-association of both proteins is required for their modifications by SUMO. Bik1p can also be sumoylated using an *in vitro* assay, resulting in two and possibly three shifted bands. Sumoylated forms of Bik1p have also been observed *in vivo* when Bik1p and SUMO were overexpressed. Ulp1p is one of the major SUMO proteases in the cell that cleaves SUMO from target proteins. In a somewhat surprising finding, I did find that inactivation of Ulp1p with a temperature-sensitive allele did not reveal SUMO-shifted forms of Bik1p. Thus, identification of a sumoylated form of Bik1p at the endogenous level has remained elusive.

Alternative hypothesis for Bik1p sumoylation

Although, inactivation of Ulp1p did not reveal SUMO-shifted forms of Bik1p, there are some possible explanations. Perhaps Bik1p is not actually conjugated by SUMO and the putative SUMO connection occurs via a non-covalent interaction. Perhaps SUMO only attaches to Bik1p when the cell is stressed. Another possibility is based on the finding that Bik1p interacts by two-hybrid analysis with the STUbL enzyme, Uls1p-Nis1p, and the SUMO-isopeptidase Wss1p. While Bik1p's interaction with this enzyme implies that it is sumoylated at some point, the difficulty of "catching" SUMO on Bik1p is nevertheless perplexing. It is also possible that

Bik1p's interaction with the STUBL results in its rapid demise by the proteasome. As Bik1p and CLIP-170 have critical functions for microtubules, further research into the SUMO-Bik1p connection is anticipated.

Acknowledgement

Sections of chapter 2 and chapter 3 of this dissertation have been accepted for publication as a literature review by the journal, *Cytoskeleton*. Annabel Alonso authored the sections of the review included in this dissertation. The article is co-authored by Alonso, A., Greenlee, M., Matts, J., Kline, J., Davis, K., and Miller, R.K. 2015. Emerging roles of sumoylation in the regulation of actin, microtubules, intermediate filaments, and septins. *Cytoskeleton.*

Figure	Contributed by…
3A, 3B	Maliha Rahman
4A, 4B, 3D, 3E, 3F	Sonia D' Silva
8A	Jake Keeling
9	Jake Kline
3C, 5, 6A, 6B, 7A, 7B, 7B, 7C, 7C', 8B, 8C	Annabel Alonso

Table 1. Author contributions for the Alonso et. al., 2012 manuscript. The manuscript Alonso et al. 2012 was performed in collaboration with the following authors: Sonia D' Silva, Maliha Rahman, Jake Keeling, Jake Kline, and Nida Meednu. The table above gives a detail outline of the figures that each author contributed to the paper.

DISCUSSION

Despite many years of research, the regulatory mechanisms that regulate the function of Pac1p have not been discovered. Since Pac1p is conserved from yeast to human, what is learned about Pac1p regulation in yeast provides valuable insight into the more complicated mammalian system. The work presented here describes the interaction of two microtubule-associated proteins, Bik1p and Pac1p, with enzymes of the sumoylation pathway in *S. Cerevisiae*. These findings suggest the hypothesis that sumoylation may regulate the functions of these MAPs. As Pac1p is one the few examples known for substrates of the STUbL, Uls1p-Nisp1p, many questions remain about its sumoylation.

Additional work is needed to see if these modifications are conserved in Pac1p's mammalian homologue, Lis1. It is also not known how sumoylation of Pac1p might regulate either the cargo selection of dynein or the motor properties of dynein. It is important as well to identify the sites of modification in order to determine the function of this modification It is well known that different types of stresses affect global levels of sumoylation. Further work is needed to identify the types of stresses that alter Pac1p modification.

Pac1p activities, as many other MAPs, are cell cycle regulated. It will be interesting to know if the SUMOylation of Pac1p is as well regulated by the cell cycle. I predict that Pac1p modification will vary through the cell cycle, especially since the localization of dynein changes as the cell cycle progresses.

The yeast homologue of the microtubule-associated protein Lis1 interacts with the sumoylation machinery and a SUMO-targeted ubiquitin ligase

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ABSTRACT Microtubules and microtubule-associated proteins are fundamental for multiple cellular processes, including mitosis and intracellular motility, but the factors that control microtubule-associated proteins (MAPs) are poorly understood. Here we show that two MAPs—the CLIP-170 homologue Bik1p and the Lis1 homologue Pac1p—interact with several proteins in the sumoylation pathway. Bik1p and Pac1p interact with Smt3p, the yeast SUMO; Ubc9p, an E2; and Nfi1p, an E3. Bik1p interacts directly with SUMO in vitro, and overexpression of Smt3p and Bik1p results in its in vivo sumoylation. Modified Pac1p is observed when the SUMO protease Ulp1p is inactivated. Both ubiquitin and Smt3p copurify with Pac1p. In contrast to ubiquitination, sumoylation does not directly tag the substrate for degradation. However, SUMO-targeted ubiquitin ligases (STUbLs) can recognize a sumoylated substrate and promote its degradation via ubiquitination and the proteasome. Both Pac1p and Bik1p interact with the STUbL Nis1p-Ris1p and the protease Wss1p. Strains deleted for *RIS1* or *WSS1* accumulate Pac1p conjugates. This suggests a novel model in which the abundance of these MAPs may be regulated via STUbLs. Pac1p modification is also altered by Kar9p and the dynein regulator She1p. This work has implications for the regulation of dynein's interaction with various cargoes, including its off-loading to the cortex.

Monitoring Editor
Fred Chang
Columbia University

Received: Mar 7, 2012
Revised: Sep 20, 2012
Accepted: Sep 27, 2012

INTRODUCTION

Microtubules are critical for a number of basic cellular processes. They are vital to the operation of the mitotic spindle. They can act as ropes that pull on kinetochores to separate the attached chromosomes. Microtubules also function as tracks that guide the transport of various cargoes to distinct destinations within the cell. Many aspects of microtubule function are regulated by distinct classes of microtubule-binding proteins.

In the yeast *Saccharomyces cerevisiae*, microtubules are critical for positioning the mitotic spindle, a process governed by two genetic systems termed the Kar9 pathway and the dynein pathway. The Kar9 pathway orients cytoplasmic microtubules into the bud, and the dynein pathway pulls on the cytoplasmic microtubules to position the spindle across the bud neck with the onset of anaphase (Kahana *et al.*, 1995; Yeh *et al.*, 1995; Carminati and Stearns, 1997; Miller *et al.*, 1998). In the dynein pathway, both Bik1p and Pac1p help localize dynein to the plus end of the microtubule before it is "off-loaded" to the cortex (Sheeman *et al.*, 2003; Lee *et al.*, 2005; Caudron *et al.*, 2008; Markus *et al.*, 2011). The microtubule-binding protein Bik1p is a member of the CLIP-170 family, and Pac1p is a member of the Lis1 family.

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E12-03-0195) on October 3, 2012.

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Abbreviations used: AD, activation domain; BD, DNA-binding domain; SC, synthetic complete; STUbL, SUMO-targeted ubiquitin ligase; TBS, Tris-buffered saline.

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Supplemental Material can be found at:
http://www.molbiolcell.org/content/suppl/2012/10/01/mbc.E12-03-0195v1.DC1.html

Bik1p is a multifunctional protein. Bik1p is found on both nuclear and cytoplasmic microtubules (Berlin *et al.*, 1990; Sheeman *et al.*, 2003; Miller *et al.*, 2006). It stabilizes microtubules, and in its absence, microtubules are very short (Berlin *et al.*, 1990; Blake-Hodek *et al.*, 2010). It also acts to tether microtubules to the cell cortex in mating cells (Molk *et al.*, 2006). Whereas the mammalian CLIP-170 binds growing microtubules only, the yeast Bik1p tracks both the growing and shrinking ends of microtubules (Carvalho *et al.*, 2004). In the nucleus, Bik1p is also part of the kinetochore, and its activity there has been linked to preanaphase kinetochore separation in polyploid strains (He *et al.*, 2001; Lin *et al.*, 2001). However, its precise role at kinetochores remains unclear (Westermann *et al.*, 2007). Bik1p localization at the plus end of cytoplasmic microtubules is mediated by the kinesin motor, Kip2p, which carries it to the plus end (Carvalho *et al.*, 2004; Caudron *et al.*, 2008).

Bik1p and Pac1p are each structurally similar to their mammalian counterparts. Bik1p is composed of an amino-terminal head domain, a central coiled-coil domain, and a carboxy-terminal tail (Miller *et al.*, 2006). In its head domain, Bik1p has one CAP-Gly domain for microtubule binding, whereas CLIP-170 has two. This is followed by a serine-rich domain, which may be used in phosphoregulation (Miller *et al.*, 2006). A 40–amino acid region at the carboxy-terminus termed the cargo-binding domain is required for interaction with dynein and Pac1p (Lin *et al.*, 2001; Sheeman *et al.*, 2003; Li *et al.*, 2005). The structure of Pac1p includes a short coiled-coil domain and a highly conserved WD40 repeat domain. Neither of the two domains alone is sufficient for microtubule binding or plus-end tracking in vivo (Markus *et al.*, 2011).

Mutations in Lis1 cause lissencephaly, a devastating disease of abnormal brain development resulting from incomplete neuronal migration (Reiner *et al.*, 2006). Lis1 has a number of activities associated with microtubules. Lis1 function is closely linked to dynein, a minus end–directed motor protein that regulates a number of different movements within the cell, including vesicle transport, mitosis, cell migration, nuclear migration, and spindle orientation (Faulkner *et al.*, 2000; Dujardin *et al.*, 2003; Shu *et al.*, 2004; Tanaka *et al.*, 2004; Tsai *et al.*, 2005, 2007, 2010; Lam *et al.*, 2010; Zhang *et al.*, 2010). As an adaptor to dynein, Lis1 modulates a subset of dynein functions (Faulkner *et al.*, 2000). Lis1 also interacts with the motor domain of dynein and affects its motor activity (McKenney *et al.*, 2010; Torisawa *et al.*, 2011; Huang *et al.*, 2012). In doing so, it increases the time that dynein interacts with the microtubule, making dynein more persistent in generating force for transport of heavy loads (McKenney *et al.*, 2010, 2011). Furthermore, Lis1 coordinates the activity of plus end–directed motors with minus end–directed motors (Yi *et al.*, 2011). Lis1 can also affect microtubule assembly, both positively or negatively (Sapir *et al.*, 1997; Han *et al.*, 2001). In the upper layers of the epithelium, Lis1 is important for the organization of cortical microtubules and the stability of desmosomes (Sumigray *et al.*, 2011). In yeast, Pac1p functions in the dynein pathway by working with Bik1p to recruit dynein to the plus end of the microtubule (Lee *et al.*, 2003; Sheeman *et al.*, 2003; Li *et al.*, 2005; Markus *et al.*, 2011). Although much is known about the various activities of Lis1, little is known about the regulation of Lis1 and/or Pac1p in each of these different contexts.

Proteins in both the Kar9p and dynein pathways have been shown to be regulated by phosphorylation (Choi *et al.*, 2000, 2002; Liakopoulos *et al.*, 2003; Maekawa and Schiebel, 2004; Moore *et al.*, 2006, 2008). However, little is known about how other protein modifications regulate spindle positioning.

Sumoylation is a small ubiquitin-like modification that regulates many divergent cellular processes and is key to several diseases (reviewed in Dasso, 2008; Gareau and Lima, 2010; Sarge and Park-Sarge, 2011; Praefcke *et al.*, 2012). In yeast, the single gene for SUMO is *SMT3*. SUMO conjugation onto target lysines occurs through a terminal glycine residue, which becomes exposed by the action of the Ulp1p protease, which removes the last three amino acids of SUMO. A cascade of enzymes analogous to, yet distinct from, ubiquitination is responsible for SUMO conjugation to its substrates. The E1 dimer Aos1p–Uba2p activates Smt3p in an ATP-dependent step. The E2-conjugating enzyme is Ubc9p. Four E3 ligases are known in yeast that also confer substrate specificity. These are Nfi1/Siz1p, Siz2p, Nse2p, and Mss21p (Johnson and Gupta, 2001; Reindle *et al.*, 2006; Duan *et al.*, 2011; Heideker *et al.*, 2011; Stephan *et al.*, 2011).

Removal of SUMO from targets is a dynamic process, and SUMO deconjugation is important for cellular health (Bekes *et al.*, 2011). Three proteases remove SUMO from its targets in yeast. These are Ulp1p, Ulp2p, and Wss1p (Mukhopadhyay and Dasso, 2007; Kolli *et al.*, 2010; Mullen *et al.*, 2010). Ulp1p is an essential protease required for cell cycle progression that localizes to the nuclear envelope and septin ring (Li and Hochstrasser, 1999; Elmore *et al.*, 2011). The Ulp2p has chain-editing activity and can cleave SUMO chains, acting to shorten their length (Bylebyl *et al.*, 2003; Mukhopadhyay *et al.*, 2007; Yeh, 2009). The protease Wss1p displays SUMO-isopeptidase activity and is reported to be associated with the proteasome (Mullen *et al.*, 2010). Ulp1p and Ulp2p are SUMO specific, whereas Wss1p may remove both ubiquitin and SUMO (Mullen *et al.*, 2010; Su and Hochstrasser, 2010).

In 2007, a new mode of cross-talk was identified between the ubiquitination and sumoylation systems (Sun *et al.*, 2007; Uzunova *et al.*, 2007; Xie *et al.*, 2007). SUMO targeted ubiquitin ligases (STUbLs) are ubiquitin ligases that recognize a sumoylated protein and polyubiquitinate it, sending the target to the proteasome for degradation. How STUbLs interact with microtubule-binding proteins is not known.

Recent work suggests that sumoylation regulates the Kar9p spindle-positioning pathway by modifying the Kar9 protein (Leisner *et al.*, 2008; Meednu *et al.*, 2008). Defects in sumoylation alter the localization of Kar9p to both spindle poles rather than just the pole destined for the bud (Leisner *et al.*, 2008; Meednu *et al.*, 2008). In the present study, we show that two proteins from the dynein pathway, Bik1p and Pac1p, interact with SUMO and several proteins from the SUMO conjugating and processing system. We also show that the SUMO protease Ulp1p controls the amount of shifted Pac1p. Both Bik1p and Pac1p also interact with the STUbL enzyme dimer Nis1p–Ris1p. Taken together, our findings suggest that these microtubule-associated proteins may be regulated by the SUMO signal transduction system. This could have implications for the regulation of CLIP-170 and Lis1 family members in other systems.

RESULTS

Both Bik1p and Pac1p interact with the yeast SUMO Smt3p

Bik1p interacts physically with Kar9p, and Kar9p is sumoylated (Moore *et al.*, 2006; Leisner *et al.*, 2008; Meednu *et al.*, 2008). We therefore investigated whether proteins in the dynein pathway might also be regulated by sumoylation. We tested for an interaction between Smt3p and two proteins that function in the dynein pathway, Bik1p and Pac1p, by two-hybrid analysis. As shown in Figure 1, both *BIK1* and *PAC1* interacted with *SMT3*. *BIK1* did not interact with ubiquitin encoded by *UBI4* or the small ubiquitin-related protein encoded by *URM1*. In contrast, *PAC1* also displayed a slight but consistently detectable interaction with ubiquitin. As previously reported, the kinesin motor protein encoded by *KIP2*

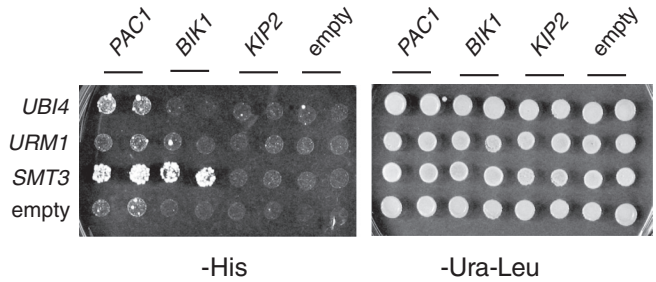

FIGURE 1: *BIK1* and *PAC1* interact with SUMO/*SMT3* by two-hybrid analysis. Yeast ubiquitin is encoded by *UBI4*, and the ubiquitin-related modifier is encoded by *URM1*. Diploid two-hybrid reporter strains were generated by crossing yRM1757/PJ65-4A containing *PAC1*-BD (pRM3604), *BIK1*-BD (pRM4924), *KIP2*-DB (pRM3595), or empty-BD (pRM1154) with yRM1756/PJ69-4A containing activation domain (AD)-*UBI4* (pRM5880), AD-*URM1* (pRM5829), AD-*SMT3* (pRM4920), or empty AD (pRM1151). Diploids were selected on SC–ura–leu and tested for interaction by growth on SC–his at 30°C for 2–3 d. Two independent diploid colonies were tested. Although *UBI4*, *URM1*, and *SMT3* are members of the same protein superfamily, only *SMT3* interacted strongly with both *BIK1* and *PAC1*.

did not interact with *SMT3* (Meednu *et al.*, 2008). Thus two-hybrid analysis of three members of the ubiquitin superfamily suggests that Bik1p and Pac1p interact strongly with Smt3p.

Bik1p and Pac1p interact with Smt3p-GG but not with Smt3p-GA

The protease Ulp1p is responsible for removing the carboxyl three amino acids from full-length Smt3p. This exposes a glycine at position 98 for conjugation to substrates. The absence of this terminal glycine precludes conjugation to target proteins (Johnson and Blobel, 1997). We therefore tested whether two mutations would abrogate the interaction. The first mutation deletes the last three amino acids (ATY), exposing the terminal diglycine motif (*SMT3*-GG). The second mutation replaces the terminal glycine with alanine (*SMT3*-GA), preventing conjugation. As shown in Figure 2A, full-length *SMT3* and the truncated form of *SMT3*, *SMT3*-GG, interacted with both *BIK1* and *PAC1*, but the *SMT3*-GA form did not. Previous work demonstrated that the *SMT3*-GA construct was expressed (Meednu *et al.*, 2008). This suggests the possibility that conjugation mediates the two-hybrid interactions of Bik1p and/or Pac1p with Smt3p.

Bik1p and Pac1p interact with other enzymes in the sumoylation pathway

Several enzymes in the sumoylation pathway facilitate the transfer of SUMO to target proteins. Kar9p was previously shown to interact with several of these by two-hybrid analysis (Meednu *et al.*, 2008). Both *BIK1* and *PAC1* interacted with *UBC9* encoding the E2 enzyme and *NFI1/SIZ2* encoding an E3 enzyme (Figure 2B). Thus two proteins from the dynein pathway interact with multiple enzymes required for sumoylation.

Bridging

A yeast two-hybrid interaction can be explained most simply by a direct binding of the bait and prey proteins. However, if a third protein binds between the bait and prey, it might activate transcription of the reporter gene and falsely suggest a direct interaction. Bik1p interacts with two other spindle-positioning proteins, Kar9p and Bim1p (Schwartz *et al.*, 1997; Moore *et al.*, 2006; Wolyniak *et al.*, 2006), both of which also interact with Smt3p (Meednu *et al.*, 2008). To investigate whether either Kar9p or Bim1p could serve as a bridge in the two-hybrid interaction between Bik1p and Smt3p, we tested the two-hybrid interaction in a reporter strain deleted for either of these genes. As shown in Figure 3, A and B, neither *KAR9* nor *BIM1* is required for the interaction. We also tested whether *PAC1* was required for the interaction of Kar9p with the SUMO pathway. As shown in Figure 3C, no difference was observed for the interaction in the *pac1*Δ and wild-type strains, suggesting that the interaction of Kar9p with sumoylation proteins does not require Pac1p.

Because Bik1p also interacts with Pac1p (Sheeman *et al.*, 2003), we next considered the simple bridging model in which Pac1p might bridge the two-hybrid interaction between Bik1p and Smt3p or vice versa. To test this, we deleted either *BIK1* or *PAC1* from the

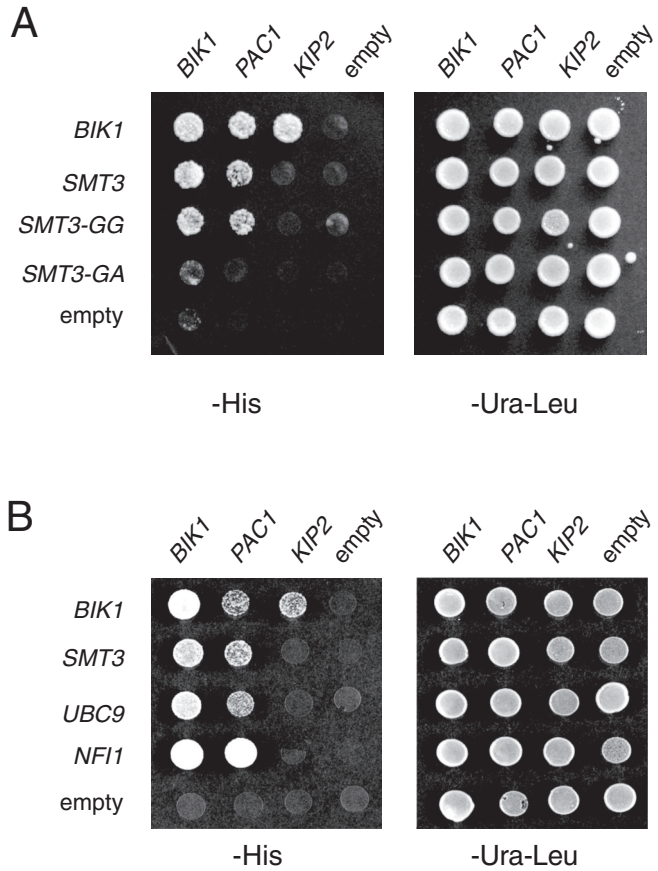

FIGURE 2: *PAC1* and *BIK1* interact with multiple genes in the sumoylation pathway by two-hybrid analysis. Two-hybrid reporter strains (yRM1757/PJ69-4A) containing *BIK1-BD* (pRM4924), *PAC1-BD* (pRM3604), *KIP2-BD* (pRM3595), or empty BD (pRM1154) were mated to reporter strains (yRM1756/ PJ69-4α) containing AD-*BIK1* (pRM2627), AD-*SMT3* (pRM4920), AD-*SMT3*-GG (pRM4382), AD-*SMT3*-GA (pRM4383), AD-*UBC9* (pRM4495), AD-*NFI1* (pRM4496), or empty AD (pRM4380) plasmids. Diploids were selected on media lacking uracil and leucine (–ura –leu) and assayed for interactions on media lacking histidine (–his). AD-*SMT*3 encodes full-length *SMT3*. In the AD-*SMT3*-GG construct, the last three amino acids have been truncated, exposing glycine 98 as the terminal amino acid. In the AD-*SMT3*-GA construct, glycine 98 has been replaced by an alanine residue. The kinesin encoded by *KIP2* transports Bik1p along microtubules (Carvalho *et al.*, 2004) and serves as an extra negative control here. The *KIP2*-BD construct is functional, as shown by the interaction between *BIK1* and *KIP2*.

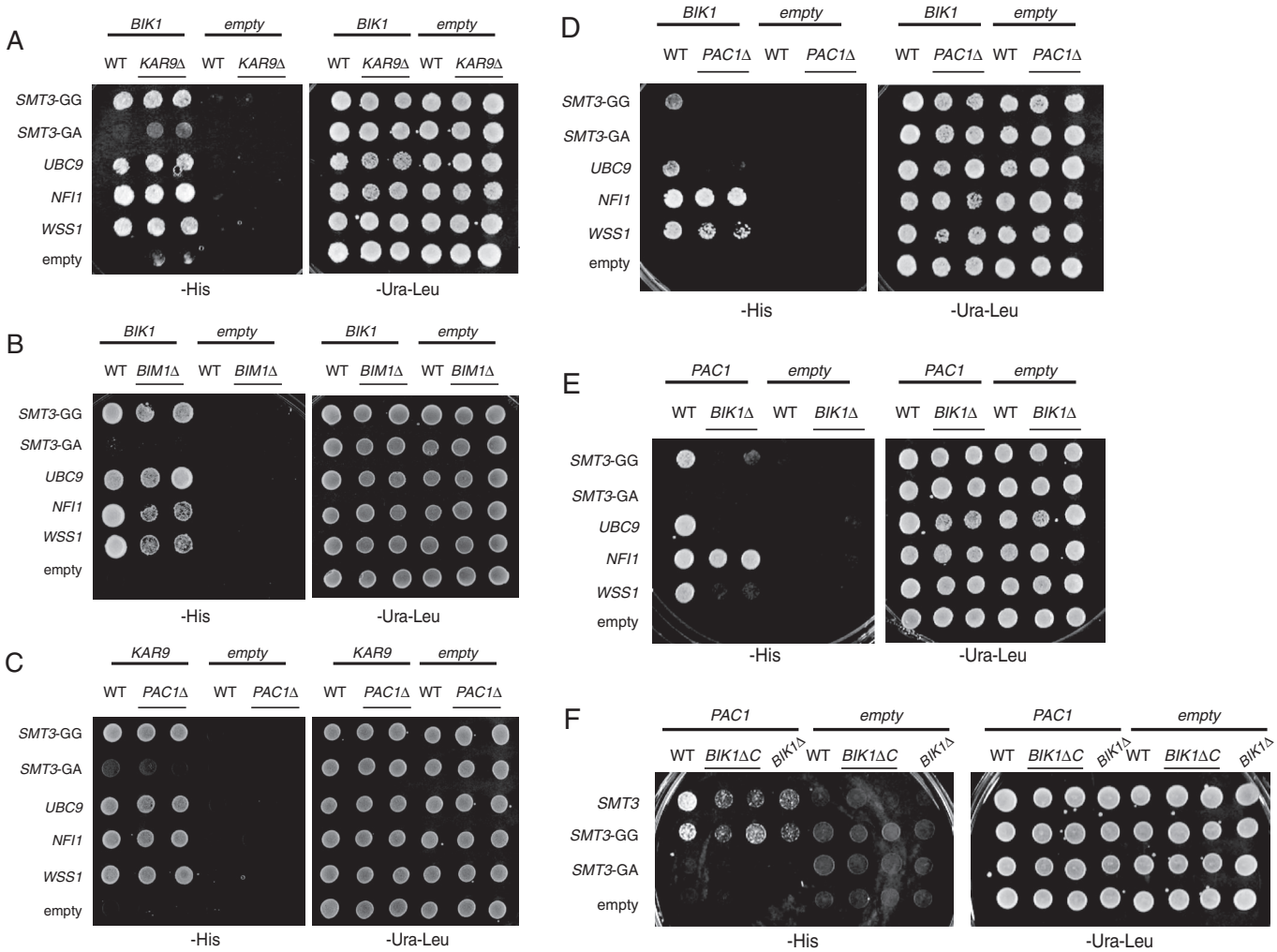

FIGURE 3: The interaction of *PAC1* with *SMT3* is enhanced by *BIK1*. (A) The interaction of *BIK1* with genes in the sumoylation pathway does not require *KAR9*. Either wild-type (yRM1757) or two-hybrid reporter strains disrupted for *KAR9* (*kar9*Δ, yRM6172) were transformed with the following constructs: *KAR9*-BD, empty BD, AD-*SMT3*, AD-*SMT3*-GG, AD-*SMT3*-GA, AD-*UBC9*, *AD-NFI*, *AD-WSS1*, or empty AD. For this assay, haploid reporter strains were used. The interactions were assayed on media lacking histidine (–his). Two colonies of *BIK1-BD* and empty BD were tested in the *kar9*Δ strain. One colony of the wild-type strain is shown for comparison. (B) The interaction of *BIK1* with genes in the sumoylation pathway does not require *BIM1*. Either the wild-type (yRM1757) or a two-hybrid reporter strain deleted for *BIM1* (yRM2057) was transformed with the plasmids described A. Two colonies of *BIK1*-BD and empty BD in *bim1*Δ were tested. (C) The *KAR9-SMT3* interaction does not require *PAC1*. The interactions of *KAR9* (pRM1493) were analyzed in either a wild-type (yRM1757) or a two-hybrid reporter strain deleted for *PAC1* (yRM6249), using the constructs and conditions described. (D) *BIK1* requires *PAC1* for interaction with *SMT3*. Either wild-type (yRM1757) or a two-hybrid reporter strain deleted for *PAC1* (yRM6249) was transformed with the indicated constructs. (E) *PAC1* requires *BIK1* for interactions with some sumoylation genes. AD-*SMT3*, AD-*SMT3*-GG, AD-*SMT3*-GA, AD-*UBC9*, AD-*NFI*, AD-*WSS1*, or empty AD was transformed into the two-hybrid reporter strain containing either *PAC1*-BD (pRM3604) or empty BD (pRM1154). Two two-hybrid reporter strains were used, wild-type strain (yRM1757) and a strain deleted for *BIK1* (yRM2258). The transformants possessing both BD and AD constructs were selected on the media lacking uracil and leucine. To test for the interaction, the transformants from both wild-type and *bik1*Δ strains were transferred to a plate with media lacking histidine (–his). One colony of the wild-type and two colonies of *bik1*Δ strain were analyzed. (F) The *PAC1-SMT3* interaction requires the cargo-binding domain of *BIK1*. Two-hybrid analysis was carried out in a haploid wild-type reporter strain (yRM1757) and an isogenic reporter strain deleted for the carboxy-terminal 40 amino acids of Bik1p, *bik1*ΔC40 (yRM6444). The cargo-binding domain is required for the Bik1p–Pac1p interaction (Sheeman *et al.*, 2003). The indicated BD fusion and AD fusions were used. Two independent colonies carrying the *bik1ΔC40* mutation are shown, as well as one colony each for the wild-type and *bik1*Δ deletion strains. The presence of the truncated Bik1p in the reporter strain was confirmed by Western blotting (unpublished data).

two-hybrid reporter strain. To our surprise, the deletion of either gene resulted in the loss of Smt3p's interaction with the reciprocal protein (Figure 3, D and E). This result is not consistent with a simple bridging model in which Bik1p bridges Pac1p and Smt3p or Pac1p bridges Bik1p and Smt3p. Instead, we speculate that the cointeraction of Bik1p with Pac1p promotes a synergistic interaction with

Smt3p. Work from the Pellman lab showed that the cargo-binding domain of Bik1p is required for its two-hybrid interaction with Pac1p (Sheeman *et al.*, 2003), suggesting that this domain may mediate the Bik1p–Pac1p interaction. To gain insight into the Pac1p– Smt3p interaction, we deleted the cargo-binding domain of Bik1p from the two-hybrid reporter strain. The absence of the cargo-binding domain greatly diminished the interaction between *PAC1* and *SMT3* (Figure 3F). This finding is consistent with Smt3p interacting with a complex of Bik1p–Pac1p. Further testing of this model will require the identification of a *PAC1* allele that does not interact with Bik1p.

Bik1p can be sumoylated in vitro and in vivo

To determine whether Bik1p is a target for sumoylation, we analyzed Bik1p in an in vitro sumoylation assay using recombinant Smt3p, Ubc9p, and Uba2p/Aos1p purified from bacteria. Bik1p isoforms were separated on SDS–PAGE and analyzed by Western blotting. Two, and possibly three, higher–molecular weight forms of Bik1p were observed in the test lane (Figure 4A, lane 1) but not in the control reactions, each lacking the respective component of the assay (lanes 2–6). These results are consistent with two possibilities. Bik1p sumoylation may occur on at least two sites or polysumoylation might occur by the formation of SUMO chains.

Sumoylation frequently occurs on a lysine residue contained within the consensus motif, ΨKxD/E, where Ψ is a hydrophobic amino acid, x is any amino acid, and D/E is an aspartic or glutamic acid. Bik1p contains one ΨKxD/E consensus sequence, WKPD at K180, and two examples of a less well-conserved KxD/E motif, GKND at K46 and KKLEE at K373 and K374. Mutation of the four lysines to arginine within these three locations, as well as a mutation in an additional lysine at position K251, did not alter the shift seen in vitro (unpublished data). This indicates that nonstandard sites may be used in the sumoylation of Bik1p.

Next we investigated whether Bik1p is sumoylated in the cell. Bik1p-hexahistidine (his6) was isolated from cells either overexpressing Smt3p or containing an empty vector. Analysis by immunoblotting revealed a shifted form of Bik1p that was present only when Smt3p was overexpressed (Figure 4B, panel 1, lanes 1 and 3). This shifted band corresponded exactly to a band revealed in an identical blot electrophoresed simultaneously and probed with polyclonal Smt3p or anti-hemagglutinin (HA). This suggests that overexpression of Smt3p results in Bik1p sumoylation.

Pac1p, but not Bik1p, is shifted by inhibition of the Ulp1p protease

Ulp1p is a SUMO protease that cleaves SUMO from sumoylated targets. Inhibition of Ulp1p is predicted to increase the sumoylation levels of Ulp1p substrates (Li and Hochstrasser, 1999, 2000). To test this idea for Bik1p, we examined the levels of shifted Bik1p in *ulp1*-ts at the nonpermissive temperature of 37°C. However, despite numerous attempts using a variety of different conditions, we could not detect higher–molecular weight forms of Bik1p in this strain, even when Smt3p was overexpressed for 2 h (unpublished data).

We next investigated whether Pac1p would shift in the *ulp1*-ts strain. Unlike Bik1p, Pac1p displayed an extensive ladder of slower-migrating forms at the nonpermissive temperature (Figure 5, compare lanes 3 and 6). When additional Smt3p expression was induced, the level of shifted forms of Pac1p was pronounced even at the permissive temperature (compare lanes 1 and 4). Few shifted forms of Pac1p were observed in a wild-type *ULP1*+ strain prepared under the same conditions (compare lanes 9 and 12). These results suggest that these higher–molecular weight forms of Pac1p are caused either directly or indirectly by the presence of SUMO.

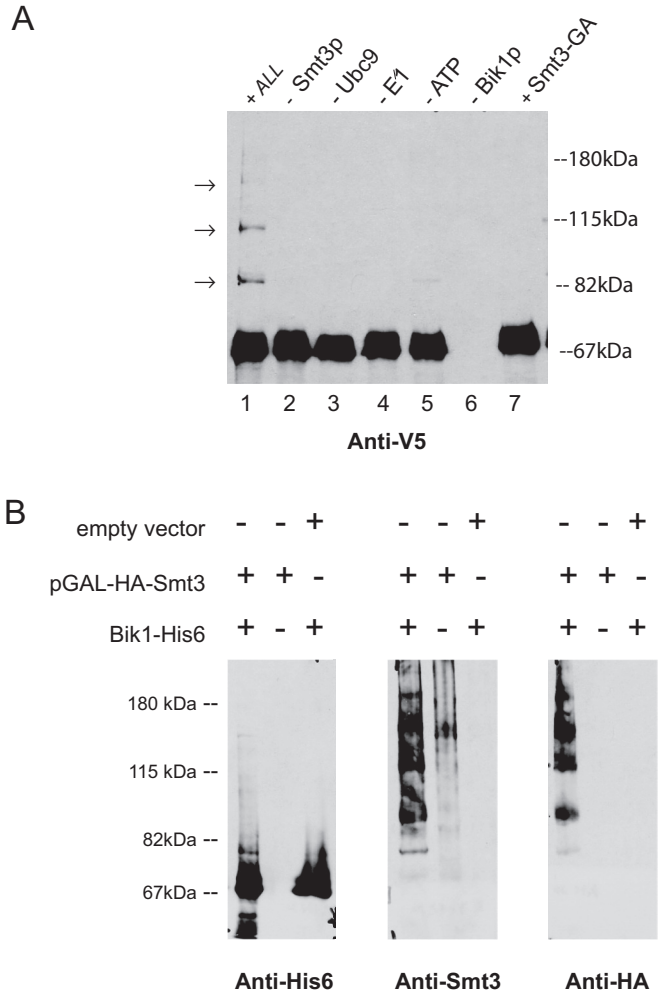

FIGURE 4: Bik1p can be conjugated by Smt3p in vitro and in vivo. (A) Bik1p can be sumoylated in vitro. Bik1p was sumoylated using an in vitro assay described previously (Meednu *et al.*, 2008). Bik1p-V5-his6 (pRM5487) was purified from yeast, and the sumoylation enzymes were purified from bacteria (see *Materials and Methods*). The indicated components were mixed and incubated at 30°C for 2 h. The reactions were prepared for SDS–PAGE and Western blot analysis using anti-V5. Half of each reaction volume was run per lane. Bik1p-V5-his6 can be conjugated by Smt3p-GG in vitro (lane 1), as indicated by the shifted band (arrow). This band was always absent in reactions lacking Smt3p-GG (lane 2), Ubc9p (lane 3), and Aos1 (lane 4). A faint band of shifted Bik1p is observed when ATP is omitted from the reaction (lane 5). Bik1p also did not shift when Smt3-GA was used in the reaction (lane 7). (B) Bik1-his6 is shifted by the overexpression of Smt3p in vivo. Bik1-his6 (pRM5487) was purified from a yeast strain (yRM3350) either expressing HA-Smt3p (pRM5251) or containing an empty vector. Identical immunoblots were probed with anti-his6, polyclonal anti-Smt3p, or anti-HA.

SUMO chaining and ubiquitination contribute to Pac1p shifts

To determine whether the shift of Pac1p mobility was due to multiple monosumoylation events or polysumoylation, we mutated to arginine the three lysine residues within Smt3p that are required for chain formation (Bylebyl *et al.*, 2003). Analysis of the Pac1p shift using *SMT3*-K11R-K15R-K19R revealed that the higher–molecular weight smears were greatly reduced (Figure 6A, compare lanes 1 and 7). This suggests that polysumoylation contributes to the higher–molecular weight shifts of Pac1p.

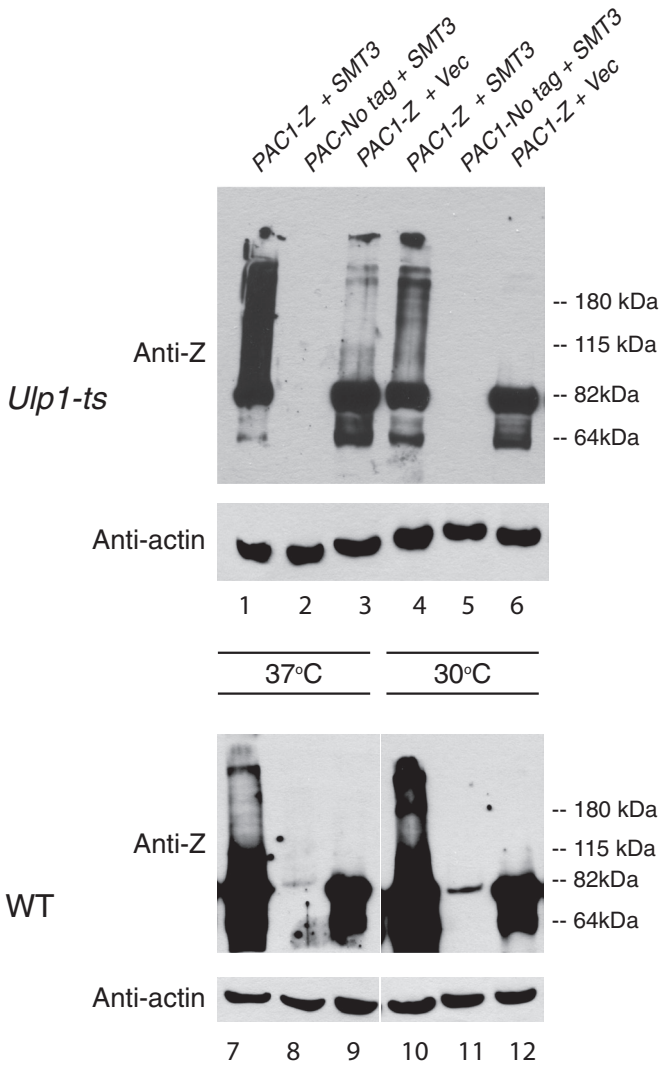

FIGURE 5: Inhibition of the SUMO protease Ulp1p shifts Pac1p in vivo. Whole-cell extracts were prepared as described in *Materials and Methods* from a *ulp1*-ts strain (yRM8139; top) or a wild-type strain (yRM3403; bottom) expressing Pac1p-4Z on a CEN plasmid under the control of its own promoter (pRM3573) and Smt3p under the control of a copper-inducible promoter (pRM8023). The appropriate empty CEN vectors allowed all strains to be grown in SC–ura–leu. Smt3p was induced for 2 h at the indicated temperatures. The white line in the Western blot of at the bottom indicates that this blot was rearranged to match the layout of the top. Lanes 7–9 are directly comparable to lanes 10–12. Rabbit anti-human immunoglobulin–horseradish peroxidase (IgG-HRP) antibody (Santa Cruz Biotechnology) was used to detect Pac1p-4Z. The stacker gel in this experiment was removed before immunoblotting.

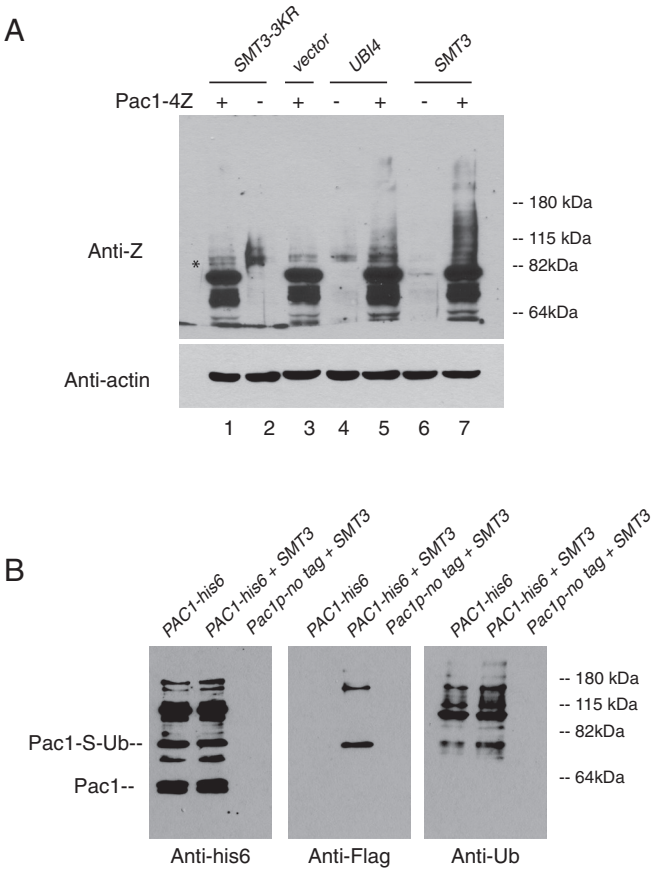

FIGURE 6: Both SUMO chaining and ubiquitination contribute to the higher–molecular weight forms of Pac1p. (A) The laddering effect of Pac1p is due to SUMO chain formation and ubiquitination. Plasmids expressing *SMT3gg*-his6-HA (pRM8023), *smt3gg-K11, 15, 19*R-his6-HA (*smt3*-3KR, pRM8836), or an empty *URA3* vector (pRM2205) were transformed into *ULP1*-TS strain (yRM8139). To induce the *SMT3* constructs, strains were treated with 1 mM $CuSO_4$ for 2 h at 37°C. Whole-cell lysates were prepared for each strain, as described in *Materials and Methods*, and analyzed by 8% SDS–PAGE. Membrane was immunoblotted with rabbit anti-human IgG-HRP antibody (Santa Cruz Biotechnology) to identify Pac1p-4Z. Pac1p laddering is due in part to ubiquitination. Plasmids expressing *UBI4*-myc (Ub, pRM8388) or an empty *URA3*-vector (pRM2205) were transformed into a *ULP1*-TS strain (yRM8139). Asterisk denotes a background band. *UBI4* encodes ubiquitin (Ub). In this experiment, the stacker gel was removed before immunoblotting. (B) SUMO and ubiquitin copurify with Pac1p. Pac1p-his6 (pRM9232) was integrated at the genomic *PAC1* locus of *ulp1*-TS strains. The resulting Pac1p-his6 is expressed at endogenous levels under its own promoter. These strains contained either FLAG-Smt3-GG (yRM8011) or an empty vector. The strains were shifted to 37°C for 2 h, and whole-cell extracts were prepared (14 ml at 1.4 mg/ml). Pac1p-his6 was purified from the cell extract using nickel beads, as described in *Materials and Methods*. The his6 epitope was detected using mouse anti-his6, Smt3p with mouse anti-FLAG, and ubiquitin with mouse anti-ubiquitin, as described in *Material and Methods*.

Although Pac1p shifts in a Ulp1p-dependent manner, this does not eliminate the possibility that other modifications might also play a role. To test whether ubiquitin might also contribute to the shifts, we transformed a ubiquitin plasmid into the *ulp1*-ts strain containing Pac1p. This resulted in some higher–molecular weight bands of Pac1p, but the shift was not as extensive as when *SMT3* was present (Figure 6A, compare lanes 5 and 7). Taken together, these findings suggest that both polysumoylation and ubiquitination contribute to the higher–molecular weight forms of Pac1p.

SUMO and ubiquitin copurify with Pac1p

To further explore whether the Pac1p shifts observed in the *ulp1*-ts strain are due to SUMO conjugation, we pulled down Pac1p-his6 on nickel beads from a yeast strain expressing Smt3-GG, the processed form (see *Materials and Methods*). The unmodified form of Pac1p appeared as a doublet, perhaps indicating that Pac1p is phosphorylated. Four to seven higher–molecular weight forms of Pac1p were

observed after the pull down (Figure 6B). Two of these cross-reacted with anti-FLAG, which marks SUMO, indicating that these two shifts are due to SUMO conjugation of Pac1p (Pac1-S). To investigate whether the nonreactive bands might be due to another modification, we probed a third identical panel with anti-ubiquitin. This revealed that both of the anti-FLAG bands also reacted with anti-ubiquitin. These findings suggest that Pac1p is altered by at least two types of posttranslational modification—ubiquitin and SUMO. Moreover, two of the bands that were nonreactive with anti-FLAG did react with anti-ubiquitin. This suggests the possibility that some forms of Pac1p may contain only ubiquitin.

She1p and Kar9p inhibit the modification of Pac1p

Pac1p functions in the dynein pathway to help recruit dynein to the plus end of the cytoplasmic microtubule before its off-loading to the cortex (Li *et al.*, 2005; Markus *et al.*, 2011). To determine whether the integrity of the dynein pathway affects the modification of Pac1p, we examined Pac1p in several deletion strains related to various aspects of dynein function. *KIP2* encodes a kinesin that transports Bik1p to the plus end of the microtubule (Carvalho *et al.*, 2004). *DYN1* encodes the dynein motor itself, and *JNM1* encodes a component of the dynactin complex (McMillan and Tatchell, 1994). *NDL1* encodes a NudE homologue that helps localize dynein to the plus end (Li *et al.*, 2005; Moore *et al.*, 2008). *NUM1* encodes a cortical protein important for the off-loading of dynein (Heil-Chapdelaine *et al.*, 2000; Farkasovsky and Küntzel, 2001). In a *NUM1*-deleted strain, the off-loading of dynein to the cortex is blocked (Lee *et al.*, 2005). In each of these delete strains, little or no additional shift in Pac1p was observed (Figure 7A). We next tested a strain deleted for *SHE1*, which encodes a regulator of the dynein–dynactin interaction (Woodruff *et al.*, 2009; Markus *et al.*, 2011; Bergman *et al.*, 2012). Surprisingly, Pac1p shifted significantly. This finding suggests that She1p is a novel inhibitor of Pac1p modifications.

To investigate further, we analyzed the shift of Pac1p in strains deleted for members of the Kar9 pathway, which orients the cytoplasmic microtubule into the bud (Miller and Rose, 1998; Miller *et al.*, 1998). *BIM1* encodes an EB1 homologue and microtubule-binding protein that binds Kar9p (Lee *et al.*, 2000; Miller *et al.*, 2000). *KAR9* encodes a linker protein between *BIM1* and the type V myosin Myo2p (Miller *et al.*, 1999, 2000; Hwang *et al.*, 2003). The movement of Myo2p along an actin cable serves to guide the attached microtubule into the bud (Beach *et al.*, 2000; Yin *et al.*, 2000). *KIP3* encodes a type 8 kinesin in the Kar9 pathway that controls microtubule length (Miller *et al.*, 1998; Su *et al.*, 2011). Surprisingly, an increase was observed in the shift of Pac1p (Figure 7B). This suggests that the Kar9 pathway inhibits the interaction of Pac1p with Smt3p and/or ubiquitin. This is consistent with earlier findings that Bim1p interacts with She1p by two-hybrid analysis (Wong *et al.*, 2007).

The results from Figure 7A could in theory be explained by She1p blocking the addition of either SUMO or ubiquitin. To distinguish between these two possible mechanisms, Pac1p-his6 from *she1*Δ and wild-type strains was enriched on nickel beads, and identical immunoblots were analyzed by probing with anti-SUMO or anti-ubiquitin (Figure 7C and 7C′). Consistently, larger amounts of Pac1p were purified from equal amounts of protein extract in the *she1*Δ and *ulp*1 strains than from wild type. This indicates, but does not prove, that these modifications may affect the steady-state stability of Pac1p. Furthermore, when anti-SUMO was used, nearly identical amounts of reactivity were observed in the *she1*Δ and wild-type (WT) strains. This suggests that increased sumoylation is not the cause of the higher–molecular weight forms of Pac1p observed in the *she1*Δ strains. However, when probed with anti-ubiquitin, additional reactivity was observed. This suggests that the addition of ubiquitin contributes to the higher forms of Pac1p seen in *she1*Δ. How She1p blocks this addition of ubiquitin remains an active avenue of further investigation.

PAC1 and *BIK1* interact with *WSS1*, a SUMO isopeptidase, and *NIS1-RIS1*, a STUbL enzyme complex

SUMO-targeted ubiquitin ligases can link the sumoylation and ubiquitination pathways by ubiquitinating proteins already modified by sumoylation, thus promoting their removal by the proteasome (Sun *et al.*, 2007; Uzunova *et al.*, 2007; Xie *et al.*, 2007). Wss1p was originally identified as a weak suppressor of a temperature-sensitive allele of Smt3p (Biggins *et al.*, 2001). Recently Wss1p was shown to be a SUMO-dependent isopeptidase that promotes the targeting of SUMO-conjugated proteins to the proteasome (Mullen *et al.*, 2010). Our previous work showed that Kar9p interacted with both *WSS1* and the complex encoded by the *RIS1 and NIS1* genes (Meednu *et al.*, 2008). Therefore we tested whether Bik1p or Pac1p would also interact with the STUbL enzyme Nis1p–Ris1p and the SUMO iospeptidase Wss1p by two-hybrid analysis. Indeed, both did (Figure 8A).

These findings suggest the possibility that the shifts of Pac1p may be modulated by a STUbL enzyme and the proteasome. If such a model is correct, higher–molecular weight forms of Pac1p should be present when the STUbL is absent from the cell. To assess this, we examined the levels of shifted Pac1p in strains deleted for *WSS1*, *RIS1–NIS1*, or another STUbL enzyme complex, *SLX5–SLX8*. As shown in Figure 8B, the shifted forms of Pac1p were significantly increased in strains lacking the catalytic subunit of the STUbL Ris1p and the Wss1p isopeptidase. No difference in the shift pattern was detected in strains deleted for *SLX5* or *SLX8*, suggesting that the shift in Pac1p is specific to only one of the two STUbLs present in yeast. These findings also indicate that Pac1p may be a substrate of the Ris1p STUbL enzyme. Further work is required to determine whether STUbLs can exert an effect on the unmodified levels and/or steady-state levels of Pac1p.

Together these results suggest the possibility that ubiquitination of Pac1p by the Nis1p–Ris1p STUbL modulates the turnover of sumoylated Pac1p. Wss1p may aid in the delivery to or the processing of Pac1p by the proteasome. To investigate this, we treated cells with a proteasome inhibitor, MG132. Because yeast cells can pump the MG132 drug out of the cell, this study was conducted in a strain deleted for *PDR5*, which encodes a drug pump (Fleming *et al.*, 2002). As seen Figure 8C, higher–molecular weight forms of Pac1p are present in the stacker portion of the gel when the proteasome is inhibited with MG132 (compare lanes 2 and 3, asterisk). Taken together, these results suggest that the STUbL enzyme Nis1p–Ris1p controls the levels of posttranslational modifications attached to Pac1p.

DISCUSSION

In this study, we show that the Lis1 homologue Pac1p and the CLIP-170 homologue Bik1p interact with SUMO and several enzymes of the SUMO modification pathway. Pac1p also interacts with ubiquitin. We also show that Bik1p and Pac1p interact with the STUbL enzyme Nis1p–Ris1p and that the posttranslational modifications of Pac1p are controlled by this STUbL enzyme. In summary, we showed that two new classes of conserved microtubule-associated proteins interact with and are likely to be regulated by SUMO. With this work, four different spindle-positioning proteins have now been shown to interact with SUMO.

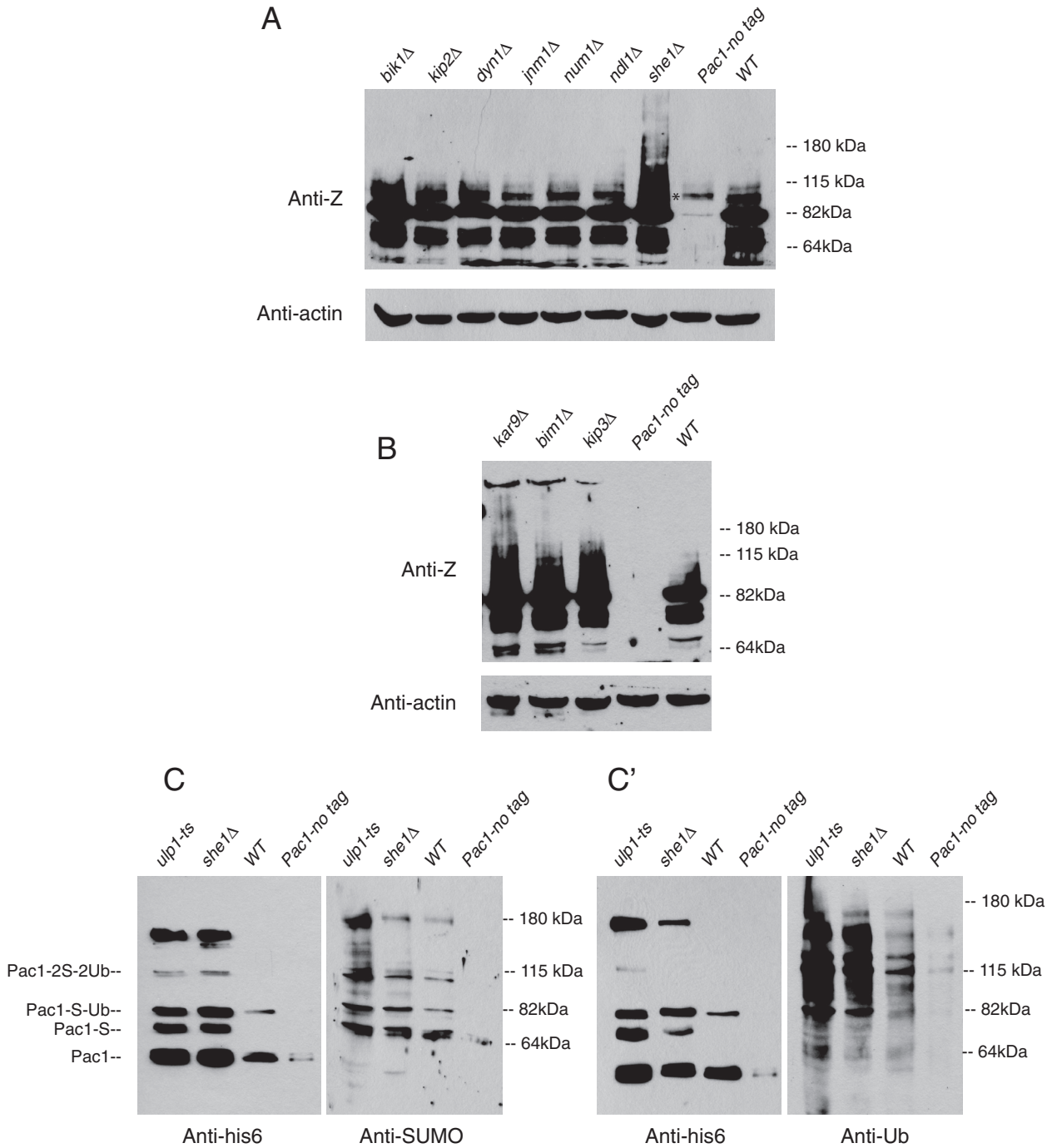

FIGURE 7: She1p and Kar9p inhibit Pac1p modification. (A) Strains deleted for *SHE1* in the dynein pathway display higher–molecular weight forms of Pac1p. Strains deleted for *BIK1* (yRM3350), *KIP2* (yRM3355), *DYN1* (yRM3346), *JNM1* (yRM7116), *NUM1* (yRM3137), *NDL1* (yRM9050), *SHE1* (yRM9051), and WT (yRM3403) were transformed with plasmids expressing Pac1p-4z (pRM3573) or nontagged Pac1p (pRM3574). Whole-cell lysates were prepared from each strain, as described in *Materials and Methods*, and analyzed by 8% SDS–PAGE. Rabbit anti-human IgG-HRP antibody (Santa Cruz Biotechnology) was used for detection of Pac1p-4z. An asterisk is used to denote a background band revealed in the Pac1-no-tag control lane. (B) Kar9p inhibits the shift of Pac1p. Whole-cell extracts prepared from strains deleted for *KAR9* (yRM3404), *BIM1* (yRM3352), *KIP3* (yRM3140), and WT (yRM3403) were transformed with plasmids expressing Pac1p-4z (pRM3573) or nontagged Pac1p (in WT; pRM3574). Rabbit anti-human IgG-HRP antibody was used for detection. (C) She1p inhibits the ubiquitination of Pac1p. Pac1p was tagged with his6 by integrating the C-terminally tagged construct ΔN-Pac1-his6 (pRM9232) to produce Pac1p-his6 expressed at endogenous levels under its own promoter. Pac1p-his6 was purified on nickel beads from equal amounts of protein extract (14 ml at 1.6 mg/ml) prepared from the indicated strains, *ulp1*-ts (yRM8139), *she1*Δ (yRM9051), and WT (yRM3403), grown at 30°C. Mouse anti-his6 was used to detect the his6 epitope. Smt3p was detected with rabbit anti-smt3, and ubiquitin with mouse anti-ubiquitin, as described in *Materials and Methods*. Note that the pattern of shifted Pac1p appears slightly different in this pull down than in Figure 6B because the pull downs were performed at different temperatures.

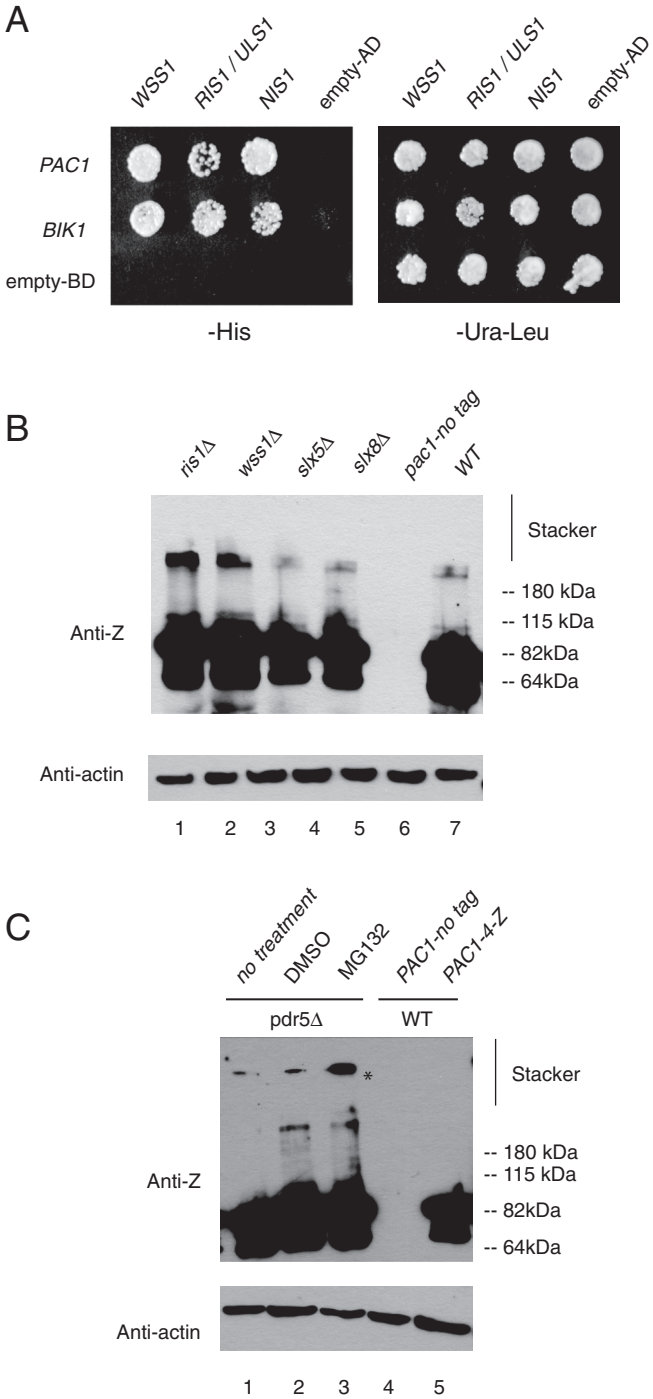

FIGURE 8: A STUbL complex alters Pac1p modification. (A) Pac1p interacts with the STUbL enzyme Nis1p–Ris1p and the SUMO isopeptidase Wss1p. *BIK1-BD* and *PAC1-BD* were tested for interaction against the SUMO peptidase encoded by *WSS1* (pRM4597) and the STUbL complex composed of *RIS1* (pRM4596), *NIS1 (pRM4595),* or empty AD (pRM4380). Diploids were selected on media lacking uracil and leucine (–ura–leu) and assayed for interactions on media lacking histidine (–his). (B) Strains deleted for the Ris1p STUbL display higher-molecular weight forms of Pac1p. Strains deleted for *RIS1* (yRM8574), *WSS1* (yRM4636), *SLX5* (yRM7887), *SLX8* (yRM7888), and WT (yRM3403) were transformed with a plasmid expressing Pac1-4Z (pRM3573) and nontagged Pac1p (pRM3574). Whole-cell extracts from the yeast strains were resolved by 8% SDS–PAGE and analyzed by anti-Z immunoblotting. An increase in SUMO conjugate levels can be seen in the *ris1*Δ and *wss1*Δ mutant cells compared with WT. (C) Inhibition of the proteasome results in accumulation of higher–molecular weight forms of Pac1p. Plasmids expressing Pac1p-4z (pRM3573) or nontagged Pac1p (pRM3574) were transformed into WT (yRM3403) and *pdr5*Δ (yRM8571) strains. Cells were treated at 30°C for 2 h with dimethyl sulfoxide (DMSO) alone or 50 μM MG132 (dissolved in DMSO) or not treated, as indicated. Whole-cell lysates were prepared as described in *Materials and Methods* and analyzed by 8% SDS–PAGE for anti–Pac1p-4z.

We use several different approaches to show that Pac1p interacts with SUMO. First, the two-hybrid analysis shows that Pac1p interacts with the GG but not the GA form of SUMO. This implies, but does not prove, that the interaction is due to a conjugation event. Second, inhibition of the SUMO-specific protease Ulp1p results in higher–molecular weight forms of Pac1p. This suggests that at least some of the higher–molecular weight forms are caused by SUMO moieties conjugated onto Pac1p. Indeed, when an additional SUMO is provided to the cell on a plasmid, a similar but significantly stronger banding pattern is seen. The higher–molecular weight forms of Pac1p are likely due to poly-SUMO chain formation, because the shifts are greatly diminished by the presence of a nonchainable form of SUMO. Pac1p also interacts with the STUbL enzyme Nis1p–Ris1p, an enzyme that recognizes sumoylated proteins. A pull-down assay with Pac1p suggests that the higher–molecular weight forms of Pac1p contain covalently attached SUMO. Taken together, these experiments strongly support our assertion that Pac1p is conjugated with SUMO.

Our conclusion that the interaction of Pac1p with SUMO may also involve cross-talk with ubiquitin is derived from four lines of investigation. First, we see a weak two-hybrid interaction of *PAC1* with ubiquitin, encoded by *UBI4*. Pac1p is also likely to be modified by ubiquitin, because higher–molecular weight forms of Pac1p can be generated by the inclusion of a plasmid encoding ubiquitin. Third, ubiquitin copurifies with Pac1p, with some shifted bands cross-reacting with both anti-ubiquitin and anti-SUMO. Fourth, Pac1p interacts with the ubiquitin ligase complex, Ris1p–Nis1p, and deletion of *RIS1* results in higher–molecular weight forms of Pac1p. This is consistent with our previous finding that Kar9p and Bim1p interact with Wss1p and the same STUbL (Meednu *et al.*, 2008). The finding that multiple spindle-positioning proteins interact with SUMO suggests the possibility that the Nis1p–Ris1p STUbL enzyme may regulate spindle positioning.

This is the first report that a member of the Lis1 family or a member of the CAP-Gly domain family is modified by SUMO. This has significant implications for the regulation of these two classes of microtubule-associated proteins, which are widely conserved across evolution. Prior to this work, only four other microtubule-associated proteins have been shown to be modified by SUMO. These are Tau (Dorval and Fraser, 2006; Takahashi *et al.*, 2008), Ndc80p (Montpetit *et al.*, 2006), CENP-E (Zhang *et al.*, 2008), and Kar9p (Leisner *et al.*, 2008; Meednu *et al.*, 2008). We also showed that the EB1 homologue Bim1p interacts with SUMO by two-hybrid analysis, but details of this interaction and whether this interaction represents an actual conjugation by SUMO remain to be elucidated (Meednu *et al.*, 2008). Our findings are also notable in that Pac1p was not found in any of the previous genome-wide screens for sumoylated proteins (Zhou *et al.*, 2004), indicating that yeast SUMO-ome may not yet be complete.

Previous reports suggest that the Kar9 pathway for spindle positioning is regulated by sumoylation (Leisner *et al.*, 2008; Meednu *et al.*, 2008). Leisner *et al.* (2008) showed that the spindle-positioning defect seen in the nonsumoylatable *kar9*-4K-R mutant is not as severe as the *smt3-331* defect. This suggests that there are

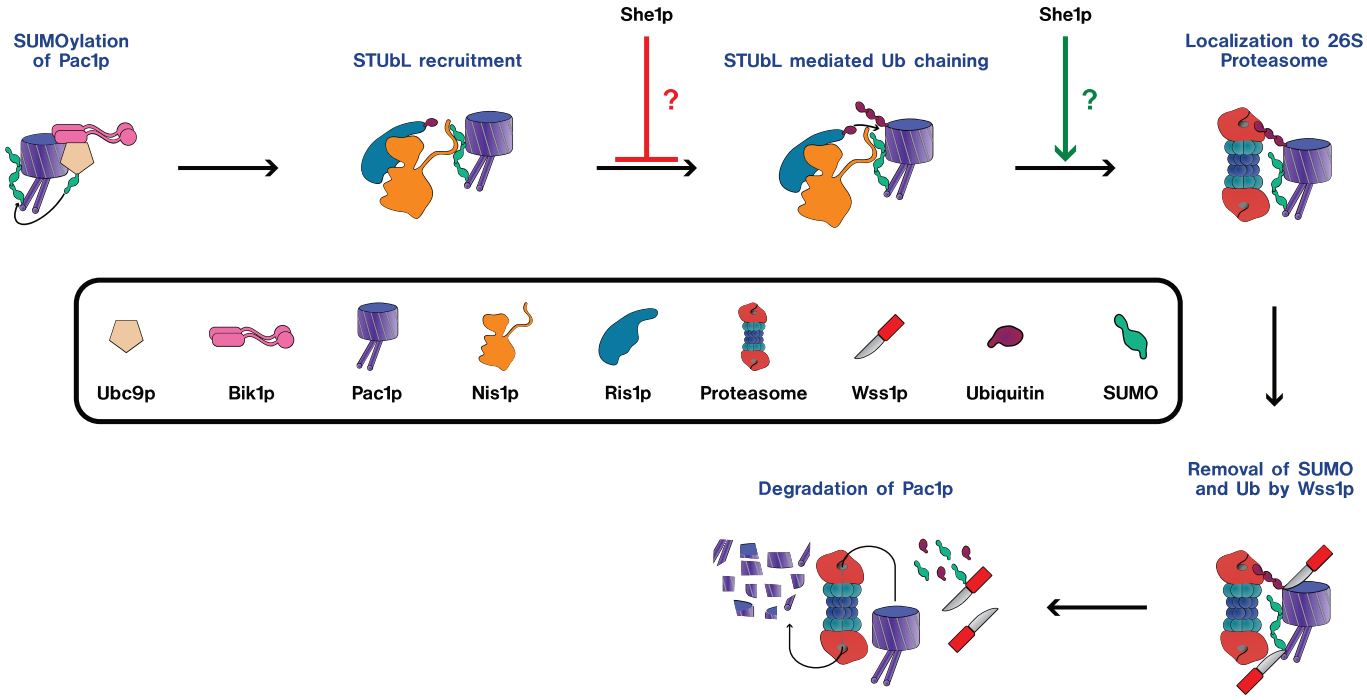

FIGURE 9: Model of Bik1p and Pac1p interaction with a STUbL and Wss1p.

additional proteins required for spindle positioning that are also regulated by sumoylation. The findings reported here suggest that Pac1p and/or Bik1p may be the additional protein(s). Thus it is likely that both spindle-positioning pathways in yeast may be regulated by sumoylation. Future studies should elucidate whether this signal transduction system regulates each pathway separately or whether they are coordinated as a unit.

Our two-hybrid bridging data suggest that Bik1p and Pac1p are both required for their mutual interaction with SUMO and Ubc9p. However, this relationship was not observed in the in vitro shift assay using purified Bik1p. This suggests that Pac1p is not required for the in vitro shift of Bik1p. The apparent discrepancy between the two assays may be reconciled if Pac1p enhances the sumoylation of Bik1p. Consistent with this idea is our observation that Bik1p from partially fractionated extracts shifted better in vitro than purified Bik1p (unpublished data). Future work to test this hypothesis is warranted.

Furthermore, the bridging data in which Bik1p is required for the Wss1p's interaction with Pac1p (but not vice versa) suggest that Bik1p may recruit Wss1p to the Bik1p–Pac1p complex (Figure 9). This is consistent with our data that Pac1p is hypermodified in a strain deleted for *WSS1*.

We propose a model in which Pac1p sumoylation and its subsequent interaction with a STUbL promotes its degradation via the proteasome (Figure 9). In this model, Wss1p aids in debranching of polysumoylated and/or polyubiquitinated Pac1p at the proteasome. Because She1p regulates the association of dynactin with dynein (Woodruff *et al.*, 2009), our finding that She1p inhibits the modification of Pac1p suggests the possibility that sumoylation and/or ubiquitination may regulate the interaction of dynein with its accessory proteins. In our model, She1 protein blocks the ubiquitination of Pac1p. It might also promote the proteasome-mediated degradation of modified Pac1p. We speculate that the sumoylation of Pac1p may play a role in regulating the off-loading of dynein to the cell cortex.

Several questions remain. SUMO is induced by various types of cellular stress. Future work is needed to show whether the sumoylation of these key classes of microtubule-associated proteins represents a mechanism by which microtubule-dependent processes are inactivated under conditions that are inhospitable for cell division. Furthermore, both Pac1p and Lis1 serve as adaptors of dynein. Lis1 regulates some but not all of dynein's functions. It remains to be determined whether Lis1, either in neurons or epithelial cells, is also sumoylated. Our findings have implications for how this class of dynein adaptor may regulate dynein function. Because dynein is critical to a number of fundamental processes important for life, it will be important for future studies to elucidate the widespread utility of this modification in other systems.

MATERIALS AND METHODS

Two-hybrid analysis

Two-hybrid analysis was carried out as previously described (Moore and Miller, 2007; Moore *et al.*, 2008 ; Meednu *et al.*, 2008). All analysis was carried out after 2–3 d of growth at 30°C. *PAC1*-DNA-binding domain (BD) was constructed by amplifying *PAC1* from pRM3574/pJG13 with *Bam*HI and *Pst*I ends and ligating it into pRM1154/GBDU-C1 cleaved with the same enzymes. This generated pRM3604 (Table 1), which was sequenced to confirm the absence of unintended PCR errors.

PAC1 was deleted from the two-hybrid reporter strain yRM1757 by one-step gene replacement using a PCR product derived from genomic DNA from the *pac1*Δ::*KAN*^{R} deletion strain (Open Biosystems, Huntsville, AL), yRM3138. This generated pRM6249, which was confirmed by PCR.

BIK1-BD was generated by excising the *BIK1* coding fragment with *Eco*RI and *Bam*HI from pRM2627 and ligating it to the BD vector, pRM1156, cut with the same enzymes. This generated pRM4924.

Yeast strain	Genotype/comments	Source
yRM1756/PJ69-4α	*MATα trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	James *et al.* (1996)
yRM1757/PJ69-4A	*MAT***a** *trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	James *et al.* (1996)
yRM2057	*MAT***a** *bim1Δ::KAN trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	Miller *et al.* (2000)
yRM2258	*MAT***a** *bik1Δ::TRP trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	Moore *et al.* (2006)
yRM3137	*MAT***a** *num1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3138	*MAT***a** *pac1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3140	*MAT***a** *kip3Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3346	*MAT***a** *dyn1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3350	*MAT***a** *bik1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3352	*MAT***a** *bim1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3355	*MAT***a** *kip2Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM3403	*MAT***a** *his3Δ1 leu2Δ1 met15Δ ura3Δ*	This study
yRM3404	*MAT***a** *kar9Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM4636	*MAT***a** *wss1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM6172	*MAT***a** *kar9Δ::KAN trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	Meednu *et al.* (2008)
yRM6249	*MAT***a** *pac1Δ::KAN*^{R} *trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	This study
yRM6444	*MATα bik1ΔC40::TRP1*$^{+}$ *trp1-901 leu2-3 leu2-112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	This study
yRM7205	*MAT***a** *bik1Δ::KAN*^{R} *leu2Δ ura3Δ his3Δ met15Δ {pRM5487 = pGAL-BIK1-his6* Amp*R URA3}*	This study
	{pRM5251 = pGAL-3HA-FLAG-SMT3 Amp^{R} *HIS3}*	
yRM7116	*MAT***a** *jnm1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM7213	*MAT***a** *bik1Δ::KAN*^{R} *leu2Δ ura3Δ his3Δ met15Δ*	This study
	{pRM5251 = pGAL-3HA-FLAG-SMT3 Amp^{R} *HIS3}*	
yRM7600	*MAT***a** *bik1Δ::KAN*^{R} *leu2Δ ura3Δ his3Δ met15Δ*	This study
	{pRM5487 = pGAL-BIK1-his6 Amp*R URA3}*	
	{pRM4878 = pESC HIS3 with c-myc eliminated}	
yRM7887	*MAT***a** *slx5Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM7888	*MAT***a** *slx8Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM8011/YOK428	*MATα ulp1::KAN ulp1*^{ts}*-NAT-TRP his3Δ1 leu2Δ ura3Δ*	Elmore *et al.* (2011)
	{pRS425 GPD flag-SMT3-gg LEU2 2μ *Amp*^{R}*}*	
yRM8012/YOK430	*MATα ulp1::KAN ulp1*^{ts}*-NAT-TRP his3Δ1 leu2Δ ura3Δ*	Elmore *et al.* (2011)
	{pRS425 GPD-SMT3-gg LEU2 2μ *Amp*^{R}*}*	
yRM8139	*MATαulp1::KAN ulp1*^{ts}*-NAT-TRP his3Δ1 leu2Δ ura3Δ*	This study
yRM8571	*MAT***a** *pdr5Δ::KAN his3Δ leu2 Δ met15 Δ ura3 Δ*	Open Biosystems
yRM8574	*MAT***a** *ris1Δ::KAN his3Δ leu2 Δ met15 Δ ura3 Δ*	Open Biosystems
yRM9050	*MAT***a** *ndl1Δ::KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM9051	*MAT***a** *she1Δ:: KAN his3Δ leu2Δ met15Δ ura3Δ*	Open Biosystems
yRM9248	*MATα ulp1::KAN ulp1*^{ts}*-NAT-TRP his3Δ1 leu2Δ ura3Δ PAC1::his6:: HIS3+*	This study
	{pRS524 GPD-FLAG-Smt3-gg-LEU2 2 μ Amp^{R}*}*	

TABLE 1: Strains and plasmids used in this study.

Continues

Plasmid	Genotype/comments	Source
pRM1151	*GAD empty LEU2* 2μ Amp^R	James *et al.* (1996)
pRM1154	*GBDU* empty *URA3* 2μ Amp^R	James *et al.* (1996)
pRM1493	*GBDU-KAR9 URA3* 2μ Amp^R	Miller *et al.* (2000)
pRM2200/pRS415	*LEU2 CEN* Amp^R	Sikorski and Hieter (1989)
pRM2205/pRS426	*URA3* 2μ Amp^R	Sikorski and Hieter (1989)
pRM2627	*GAD-BIK1 LEU2* 2μ Amp^R	Moore *et al.* (2006)
pRM2908	*pGAL URA3* 2μ Amp^R	This study
pRM3573/pJG423	*PAC1-4Z CEN6 LEU2* Amp^R	Sheeman *et al.* (2003)
pRM3574/pJG213	*PAC1 CEN6 LEU2* Amp^R	Sheeman *et al.* (2003)
pRM3595	*GBDU-KIP2 URA3* 2μ Amp^R	Meednu *et al.* (2008)
pRM3604	*GBDU-PAC1 URA3* 2μ Amp^R	This study
pRM4380	*GAD424 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4382/pLAJ20	*GAD-SMT3-GG LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4383/pLAJ21	*GAD-SMT3-GA LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4495	*GAD-UBC9 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4496	*GAD-NFI1 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4595	*GAD-NIS1 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4596	*GAD-RIS1/ ULS1 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4597	*GAD-WSS1 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4920/pLAJ19	*GAD-SMT3 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM4924	*GBDU-BIK1 URA3* 2μ Amp^R	This study
pRM5169	*his6-UBC9* Amp^R	Johnson and Blobel (1997)
pRM5251	*pGAL-3HA-FLAG-SMT3 HIS3* Amp^R	This study
pRM5487	*pGAL-BiK1-V5-his6 URA3* Amp^R	This study
pRM5829	*GAD-URM1 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM5880.	*GAD-UBI4 LEU2* 2μ Amp^R	Meednu *et al.* (2008)
pRM6713	*his6-S-tag-Smt3p-gg Kan^R*	This study
pRM6760	*GST-AOS1/UBA2* 2μ Amp^R	Bencsath *et al.* (2002)
pRM8023	*Cu^{2+} promoter - HA-his6-SMT3-gg URA3* 2μ Amp^R	Elmore *et al.* (2011)
pRM8388/pUB175	*Cu^{2+} promoter - myc-UBI4 URA3* 2μ Amp^R	Finley *et al.* (1989)
pRM8836	*Cu2+ promoter - HA- his6-smt3-gg-K11R-K15R-K19R URA3* 2μ Amp^R	This study
pRM9232	*ΔN-Pac1-his6 HIS3 YIP* Amp^R	This study

TABLE 1: Strains and plasmids used in this study. Continued

In vitro sumoylation

Bik1p was sumoylated using a previously described protocol (Meednu *et al.*, 2008). Strains expressing Bik1p-V5-his6 were grown at 30°C in synthetic complete (SC) media lacking uracil (–ura) containing 2% sucrose, and Bik1p expression was induced by the addition of 2% galactose for 2 h. To prepare yeast extracts, cells were washed once with water, resuspended in cold 1× binding buffer (20 mM Tris-HCl, pH 7.9, 5 mM imidazole, 0.5 M NaCl) containing 0.2% protease inhibitor P8215 (Sigma-Aldrich, St. Louis, MO) and 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich). Cells were lysed by vortexing with glass beads. Crude extracts were clarified by centrifugation at 13,000 rpm for 30 min, applied to nickel resin (Novagen/EMD, Darmstadt, Germany), and washed with 25 column volumes of 1× binding buffer containing 50 mM imidazole. Bound protein was eluted with 1× binding buffer containing 300 mM imidazole and dialyzed overnight into sumoylation assay buffer (50 mM Tris, pH 7.6, 5 mM $MgCl_2$, 15% glycerol) at 4°C.

Sumoylation enzymes were purified from bacteria as previously described (Meednu *et al.*, 2008). Briefly, the processed form of SUMO his6-Smt3p-GG (pRM6713) and Ubc9p-his6 (pRM5169) were purified from bacteria using nickel affinity chromatography (Johnson and Blobel, 1997). The E1 components, Aos1p and Uba2p, located on a polycistronic plasmid (pRM6760) were copurified from bacteria using glutathione affinity chromatography (Bencsath *et al.*, 2002). The assay was carried out using purified sumoylation enzymes (2 μg of Smt3p-his6, 1 μg of Ubc9p-his6, 1 μg of GST-Uba2p/Aos1p) mixed with Bik1p-V5-his6 (1 μg) in the presence and absence of ATP and an ATP regeneration system at 30°C for 2 h.

Bik1p in vivo shift assay

Yeast strains (yRM3350) containing pGAL-*BIK1*-his6 (pRM5487) and 3HA-FLAG-*SMT3* (pRM5251) or an empty p*GAL* plasmid (pRM2908) were grown to mid–exponential phase in SC media lacking uracil and histidine (–ura –his) containing 2% sucrose and then induced with 2% galactose. Extracts were prepared by disrupting cells with glass beads, followed by centrifugation at 13,000 rpm in a 4°C cold room for 30 min. Bik1p was enriched from 3 mg of extract on a nickel column resin (Novagen/EMD). Beads were washed eight times with B150 buffer and twice with B150 buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.2% Triton X-100) containing 50 mM imidazole (Sigma-Aldrich). Bound proteins were eluted by the addition of 50 µl of 3× Laemmli buffer and boiled for 5 min. Samples were analyzed by SDS–PAGE (8%) and immunoblotting. Bik1-his6 was detected with mouse anti-his6 (Novagen/EMD). HA-tagged proteins were detected using mouse anti-HA (Santa Cruz Biotechnology, Santa Cruz, CA), and Smt3p was detected with rabbit anti-Smt3p. Secondary antibodies were obtained from Santa Cruz Biotechnology.

Preparation of whole-cell extracts in *ulp1*-ts strains.

Strains expressing *SMT3* under the control of a copper-inducible promoter (pRM8023) were grown to mid–exponential phase to an OD_{600} of <0.4 in liquid SC media lacking uracil and leucine (–ura –leu). Cells were shifted to either 30 or 37°C and simultaneously induced for *SMT3* expression by the addition of 1 mM $CuSO_4$ for 2 h. Cells were collected by low-speed centrifugation, washed, and resuspended in cold B150 breaking buffer; excess fluid was removed, and cells were resuspended in B150 breaking buffer containing 1 mM PMSF, 20 mM *N*-ethylmaleimide, 20 mM 2-iodoacetamide, and 1% Sigma-Aldrich protease inhibitor. Cells were lysed by vortexing with glass beads for 10 min. It should be noted that the inclusion of the alkylating agent 2-iodoacetomide was crucial for obtaining consistent results. Extracts were clarified by centrifugation at 13,000 × *g* for 20 min at 4°C. Protein concentrations were determined by Bradford Protein Assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard.

Pull-down assay

Yeast extracts were prepared as described earlier by bead beating cells in binding buffer (20 mM Tris-HCl, pH 7.9, 0.3 M NaCl, 10 mM imidazole, 1% protease inhibitor [#P8849; Sigma-Aldrich]). The volume was increased to 14 ml with binding buffer. Extracts were incubated with nickel beads at 4°C on a rotisserie for 1 h. Beads were collected and washed 7× with cold binding buffer containing 50 mM imidazole and 0.5 M NaCl. Samples were prepared for SDS–PAGE and Western blot analysis. The his6 epitope was detected using mouse anti-his6 (Novagen, Madison, WI) at 1:1500 in Tris-buffered saline (TBS) for 3 h at RT. Smt3p was detected using anti-Smt3p (Rockland, Gilbertsville, PA) at 1:2000 in phosphate-buffered saline (PBS) for 3 h at RT. Mouse anti-ubiquitin was obtained from Covance (Princeton, NJ) and used at 1:1500 in TBS for 3 h at room temperature. FLAG-tagged Smt3p was detected using mouse anti-FLAG (#3165; Sigma-Aldrich) at 1:5000 in TBS for 3 h at room temperature.

Western blotting

Western blotting was carried out using 8% SDS–PAGE as previously described (Moore *et al*., 2006; Meednu *et al*., 2008), except that 0.2% Tropix I-Block reagent (Applied Biosystems, Foster City, CA) with 0.1% Tween-20 in TBS was used as the blocking agent.

Plasmid construction

Nonchainable SUMO. Point mutations to generate lysine-to-arginine substitutions were introduced into HA-his6-Smt3 plasmid (pRM8023) by site-directed mutagenesis using the QuikChange Kit (Stratagene, La Jolla, CA). The mutant K11R was generated using primers #837 and #838. K15R was generated using primers #839 and #840. K19R was generated using primers #841 and #842. The final construct (pRM8836) was sequenced to confirm the absence of additional mutations. The sequences of these primers are given in the Supplemental Material.

PAC1-his6. An integration vector for Pac1p was constructed with a his6 tag fused in-frame at its 3′ prime end. A PCR fragment starting at *PAC1* bp699 was synthesized by PCR with *Xho*I and *Bam*HI restriction sites at its termini. This was ligated into the corresponding sites of the vector, pRM2194. This created plasmid pRM9232, which was sequenced to confirm the intended sequence. For integration by one-step gene tagging, the plasmid was linearized with *Cla*I, and transformants were selected on SC media lacking histidine.

ACKNOWLEDGMENTS

We thank Jake Kline for his artistic interpretations and creativity in designing the artwork for the model. This work was supported in part by funding from the National Science Foundation (MCB-0414768 and MCB-1052174), the Oklahoma Health Research Program of the Oklahoma Center for the Advancement of Science and Technology (OCAST HR09-150S), and start-up funds to R.K.M from Oklahoma State University and the Oklahoma Agricultural Experiment Station. A.A. is supported in part by a fellowship from the Sloan Foundation. J.W.P.K. was supported by a Niblack Research Scholarship and a Wentz Research Scholarship. We also thank the manuscript reviewers for their insightful comments.

REFERENCES

Beach DL, Thibodeaux J, Maddox P, Yeh E, Bloom K (2000). The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. Curr Biol 10, 1497–1506.

Bekes M, Prudden J, Srikumar T, Raught B, Boddy MN, Salvesen GS (2011). The dynamics and mechanism of SUMO chain deconjugation by SUMO-specific proteases. J Biol Chem 286, 10238–10247.

Bencsath KP, Podgorski MS, Pagala VR, Slaughter CA, Schulman BA (2002). Identification of a multifunctional binding site on Ubc9p required for Smt3p conjugation. J Biol Chem 277, 47938–47945.

Bergman ZJ, Xia X, Amaro IA, Huffaker TC (2012). Constitutive dynein activity in She1 mutants reveals differences in microtubule attachment at the yeast spindle pole body. Mol Biol Cell 23, 2319–2326.

Berlin V, Styles CA, Fink GR (1990). BIK1, a protein required for microtubule function during mating and mitosis in *Saccharomyces cerevisiae*, colocalizes with tubulin. J Cell Biol 111, 2573–2586.

Biggins S, Bhalla N, Chang A, Smith DL, Murray AW (2001). Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerevisiae*. Genetics 159, 453–470.

Blake-Hodek KA, Cassimeris L, Huffaker TC (2010). Regulation of microtubule dynamics by Bim1 and Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. Mol Biol Cell 21, 2013–2023.

Bylebyl GR, Belichenko I, Johnson ES (2003). The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. J Biol Chem 278, 44113–44120.

Carminati JL, Stearns T (1997). Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. J Cell Biol 138, 629–641.

Carvalho P, Gupta MLJ, Hoyt MA, Pellman D (2004). Cell cycle control of kinesin-mediated transport of Bik1(CLIP-170) regulates microtubule stability and dynein activation. Dev Cell 6, 815–829.

Caudron F, Andrieux A, Job D, Boscheron C (2008). A new role for kinesin-directed transport of Bik1p (CLIP-170) in *Saccharomyces cerevisiae*. J Cell Sci 121, 1506–1513.

Choi JH, Adames NR, Chan TF, Zeng C, Cooper JA, Zheng XFS (2000). TOR signaling regulates microtubule structure and function. Curr Biol 10, 861–864.
Choi JH, Bertram PG, Drenan R, Carvalho J, Zhou HH, Zheng XFS (2002). The FKBP12-rapamycin-associated protein (FRAP) is a CLIP-170 kinase. EMBO Rep 3, 988–994.
Dasso M (2008). Emerging roles of the SUMO pathway in mitosis. Cell Division 3, 5.
Dorval V, Fraser PE (2006). Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. J Biol Chem 281, 9919–9924.
Duan X, Holmes WB, Ye H (2011). Interaction mapping between *Saccharomyces cerevisiae* Smc5 and SUMO E3 ligase Mms21. Biochemistry 50, 10182–10188.
Dujardin DL, Barnhart LE, Stehman SA, Gomes ER, Gundersen GG, Vallee RB (2003). A role for cytoplasmic dynein and LIS1 in directed cell movement. J Cell Biol 163, 1205–1211.
Elmore ZC, Donaher M, Matson BC, Murphy H, Westerbeck JW, Kerscher O (2011). SUMO-dependent substrate targeting of the SUMO protease Ulp1. BMC Biol 9, 74.
Farkasovsky M, Küntzel H (2001). Cortical Num1p interacts with the dynein intermediate chain Pac11p and cytoplasmic microtubules in budding yeast. J Cell Biol 152, 251–262.
Faulkner NE, Dujardin DL, Tai C-Y, Vaughan KT, O'Connell CB, Wang YL, Vallee RB (2000). A role for the lissencephaly gene *LIS1* in mitosis and cytoplasmic dynein function. Nat Cell Biol 2, 784–791.
Finley, Bartel DB, Varshavsky A (1989). The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. Nature 338, 394–401.
Fleming JA, Lightcap ES, Sadis S, Thoroddsen V, Bulawa CE, Blackman RK (2002). Complementary whole-genome technologies reveal the cellular response to proteasome inhibition by PS-341. Proc Natl Acad Sci USA 99, 1461–1466.
Gareau JR, Lima CD (2010). The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. Nat Rev Mol Cell Biol 11, 861–871.
Han G, Liu B, Zhang J, Zuo W, Morris NR, Xiang X (2001). The *Aspergillus* cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. Curr Biol 11, 719–724.
He X, Rines DR, Espelin CW, Sorger PK (2001). Molecular analysis of kinetochore-microtubule attachment in budding yeast. Cell 106, 195–206.
Heideker J, Prudden J, Perry JJP, Tainer JA, Boddy MN (2011). SUMO-targeted ubiquitin ligase, Rad60, and Nse2 SUMO ligase suppress spontaneous Top1-mediated DNA damage and genome instability. PLoS Genet 7, e10001320.
Heil-Chapdelaine RA, Oberle JR, Cooper JA (2000). The cortical protein Num1p is essential for dynein-dependent interactions of microtubules with the cortex. J Cell Biol 151, 1337–1344.
Huang J, Roberts AJ, Leschziner AE, Reck-Peterson SL (2012). Lis1 acts as a "clutch" between the ATPase and microtubule binding domains of the dynein motor. Cell 150, 975–986.
Hwang E, Kusch EJ, Barral Y, Huffaker TC (2003). Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. J Cell Biol 161, 483–488.
James P, Halladay J, Craig EA (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics 144, 1425–1436.
Johnson ES, Blobel G (1997). Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. J Biol Chem 272, 26799–26802.
Johnson ES, Gupta AA (2001). An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 106, 735–744.
Kahana JA, Schnapp BJ, Silver PA (1995). Kinetics of spindle pole body separation in budding yeast. Proc Natl Acad Sci USA 92, 9707–9711.
Kolli N, Mikolajczyk J, Drag M, Mukhopadhyay D, Moffatt N, Dasso M, Salvesen G, Wilkinson KD (2010). Distribution and paralogue specificity of mammalian deSUMOylating enzymes. Biochem J 430, 335–344.
Lam C, Vergnolle MA, Thorpe L, Woodman PG, Allan VJ (2010). Functional interplay between LIS1, NDE1 and NDEL1 in dynein-dependent organelle positioning. J Cell Sci 123, 202–212.
Lee L, Tirnauer JS, Li J, Schuyler SC, Liu JY, Pellman D (2000). Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. Science 287, 2260–2262.
Lee WL, Kaiser MA, Cooper JA (2005). The offloading model for dynein function: differential function of motor subunits. J Cell Biol 168, 201–207.
Lee WL, Oberle JR, Cooper JA (2003). The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. J Cell Biol 160, 355–364.
Leisner C, Kammerer D, Denoth A, Britschi M, Barral Y, Liakopoulos D (2008). Regulation of mitotic spindle asymmetry by SUMO and the spindle-assembly checkpoint in yeast. Curr Biol 18, 1249–1255.
Li J, Lee WL, Cooper JA (2005). NudEL targets dynein to microtubule ends through LIS1. Nat Cell Biol 7, 686–690.
Li SJ, Hochstrasser M (1999). A new protease required for cell-cycle progression in yeast. Nature 398, 246–251.
Li SJ, Hochstrasser M (2000). The yeast *ULP2(SMT4)* gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. Mol Cell Biol 20, 2367–2377.
Liakopoulos D, Kusch J, Grava S, Vogel J, Barral Y (2003). Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. Cell 112, 561–574.
Lin H, de Carvalho P, Kho D, Tai CY, Pierre P, Fink GR, Pellman D (2001). Polyploids require Bik1 for kinetochore-microtubule attachment. J Cell Biol 155, 1173–1184.
Maekawa H, Schiebel E (2004). Cdk1-Clb4 controls the interaction of astral microtubule plus ends with subdomains of the daughter cell cortex. Genes Dev 18, 1709–1724.
Markus SM, Plevock KM, St. Germain BJ, Punch JJ, Meaden CW, Lee WL (2011). Quantitative analysis of Pac1/Lis1-mediated dynein targeting: Implications for regulation of dynein activity in budding yeast. Cytoskeleton 68, 157–174.
McKenney RJ, Vershinin J, Kunwar A, Vallee RB, Gross SP (2010). Lis1 and NudE induce a persistent dynein force-producing state. Cell 141, 304–314.
McKenney RJ, Weil SJ, Scherer J, Vallee RB (2011). Mutually exclusive cytoplasmic dynein regulation by NudE-Lis1 and dynactin. J Biol Chem 286, 39615–39622.
McMillan JN, Tatchell K (1994). The *JNM1* gene in the yeast *Saccharomyces cerevisiae* is required for nuclear migration and spindle orientation during the mitotic cell cycle. J Cell Biol 125, 143–158.
Meednu N, Hoops H, D'Silva S, Pogorzala L, Wood S, Farkas D, Sorrentino M, Sia E, Meluh P, Miller RK (2008). The spindle positioning protein Kar9p interacts with the sumoylation machinery in *Saccharomyces cerevisiae*. Genetics 180, 2033–2055.
Miller RK, Cheng S-C, Rose MD (2000). Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. Mol Biol Cell 11, 2949–2959.
Miller RK, Heller KK, Frisen L, Wallack DL, Loayza D, Gammie AE, Rose MD (1998). The kinesin-related proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. Mol Biol Cell 9, 2051–2068.
Miller RK, Matheos D, Rose MD (1999). The cortical localization of the microtubule orientation protein, Kar9p, is dependent upon actin and proteins required for polarization. J Cell Biol 144, 963–975.
Miller RK, Moore JK, D'Silva S, Goodson HV (2006). The CLIP-170 orthologue Bik1p and positioning the mitotic spindle in yeast. Curr Topics Dev Biol 49–87.
Miller RK, Rose MD (1998). Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J Cell Biol 140, 377–390.
Molk JN, Salmon ED, Bloom K (2006). Nuclear congression is driven by cytoplasmic microtubule plus end interactions in *S. cerevisiae*. J Cell Biol 172, 27–39.
Montpetit B, Hazbun TR, Fields S, Hieter P (2006). Sumoylation of the budding yeast kinetochore protein Ndc10 is required for Ndc10 spindle localization and regulation of anaphase spindle elongation. J Cell Biol 174, 653–663.
Moore JK, D'Silva S, Miller RK (2006). The CLIP-170 homologue Bik1p promotes the phosphorylation and asymmetric localization of Kar9p. Mol Biol Cell 17, 178–191.
Moore JK, Li J, Cooper JA (2008). Dynactin function in mitotic spindle positioning. Traffic 9, 510–527.
Moore JK, Miller RK (2007). The cyclin-dependent kinase Cdc28p regulates multiple aspects of Kar9p function in yeast. Mol Biol Cell 18, 1187–1202.
Mukhopadhyay D, Dasso M (2007). Modification in reverse: the SUMO proteases. Trends Biochem Sci 32, 286–295.
Mullen JR, Chem CF, Brill SJ (2010). Wss1 is a SUMO-dependent isopeptidase that interacts genetically with the Slx5-Slx8 SUMO-targeted ubiquitin ligase. Mol Cell Biol 30, 3737–3748.
Praefcke GJ, Hofmann K, Dohmen RJ (2012). SUMO playing tag with ubiquitin. Trends Biochem Sci 37, 23–31.

Reindle A, Belichenko I, Bylebyl GR, Chen XL, Gandhi N, Johnson ES (2006). Multiple domains in Siz SUMO ligases contribute to substrate selectivity. J Cell Sci 119, 4749–4757.
Reiner O, Sapoznik S, Sapir T (2006). Lissencephaly 1 linking to multiple diseases: mental retardation, neurodegeneration, schizophrenia, male sterility, and more. Neuromolecular Med 8, 547–565.
Sapir T, Elbaum M, Reiner O (1997). Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. EMBO J 16, 6977–6984.
Sarge KD, Park-Sarge OK (2011). SUMO and its role in human diseases. Int Rev Cell Mol Biol 288, 167–183.
Schwartz K, Richards K, Botstein D (1997). *BIM1* encodes a microtubule-binding protein in yeast. Mol Biol Cell 8, 2677–2691.
Sheeman B, Carvalho P, Sagot I, Geiser J, Kho D, Hoyt MA, Pellman D (2003). Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. Curr Biol 13, 364–372.
Shu T, Ayala R, Nguyen MD, Xie Z, Gleeson JG, Tsai LH (2004). Ndel1 operates in a common pathway with LIS1 and cytoplasmic dynein to regulate cortical neuronal positioning. Neuron 44, 263–277.
Sikorski RS, Hieter P (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122, 19–27.
Stephan AK, Kliszczak M, Morrison CG (2011). The Nse/Mms21 SUMO ligase of the Smc5/6 complex in the maintenance of genome stability. FEBS Lett 585, 2907–2913.
Su D, Hochstrasser M (2010). A WLM protein with SUMO-directed protease activity. Mol Cell Biol 30, 3734–3736.
Su X, Qiu W, Gupta Jr ML, Pereira-Leal JB, Reck-Peterson SL, Pellman D (2011). Mechanisms underlying the dual-mode regulation of microtubule dynamics by Kip3/kinesin-8. Mol Cell 43, 751–763.
Sumigray KD, Chen H, Lechler T (2011). Lis1 is essential for cortical microtubule organization and desmosome stability in the epidermis. J Cell Biol 194, 631–642.
Sun H, Leverson JD, Hunter T (2007). Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. EMBO J 26, 4102–4112.
Takahashi K, Ishida M, Komano H, Takahashi H (2008). SUMO-1 immunoreactivity co-localizes with phospho-Tau in APP transgenic mice but not in mutant Tau transgenic mice. Neurosci Lett 441, 90–93.
Tanaka T, Serneo FF, Higgins C, Gambello MJ, Wynshaw-Boris A, Gleeson JG (2004). Lis1 and doublecortin function with dynein to mediate coupling of the nucleus to the centrosome in neuronal migration. J Cell Biol 165, 709–721.
Torisawa T, Nakayama A, Furuta K, Yamada M, Hirotsune S, Toyoshima YY (2011). Functional dissection of LIS1 and NDEL1 towards understanding the molecular mechanisms of cytoplasmic dynein regulation. J Biol Chem 286, 1959–1965.
Tsai JW, Bremner KH, Vallee RB (2007). Dual subcellular roles for LIS1 and dynein in radial neuronal migration in live brain tissue. Nat Neurosci 10, 970–979.
Tsai JW, Chen Y, Kriegstein AR, Vallee RB (2005). LIS1 RNA interference blocks neural stem cell division, morphogenesis, and motility at multiple stages. J Cell Biol 170, 935–945.
Tsai JW, Lian WN, Kemal S, Kriegstein AR, Vallee RB (2010). Kinesin 3 and cytoplasmic dynein mediate interkinetic nuclear migration in neural stem cells. Nat Neurosci 13, 1463–1471.
Uzunova K *et al*. (2007). Ubiquitin-dependent proteolytic control of SUMO conjugates. J Biol Chem 282, 34167–34175.
Westermann S, Drubin DG, Barnes G (2007). Structures and functions of yeast kinetochore complexes. Annu Rev Biochem 76, 563–591.
Wolyniak MJ, Blake-Hodek K, Kosco K, Hwang E, You L, Huffaker TC (2006). The regulation of microtubule dynamics in *Saccharomyces cerevisiae* by three interacting plus end tracking proteins. Mol Biol Cell 17, 2789–2798.
Woodruff JB, Drubin DG, Barnes G (2009). Dynein-driven mitotic spindle positioning restricted to anaphase by She1p inhibition of dynactin recruitment. Mol Biol Cell 20, 3003–3011.
Wong J *et al*. (2007). A protein interaction map of the mitotic spindle. Mol Biol Cell 18, 3800–3809.
Xie Y, Kerscher O, Kroetz MB, McConchies HF, Sung P, Hochstrasser M (2007). The yeast Hex3-Slx8 heterodimer is a ubiquitin ligase stimulated by substrate sumoylation. J Biol Chem 282, 34176–34184.
Yeh E, Skibbens RV, Cheng JW, Salmon ED, Bloom K (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. J Cell Biol 130, 687–700.
Yeh ET (2009). SUMOylation and De-SUMOylation: wrestling with life's processes. J Biol Chem 284, 8223–8227.
Yi JY, Ori-McKenny KM, McKenney RJ, Vershinin M, Gross SP, Vallee RB (2011). High-resolution imaging reveals indirect coordination of opposite motors and a role for LIS1 in high-load axonal transport. J Cell Biol 195, 193–201.
Yin HDP, Huffaker TC, Bretscher A (2000). Myosin V orientates the mitotic spindle in yeast. Nature 406, 1013–1015.
Zhang J, Zhuang L, Lee Y, Abenza JF, Penalva MA, Xiang X (2010). The microtubule plus-end localization of *Aspergillus* dynein is important for dynein-early-endosome interaction but not for dynein ATPase activation. J Cell Sci 123, 3596–3604.
Zhang XD, Goeres J, Zhang H, Yen TJ, Porter AC, Matunis MJ (2008). SUMO-2/3 modification and binding regulate the association of CENP-E with kinetochores and progression through mitosis. Mol Cell 29, 729–741.
Zhou W, Ryan JJ, Zhou H (2004). Global analyses of sumoylated proteins in *Saccharomyces cerevisiae*: Induction of protein sumoylation by cellular stresses. J Biol Chem 279, 32262–32268.

REFERENCES

Adames, N.R., and J.A. Cooper. 2000. Microtubule interactions with the cell cortex causing nuclear movements in Saccharomyces cerevisiae. *J Cell Biol*. 149:863-874.

Ahner, A., X. Gong, B.Z. Schmidt, K.W. Peters, W.M. Rabeh, P.H. Thibodeau, G.L. Lukacs, and R.A. Frizzell. 2013. Small heat shock proteins target mutant cystic fibrosis transmembrane conductance regulator for degradation via a small ubiquitin-like modifier-dependent pathway. *Molecular biology of the cell*. 24:74-84.

Akhmanova, A., and C.C. Hoogenraad. 2005. Microtubule plus-end-tracking proteins: mechanisms and functions. *Current opinion in cell biology*. 17:47-54.

Akhmanova, A., and M.O. Steinmetz. 2008. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nature reviews. Molecular cell biology*. 9:309-322.

Al-Bassam, J., R.S. Ozer, D. Safer, S. Halpain, and R.A. Milligan. 2002. MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. *The Journal of cell biology*. 157:1187-1196.

Albuquerque, C.P., G. Wang, N.S. Lee, R.D. Kolodner, C.D. Putnam, and H. Zhou. 2013. Distinct SUMO ligases cooperate with Esc2 and Slx5 to suppress duplication-mediated genome rearrangements. *PLoS genetics*. 9:e1003670.

Allen, C., and G.G. Borisy. 1974. Structural polarity and directional growth of microtubules of Chlamydomonas flagella. *J Mol Biol*. 90:381-402.

Alonso, A., S. D'Silva, M. Rahman, P.B. Meluh, J. Keeling, N. Meednu, H.J. Hoops, and R.K. Miller. 2012. The yeast homologue of the microtubule-associated protein Lis1 interacts with the sumoylation machinery and a SUMO-targeted ubiquitin ligase. *Molecular biology of the cell*. 23:4552-4566.

Alonso, A., Greenlee, M., Matts, J., Kline, J., Davis, K., and Miller, R.K. 2015.Emerging roles of sumoylation in the regulation of actin, microtubules, intermediate filaments, and septins. *Cytoskeleton*.

Amos, L.A., and D. Schlieper. 2005. Microtubules and maps. *Advances in protein chemistry*. 71:257-298.

Arnaud, L.T., N. Myeku, and M.E. Figueiredo-Pereira. 2009. Proteasome-caspase-cathepsin sequence leading to tau pathology induced by prostaglandin J2 in neuronal cells. *Journal of neurochemistry*. 110:328-342.

Asante, D., N.L. Stevenson, and D.J. Stephens. 2014. Subunit composition of the human cytoplasmic dynein-2 complex. *Journal of cell science*.

Aumais, J.P., J.R. Tunstead, R.S. McNeil, B.T. Schaar, S.K. McConnell, S.H. Lin, G.D. Clark, and L.Y. Yu-Lee. 2001. NudC associates with Lis1 and the dynein motor at the leading pole of neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 21:RC187.

Bader, J.R., and K.T. Vaughan. 2010. Dynein at the kinetochore: Timing, Interactions and Functions. *Seminars in cell & developmental biology*. 21:269-275.

Bancher, C., I. Grundke-Iqbal, K. Iqbal, V.A. Fried, H.T. Smith, and H.M. Wisniewski. 1991. Abnormal phosphorylation of tau precedes ubiquitination in neurofibrillary pathology of Alzheimer disease. *Brain research*. 539:11-18.

Bawa-Khalfe, T., and E.T. Yeh. 2010. SUMO Losing Balance: SUMO Proteases Disrupt SUMO Homeostasis to Facilitate Cancer Development and Progression. *Genes & cancer*. 1:748-752.

Bayer, P., A. Arndt, S. Metzger, R. Mahajan, F. Melchior, and J. Becker. 1998. Structure determination of the small ubiquitin-related modifier SUMO-1. *J. Mol. Biol.* 280:275-286.

Beach, D.L., J. Thibodeaux, P. Maddox, E. Yeh, and K. Bloom. 2000. The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. *Curr. Biol*. 10:1497-1506.

Belmont, L.D., and T.J. Mitchison. 1996. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. *Cell*. 84:623-631.

Bencsath, K.P., M.S. Podgorski, V.R. Pagala, C.A. Slaughter, and B.A. Schulman. 2002. Identification of a multifunctional binding site on Ubc9p required for Smt3p conjugation. *J Biol Chem*. 277:47938-47945.

Bergen, L.G., and G.G. Borisy. 1980. Head-to-tail polymerization of microtubules in vitro. Electron microscope analysis of seeded assembly. *J Cell Biol*. 84:141-150.

Berlin, V., C.A. Styles, and G.R. Fink. 1990. BIK1, a protein required for microtubule function during mating and mitosis in Saccharomyces cerevisiae, colocalizes with tubulin. *J Cell Biol*. 111:2573-2586.

Bhabha, G., H.C. Cheng, N. Zhang, A. Moeller, M. Liao, J.A. Speir, Y. Cheng, and R.D. Vale. 2014. Allosteric communication in the Dynein motor domain. *Cell*. 159:857-868.

Bienz, M. 2001. Spindles cotton on to junctions, APC and EB1. *Nature cell biology*. 3:E67-68.

Biggins, S., N. Bhalla, A. Chang, D.L. Smith, and A.W. Murrary. 2001. Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerevisiae*. *Genetics*. 159:453-470.

Binder, L.I., A. Frankfurter, and L.I. Rebhun. 1985. The distribution of tau in the mammalian central nervous system. *J Cell Biol*. 101:1371-1378.

Blake-Hodek, K.A., L. Cassimeris, and T.C. Huffaker. 2010. Regulation of microtubule dynamics by Bim1 and Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. *Mol Biol Cell*. 21:2013-2023.

Blennow, K., H. Zetterberg, and A.M. Fagan. 2012. Fluid biomarkers in Alzheimer disease. *Cold Spring Harbor perspectives in medicine*. 2:a006221.

Blomster, H.A., V. Hietakangas, J. Wu, P. Kouvonen, S. Hautaniemi, and L. Sistonen. 2009. Novel proteomics strategy brings insight into the prevalence of SUMO-2 target sites. *Molecular & cellular proteomics : MCP*. 8:1382-1390.

Blomster, H.A., S.Y. Imanishi, J. Siimes, J. Kastu, N.A. Morrice, J.E. Eriksson, and L. Sistonen. 2010. In vivo identification of sumoylation sites by a signature tag and cysteine-targeted affinity purification. *The Journal of biological chemistry*. 285:19324-19329.

Bonacci, T., S. Audebert, L. Camoin, E. Baudelet, G. Bidaut, M. Garcia, Witzel, II, N.D. Perkins, J.P. Borg, J.L. Iovanna, and P. Soubeyran. 2014. Identification of new mechanisms of cellular response to chemotherapy by tracking changes in post-translational modifications by ubiquitin and ubiquitin-like proteins. *Journal of proteome research*. 13:2478-2494.

Bu, P., K.Y. Chen, S.M. Lipkin, and X. Shen. 2013. Asymmetric division: a marker for cancer stem cells in early stage tumors? *Oncotarget*. 4:950-951.

Bulbarelli, A., E. Lonati, E. Cazzaniga, M. Gregori, and M. Masserini. 2009. Pin1 affects Tau phosphorylation in response to Abeta oligomers. *Molecular and cellular neurosciences*. 42:75-80.

Bylebyl, G.R., I. Belichenko, and E.S. Johnson. 2003. The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. *Journal of Biological Chemistry*. 278:44113-44120.

Cal-Bakowska, M., I. Litwin, T. Bocer, R. Wysocki, and D. Dziadkowiec. 2011. The Swi2-Snf2-like protein Uls1 is involved in replication stress response. *Nucleic acids research*. 39:8765-8777.

Carter, A.P., C. Cho, L. Jin, and R.D. Vale. 2011. Crystal structure of the dynein motor domain. *Science*. 331:1159-1165.

Carter, A.P., J.E. Garbarino, E.M. Wilson-Kubalek, W.E. Shipley, C. Cho, R.A. Milligan, R.D. Vale, and I.R. Gibbons. 2008. Structure and functional role of dynein's microtubule-binding domain. *Science*. 322:1691-1695.

Carvalho, P., M.L.J. Gupta, M.A. Hoyt, and D. Pellman. 2004. Cell cycle control of kinesin-mediated transport of Bik1(CLIP170) regulates microtubule stability and dynein activation. *Dev. Cell*. 6:815-829.

Cassimeris, L., N.K. Pryer, and E.D. Salmon. 1988. Real-time observations of microtubule dynamic instability in living cells. *J Cell Biol*. 107:2223-2231.

Caudron, F., A. Andrieux, D. Job, and c. Boscheron. 2008. A new role for kinesin-directed transport of Bik1p (CLIP-170) in *Saccharomyces cerevisiae*. *J. Cell Sci.* 121:1506-1513.

Chapin, S.J., and J.C. Bulinski. 1991. Non-neuronal 210 x 10(3) Mr microtubule-associated protein (MAP4) contains a domain homologous to the microtubule-binding domains of neuronal MAP2 and tau. *J Cell Sci*. 98 (Pt 1):27-36.

Cheerambathur, D.K., and A. Desai. 2014. Linked in: formation and regulation of microtubule attachments during chromosome segregation. *Current opinion in cell biology*. 26:113-122.

Cheeseman, I.M., J.S. Chappie, E.M. Wilson-Kubalek, and A. Desai. 2006. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell*. 127:983-997.

Chen, Y.Z., Z. Chen, Y.A. Gong, and G. Ying. 2012. SUMOhydro: a novel method for the prediction of sumoylation sites based on hydrophobic properties. *PloS one*. 7:e39195.

Cheng, C.H., Y.H. Lo, S.S. Liang, S.C. Ti, F.M. Lin, C.H. Yeh, H.Y. Huang, and T.F. Wang. 2006. SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of Saccharomyces cerevisiae. *Genes & development*. 20:2067-2081.

Chretien, D., S.D. Fuller, and E. Karsenti. 1995. Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates. *J Cell Biol*. 129:1311-1328.

Chretien, D., I. Jainosi, J.C. Taveau, and H. Flyvbjerg. 1999. Microtubule's conformational cap. *Cell structure and function*. 24:299-303.

Citro, S., and S. Chiocca. 2013. Sumo paralogs: redundancy and divergencies. *Front Biosci (Schol Ed)*. 5:544-553.

Clark, S.W., O. Staub, I.B. Clark, E.L. Holzbaur, B.M. Paschal, R.B. Vallee, and D.I. Meyer. 1994. Beta-centractin: characterization and distribution of a new member of the centractin family of actin-related proteins. *Molecular biology of the cell*. 5:1301-1310.

Cleveland, D.W., S.Y. Hwo, and M.W. Kirschner. 1977. Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. *J Mol Biol*. 116:207-225.

Collins, E.S., S.K. Balchand, J.L. Faraci, P. Wadsworth, and W.L. Lee. 2012. Cell cycle-regulated cortical dynein/dynactin promotes symmetric cell division by differential pole motion in anaphase. *Molecular biology of the cell*. 23:3380-3390.

Conde, C., and A. Caceres. 2009. Microtubule assembly, organization and dynamics in axons and dendrites. *Nature reviews. Neuroscience*. 10:319-332.

Cook, C.E., M. Hochstrasser, and O. Kerscher. 2009. The SUMO-targeted ubiquitin ligase subunit Slx5 resides in nuclear foci and at sites of DNA breaks. *Cell cycle*. 8:1080-1089.

Cubenas-Potts, C., J.D. Goeres, and M.J. Matunis. 2013. SENP1 and SENP2 affect spatial and temporal control of sumoylation in mitosis. *Molecular biology of the cell*. 24:3483-3495.
D'Silva, S. 2008. A Structure-function analysis of the CLIP-170 homologue, Bik1p, in Saccharomyces cerevisiae. *In* University of Rochester-Biology Vol. Ph.D. Biology. University of Rochester.
Dasso, M. 2008. Emerging roles of the SUMO pathway in mitosis *Cell Division*. 3:5.
David, D.C., R. Layfield, L. Serpell, Y. Narain, M. Goedert, and M.G. Spillantini. 2002. Proteasomal degradation of tau protein. *Journal of neurochemistry*. 83:176-185.
de The, H., M. Le Bras, and V. Lallemand-Breitenbach. 2012. The cell biology of disease: Acute promyelocytic leukemia, arsenic, and PML bodies. *The Journal of cell biology*. 198:11-21.
Delacourte, A., J.P. David, N. Sergeant, L. Buee, A. Wattez, P. Vermersch, F. Ghozali, C. Fallet-Bianco, F. Pasquier, F. Lebert, H. Petit, and C. Di Menza. 1999. The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology*. 52:1158-1165.
Desai, A., and T.J. Mitchison. 1997. Microtubule polymerization dynamics. *Annual review of cell and developmental biology*. 13:83-117.
Desterro, J.M., M.S. Rodriguez, and R.T. Hay. 1998. SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. *Molecular cell*. 2:233-239.
Desterro, J.M., M.S. Rodriguez, G.D. Kemp, and R.T. Hay. 1999. Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J Biol Chem*. 274:10618-10624.
Desterro, J.M., J. Thomson, and R.T. Hay. 1997. Ubch9 conjugates SUMO but not ubiquitin. *FEBS Lett*. 417:297-300.
Di Bacco, A., J. Ouyang, H.Y. Lee, A. Catic, H. Ploegh, and G. Gill. 2006. The SUMO-specific protease SENP5 is required for cell division. *Molecular and cellular biology*. 26:4489-4498.
Diamantopoulos, G.S., F. Perez, H.V. Goodson, G. Batelier, R. Melki, T.E. Kreis, and J.E. Rickard. 1999. Dynamic localization of CLIP-170 to microtubule plus ends is coupled to microtubule assembly. *J Cell Biol*. 144:99-112.
Dohmen, R.J., R. Stappen, J.P. McGrath, H. Forrova, J. Kolarov, A. Goffeau, and A. Varshavsky. 1995. An essential yeast gene encoding a homolog of ubiquitin-activating enzyme. *J Biol Chem*. 270:18099-18109.
Dorval, V., and P.E. Fraser. 2006. Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. *J. Biol. Chem.* 281:9919-9924.
Dorval, V., and P.E. Fraser. 2007. SUMO on the road to neurodegeneration. *Biochimica et biophysica acta*. 1773:694-706.
Drag, M., and G.S. Salvesen. 2008. DeSUMOylating enzymes--SENPs. *IUBMB life*. 60:734-742.
Drechsel, D.N., A.A. Hyman, M.H. Cobb, and M.W. Kirschner. 1992. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Molecular biology of the cell*. 3:1141-1154.
Drewes, G., B. Trinczek, S. Illenberger, J. Biernat, G. Schmitt-Ulms, H.E. Meyer, E.M. Mandelkow, and E. Mandelkow. 1995. Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. *J Biol Chem*. 270:7679-7688.
Drubin, D.G., and M.W. Kirschner. 1986. Tau protein function in living cells. *The Journal of cell biology*. 103:2739-2746.
Duan, X., W.B. Holmes, and H. Ye. 2011. Interaction mapping between Saccharomyces cerevisiae Smc5 and SUMO E3 ligase Mms21. *Biochemistry*. 50:10182-10188.

Eckley, D.M., S.R. Gill, K.A. Melkonian, J.B. Bingham, H.V. Goodson, J.E. Heuser, and T.A. Schroer. 1999. Analysis of dynactin subcomplexes reveals a novel actin-related protein associated with the arp1 minifilament pointed end. *J Cell Biol*. 147:307-320.

Efimov, V.P., and N.R. Morris. 2000. The LIS1-related NUDF protein of Aspergillus nidulans interacts with the coiled-coil domain of the NUDE/RO11 protein. *J Cell Biol*. 150:681-688.

Elmore, Z.C., M. Donaher, B.C. Matson, H. Murphy, J.W. Westerbeck, and O. Kerscher. 2011. SUMO-dependent substrate targeting of the SUMO protease Ulp1. *BMC Biol.* 9:74.

Erker, Y., H. Neyret-Kahn, J.S. Seeler, A. Dejean, A. Atfi, and L. Levy. 2013. Arkadia, a novel SUMO-targeted ubiquitin ligase involved in PML degradation. *Molecular and cellular biology*. 33:2163-2177.

Eshel, D., L.A. Urrestarazu, S. Vissers, J.C. Jauniaux, J.C. van Vliet-Reedijk, R.J. Planta, and I.R. Gibbons. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc Natl Acad Sci U S A*. 90:11172-11176.

Etienne-Manneville, S. 2010. From signaling pathways to microtubule dynamics: the key players. *Current opinion in cell biology*. 22:104-111.

Etienne-Manneville, S. 2013. Microtubules in cell migration. *Annual review of cell and developmental biology*. 29:471-499.

Fang, S., and A.M. Weissman. 2004. A field guide to ubiquitylation. *Cellular and molecular life sciences : CMLS*. 61:1546-1561.

Faulkner, N.E., D.L. Dujardin, T. C.-Y., K.T. Vaughan, C.B. O'Connell, Y.-L. Wang, and R.B. Vallee. 2000. A role for the lissencephaly gene *LIS1* in mitosis and cytoplasmic dynein function. *Nature cell biology*. 2:784-791.

Ferreira, J.G., A.L. Pereira, and H. Maiato. 2014. Microtubule plus-end tracking proteins and their roles in cell division. *International review of cell and molecular biology*. 309:59-140.

Fischer, D., M.D. Mukrasch, J. Biernat, S. Bibow, M. Blackledge, C. Griesinger, E. Mandelkow, and M. Zweckstetter. 2009. Conformational changes specific for pseudophosphorylation at serine 262 selectively impair binding of tau to microtubules. *Biochemistry*. 48:10047-10055.

Folker, E.S., B.M. Baker, and H.V. and Goodson. 2005. Interactions between CLIP-170, Tubulin, and Microtubules: Implications for the Mechanism of CLIP-170 Plus-End Tracking Behavior. *Molecular biology of the cell*. 16:5373-5384.

Gaglio, T., M.A. Dionne, and D.A. Compton. 1997. Mitotic spindle poles are organized by structural and motor proteins in addition to centrosomes. *The Journal of cell biology*. 138:1055-1066.

Galanty, Y., R. Belotserkovskaya, J. Coates, and S.P. Jackson. 2012. RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes & development*. 26:1179-1195.

Gan-Erdene, T., K. Nagamalleswari, L. Yin, K. Wu, Z.Q. Pan, and K.D. Wilkinson. 2003. Identification and characterization of DEN1, a deneddylase of the ULP family. *The Journal of biological chemistry*. 278:28892-28900.

Garces, J.A., I.B. Clark, D.I. Meyer, and R.B. Vallee. 1999. Interaction of the p62 subunit of dynactin with Arp1 and the cortical actin cytoskeleton. *Current biology : CB*. 9:1497-1500.

Gardner, M.K., A.J. Hunt, H.V. Goodson, and D.J. Odde. 2008. Microtubule assembly dynamics: new insights at the nanoscale. *Current opinion in cell biology*. 20:64-70.

Gee, M.A., J.E. Heuser, and R.B. Vallee. 1997. An extended microtubule-binding structure within the dynein motor domain. *Nature*. 390:636-639.

Geiser, J.R., E.J. Schott, T.J. Kingsbury, N.B. Cole, L.J. Totis, G. Bhattacharyya, L. He, and M.A. Hoyt. 1997. Saccharomyces cerevisiae genes required in the absence of the CIN8-

encoded spindle motor act in functionally diverse mitotic pathways. *Molecular biology of the cell*. 8:1035-1050.
Gibbons, I.R. 1995. Dynein family of motor proteins: present status and future questions. *Cell motility and the cytoskeleton*. 32:136-144.
Gillett, E.S., C.W. Espelin, and P.K. Sorger. 2004. Spindle checkpoint proteins and chromosome-microtubule attachment in budding yeast. *J Cell Biol*. 164:535-546.
Gillies, J., and M. Hochstrasser. 2012. A new class of SUMO proteases. *EMBO reports*. 13:284-285.
Goedert, M. 2001. The significance of tau and alpha-synuclein inclusions in neurodegenerative diseases. *Current opinion in genetics & development*. 11:343-351.
Goedert, M., C.P. Baur, J. Ahringer, R. Jakes, M. Hasegawa, M.G. Spillantini, M.J. Smith, and F. Hill. 1996. PTL-1, a microtubule-associated protein with tau-like repeats from the nematode Caenorhabditis elegans. *J Cell Sci*. 109 (Pt 11):2661-2672.
Goedert, M., M.G. Spillantini, R. Jakes, D. Rutherford, and R.A. Crowther. 1989a. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*. 3:519-526.
Goedert, M., M.G. Spillantini, M.C. Potier, J. Ulrich, and R.A. Crowther. 1989b. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J*. 8:393-399.
Goeres, J., P.K. Chan, D. Mukhopadhyay, H. Zhang, B. Raught, and M.J. Matunis. 2011. The SUMO-specific isopeptidase SENP2 associates dynamically with nuclear pore complexes through interactions with karyopherins and the Nup107-160 nucleoporin subcomplex. *Molecular biology of the cell*. 22:4868-4882.
Gong, L., B. Li, S. Millas, and E.T. Yeh. 1999. Molecular cloning and characterization of human AOS1 and UBA2, components of the sentrin-activating enzyme complex. *FEBS Lett*. 448:185-189.
Goodson, H.V., S.B. Skube, R. Stalder, C. Valetti, T.E. Kreis, E.E. Morrison, and T.A. Schroer. 2003. CLIP-170 interacts with dynactin complex and the APC-binding protein EB1 by different mechanisms. *Cell Motility & the Cytoskeleton*. 55:156-173.
Grissom, P.M., E.A. Vaisberg, and J.R. McIntosh. 2002. Identification of a novel light intermediate chain (D2LIC) for mammalian cytoplasmic dynein 2. *Molecular biology of the cell*. 13:817-829.
Groden, J., A. Thliveris, W. Samowitz, M. Carlson, L. Gelbert, H. Albertsen, G. Joslyn, J. Stevens, L. Spirio, M. Robertson, and et al. 1991. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*. 66:589-600.
Grundke-Iqbal, I., K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, and L.I. Binder. 1986. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A*. 83:4913-4917.
Grunstein, M. 1997. Molecular model for telomeric heterochromatin in yeast. *Current opinion in cell biology*. 9:383-387.
Guimaraes, G.J., Y. Dong, B.F. McEwen, and J.G. Deluca. 2008. Kinetochore-microtubule attachment relies on the disordered N-terminal tail domain of Hec1. *Current biology : CB*. 18:1778-1784.
Gundersen, G.G. 2002. Evolutionary conservation of microtubule-capture mechanisms. *Nature reviews. Molecular cell biology*. 3:296-304.
Gundersen, G.G., and A. Bretscher. 2003. Microtubule Asymmetry. *Science*. 300:2040.
Guo, D., M. Li, Y. Zhang, P. Yang, S. Eckenrode, D. Hopkins, W. Zheng, S. Purohit, R.H. Podolsky, A. Muir, J. Wang, Z. Dong, T. Brusko, M. Atkinson, P. Pozzilli, A. Zeidler, L.J. Raffel, C.O. Jacob, Y. Park, M. Serrano-Rios, M.T. Larrad, Z. Zhang, H.J. Garchon, J.F. Bach, J.I. Rotter, J.X. She, and C.Y. Wang. 2004. A functional variant of SUMO4, a

new I kappa B alpha modifier, is associated with type 1 diabetes. *Nature genetics*. 36:837-841.

Gupta, A., L.H. Tsai, and A. Wynshaw-Boris. 2002. Life is a journey: a genetic look at neocortical development. *Nature reviews. Genetics*. 3:342-355.

Gupta, K.K., E.O. Alberico, I.S. Nathke, and H.V. Goodson. 2014. Promoting microtubule assembly: A hypothesis for the functional significance of the +TIP network. *BioEssays : news and reviews in molecular, cellular and developmental biology*. 36:818-826.

Gupta, K.K., M.V. Joyce, A.R. Slabbekoorn, Z.C. Zhu, B.A. Paulson, B. Boggess, and H.V. Goodson. 2010. Probing interactions between CLIP-170, EB1, and microtubules. *Journal of molecular biology*. 395:1049-1062.

Gupta, K.K., C. Li, A. Duan, E.O. Alberico, O.V. Kim, M.S. Alber, and H.V. Goodson. 2013. Mechanism for the catastrophe-promoting activity of the microtubule destabilizer Op18/stathmin. *Proceedings of the National Academy of Sciences of the United States of America*. 110:20449-20454.

Guzzo, C.M., C.E. Berndsen, J. Zhu, V. Gupta, A. Datta, R.A. Greenberg, C. Wolberger, and M.J. Matunis. 2012. RNF4-dependent hybrid SUMO-ubiquitin chains are signals for RAP80 and thereby mediate the recruitment of BRCA1 to sites of DNA damage. *Science signaling*. 5:ra88.

Hang, J., and M. Dasso. 2002. Association of the human SUMO-1 protease SENP2 with the nuclear pore. *The Journal of biological chemistry*. 277:19961-19966.

Hannich, J.T., A. Lewis, M.B. Kroetz, S.J. Li, H. Heide, A. Emili, and M. Hochstrasser. 2005. Defining the SUMO-modified proteome by multiple approaches in *Saccharomyces cerevisiae*. *J. Biol. Chem*. 280:4102-4110.

Hattori, M., H. Adachi, M. Tsujimoto, H. Arai, and K. Inoue. 1994. Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected]. *Nature*. 370:216-218.

Hay, R.T. 2013. Decoding the SUMO signal. *Biochemical Society transactions*. 41:463-473.

Hecker, C.M., M. Rabiller, K. Haglund, P. Bayer, and I. Dikic. 2006. Specification of SUMO1- and SUMO2-interacting motifs. *The Journal of biological chemistry*. 281:16117-16127.

Heideker, J., J. Prudden, J.J. Perry, J.A. Tainer, and M.N. Boddy. 2011. SUMO-targeted ubiquitin ligase, Rad60, and Nse2 SUMO ligase suppress spontaneous Top1-mediated DNA damage and genome instability. *PLoS genetics*. 7:e1001320.

Heil-Chapdelaine, R.A., J.R. Oberle, and J.A. Cooper. 2000. The cortical protein Num1p is essential for dynein-dependent interactions of microtubules with the cortex. *J Cell Biol*. 151:1337-1344.

Helfand, B.T., A. Mikami, R.B. Vallee, and R.D. Goldman. 2002. A requirement for cytoplasmic dynein and dynactin in intermediate filament network assembly and organization. *J Cell Biol*. 157:795-806.

Hickey, C.M., N.R. Wilson, and M. Hochstrasser. 2012. Function and regulation of SUMO proteases. *Nature reviews. Molecular cell biology*. 13:755-766.

Hietakangas, V., J. Anckar, H.A. Blomster, M. Fujimoto, J.J. Palvimo, A. Nakai, and L. Sistonen. 2006. PDSM, a motif for phosphorylation-dependent SUMO modification. *Proc Natl Acad Sci U S A*. 103:45-50.

Ho, C.W., H.T. Chen, and J. Hwang. 2011. UBC9 autosumoylation negatively regulates sumoylation of septins in Saccharomyces cerevisiae. *J Biol Chem*. 286:21826-21834.

Hochstrasser, M. 2001. SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. *Cell*. 107:5-8.

Hoege, C., B. Pfander, G.L. Moldovan, G. Pyrowolakis, and S. Jentsch. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*. 419:135-141.

Holzbaur, E.L., and R.B. Vallee. 1994. DYNEINS: molecular structure and cellular function. *Annual review of cell biology*. 10:339-372.
Horiguchi, T., K. Uryu, B.I. Giasson, H. Ischiropoulos, R. LightFoot, C. Bellmann, C. Richter-Landsberg, V.M. Lee, and J.Q. Trojanowski. 2003. Nitration of tau protein is linked to neurodegeneration in tauopathies. *The American journal of pathology*. 163:1021-1031.
Howard, J., and A.A. Hyman. 2003. Dynamics and mechanics of the microtubule plus end. *Nature*. 422:753-758.
Howell, B.J., B.F. McEwen, J.C. Canman, D.B. Hoffman, E.M. Farrar, C.L. Rieder, and E.D. Salmon. 2001. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *The Journal of cell biology*. 155:1159-1172.
Hsieh, Y.L., H.Y. Kuo, C.C. Chang, M.T. Naik, P.H. Liao, C.C. Ho, T.C. Huang, J.C. Jeng, P.H. Hsu, M.D. Tsai, T.H. Huang, and H.M. Shih. 2013. Ubc9 acetylation modulates distinct SUMO target modification and hypoxia response. *The EMBO journal*. 32:791-804.
Huang, J., A.J. Roberts, A.E. Leschziner, and S.L. Reck-Peterson. 2012. Lis1 acts as a "clutch" between the ATPase and microtubule-binding domains of the dynein motor. *Cell*. 150:975-986.
Huang, T.T., S.M. Wuerzberger-Davis, Z.H. Wu, and S. Miyamoto. 2003. Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. *Cell*. 115:565-576.
Huttner, W.B., and Y. Kosodo. 2005. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. *Current opinion in cell biology*. 17:648-657.
Hwang, E.S., J. Kusch, Y. Barral, and T.C. Huffaker. 2003. Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. *J. Cell Biol.* 161:483-488.
Hyman, A.A., S. Salser, D.N. Drechsel, N. Unwin, and T.J. Mitchison. 1992. Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP. *Molecular biology of the cell*. 3:1155-1167.
Ihara, Y., N. Nukina, R. Miura, and M. Ogawara. 1986. Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J Biochem*. 99:1807-1810.
Inoue, S., and E.D. Salmon. 1995. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Molecular biology of the cell*. 6:1619-1640.
Irazoqui, J.E., and D.J. Lew. 2004. Polarity establishment in yeast. *J Cell Sci*. 117:2169-2171.
Iyer, L.M., E.V. Koonin, and L. Aravind. 2004. Novel predicted peptidases with a potential role in the ubiquitin signaling pathway. *Cell cycle*. 3:1440-1450.
Jain, D., and J.P. Cooper. 2010. Telomeric strategies: means to an end. *Annual review of genetics*. 44:243-269.
Jaspersen, S.L., and M. Winey. 2004. The budding yeast spindle pole body: structure, duplication, and function. *Annual review of cell and developmental biology*. 20:1-28.
Johansson, M., N. Rocha, W. Zwart, I. Jordens, L. Janssen, C. Kuijl, V.M. Olkkonen, and J. Neefjes. 2007. Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor betalll spectrin. *The Journal of cell biology*. 176:459-471.
Johnson, E.S. 2004. Protein Modification by SUMO. *Annu. Rev. Biochem.* 73:355-382.
Johnson, E.S., and G. Blobel. 1997. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* 272:26799-26802.
Johnson, E.S., and G. Blobel. 1999. Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J. Cell Biol.* 147:981-994.
Johnson, E.S., and A.A. Gupta. 2001. An E3-like factor that promotes SUMO conjugation to the yeast septins. *Cell*. 106:735-744.

Johnson, E.S., Schwienhorst, R.J. Dohmen, and G. and Blobel. 1997. The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *The EMBO journal*. 16:5509-5519.

Jones, D., E. Crowe, T.A. Stevens, and E.P. Candido. 2002. Functional and phylogenetic analysis of the ubiquitylation system in Caenorhabditis elegans: ubiquitin-conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like proteins. *Genome Biol*. 3:RESEARCH0002.

Joseph, J., S.T. Liu, S.A. Jablonski, T.J. Yen, and M. Dasso. 2004. The RanGAP1-RanBP2 complex is essential for microtubule-kinetochore interactions in vivo. *Current biology : CB*. 14:611-617.

Joseph, J., S.H. Tan, T.S. Karpova, J.G. McNally, and M. Dasso. 2002. SUMO-1 targets RanGAP1 to kinetochores and mitotic spindles. *J Cell Biol*. 156:595-602.

Kagey, M.H., T.A. Melhuish, and D. Wotton. 2003. The polycomb protein Pc2 is a SUMO E3. *Cell*. 113:127-137.

Kahana, J.A., G. Schlenstedt, D.M. Evanchuk, J.R. Geiser, M.A. Hoyt, and P.A. Silver. 1998. The yeast dynactin complex is involved in partitioning the mitotic spindle between mother and daughter cells during anaphase B. *Molecular biology of the cell*. 9:1741-1756.

Kahana, J.A., B.J. Schnapp, and P.A. Silver. 1995. Kinetics of spindle pole body separation in budding yeast. *Proc Natl Acad Sci U S A*. 92:9707-9711.

Kamitani, T., H.P. Nguyen, and E.T.H. Yeh. 1997. Preferential modification of nuclear proteins by a novel ubiquitin-like molecule. *J. Biol. Chem.* 272:14001-14004.

Kanai, Y., J. Chen, and N. Hirokawa. 1992. Microtubule bundling by tau proteins in vivo: analysis of functional domains. *The EMBO journal*. 11:3953-3961.

Kardon, J.R., and R.D. Vale. 2009. Regulators of the cytoplasmic dynein motor. *Nature reviews. Molecular cell biology*. 10:854-865.

Kato, M., and W.B. Dobyns. 2003. Lissencephaly and the molecular basis of neuronal migration. *Human molecular genetics*. 12 Spec No 1:R89-96.

Kerscher, O. 2007. SUMO junction-what's your function? New insights through SUMO-interacting motifs. *EMBO reports*. 8:550-555.

Kim, J.H., and S.H. Baek. 2009. Emerging roles of desumoylating enzymes. *Biochim Biophys Acta*. 1792:155-162.

King, S.J., M. Bonilla, M.E. Rodgers, and T.A. Schroer. 2002. Subunit organization in cytoplasmic dynein subcomplexes. *Protein science : a publication of the Protein Society*. 11:1239-1250.

Kirschner, M.W. 1978. Microtubule assembly and nucleation. *International review of cytology*. 54:1-71.

Kita, K., T. Wittmann, I.S. Nathke, and C.M. Waterman-Storer. 2006. Adenomatous polyposis coli on microtubule plus ends in cell extensions can promote microtubule net growth with or without EB1. *Molecular biology of the cell*. 17:2331-2345.

Klein, U.R., M. Haindl, E.A. Nigg, and S. Muller. 2009. RanBP2 and SENP3 function in a mitotic SUMO2/3 conjugation-deconjugation cycle on Borealin. *Molecular biology of the cell*. 20:410-418.

Klug, H., M. Xaver, V.K. Chaugule, S. Koidl, G. Mittler, F. Klein, and A. Pichler. 2013. Ubc9 sumoylation controls SUMO chain formation and meiotic synapsis in Saccharomyces cerevisiae. *Molecular cell*. 50:625-636.

Knipscheer, P., A. Flotho, H. Klug, J.V. Olsen, W.J. van Dijk, A. Fish, E.S. Johnson, M. Mann, T.K. Sixma, and A. Pichler. 2008. Ubc9 sumoylation regulates SUMO target discrimination. *Molecular cell*. 31:371-382.

Kolli, N., J. Mikolajczyk, M. Drag, D. Mukhopadhyay, N. Moffatt, M. Dasso, and G. Salvesen. 2010. Distribution and paralogue specificity of mammalian deSUMOylating enzymes. *Biochem J.* 430:335-344.

Kon, T., M. Nishiura, R. Ohkura, Y.Y. Toyoshima, and K. Sutoh. 2004. Distinct functions of nucleotide-binding/hydrolysis sites in the four AAA modules of cytoplasmic dynein. *Biochemistry*. 43:11266-11274.

Kopeikina, K.J., B.T. Hyman, and T.L. Spires-Jones. 2012. Soluble forms of tau are toxic in Alzheimer's disease. *Translational neuroscience*. 3:223-233.

Korinek, W.S., M.J. Copeland, A. Chaudhuri, and J. Chant. 2000. Molecular linkage underlying microtubule orientation toward cortical sites in yeast. *Science*. 287:2257-2259.

Kotak, S., C. Busso, and P. Gonczy. 2014. NuMA interacts with phosphoinositides and links the mitotic spindle with the plasma membrane. *The EMBO journal*. 33:1815-1830.

Kotak, S., and P. Gonczy. 2013. Mechanisms of spindle positioning: cortical force generators in the limelight. *Current opinion in cell biology*. 25:741-748.

Kroetz, M.B., and M. Hochstrasser. 2009. Identification of SUMO-interacting proteins by yeast two-hybrid analysis. *Methods in molecular biology*. 497:107-120.

Kuhla, B., C. Haase, K. Flach, H.J. Luth, T. Arendt, and G. Munch. 2007. Effect of pseudophosphorylation and cross-linking by lipid peroxidation and advanced glycation end product precursors on tau aggregation and filament formation. *J Biol Chem*. 282:6984-6991.

Kumar, A., U. Ray, and S. Das. 2013. Human La Protein Interaction with GCAC near the Initiator AUG Enhances Hepatitis C Virus RNA Replication by Promoting Linkage between 5' and 3' Untranslated Regions. *Journal of virology*. 87:6713-6726.

Kurihara, L.J., C.T. Beh, M. Latterich, R. Schekman, and M.D. Rose. 1994. Nuclear congression and membrane fusion: two distinct events in the yeast karyogamy pathway. *J Cell Biol*. 126:911-923.

Lambert de Rouvroit, C., and A.M. Goffinet. 2001. Neuronal migration. *Mechanisms of development*. 105:47-56.

LaMonte, B.H., K.E. Wallace, B.A. Holloway, S.S. Shelly, J. Ascano, M. Tokito, T. Van Winkle, D.S. Howland, and E.L. Holzbaur. 2002. Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration. *Neuron*. 34:715-727.

Lampert, F., and S. Westermann. 2011. A blueprint for kinetochores - new insights into the molecular mechanics of cell division. *Nature reviews. Molecular cell biology*. 12:407-412.

Landino, L.M., T.E. Skreslet, and J.A. Alston. 2004. Cysteine oxidation of tau and microtubule-associated protein-2 by peroxynitrite: modulation of microtubule assembly kinetics by the thioredoxin reductase system. *J Biol Chem*. 279:35101-35105.

Lansbergen, G., and A. Akhmanova. 2006. Microtubule plus end: a hub of cellular activities. *Traffic*. 7:499-507.

Larsen, C.N., B.A. Krantz, and K.D. Wilkinson. 1998. Substrate specificity of deubiquitinating enzymes: ubiquitin C-terminal hydrolases. *Biochemistry*. 37:3358-3368.

Lee, G.W., F. Melchior, M.J. Matunis, R. Mahajan, Q. Tian, and P. Anderson. 1998. Modification of Ran GTPase-activating protein by the small ubiquitin-related modifier SUMO-1 requires Ubc9, an E2-type ubiquitin-conjugating enzyme homologue. *J Biol Chem*. 273:6503-6507.

Lee, W.-L., J.R. Oberle, and J.A. Cooper. 2003. The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. *The Journal of Cell Biology*. 160:355-364.

Lee, W.L., M.A. Kaiser, and J.A. Cooper. 2005. The offloading model for dynein function: differential function of motor subunits. *J. Cell Biol.* 168:201-207.

Leisner, C., D. Kammerer, A. Denoth, M. Britschi, Y. Barral, and D. Liakopoulos. 2008. Regulation of mitotic spindle asymmetry by SUMO and the spindle-assembly checkpoint in yeast. *Current Biology*. 18:1249-1255.

Lescasse, R., S. Pobiega, I. Callebaut, and S. Marcand. 2013. End-joining inhibition at telomeres requires the translocase and polySUMO-dependent ubiquitin ligase Uls1. *EMBO J*. 32:805-815.

Leventer, R.J., C. Cardoso, D.H. Ledbetter, and W.B. Dobyns. 2001a. LIS1 missense mutations cause milder lissencephaly phenotypes including a child with normal IQ. *Neurology*. 57:416-422.

Leventer, R.J., C. Cardoso, D.H. Ledbetter, and W.B. Dobyns. 2001b. LIS1: from cortical malformation to essential protein of cellular dynamics. *Trends in neurosciences*. 24:489-492.

Levy, J.R., and E.L. Holzbaur. 2006. Cytoplasmic dynein/dynactin function and dysfunction in motor neurons. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*. 24:103-111.

Li, J., W.L. Lee, and J.A. Cooper. 2005. NudEL targets dynein to microtubule ends through LIS1. *Nature cell biology*. 7:686-690.

Li, S.-J., and M. Hochstrasser. 1999. A new protease required for cell-cycle progression in yeast. *Nature*. 398:246-251.

Li, S.J., and M. Hochstrasser. 2000. The yeast *ULP2(SMT4*) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol. and Cell. Biol.* 20:2367-2377.

Li, W., and Y. Ye. 2008. Polyubiquitin chains: functions, structures, and mechanisms. *Cellular and molecular life sciences : CMLS*. 65:2397-2406.

Liakopoulos, D., J. Kusch, S. Grava, J. Vogel, and Y. Barral. 2003. Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. *Cell*. 112:561-574.

Lima, C.D., and D. Reverter. 2008. Structure of the human SENP7 catalytic domain and poly-SUMO deconjugation activities for SENP6 and SENP7. *The Journal of biological chemistry*. 283:32045-32055.

Lin, H., P. de Carvalho, D. Kho, C.Y. Tai, P. Pierre, G.R. Fink, and D. Pellman. 2001. Polyploids require Bik1 for kinetochore-microtubule attachment. *J Cell Biol*. 155:1173-1184.

Litersky, J.M., G.V. Johnson, R. Jakes, M. Goedert, M. Lee, and P. Seubert. 1996. Tau protein is phosphorylated by cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase II within its microtubule-binding domains at Ser-262 and Ser-356. *The Biochemical journal*. 316 (Pt 2):655-660.

Liu, D., X. Ding, J. Du, X. Cai, Y. Huang, T. Ward, A. Shaw, Y. Yang, R. Hu, C. Jin, and X. Yao. 2007. Human NUF2 interacts with centromere-associated protein E and is essential for a stable spindle microtubule-kinetochore attachment. *J Biol Chem*. 282:21415-21424.

Liu, J.S. 2011. Molecular genetics of neuronal migration disorders. *Current neurology and neuroscience reports*. 11:171-178.

Liu, J.X., G.B. Zhou, S.J. Chen, and Z. Chen. 2012. Arsenic compounds: revived ancient remedies in the fight against human malignancies. *Current opinion in chemical biology*. 16:92-98.

Liu, Y.H., W. Wei, J. Yin, G.P. Liu, Q. Wang, F.Y. Cao, and J.Z. Wang. 2009. Proteasome inhibition increases tau accumulation independent of phosphorylation. *Neurobiology of aging*. 30:1949-1961.

Lo, K.W., S. Naisbitt, J.S. Fan, M. Sheng, and M. Zhang. 2001. The 8-kDa dynein light chain binds to its targets via a conserved (K/R)XTQT motif. *J Biol Chem*. 276:14059-14066.

Luo, K., H. Zhang, L. Wang, J. Yuan, and Z. Lou. 2012. Sumoylation of MDC1 is important for proper DNA damage response. *The EMBO journal*. 31:3008-3019.

Ma, S., L. Trivinos-Lagos, R. Graf, and R.L. Chisholm. 1999. Dynein intermediate chain mediated dynein-dynactin interaction is required for interphase microtubule organization and centrosome replication and separation in Dictyostelium. *J Cell Biol*. 147:1261-1274.

Maccioni, R.B., L. Tapia, and V. Cambiazo. 1995. Functional organization of tau proteins during neuronal differentiation and development. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.]*. 28:827-841.

Mahadevan, L., and T.J. Mitchison. 2005. Cell biology: powerful curves. *Nature*. 435:895-897.

Maiato, H., J. DeLuca, E.D. Salmon, and W.C. Earnshaw. 2004. The dynamic kinetochore-microtubule interface. *J Cell Sci*. 117:5461-5477.

Mao, Y., D. Varma, and R. Vallee. 2010. Emerging functions of force-producing kinetochore motors. *Cell Cycle*. 9:715-719.

Markowitz, S.D., and M.M. Bertagnolli. 2009. Molecular origins of cancer: Molecular basis of colorectal cancer. *The New England journal of medicine*. 361:2449-2460.

Markus, S.M., and W.L. Lee. 2011. Regulated offloading of cytoplasmic dynein from microtubule plus ends to the cortex. *Developmental cell*. 20:639-651.

Markus, S.M., K.M. Plevock, B.J. St Germain, J.J. Punch, C.W. Meaden, and W.L. Lee. 2011. Quantitative analysis of Pac1/LIS1-mediated dynein targeting: Implications for regulation of dynein activity in budding yeast. *Cytoskeleton (Hoboken)*. 68:157-174.

Maroui, M.A., S. Kheddache-Atmane, F. El Asmi, L. Dianoux, M. Aubry, and M.K. Chelbi-Alix. 2012. Requirement of PML SUMO interacting motif for RNF4- or arsenic trioxide-induced degradation of nuclear PML isoforms. *PloS one*. 7:e44949.

Martin, N., K. Schwamborn, V. Schreiber, A. Werner, C. Guillier, X.D. Zhang, O. Bischof, J.S. Seeler, and A. Dejean. 2009. PARP-1 transcriptional activity is regulated by sumoylation upon heat shock. *The EMBO journal*. 28:3534-3548.

Matafora, V., A. D'Amato, S. Mori, F. Blasi, and A. Bachi. 2009. Proteomics analysis of nucleolar SUMO-1 target proteins upon proteasome inhibition. *Mol Cell Proteomics*. 8:2243-2255.

Mateja, A., T. Cierpicki, M. Paduch, Z.S. Derewenda, and J. Otlewski. 2006. The dimerization mechanism of LIS1 and its implication for proteins containing the LisH motif. *J Mol Biol*. 357:621-631.

Matic, I., J. Schimmel, I.A. Hendriks, M.A. van Santen, F. van de Rijke, H. van Dam, F. Gnad, M. Mann, and A.C. Vertegaal. 2010. Site-specific identification of SUMO-2 targets in cells reveals an inverted SUMOylation motif and a hydrophobic cluster SUMOylation motif. *Mol Cell*. 39:641-652.

Matic, I., M. van Hagen, J. Schimmel, B. Macek, S.C. Ogg, M.H. Tatham, R.T. Hay, A.I. Lamond, M. Mann, and A.C. Vertegaal. 2008. In vivo identification of human small ubiquitin-like modifier polymerization sites by high accuracy mass spectrometry and an in vitro to in vivo strategy. *Mol Cell Proteomics*. 7:132-144.

Matsumoto, N., R.J. Leventer, J.A. Kuc, S.K. Mewborn, L.L. Dudlicek, M.B. Ramocki, D.T. Pilz, P.L. Mills, S. Das, M.E. Ross, D.H. Ledbetter, and W.B. Dobyns. 2001. Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. *European journal of human genetics : EJHG*. 9:5-12.

Mazumdar, M., A. Mikami, M.A. Gee, and R.B. Vallee. 1996. In vitro motility from recombinant dynein heavy chain. *Proc Natl Acad Sci U S A*. 93:6552-6556.

McCarthy, E.K., and B. Goldstein. 2006. Asymmetric spindle positioning. *Current opinion in cell biology*. 18:79-85.

McKenney, R.J., J. Vershinin, A. Kunwar, R.B. Vallee, and S.P. Gross. 2010. Lis1 and NudE induce a persistent dynein force-producing state. *Cell*. 141:304-314.

McKenney, R.J., S.J. Weil, J. Scherer, and R.B. Vallee. 2011. Mutually exclusive cytoplasmic dynein regulation by NudE-Lis1 and dynactin. *J Biol Chem*. 286:39615-39622.

McMillan, J.N., and K. Tatchell. 1994. The JNM1 gene in the yeast Saccharomyces cerevisiae is required for nuclear migration and spindle orientation during the mitotic cell cycle. *J Cell Biol*. 125:143-158.

McNally, F.J., and R.D. Vale. 1993. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell*. 75:419-429.

Meednu, N., H. Hoops, S. D'Silva, L. Pogorzala, S. Wood, D. Farkas, M. Sorrentino, E. Sia, P. Meluh, and R.K. Miller. 2008. The spindle positioning protein Kar9p interacts with the sumoylation machinery in Saccharomyces cerevisiae. *Genetics*. 180:2033-2055.

Melchior, F. 2000. SUMO-nonclassical ubiquitin. *Annu. Rev. Cell Dev. Biol.* 16:591-626.

Meluh, P.B., and D. Koshland. 1995. Evidence that the MIF2 gene of Saccharomyces cerevisiae encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Molecular biology of the cell*. 6:793-807.

Meraz-Rios, M.A., K.I. Lira-De Leon, V. Campos-Pena, M.A. De Anda-Hernandez, and R. Mena-Lopez. 2010. Tau oligomers and aggregation in Alzheimer's disease. *Journal of neurochemistry*. 112:1353-1367.

Merlo, L.M., L.S. Wang, J.W. Pepper, P.S. Rabinovitch, and C.C. Maley. 2010. Polyploidy, aneuploidy and the evolution of cancer. *Advances in experimental medicine and biology*. 676:1-13.

Mikami, A., S.H. Tynan, T. Hama, K. Luby-Phelps, T. Saito, J.E. Crandall, J.C. Besharse, and R.B. Vallee. 2002. Molecular structure of cytoplasmic dynein 2 and its distribution in neuronal and ciliated cells. *J Cell Sci*. 115:4801-4808.

Mikolajczyk, J., M. Drag, M. Bekes, J.T. Cao, Z. Ronai, and G.S. Salvesen. 2007. Small ubiquitin-related modifier (SUMO)-specific proteases: profiling the specificities and activities of human SENPs. *The Journal of biological chemistry*. 282:26217-26224.

Miller, R.K., S.C. Cheng, and M.D. Rose. 2000. Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. *Molecular biology of the cell*. 11:2949-2959.

Miller, R.K., D. Matheos, and M.D. Rose. 1999. The cortical localization of the microtubule orientation protein, Kar9p, is dependent upon actin and proteins required for polarization. *J Cell Biol*. 144:963-975.

Miller, R.K., J.K. Moore, S. D'Silva, and H.V. Goodson. 2006. The CLIP-170 orthologue Bik1p and positioning the mitotic spindle in yeast. *Current Topics in Developmental Biology.*:49-87.

Miller, R.K., and M.D. Rose. 1998. Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. *J Cell Biol*. 140:377-390.

Miller, S.A., M.L. Johnson, and P.T. Stukenberg. 2008. Kinetochore attachments require an interaction between unstructured tails on microtubules and Ndc80(Hec1). *Current biology : CB*. 18:1785-1791.

Minty, A., X. Dumont, M. Kaghad, and D. Caput. 2000. Covalent modification of p73alpha by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. *The Journal of biological chemistry*. 275:36316-36323.

Mitchison, T., and M. Kirschner. 1984a. Dynamic instability of microtubule growth. *Nature*. 312:237-242.

Mitchison, T., and M. Kirschner. 1984b. Microtubule assembly nucleated by isolated centrosomes. *Nature*. 312:232-237.

Mocz, G., and I.R. Gibbons. 2001. Model for the motor component of dynein heavy chain based on homology to the AAA family of oligomeric ATPases. *Structure*. 9:93-103.

Mok, Y.K., K.W. Lo, and M. Zhang. 2001. Structure of Tctex-1 and its interaction with cytoplasmic dynein intermediate chain. *J Biol Chem*. 276:14067-14074.

Montpetit, B., T.R. Hazbun, S. Fields, and P. Hieter. 2006. Sumoylation of the budding yeast kinetochore protein Ndc10 is required for Ndc10 spindle localizatio and regulation of anaphase spindle elongation. *J. Cell Biol.* 174:653-663.

Moore, J.K., and J.A. Cooper. 2010. Coordinating mitosis with cell polarity: Molecular motors at the cell cortex. *Seminars in cell & developmental biology*. 21:283-289.

Moore, J.K., S. D'Silva, and R.K. Miller. 2006. The CLIP-170 homologue Bik1p promotes the phosphorylation and asymmetric localization of Kar9p. *Molecular biology of the cell*. 17:178-191.

Moore, J.K., and R.K. Miller. 2007. The cyclin-dependent kinase Cdc28p regulates multiple aspects of Kar9p function in yeast. *Molecular biology of the cell*. 18:1187-1202.

Moughamian, A.J., and E.L. Holzbaur. 2012. Dynactin is required for transport initiation from the distal axon. *Neuron*. 74:331-343.

Muhua, L., T.S. Karpova, and J.A. Cooper. 1994. A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. *Cell*. 78:669-679.

Mukhopadhyay, D., and M. Dasso. 2007. Modification in reverse: the SUMO proteases. *Trends in Biochem Sci*. 32:286-295.

Mukhopadhyay, D., and M. Dasso. 2010. The fate of metaphase kinetochores is weighed in the balance of SUMOylation during S phase. *Cell cycle*. 9:3194-3201.

Mullen, J.R., C.F. Chem, and S.J. Brill. 2010. Wss1 is a SUMO-dependent isopeptidase that interacts genetically with the Slx5-Slx8 SUMO-targeted Ub ligase. *Mol. Cell Biol.* 30:3737-3748.

Mullen, J.R., V. Kaliraman, S.S. Ibrahim, and S.J. Brill. 2001. Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in Saccharomyces cerevisiae. *Genetics*. 157:103-118.

Munoz-Galvan, S., S. Jimeno, R. Rothstein, and A. Aguilera. 2013. Histone H3K56 acetylation, Rad52, and non-DNA repair factors control double-strand break repair choice with the sister chromatid. *PLoS genetics*. 9:e1003237.

Murthy, S.N., J.H. Wilson, T.J. Lukas, J. Kuret, and L. Lorand. 1998. Cross-linking sites of the human tau protein, probed by reactions with human transglutaminase. *Journal of neurochemistry*. 71:2607-2614.

Nacharaju, P., L. Ko, and S.H. Yen. 1997. Characterization of in vitro glycation sites of tau. *Journal of neurochemistry*. 69:1709-1719.

Nagai, S., N. Davoodi, and S.M. Gasser. 2011. Nuclear organization in genome stability: SUMO connections. *Cell research*. 21:474-485.

Necula, M., and J. Kuret. 2004. Pseudophosphorylation and glycation of tau protein enhance but do not trigger fibrillization in vitro. *J Biol Chem*. 279:49694-49703.

Neuwald, A.F., L. Aravind, J.L. Spouge, and E.V. Koonin. 1999. AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome research*. 9:27-43.

Okuma, T., R. Honda, G. Ichikawa, N. Tsumagari, and H. Yasuda. 1999. In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. *Biochem Biophys Res Commun*. 254:693-698.

Olsen, S.K., A.D. Capili, X. Lu, D.S. Tan, and C.D. Lima. 2010. Active site remodelling accompanies thioester bond formation in the SUMO E1. *Nature*. 463:906-912.

Ozkaynak, E., D. Finley, M.J. Solomon, and A. Varshavsky. 1987. The yeast ubiquitin genes: a family of natural gene fusions. *The EMBO journal*. 6:1429-1439.

Panse, V.G., U. Hardeland, T. Werner, B. Kuster, and E. Hurt. 2004. A proteome-wide approach identifies sumoylated substrate proteins in yeast. *Journal of Biological Chemistry*. 279:41346-41351.

Panse, V.G., B. Kuster, T. Gerstberger, and E. Hurt. 2003. Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nature cell biology*. 5:21-27.

Parker, J.L., and H.D. Ulrich. 2012. A SUMO-interacting motif activates budding yeast ubiquitin ligase Rad18 towards SUMO-modified PCNA. *Nucleic acids research*. 40:11380-11388.

Parker, J.L., and H.D. Ulrich. 2014. SIM-dependent enhancement of substrate-specific SUMOylation by a ubiquitin ligase in vitro. *The Biochemical journal*. 457:435-440.

Paschal, B.M., H.S. Shpetner, and R.B. Vallee. 1987. MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. *The Journal of cell biology*. 105:1273-1282.

Pazour, G.J., B.L. Dickert, and G.B. Witman. 1999. The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J Cell Biol*. 144:473-481.

Perry, J.J., J.A. Tainer, and M.N. Boddy. 2008. A SIM-ultaneous role for SUMO and ubiquitin. *Trends in biochemical sciences*. 33:201-208.

Petrucelli, L., D. Dickson, K. Kehoe, J. Taylor, H. Snyder, A. Grover, M. De Lucia, E. McGowan, J. Lewis, G. Prihar, J. Kim, W.H. Dillmann, S.E. Browne, A. Hall, R. Voellmy, Y. Tsuboi, T.M. Dawson, B. Wolozin, J. Hardy, and M. Hutton. 2004. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Human molecular genetics*. 13:703-714.

Pichler, A., A. Gast, J.S. Seeler, A. Dejean, and F. Melchior. 2002. The nucleoporin RanBP2 has SUMO1 E3 ligase activity. *Cell*. 108:109-120.

Poulsen, S.L., R.K. Hansen, S.A. Wagner, L. van Cuijk, G.J. van Belle, W. Streicher, M. Wikstrom, C. Choudhary, A.B. Houtsmuller, J.A. Marteijn, S. Bekker-Jensen, and N. Mailand. 2013. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *The Journal of cell biology*. 201:797-807.

Pountney, D.L., Y. Huang, R.J. Burns, E. Haan, P.D. Thompson, P.C. Blumbergs, and W.P. Gai. 2003. SUMO-1 marks the nuclear inclusions in familial neuronal intranuclear inclusion disease. *Experimental neurology*. 184:436-446.

Praefcke, G.J., K. Hofmann, and R.J. Dohmen. 2012. SUMO playing tag with ubiquitin. *Trends Biochemical Sciences*. 37:23-31.

Prakash, S., and L. Prakash. 1977. Increased spontaneous mitotic segregation in MMS-sensitive mutants of Saccharomyces cerevisiae. *Genetics*. 87:229-236.

Pringle, J.R., E. Bi, H.A. Harkins, J.E. Zahner, C. De Virgilio, J. Chant, K. Corrado, and H. Fares. 1995. Establishment of cell polarity in yeast. *Cold Spring Harbor symposia on quantitative biology*. 60:729-744.

Prudden, J., S. Pebernard, G. Raffa, D.A. Slavin, J.J. Perry, J.A. Tainer, C.H. McGowan, and M.N. Boddy. 2007. SUMO-targeted ubiquitin ligases in genome stability. *EMBO J*. 26:4089-4101.

Quintyne, N.J., S.R. Gill, D.M. Eckley, C.L. Crego, D.A. Compton, and T.A. Schroer. 1999. Dynactin is required for microtubule anchoring at centrosomes. *J Cell Biol*. 147:321-334.

Rai, R., S.P. Varma, N. Shinde, S. Ghosh, S.P. Kumaran, G. Skariah, and S. Laloraya. 2011. Small ubiquitin-related modifier ligase activity of Mms21 is required for maintenance of chromosome integrity during the unperturbed mitotic cell division cycle in Saccharomyces cerevisiae. *The Journal of biological chemistry*. 286:14516-14530.

Reindle, A., I. Belichenko, G.R. Bylebyl, X.L. Chen, N. Gandhi, and E.S. Johnson. 2006. Multiple domains in Siz SUMO ligases contribute to substrate selectivity. *Journal of cell science*. 119:4749-4757.

Reiner, O., and T. Sapir. 2013. LIS1 functions in normal development and disease. *Current opinion in neurobiology*. 23:951-956.

Reiner, O., S. Sapoznik, and T. Sapir. 2006. Lissencephaly 1 linking to multiple diseases: mental retardation, neurodegeneration, schizophrenia, male sterility, and more. *Neuro Molecular Med.* 8:547-565.

Rhyu, M.S., L.Y. Jan, and Y.N. Jan. 1994. Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell*. 76:477-491.

Rickard, J.E., and T.E. Kreis. 1991. Binding of pp170 to microtubules is regulated by phosphorylation. *J Biol Chem*. 266:17597-17605.

Rocha, N., C. Kuijl, R. van der Kant, L. Janssen, D. Houben, H. Janssen, W. Zwart, and J. Neefjes. 2009. Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. *The Journal of cell biology*. 185:1209-1225.

Rojas-Fernandez, A., A. Plechanovova, N. Hattersley, E. Jaffray, M.H. Tatham, and R.T. Hay. 2014. SUMO chain-induced dimerization activates RNF4. *Molecular cell*. 53:880-892.

Rout, M.P., and J.V. Kilmartin. 1990. Components of the yeast spindle and spindle pole body. *J Cell Biol*. 111:1913-1927.

Saitoh, H., and J. Hinchey. 2000. Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. *J Biol Chem*. 275:6252-6258.

Sammak, P.J., and G.G. Borisy. 1988. Direct observation of microtubule dynamics in living cells. *Nature*. 332:724-726.

Sapir, T., M. Eisenstein, H.A. Burgess, D. Horesh, A. Cahana, J. Aoki, M. Hattori, H. Arai, K. Inoue, and O. Reiner. 1999. Analysis of lissencephaly-causing LIS1 mutations. *Eur J Biochem*. 266:1011-1020.

Sapir, T., M. Elbaum, and O. Reiner. 1997. Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. *EMBO J*. 16:6977-6984.

Sardar, H.S., V.G. Luczak, M.M. Lopez, B.C. Lister, and S.P. Gilbert. 2010. Mitotic kinesin CENP-E promotes microtubule plus-end elongation. *Current biology : CB*. 20:1648-1653.

Scherer, J., and R.B. Vallee. 2011. Adenovirus recruits dynein by an evolutionary novel mechanism involving direct binding to pH-primed hexon. *Viruses*. 3:1417-1431.

Schmidts, M., H.H. Arts, E.M. Bongers, Z. Yap, M.M. Oud, D. Antony, L. Duijkers, R.D. Emes, J. Stalker, J.B. Yntema, V. Plagnol, A. Hoischen, C. Gilissen, E. Forsythe, E. Lausch, J.A. Veltman, N. Roeleveld, A. Superti-Furga, A. Kutkowska-Kazmierczak, E.J. Kamsteeg, N. Elcioglu, M.C. van Maarle, L.M. Graul-Neumann, K. Devriendt, S.F. Smithson, D. Wellesley, N.E. Verbeek, R.C. Hennekam, H. Kayserili, P.J. Scambler, P.L. Beales, N.V. Knoers, R. Roepman, and H.M. Mitchison. 2013a. Exome sequencing identifies DYNC2H1 mutations as a common cause of asphyxiating thoracic dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. *Journal of medical genetics*. 50:309-323.

Schmidts, M., J. Vodopiutz, S. Christou-Savina, C.R. Cortes, A.M. McInerney-Leo, R.D. Emes, H.H. Arts, B. Tuysuz, J. D'Silva, P.J. Leo, T.C. Giles, M.M. Oud, J.A. Harris, M. Koopmans, M. Marshall, N. Elcioglu, A. Kuechler, D. Bockenhauer, A.T. Moore, L.C. Wilson, A.R. Janecke, M.E. Hurles, W. Emmet, B. Gardiner, B. Streubel, B. Dopita, A. Zankl, H. Kayserili, P.J. Scambler, M.A. Brown, P.L. Beales, C. Wicking, E.L. Duncan, and H.M. Mitchison. 2013b. Mutations in the gene encoding IFT dynein complex component WDR34 cause Jeune asphyxiating thoracic dystrophy. *American journal of human genetics*. 93:932-944.

Schroer, T.A. 2004. Dynactin. *Annual review of cell and developmental biology*. 20:759-779.

Schulze, E., and M. Kirschner. 1988. New features of microtubule behaviour observed in vivo. *Nature*. 334:356-359.

Schuyler, S.C., and D. Pellman. 2001. Microtubule "plus-end-tracking proteins": The end is just the beginning. *Cell*. 105:421-424.

Schwartz, K., K. Richards, and D. Botstein. 1997. BIM1 encodes a microtubule-binding protein in yeast. *Molecular biology of the cell*. 8:2677-2691.

Schwarz, S.E., K. Matuschewski, D. Liakopoulos, M. Scheffner, and S. Jentsch. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. *Proc. Natl. Acad. Sci. USA*. 95:560-564.

Schweers, O., E.M. Mandelkow, J. Biernat, and E. Mandelkow. 1995. Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein tau controls the in vitro assembly of paired helical filaments. *Proc Natl Acad Sci U S A*. 92:8463-8467.

Schwienhorst, I., E.S. Johnson, and R.J. Dohmen. 2000. SUMO conjugation and deconjugation. *Mol Gen Genet*. 263:771-786.

Serrentino, M.E., E. Chaplais, V. Sommermeyer, and V. Borde. 2013. Differential association of the conserved SUMO ligase Zip3 with meiotic double-strand break sites reveals regional variations in the outcome of meiotic recombination. *PLoS genetics*. 9:e1003416.

Sheeman, B., P. Carvalho, I. Sagot, J. Geiser, D. Kho, M.A. Hoyt, and D. Pellman. 2003. Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. *Current Biology*. 13:364-372.

Sheng, W., and X. Liao. 2002. Solution structure of a yeast ubiquitin-like protein Smt3: the role of structurally less defined sequences in protein-protein recognitions. *Protein science : a publication of the Protein Society*. 11:1482-1491.

Shin, E.J., H.M. Shin, E. Nam, W.S. Kim, J.H. Kim, B.H. Oh, and Y. Yun. 2012. DeSUMOylating isopeptidase: a second class of SUMO protease. *EMBO Rep*. 13:339-346.

Shin, Y.C., S.J. Tang, J.H. Chen, P.H. Liao, and S.C. Chang. 2011. The molecular determinants of NEDD8 specific recognition by human SENP8. *PloS one*. 6:e27742.

Shuai, K. 2000. Modulation of STAT signaling by STAT-interacting proteins. *Oncogene*. 19:2638-2644.

Signor, D., K.P. Wedaman, J.T. Orozco, N.D. Dwyer, C.I. Bargmann, L.S. Rose, and J.M. Scholey. 1999. Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living Caenorhabditis elegans. *J Cell Biol*. 147:519-530.

Siller, K.H., and C.Q. Doe. 2008. Lis1/dynactin regulates metaphase spindle orientation in Drosophila neuroblasts. *Dev Biol*. 319:1-9.

Sitaram, P., M.A. Anderson, J.N. Jodoin, E. Lee, and L.A. Lee. 2012. Regulation of dynein localization and centrosome positioning by Lis-1 and asunder during Drosophila spermatogenesis. *Development*. 139:2945-2954.

Song, J., L.K. Durrin, T.A. Wilkinson, T.G. Krontiris, and Y. Chen. 2004. Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. *Proc Natl Acad Sci U S A*. 101:14373-14378.

Splinter, D., D.S. Razafsky, M.A. Schlager, A. Serra-Marques, I. Grigoriev, J. Demmers, N. Keijzer, K. Jiang, I. Poser, A.A. Hyman, C.C. Hoogenraad, S.J. King, and A. Akhmanova. 2012. BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. *Molecular biology of the cell*. 23:4226-4241.

Srikumar, T., M.C. Lewicki, and B. Raught. 2013. A global S. cerevisiae small ubiquitin-related modifier (SUMO) system interactome. *Molecular systems biology*. 9:668.

Steffan, J.S., N. Agrawal, J. Pallos, E. Rockabrand, L.C. Trotman, N. Slepko, K. Illes, T. Lukacsovich, Y.-Z. Zhu, E. Cattaneo, P.P. Pandolfi, L.M. Thompson, and J.L. Marsh. 2004. SUMO modification of Huntingtin and Huntington's disease pathology. *Science*. 304:100-103.

Stephan, A.K., M. Kliszczak, and C.G. Morrison. 2011. The Nse2/Mms21 SUMO ligase of the Smc5/6 complex in the maintenance of genome stability. *FEBS Lett*. 585:2907-2913.
Storchova, Z., and D. Pellman. 2004. From polyploidy to aneuploidy, genome instability and cancer. *Nature reviews. Molecular cell biology*. 5:45-54.
Su, D., and M. Hochstrasser. 2010. A WLM protein with SUMO-directed protease activity. *Molecular and cellular biology*. 30:3734-3736.
Su, Y.F., T. Yang, H. Huang, L.F. Liu, and J. Hwang. 2012. Phosphorylation of Ubc9 by Cdk1 enhances SUMOylation activity. *PloS one*. 7:e34250.
Suh, H.Y., J.H. Kim, J.S. Woo, B. Ku, E.J. Shin, Y. Yun, and B.H. Oh. 2012. Crystal structure of DeSI-1, a novel deSUMOylase belonging to a putative isopeptidase superfamily. *Proteins*. 80:2099-2104.
Sumigray, K.D., H. Chen, and T. Lechler. 2011. Lis1 is essential for cortical microtubule organization and desmosome stability in the epidermis. *J Cell Biol*. 194:631-642.
Sumigray, K.D., and T. Lechler. 2011. Control of cortical microtubule organization and desmosome stability by centrosomal proteins. *Bioarchitecture*. 1:221-224.
Sun, H., J.D. Leverson, and T. Hunter. 2007. Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. *The EMBO J.* 26:4102-4112.
Swan, A., T. Nguyen, and B. Suter. 1999. Drosophila Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. *Nature cell biology*. 1:444-449.
Szymanski, E.P., and O. Kerscher. 2013. Budding yeast protein extraction and purification for the study of function, interactions, and post-translational modifications. *Journal of visualized experiments : JoVE*:e50921.
Tai, C.-Y., D.L. Dujardin, N.E. Faulkner, and R.B. Vallee. 2002. The role of dynein, dynactin, and CLIP-170 interactions in LIS1 kinetochore function. *The Journal of cell biology*. 156:959-968.
Takahashi, K., M. Ishida, H. Komano, and H. Takahashi. 2008. SUMO-1 immunoreactivity co-localizes with phospho-Tau in APP transgenic mice but not in mutant Tau transgenic mice. *Neuroscience letters*. 441:90-93.
Takahashi, M., Y. Tsujioka, T. Yamada, Y. Tsuboi, H. Okada, T. Yamamoto, and Z. Liposits. 1999. Glycosylation of microtubule-associated protein tau in Alzheimer's disease brain. *Acta neuropathologica*. 97:635-641.
Takahashi, Y., T. Kahyo, E.A. Toh, H. Yasuda, and Y. Kikuchi. 2001. Yeast Ull1/Siz1 is a novel SUMO1/Smt3 ligase for septin components and functions as an adaptor between conjugating enzyme and substrates. *J. Biol. Chem.* 276:48973-48977.
Takahashi, Y., J. Mizoi, E.A. Toh, and Y. Kikuchi. 2000. Yeast Ulp1, an Smt3-specific protease, associates with nucleoporins. *J Biochem*. 128:723-725.
Tan, S.C., J. Scherer, and R.B. Vallee. 2011. Recruitment of dynein to late endosomes and lysosomes through light intermediate chains. *Molecular biology of the cell*. 22:467-477.
Tanaka, K., J. Nishide, K. Okazaki, H. Kato, O. Niwa, T. Nakagawa, H. Matsuda, M. Kawamukai, and Y. Murakami. 1999. Characterization of a fission yeast SUMO-1 homologue, pmt3p, required for multiple nuclear events, including the control of telomere length and chromosome segregation. *Mol Cell Biol*. 19:8660-8672.
Tatham, M.H., M.C. Geoffroy, L. Shen, A. Plechanovova, N. Hattersley, E.G. Jaffray, J.J. Palvimo, and R.T. Hay. 2008. RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. *Nature cell biology*. 10:538-546.
Tatham, M.H., E. Jaffray, O.A. Vaughan, J.M. Desterro, C.H. Botting, J.H. Naismith, and R.T. Hay. 2001. Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J Biol Chem*. 276:35368-35374.
Teng, S., H. Luo, and L. Wang. 2012. Predicting protein sumoylation sites from sequence features. *Amino acids*. 43:447-455.

Tien, J.F., K.K. Fong, N.T. Umbreit, C. Payen, A. Zelter, C.L. Asbury, M.J. Dunham, and T.N. Davis. 2013. Coupling unbiased mutagenesis to high-throughput DNA sequencing uncovers functional domains in the Ndc80 kinetochore protein of Saccharomyces cerevisiae. *Genetics*. 195:159-170.

Torisawa, T., A. Nakayama, K. Furuta, M. Yamada, S. Hirotsune, and Y.Y. Toyoshima. 2011. Functional dissection of LIS1 and NDEL1 towards understanding the molecular mechanisms of cytoplasmic dynein regulation. *The Journal of biological chemistry*. 286:1959-1965.

Toropova, K., S. Zou, A.J. Roberts, W.B. Redwine, B.S. Goodman, S.L. Reck-Peterson, and A.E. Leschziner. 2014. Lis1 regulates dynein by sterically blocking its mechanochemical cycle. *eLife*. 3.

Trotta, R., T. Vignudelli, O. Candini, R.V. Intine, L. Pecorari, C. Guerzoni, G. Santilli, M.W. Byrom, S. Goldoni, L.P. Ford, M.A. Caligiuri, R.J. Maraia, D. Perrotti, and B. Calabretta. 2003. BCR/ABL activates mdm2 mRNA translation via the La antigen. *Cancer cell*. 3:145-160.

Ulrich, H.D. 2005. Mutual interactions between the SUMO and ubiquitin systems: a plea of no contest. *Trends in cell biology*. 15:525-532.

Ulrich, H.D. 2009. The SUMO system: an overview. *Methods in molecular biology*. 497:3-16.

Uzunova, K., K. Gottsche, M. Miteva, S.R. Weisshaar, C. Glanemann, M. Schnellhardt, M. Niessen, H. Scheel, K. Hofmann, E.S. Johnson, G.J. Praefcke, and R.J. Dohmen. 2007. Ubiquitin-dependent proteolytic control of SUMO conjugates. *J Biol Chem*. 282:34167-34175.

Valiron, O., N. Caudron, and D. Job. 2001. Microtubule dynamics. *Cellular and molecular life sciences : CMLS*. 58:2069-2084.

Vallee, R.B., C. Tai, and N.E. Faulkner. 2001. LIS1: cellular function of a disease-causing gene. *Trends in cell biology*. 11:155-160.

van de Pasch, L.A., A.J. Miles, W. Nijenhuis, N.A. Brabers, D. van Leenen, P. Lijnzaad, M.K. Brown, J. Ouellet, Y. Barral, G.J. Kops, and F.C. Holstege. 2013. Centromere binding and a conserved role in chromosome stability for SUMO-dependent ubiquitin ligases. *PloS one*. 8:e65628.

van Heusden, G.P., and H.Y. Steensma. 2008. The Saccharomyces cerevisiae Wss1 protein is only present in mother cells. *FEMS microbiology letters*. 282:100-104.

van Niekerk, E.A., D.E. Willis, J.H. Chang, K. Reumann, T. Heise, and J.L. Twiss. 2007. Sumoylation in axons triggers retrograde transport of the RNA-binding protein La. *Proceedings of the National Academy of Sciences of the United States of America*. 104:12913-12918.

Vaughan, K.T. 2004. Surfing, regulating and capturing: are all microtubule-tip-tracking proteins created equal? *Trends in cell biology*. 14:491-496.

Vaughan, K.T., S.H. Tynan, N.E. Faulkner, C.J. Echeverri, and R.B. Vallee. 1999. Colocalization of cytoplasmic dynein with dynactin and CLIP-170 at microtubule distal ends. *J Cell Sci*. 112 (Pt 10):1437-1447.

Vaughan, K.T., and R.B. Vallee. 1995. Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. *J Cell Biol*. 131:1507-1516.

Vijay-Kumar, S., C.E. Bugg, and W.J. Cook. 1987. Structure of ubiquitin refined at 1.8 A resolution. *J Mol Biol*. 194:531-544.

Wan, Y., X. Zuo, Y. Zhuo, M. Zhu, S.A. Danziger, and Z. Zhou. 2013. The functional role of SUMO E3 ligase Mms21p in the maintenance of subtelomeric silencing in budding yeast. *Biochemical and biophysical research communications*. 438:746-752.

Wang, D.S., D.W. Dickson, and J.S. Malter. 2008. Tissue transglutaminase, protein cross-linking and Alzheimer's disease: review and views. *International journal of clinical and experimental pathology*. 1:5-18.

Wang, J.Z., I. Grundke-Iqbal, and K. Iqbal. 1996. Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease. *Nature medicine*. 2:871-875.

Wang, S., S.A. Ketcham, A. Schon, B. Goodman, Y. Wang, J. Yates, 3rd, E. Freire, T.A. Schroer, and Y. Zheng. 2013. Nudel/NudE and Lis1 promote dynein and dynactin interaction in the context of spindle morphogenesis. *Molecular biology of the cell*. 24:3522-3533.

Waterman-Storer, C.M., J. Gregory, S.F. Parsons, and E.D. Salmon. 1995a. Membrane/microtubule tip attachment complexes (TACs) allow the assembly dynamics of plus ends to push and pull membranes into tubulovesicular networks in interphase Xenopus egg extracts. *J Cell Biol*. 130:1161-1169.

Waterman-Storer, C.M., S. Karki, and E.L. Holzbaur. 1995b. The p150Glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1). *Proc Natl Acad Sci U S A*. 92:1634-1638.

Watson, P., R. Forster, K.J. Palmer, R. Pepperkok, and D.J. Stephens. 2005. Coupling of ER exit to microtubules through direct interaction of COPII with dynactin. *Nature cell biology*. 7:48-55.

Wei, R., B. Ngo, G. Wu, and W.H. Lee. 2011. Phosphorylation of the Ndc80 complex protein, HEC1, by Nek2 kinase modulates chromosome alignment and signaling of the spindle assembly checkpoint. *Molecular biology of the cell*. 22:3584-3594.

Wen, D., Z. Xu, L. Xia, X. Liu, Y. Tu, H. Lei, W. Wang, T. Wang, L. Song, C. Ma, H. Xu, W. Zhu, G. Chen, and Y. Wu. 2014. Important role of SUMOylation of Spliceosome factors in prostate cancer cells. *Journal of proteome research*. 13:3571-3582.

Westerbeck, J.W., N. Pasupala, M. Guillotte, E. Szymanski, B.C. Matson, C. Esteban, and O. Kerscher. 2014. A SUMO-targeted ubiquitin ligase is involved in the degradation of the nuclear pool of the SUMO E3 ligase Siz1. *Molecular biology of the cell*. 25:1-16.

Wilkinson, K.D. 1997. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 11:1245-1256.

Wu, K., K. Yamoah, G. Dolios, T. Gan-Erdene, P. Tan, A. Chen, C.G. Lee, N. Wei, K.D. Wilkinson, R. Wang, and Z.Q. Pan. 2003. DEN1 is a dual function protease capable of processing the C terminus of Nedd8 and deconjugating hyper-neddylated CUL1. *The Journal of biological chemistry*. 278:28882-28891.

Wykoff, D.D., and E.K. O'Shea. 2005. Identification of sumoylated proteins by systematic immunoprecipitation of the budding yeast proteome. *Molecular & Cellular Proteomics*. 4:73-83.

Xiang, X. 2003. LIS1 at the microtubule plus end and its role in dynein-mediated nuclear migration. *J Cell Biol*. 160:289-290.

Xiang, X., A.H. Osmani, S.A. Osmani, C.H. Roghi, D.A. Willins, S. Beckwith, G. Goldman, Y. Chiu, M. Xin, B. Liu, and et al. 1995a. Analysis of nuclear migration in Aspergillus nidulans. *Cold Spring Harbor symposia on quantitative biology*. 60:813-819.

Xiang, X., A.H. Osmani, S.A. Osmani, M. Xin, and N.R. Morris. 1995b. NudF, a nuclear migration gene in Aspergillus nidulans, is similar to the human LIS-1 gene required for neuronal migration. *Molecular biology of the cell*. 6:297-310.

Xie, Y., O. Kerscher, M.B. Kroetz, H.F. McConchies, P. Sung, and M. Hochstrasser. 2007. The Yeast Hex3*Slx8 Heterodimer is a Ubiquitin Ligase Stimulated by Substrate Sumoylation. *J. Biol. Chem.* 282:34176-34184.

Xie, Y., E.M. Rubenstein, T. Matt, and M. Hochstrasser. 2010. SUMO-independent in vivo activity of a SUMO-targeted ubiquitin ligase toward a short-lived transcription factor. *Genes & development*. 24:893-903.

Xu, J., Y. He, B. Qiang, J. Yuan, X. Peng, and X.M. Pan. 2008. A novel method for high accuracy sumoylation site prediction from protein sequences. *BMC bioinformatics*. 9:8.

Yang, L., J.R. Mullen, and S.J. Brill. 2006. Purification of the yeast Slx5-Slx8 protein complex and characterization of its DNA-binding activity. *Nucleic acids research*. 34:5541-5551.
Yang, S.H., E. Jaffray, B. Senthinathan, R.T. Hay, and A.D. Sharrocks. 2003. SUMO and transcriptional repression: dynamic interactions between the MAP kinase and SUMO pathways. *Cell cycle*. 2:528-530.
Yang, S.H., and A.D. Sharrocks. 2010. The SUMO E3 ligase activity of Pc2 is coordinated through a SUMO interaction motif. *Molecular and cellular biology*. 30:2193-2205.
Yang, Y.C., Y. Yoshikai, S.W. Hsu, H. Saitoh, and L.K. Chang. 2013. Role of RNF4 in the ubiquitination of Rta of Epstein-Barr virus. *The Journal of biological chemistry*. 288:12866-12879.
Yasugi, T., and P.M. Howley. 1996. Identification of the structural and functional human homolog of the yeast ubiquitin conjugating enzyme UBC9. *Nucleic acids research*. 24:2005-2010.
Yeh, E., R.V. Skibbens, J.W. Cheng, E.D. Salmon, and K. Bloom. 1995. Spindle dynamics and cell cycle regulation of dynein in the budding yeast, Saccharomyces cerevisiae. *J Cell Biol*. 130:687-700.
Yen, T.J., D.A. Compton, D. Wise, R.P. Zinkowski, B.R. Brinkley, W.C. Earnshaw, and D.W. Cleveland. 1991. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. *EMBO J*. 10:1245-1254.
Yin, H., D. Pruyne, T.C. Huffaker, and A. Bretscher. 2000. Myosin V orientates the mitotic spindle in yeast. *Nature*. 406:1013-1015.
Yin, Y., A. Seifert, J.S. Chua, J.F. Maure, F. Golebiowski, and R.T. Hay. 2012. SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes & development*. 26:1196-1208.
Yong-Gonzales, V., L.E. Hang, F. Castellucci, D. Branzei, and X. Zhao. 2012. The Smc5-Smc6 complex regulates recombination at centromeric regions and affects kinetochore protein sumoylation during normal growth. *PLoS One*. 7:e51540.
Yun, C., Y. Wang, D. Mukhopadhyay, P. Backlund, N. Kolli, A. Yergey, K.D. Wilkinson, and M. Dasso. 2008. Nucleolar protein B23/nucleophosmin regulates the vertebrate SUMO pathway through SENP3 and SENP5 proteases. *The Journal of cell biology*. 183:589-595.
Zhang, J.Y., S.J. Liu, H.L. Li, and J.Z. Wang. 2005. Microtubule-associated protein tau is a substrate of ATP/Mg(2+)-dependent proteasome protease system. *Journal of neural transmission*. 112:547-555.
Zhang, X., Q. Yu, L. Olsen, and X. Bi. 2012. Functions of protosilencers in the formation and maintenance of heterochromatin in Saccharomyces cerevisiae. *PloS one*. 7:e37092.
Zhang, X.-D., J. Goeres, H. Zhang, T.J. Yen, A.C.G. Porter, and M.J. Matunis. 2008. SUMO-2/3 modification and binding regulate the association of CENP-E with kietochores and progression through mitosis. *Molecular cell*. 29:729-741.
Zhou, W., J.J. Ryan, and H. Zhou. 2004. Global analyses of sumoylated proteins in *Saccharomyces cerevisiae*: Induction of protein sumoylation by cellular stresses. *J. Biol. Chem*. 279:32262-32268.
Zhou, X.Z., O. Kops, A. Werner, P.J. Lu, M. Shen, G. Stoller, G. Kullertz, M. Stark, G. Fischer, and K.P. Lu. 2000. Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. *Mol Cell*. 6:873-883.
Zimdahl, B., T. Ito, A. Blevins, J. Bajaj, T. Konuma, J. Weeks, C.S. Koechlein, H.Y. Kwon, O. Arami, D. Rizzieri, H.E. Broome, C. Chuah, V.G. Oehler, R. Sasik, G. Hardiman, and T. Reya. 2014. Lis1 regulates asymmetric division in hematopoietic stem cells and in leukemia. *Nature genetics*. 46:245-252.

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