



Emerging Roles of Sumoylation in the Regulation of Actin, Microtubules, Intermediate Filaments, and Septins

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Sumoylation is a powerful regulatory system that controls many of the critical processes in the cell, including DNA repair, transcriptional regulation, nuclear transport, and DNA replication. Recently, new functions for SUMO have begun to emerge. SUMO is covalently attached to components of each of the four major cytoskeletal networks, including microtubule-associated proteins, septins, and intermediate filaments, in addition to nuclear actin and actin-regulatory proteins. However, knowledge of the mechanisms by which this signal transduction system controls the cytoskeleton is still in its infancy. One story that is beginning to unfold is that SUMO may regulate the microtubule motor protein dynein by modification of its adaptor Lis1. In other instances, cytoskeletal elements can both bind to SUMO non-covalently and also be conjugated by it. The molecular mechanisms for many of these new functions are not yet clear, but are under active investigation. One emerging model links the function of MAP sumoylation to protein degradation through SUMO-targeted ubiquitin ligases, also known as STUbL enzymes. Other possible functions for cytoskeletal sumoylation are also discussed. © 2015 The Authors. Cytoskeleton

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Key Words: microfilaments; septins; MT; IF; SUMO; microtubule-associated proteins; MAPs

Abbreviations used: MAP, microtubule-associated protein; SIMs, SUMO interacting motifs;; SPB, spindle pole body; STUbL, SUMO-targeted ubiquitin ligase.

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Introduction

Sumoylation is a fascinating regulatory system that displays several similarities to the more well-known process of ubiquitination, but is nevertheless a distinct signal transduction system. Like ubiquitin, the SUMO moiety is conjugated to target proteins on lysine residues and this can drastically alter that protein's localization, protein-protein interactions, and even its stability. Sumoylation regulates many of the basic cellular processes, including DNA replication, translation, ribosomal maturation [Finkbeiner et al., 2011], DNA repair [Prudden et al., 2011], PML (promyelocytic leukemia) nuclear body formation [Nagai et al., 2011], nucleo-cytoplasmic trafficking [Wang et al., 2012], kinetochore function and chromosome segregation [Stead et al., 2003; Yong-Gonzales et al., 2012; Pinder et al., 2013], transcription and transcriptional repression [Garcia-Domiguez and Reyes, 2009; Ouyang et al., 2009]. These have been expertly reviewed elsewhere [Dasso 2008; Bergink and Jentsch, 2009; Stehmeier and Muller, 2009; Garreau and Lima, 2010; Nagai et al., 2011; Praefcke et al., 2012]. This review covers emerging evidence suggesting that SUMO regulates the cytoskeleton, an idea that has not been widely recognized previously.

The cytoskeleton is commonly considered to be comprised of three polymer networks; the microtubules, the actin cytoskeleton also known as microfilaments, and intermediate filaments. However, a fourth polymeric network, the septins, should be considered as part of the cytoskeleton as well [Gladfelter, 2010; Mostowy and Cossart, 2012] and we include a discussion of its sumoylation in this review. Microtubules are well recognized as a major component of the mitotic spindle that separates genetic information found in chromosomes. Actin plays important roles in a large variety of cellular processes including cytokinesis, muscle contraction, cell motility, endocytosis, and phagocytosis. Both microtubules and microfilaments can serve as railway-like transit systems that allow the movement of various cargoes by motor proteins along these tracks to specific destinations within the cell. In contrast, intermediate filament networks and septins do not serve as tracks for motor proteins. Instead, intermediate filaments

provide structural integrity to the cell, and septins promote cytokinesis by forming a filamentous collar around the neck of dividing cells [Beise and Trimble, 2011].

It has recently been established that SUMO modifies elements of all four of the cytoskeletal networks. While some functions of the SUMO modifications are clear, a plethora of questions remain concerning the when, how, why and by what mechanism SUMO signaling controls the various elements of the cytoskeleton. Elucidating the similarities and differences in SUMO's control of the different cytoskeletal networks will assuredly illuminate new communication circuits within the cell.

A better understanding of the regulation of the cytoskeleton by SUMO is likely to provide significant impacts on human health. As the cytoskeleton is intimately involved in numerous disease processes, new knowledge of its regulation will undoubtedly lead to new ways to intervene when its function is impaired. Sumoylation is well known to transduce signals from multiple types of stress to influence various cellular processes [Tempe et al., 2008]. The idea that a similar signaling paradigm could also modulate the cytoskeleton is just beginning to emerge. Currently however, the mechanisms by which the cytoskeleton deals with these stresses are poorly understood. Indeed, sumoylation is implicated in several neurodegenerative diseases including Alzheimer's [Zhang et al., 2008; McMillan et al., 2011; Hoppe et al., 2013], Parkinson's disease [Kim et al., 2011; Krumova et al., 2011; Weetman et al., 2013], as well as cancer [Lee et al., 2006; Liu et al., 2011; Bettermann et al., 2012], and other diseases [Dorval and Fraser, 2007; Sarge and Park-Sarge, 2011]. However, how sumoylation affects the cytoskeleton in these diseases remains unclear. Clarification of these pathways could ultimately lead to new paths for therapy development, including new targets for drug screening.

While it is already widely accepted that the cytoskeleton is regulated by a multitude of different post-translational modifications, these often transduce signals from a variety of inputs and thus produce a variety of outputs. The sumoylation system may provide a single molecular mechanism to signal to the multiple polymers of the cytoskeleton simultaneously. Thus, it is possible that a particular input could result in a coordinated output for multiple cytoskeletal polymers.

The Sumoylation Machinery

SUMO is about 100 amino acids in size [Johnson, 2004]. Although SUMO and ubiquitin share only ~18% sequence identity, they are structurally quite similar [Vijay-Kumar et al., 1987; Bayer et al., 1998] (Fig. 1). Like ubiquitin, the tertiary structure of SUMO contains a 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

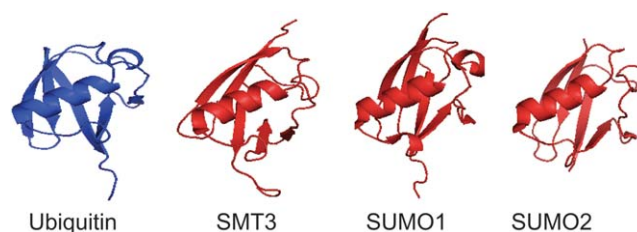


Fig. 1. Structure of ubiquitin and SUMO proteins. Ribbon drawing of ubiquitin, Smt3, SUMO1, and SUMO2. These molecules share a common secondary structure $\beta\beta\alpha\alpha\beta\beta\alpha\beta$ that assembles into a ubiquitin-like fold. Renderings were developed using the crystallography coordinates 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.

extension approximately 20 amino acids long that is absent in ubiquitin. Both are processed to yield a terminal glycine-glycine motif that is used in conjugation to target proteins [Ozkaynak et al., 1987; Wilkinson, 1997; Larsen et al., 1998; Li and Hochstrasser, 1999; Fang and Weissman, 2004; Li and Ye, 2008].

Classically, SUMO is conjugated to a lysine residue lying within the consensus sequence Ψ KxE/D, where Ψ is a large hydrophobic residue and x is any amino acid [Melchior, 2000; Johnson, 2004]. 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].

There are four SUMO paralogs in humans, SUMO1-4; but only one in the budding yeast, *Saccharomyces cerevisiae* (Smt3p); and one in the fission yeast, *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]. 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 50% amino acid sequence identity and a longer N-terminal extension [Schwarz et al., 1998; Sheng and Liao, 2002]. For any of the SUMO paralogs, SUMO is often conjugated to only a small population of the target protein at any given time [Johnson, 2004; Klug et al., 2013]. Although SUMO interacting motifs (SIMs) play a role, it still remains an outstanding question of what factors specify the conjugation of a particular paralog to a particular cytoskeletal element [Citro and Chiocca, 2013].

The enzyme cascade of the sumoylation pathway is analogous with 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

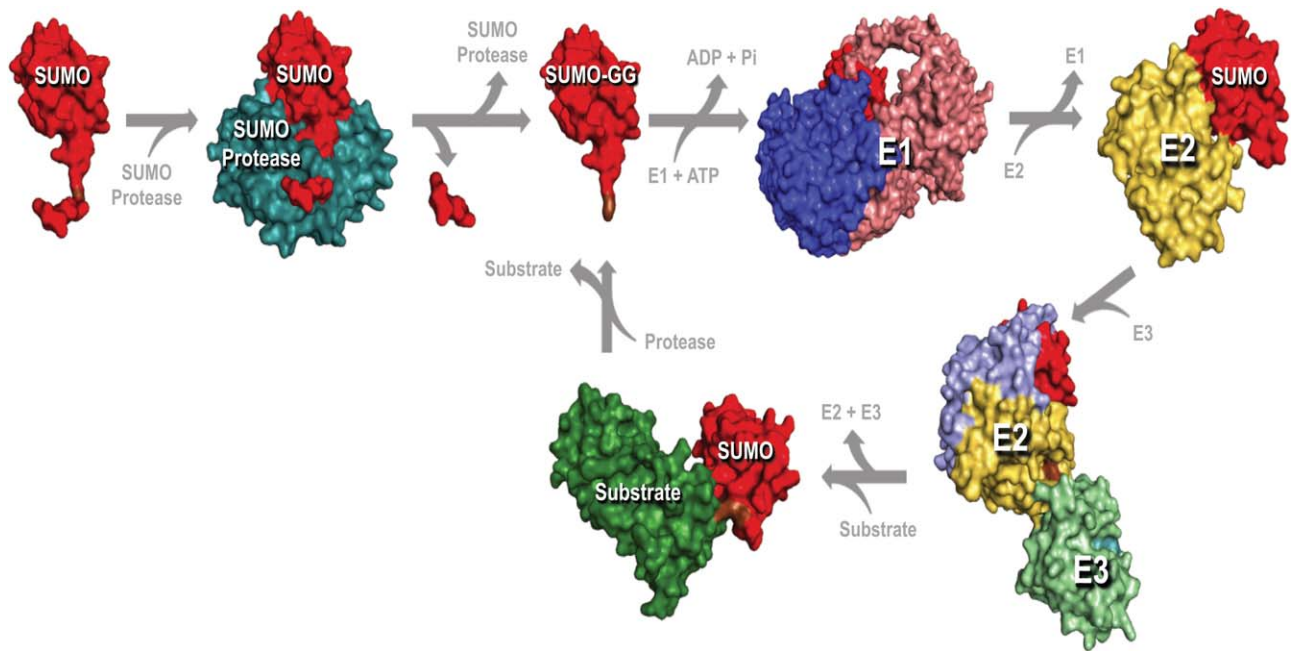


Fig. 2. SUMOylation Pathway: To portray each state in the sumoylation pathway, surface maps were developed using crystallography coordinates available from PDB with the following accession numbers: SUMO1 and Senp1 (2IY1), E1 complex (3KYC), E2 complex (2UYZ), E3 complex (3UIP), and sumoylated PCNA (3V60). The orientation of SUMO is maintained throughout the sumoylation processes depicted above.

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] (Fig. 2).

For both moieties, conjugation consists of 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, thus forming an isopeptide bond (Fig. 3A). 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].

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. This enzyme consists of a heterodimer of Aos1 and Uba2 and is conserved from yeast to human [Dohmen et al., 1995; Johnson et al., 1997; Desterro et al., 1999]. The thiol group of cysteine within the active site of Aos1-Uba2 attacks the adenylated SUMO, forming a high-energy thioester bond between the Aos1-Uba2 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 [Johnson and Blobel, 1997] (Fig. 3B).

The sole E2 SUMO-conjugating enzyme is Ubc9p, which is also highly conserved from yeast to humans [John-

son et al., 1997; Schwarz et al., 1998]. Ubc9 is regulated by multiple post-translational modifications, including sumoylation, acetylation, and phosphorylation [Ho et al., 2011; Su et al., 2012; Hsieh et al., 2013]. Phosphorylation of Ubc9p by the cyclin-dependent kinase, 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 conjugation can take place in the absence of a SUMO E3, however the E3 is thought to bring the Ubc9p into 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] (Fig. 4). There are several classes of SUMO E3s; including 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, Mms21p/Nse2p, and Cst9p/Zip3p [Johnson and Gupta, 2001; Reindle et al., 2006; Duan et al., 2011; Heideker et al., 2011; 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].

Like ubiquitin, SUMO can form chains, known as poly-sumoylation [Johnson and Gupta, 2001]. SUMO chains

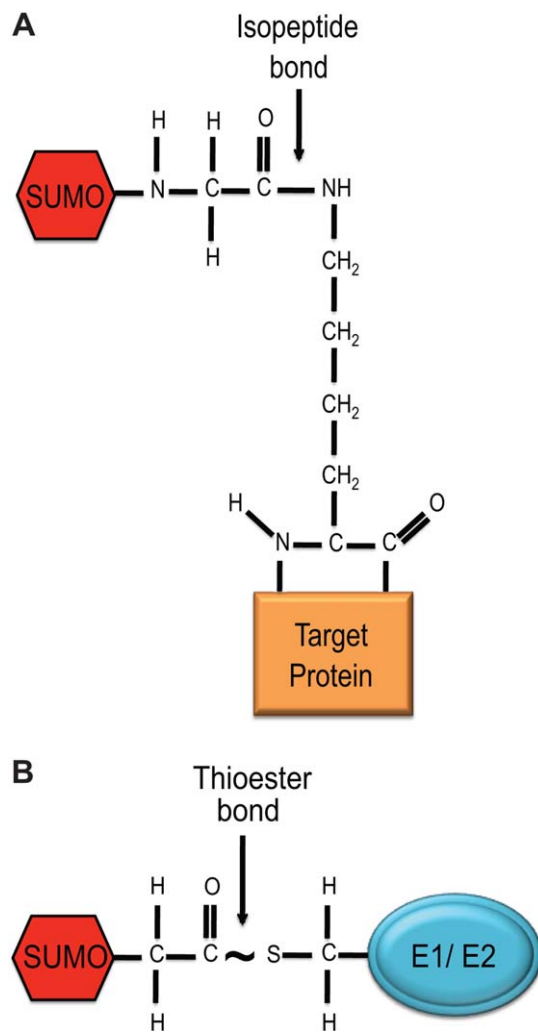


Fig. 3. Chemical bonds in the sumoylation pathway. (A) Isopeptide bond. SUMO is conjugated to the target protein via an isopeptide bond linkage between the terminal glycine residue of SUMO and the epsilon amino group of the lysine in the target. (B) Thioester Bond. Chemical linkage is highlighted between the terminal glycine carboxy group of SUMO and the active cysteine in the SUMO activating, and conjugating enzymes.

occur mainly through SUMO's flexible N-terminal extension containing a ψ KxE sequence [Tatham et al., 2001]. SUMO2/3 is more likely to form chains than SUMO1, which lacks the needed lysines [Tatham et al., 2001]. SUMO1 can also cap the end of a SUMO 2/3 chain, limiting its length [Matic et al., 2008]. The budding yeast SUMO, Smt3p, although it displays similarity to SUMO1, also forms chains [Bylebyl et al., 2003].

Two Models for Regulation by SUMO: 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 interac-

tion motifs (SIMs) on the interacting protein [Minty et al., 2000; Song et al., 2004; Kroetz and Hochstrasser, 2009]. 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].

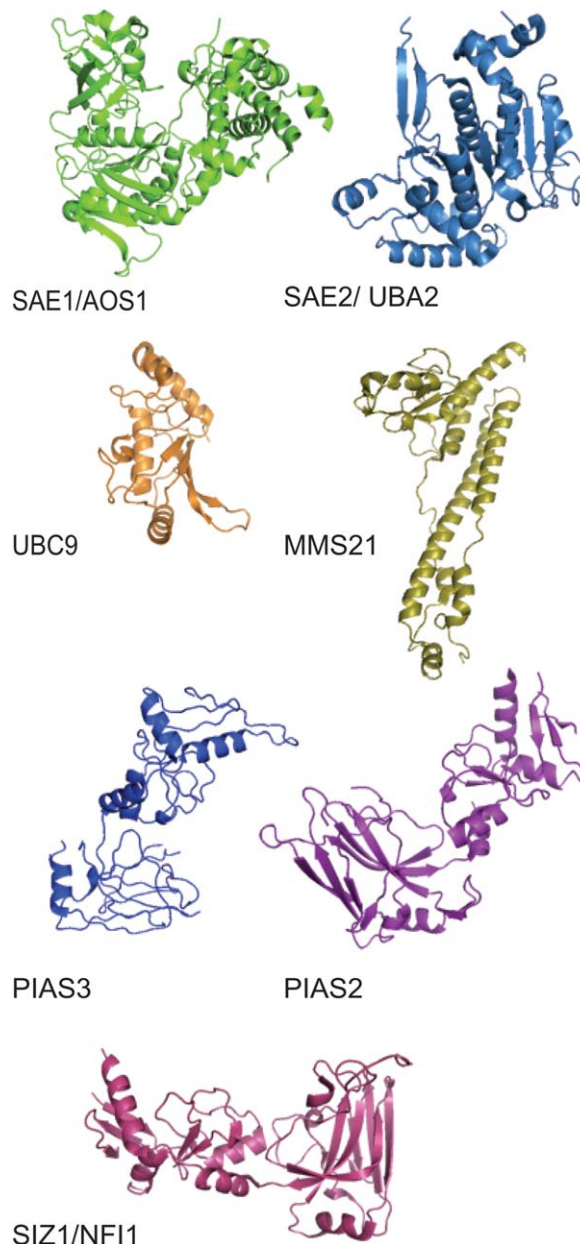


Fig. 4. Structure of SUMO E1, E2, E3 enzymes. Tertiary ribbon structure of the SUMO activating enzyme dimers Sae1 and Sae2, SUMO conjugating enzyme Ubc9, and SUMO ligating enzymes Mms21, PIAS3, PIAS2, and Siz1. These renderings were developed using the crystallography coordinates associated with the following PDB accession numbers: Sae1 (1Y8Q), Sae2 (1Y8Q), Ubc9 (2GRR), Mms2 (3HTK), PIAS3 (4MVT), PIAS2 (4FO9), and Siz1 (3I2D).

Some proteins like the kinetochore kinesin, CENP-E, can interact both ways, covalently and non-covalently [Zhang et al., 2008]. Very little is known currently about the extent to which various cytoskeletal elements interact non-covalently with SUMO.

Proteases Make Sumoylation a Reversible Process

Unlike traditional proteases, SENPs/Ulps do not degrade either SUMO or the targets. These enzymes remove SUMO from its targets by cleavage of the isopeptide bond between the glycine of SUMO and the target lysine. This allows the SUMO moiety to be recycled. 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 [Mukhopadhyay and Dasso, 2007; Drag and Salvesen, 2008; Su and Hochstrasser, 2010; Gillies and Hochstrasser, 2012; Hickey et al., 2012]. Although the different cytoskeletal polymers themselves display varying degrees of dynamic subunit turnover [Cleveland, 1982; Yoon et al., 2001; Vorobjev et al., 1999], very little is known about the rates of reversible attachment of SUMO on each of the cytoskeletal polymers.

In mammals, there are six SUMO-cleaving enzymes, SENP1, SENP2, SENP3, SENP5, SENP6, and SENP7, in addition to the recently described DeSumoylating Isopeptidase 1 (DESI) protease [Mukhopadhyay and Dasso, 2007; Shin et al., 2012; Suh et al., 2012]. In *Saccharomyces cerevisiae*, there are only three SUMO proteases, Ulp1p, Ulp2p, and Wss1p, each belonging to a distinct class [Li and Hochstrasser, 1999; Li and Hochstrasser, 2000; Bylebyl et al., 2003; Gillies and Hochstrasser, 2012].

SENPs

SENP/Ulp enzymes can 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 [Li and Hochstrasser, 1999; Mikolajczyk et al., 2007; Drag and Salvesen, 2008; Lima and Reverter, 2008]. 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; but 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, 2000; Schwienhorst et al., 2000]. These two proteases also display different subcellular localizations and virtually non-overlapping interactomes [Panse et al., 2003; Cubenas-Potts et al., 2013; Srikumar et al., 2013]. Yet surprisingly, only a few cytoskeletal substrates are known for each [Hickey et al., 2012]. The Kerscher lab and others have shown that Ulp1p in yeast desumoylates the septins [Takahashi et al., 2000; Elmore et al., 2011]. The sumoylation of septins is described more fully below. Ulp1p also de-modifies two proteins important for spindle positioning, Kar9p and Pac1p [Leisner et al., 2008; Alonso et al., 2012]. These are described below. We are not aware of any functional evidence that physically links Ulp2p to the major cytoskeletal polymers.

Wss1p

Wss1p is predicted to be a zinc-dependent metalloprotease, 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. Wss1p contains two SIMs (SUMO interacting motifs) within its extreme carboxyl-terminal domain [Uzunova et al., 2007; Mullen et al., 2010], but it also has significant conservation with deubiquitinating enzymes (DUBs) [Mullen et al., 2010]. Recent work from the Brill lab suggests the possibility that while Wss1p may have both SUMO protease and DUB types of activity, it is a much better SUMO-cleaving enzyme than a ubiquitin-cleaving one [Mullen et al., 2010].

In addition to its role in sister chromatid recombination, a type of double-strand DNA break 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 [Meednu et al., 2008; Alonso et al., 2012]. What makes this finding remarkable is that these different classes of MAPs carry out a divergent set of functions for microtubules [Berlin et al., 1990; Schwartz et al., 1997; Miller et al., 1999; Miller et al., 2000; Gundersen and Bretscher, 2003; Hwang et al., 2003; Lee et al., 2003; Sheeman et al., 2003; Miller et al., 2006; Blake-Hodek et al., 2010; Huang et al., 2012]. The effect of Wss1p on microtubule binding proteins has been examined only for the Pac1p adaptor of the dynein motor protein [Alonso et al., 2012]. These experiments show that deletion of *WSS1* results in higher molecular weight forms of Pac1p. This is consistent with the hypothesis from the Brill lab that Wss1p helps direct

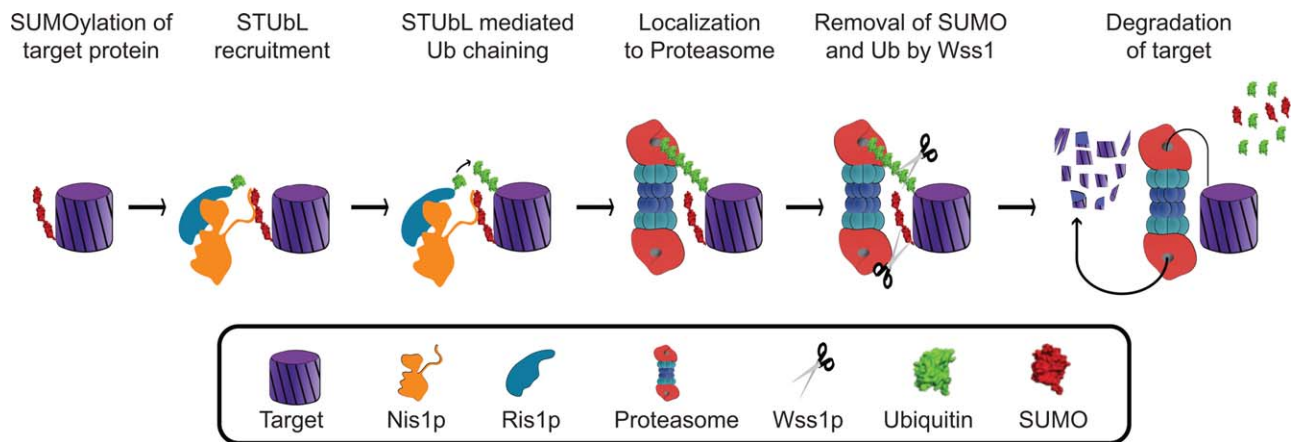


Fig. 5. STUbL Pathway Model. The Ris1p-Nis1p STUbL complex can direct a sumoylated target protein to the proteasome for degradation (Adapted from Alonso et al. 2012).

sumoylated proteins to the proteasome [Mullen et al., 2010; Alonso et al., 2012]. Further work is in progress to determine whether Wss1p alters the levels of ubiquitin on Pac1p.

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]. The punctate pattern is consistent with it also localizing on the ends of cytoplasmic microtubules in the mother cell, but this is not known definitively.

SUMO-Targeted Ubiquitin Ligases (STUbLs)

Owing that sumoylation is a reversible process, the levels of SUMO on a protein are critical and need to be maintained at an optimal homeostasis [Prudden et al., 2007; Kim and Baek, 2009; Bawa-Khalife and Yeh, 2010]. As discussed above, this can be accomplished by cleaving SUMO from targets. Another way to remove excess poly-sumoylation is to degrade the entire sumoylated protein at the proteasome. For many years, sumoylation and ubiquitination were viewed as distinct modification systems with limited cross talk [Ulrich, 2005]. In one paradigm, 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 the target is first modified 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). With this, communication between ubiquitin and SUMO became more

interesting [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 (Fig. 5).

Three STUbL families have been characterized, Uls1p-Nis1p and Slx5p-Slx8p/RNF4, and Rad18p. 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 [Xie et al., 2007; Cook et al., 2009; Szymanski and Kerscher, 2013]. Slx5p-Slx8p is the yeast homologue of the human RNF4, [Sun et al., 2007; Uzunova et al., 2007; Xie et al., 2007]. Little information is presently known about Uls1p targets, with only a few currently identified. These include the microtubule associated protein Pac1p and the DNA binding protein Rap1p [Grunstein, 1997; Jain and Cooper, 2010; Alonso et al., 2012; 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 de The et al., 2012; 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, the major treatment for acute promyelocytic leukemia, causes the sumoylation of PML-RAR α by SUMO2. RNF4 then polyubiquitinates these SUMO chains, resulting in degradation of the aberrant PML by the proteasome [Tatham et al., 2008; Liu et al., 2012; Maroui et al., 2012; Rojas-Fernandez et al., 2014]. Recently, the novel STUbL, Arkadia, was found to function similarly in PML degradation [Erker et al., 2013]. The elegant work describing PML

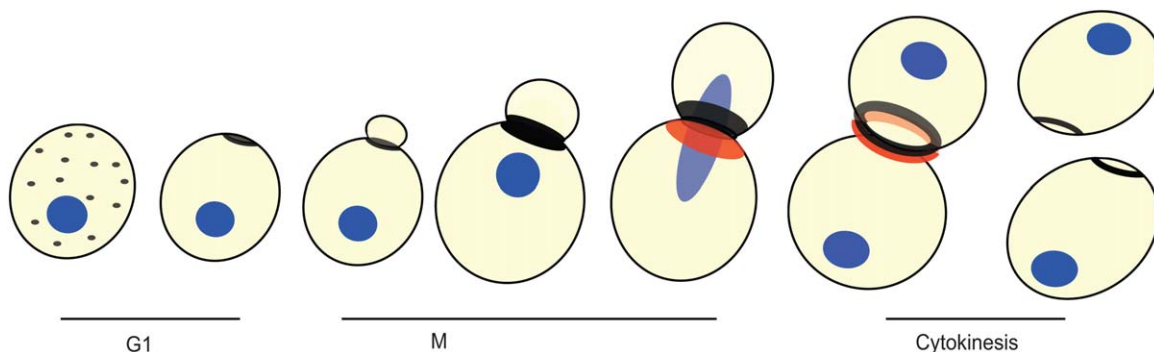


Fig. 6. Sumoylation of septins. During G1, a septin patch forms at the site of bud formation. As the bud emerges through the patch, the septins form a collar around the mother-bud neck. The five septin proteins involved in this process are Cdc3p, Cdc10p, Cdc11p, Cdc12p, and Shs1p. Prior to cytokinesis, three of these septin proteins are sumoylated, but only on the mother side of the mother-bud neck. Cdc3p is sumoylated at lysines 4, 11, 30, and 63. Cdc11p is sumoylated at lysine 412. Shs1p is sumoylated at lysines 426, and 437 [Johnson and Blobel, 1999; Takahashi et al., 1999]. The sumoylation event is color coded in red. During cytokinesis, the septin “hourglass” collar splits into two rings as the cells divide. After cytokinesis the septin rings dissociate, and the process starts again.

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.

In just a few short years, the number of targets for STUBL enzymes and processes governed by STUBLs has simply exploded, with STUBLs playing critical roles in almost as many cellular processes as SUMO itself. It is perhaps not surprising that STUBLs have now been linked to the cytoskeleton, including interactions with several microtubule-associated proteins.

SUMO and the Cytoskeleton

Septins

Septins were originally identified in yeast using screens searching for cell division cycle (CDC) genes [Hartwell, 1971; Hartwell et al., 1974]. This work identified four of the five mitotic septins, Cdc3p, Cdc10p, Cdc11p, and Cdc12p, which are essential. The name septin was later coined to describe the role of these genes in cell septation in yeast [Mostowy and Cossart, 2012]. A fifth mitotic septin, Sep7p/Shs1p was later identified as the seventh homolog of a septin [Mino et al., 1998]. Shs1p is not essential. Septins are highly conserved, found in a wide range of organisms ranging from yeast to human. However, no evidence has been found for septins in plants [Field et al., 1996; Nguyen et al., 2000; Gladfelter et al., 2001; Nishihama et al., 2011]. The reader is referred to a comprehensive review of septins that was published recently [Mostowy and Cossart, 2012].

In *Saccharomyces cerevisiae*, septins form the filaments that encircle the mother-bud neck, the site of cytokinesis in this yeast [Byers and Goetsch, 1976; Haarer and Pringle, 1987; Ford and Pringle, 1991; Bertin et al., 2012]. A septin

patch is formed initially on the cortex of the unbudded cell, just before bud emergence [reviewed in Chen et al., 2011]. As the growing bud emerges, the septins then reorganize to form an hourglass-like collar that is positioned on both sides of the mother-bud neck [Longtine and Bi, 2003; Kozubowski et al., 2005; Vrabioiu and Mitchison, 2006]. Electron microscopy studies reveal a gauze-like meshwork of filaments at the bud neck [Rodal et al., 2005] consisting of filaments running circumferentially around the neck and axial filaments running along the mother-bud axis [Garcia et al., 2011; Bertin et al., 2012; Bertin and Nogales, 2012]. At cytokinesis, the hourglass collar splits into two rings via rearrangement and reassembly mechanisms, with one ring facing the mother cell and the other facing the bud (Fig. 6) [Garcia et al., 2011; Bertin et al., 2012; Ong et al., 2014].

Notably, the two sides of the hourglass collar are not symmetric, and distinct sets of proteins are localized with the ring on the mother side and the ring on the bud side. Still other proteins localize between the two rings [Kozubowski et al., 2005]. Thus, the septins serve as scaffolds for proteins functioning in cytokinesis, bringing in and organizing components of the actomyosin constriction ring and the enzymes needed for cell wall synthesis [Gladfelter et al., 2001; McMurray et al., 2011; Kang et al., 2013].

Septins also play a role in several other cellular processes that are closely associated with membranes. These include spindle alignment and the establishment of the diffusion barrier [Kusch et al., 2002; Dobbelaere and Barral, 2004; Caudron and Barral, 2009]. Diffusion barriers block molecules in one membrane compartment from diffusing through the lipid bilayer into another compartment.

The paired filaments formed by septins are approximately 10 nm in diameter, which can also self assemble in vitro [Byers and Goetsch, 1976; Bertin et al., 2012]. In yeast, electron microscopy studies demonstrate that the basic building block of the septin filament is comprised of

the four essential septins arranged into a hetero-octomer in the order of Cdc11p-Cdc12p-Cdc3p-Cdc10p-Cdc10p-Cdc3p-Cdc12p-Cdc11p [Bertin et al., 2008]. The two halves of the octomer fit together around the two-fold rotational symmetry in the Cdc10p-Cdc10p homophilic interaction of Cdc10, creating a symmetric rod-shaped subunit that is the building block of septins [Bertin et al., 2008]. In mammalian systems, the basic building block is a hetero-hexamer, rather than an octomer, and it also has a rod-like shape [Sirajuddin et al., 2007; Garcia et al., 2011]. An alternative hetero-octomer containing Shs1p rather than Cdc11p is important for the bundling of filaments and ring formation in vitro and formation of the septin collar in vivo [Garcia et al., 2011].

Sumoylation of the Septins

Septins were the first substrates of SUMO identified in yeast [Johnson and Blobel, 1999; Takahashi et al., 1999]. Indeed, they are some of the most abundant sumoylated proteins in the cell [Johnson and Blobel, 1999; Wohlschlegel et al., 2004]. Sumoylation serves as one of the markers for the asymmetry of the two septin rings; only the septin ring on the mother side of the bud neck is sumoylated [Johnson and Blobel, 1999; Takahashi et al., 1999; Martin and Konopka, 2004]. This sumoylation occurs during mitosis, with SUMO addition occurring just before anaphase and SUMO removal occurring abruptly at cytokinesis [Johnson and Blobel, 1999]. Consistent with this, the E3 enzyme Siz1p localizes to the septin ring on the mother side of the neck at the same point in the cell cycle as the SUMO modification occurs during mitosis [Johnson and Gupta, 2001]. Additional amounts of GFP-Siz1p are found inside the nucleus as puncta [Johnson and Gupta, 2001].

Septin sumoylation has been seen to play a role in maintaining the polymerization state of septins as mutants lacking sumoylation sites display a modest delay in the disassembly of the septin rings at cytokinesis [Johnson and Blobel, 1999]. The molecular mechanism of this remains an avenue for future investigations, as the mutation of these lysines could alter other aspects of the septin proteins such as folding and stability. Several questions still remain concerning the roles of septin sumoylation [Oh and Bi, 2011]. While septins are required for cytokinesis, their sumoylation is not [Johnson and Blobel, 1999; Dobbelaere and Baral, 2004]. Septins are also not the essential substrate of sumoylation during the cell cycle, because when all the septin sumoylation sites are mutated and combined into one cell, the cells grow and do not display the cell-cycle arrest observed in SUMO deficient mutants [Johnson and Blobel, 1999].

It is notable that only a subset of the septins is sumoylated [Johnson and Blobel, 1999]. In yeast, only Cdc3p, Cdc11p, and Shs1p are modified by SUMO [Johnson and Blobel, 1999]. Cdc3p is sumoylated at four sites, Cdc11p

at one site, and Shs1p at two sites [Johnson and Blobel, 1999]. These modifications are absent in cellular extracts from a *siz1Δ* strain but not *nfi1Δ* [Johnson and Gupta, 2001]. Siz1p also enhances the in vitro sumoylation of septins. Together, these findings suggest that Siz1p is the E3 responsible for septin sumoylation [Johnson and Gupta, 2001].

Two of the sumoylated septins, Shs1p and Cdc11p, occupy the terminal position in the octomeric building-block for filament assembly. This prompts one to wonder whether this modification may modulate the specialized role of Shs1p in promoting ring formation and filament bundling [Garcia et al., 2011]. One might also speculate whether the high levels of phosphorylation on Shs1p might influence its sumoylation [Egelhofer et al., 2008; Meseroll et al., 2013]. While the precise function of septin sumoylation has been evasive, considering that Cdc11p and Cdc3p are essential, sumoylation is unlikely to play a critical role for these septins. However, the septin Shs1p is not essential, and considering that the phenotypes of Shs1p are milder than mutations in the other two septins, it is possible that the function of sumoylation is tied to this less critical septin.

Sumoylation of the septins is regulated by signals passing through the E2, Ubc9p. The sumoylation levels on the septins are inversely proportional to the levels of Ubc9p autophosphorylation [Ho et al., 2011]. Determining the extent to which Ubc9p phosphorylation by CDKs and other post-translational modifications affect septin sumoylation should prove to be a worthwhile avenue of future investigation [Su et al., 2012].

Deregulation of septins has been linked to several major diseases, including multiple cancers and neurological diseases, including Parkinson's and Alzheimer's [Ihara et al., 2007]. In Parkinson's disease, the septin SEPT4 has been shown to modulate the neurotoxicity of alpha-synuclein, but it remains to be determined whether the sumoylation of alpha-synuclein is a part of this modulation [Ihara et al., 2007]. In Alzheimer's, septins have been seen to colocalize in neurofibrillary tangles, an aberrant structure containing the MAP, Tau [Kinoshita et al., 1998]. In several types of cancer, human SEPT9 serves as a biomarker for colon cancer [reviewed in Cerveira et al., 2011; Connolly et al., 2011]. As septins are dynamic structures [Gladfelter, 2010], it is possible that sumoylation may affect their solubility and thus influence their dynamicity.

Microtubules

Microtubules are proteinaceous polymers comprised of alpha-beta tubulin dimers that make key contributions to intracellular motility and cell division [reviewed in Desai and Mitchison, 1997; Valiron et al., 2001; Howard and Hyman, 2003; Conde and Caceres, 2009; Etienne-Manneville, 2013]. They serve as tracks along which motors

move various 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 [Cassimeris et al., 1988; Sammak and Borisy, 1988; Schulze and Kirschner, 1988; Chretien et al., 1995; Akhmanova and Steinmetz, 2008; Gardner et al., 2008]. 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, 1984]. In yeast, the spindle pole body serves as the MTOC [reviewed in Rout and Kilmartin, 1990; Kahana et al., 1995; Jaspersen and Winey, 2004].

SUMO and Microtubules

Currently, sumoylation is not widely recognized as a post-translational modification of either tubulin or microtubules [Janke, 2014; Song and Brady, 2015]. To date, there is only limited evidence that tubulin itself may be modified by SUMO. Alpha and/or beta tubulins have been identified as candidates in several global sumoylation screens employing proteomics [Panse et al., 2004; Wohlschlegel et al., 2004; Rosas-Acosta et al., 2005]. However, only immunoblotting with monoclonal anti-tubulin was used to confirm the Rosas-Acosta finding of sumoylated alpha tubulin. The anti-tubulin reacted with a larger 70 kDa band in the TAP purified samples, but with only the standard 50 kDa size tubulin in the corresponding parental-control strain [Rosas-Acosta et al., 2005]. The shifted form was only observed in the SUMO3 TAP purification, but not the SUMO1 purification, indicating that SUMO3 may be responsible for modifying alpha tubulin [Rosas-Acosta et al., 2005]. As alternative explanations for these findings are possible, additional confirmatory studies are needed before other questions can be answered about how sumoylation might alter the many properties of microtubules and their dynamics.

MAPs

Many classes of microtubule-associated 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 interaction, and either stabilizing or destabilizing microtubules. Some classes of MAPs bind directly to tubulin dimers to help regulate their addition to the microtubule polymer [Etienne-Manneville, 2010; Gupta et al., 2013; Cheerambathur and Desai, 2014; Ferreira et al., 2014]. 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 shown to be modified by SUMO (Table I) and several other classes interact with SUMO either physically or by two-hybrid analysis. The MAPs that can be covalently modified include the dynein adapter Pac1p (Lis1), Bik1p (CLIP-170), the spindle positioning protein Kar9p, the Alzheimer's MAP Tau, and the kinetochore attachment protein Ndc80p [Dorval and Fraser, 2006, 2007; Montpetit et al., 2006; Leisner et al., 2008; Meednu et al., 2008; Alonso et al., 2012]. The kinetochore kinesin CENP-E is both modified by SUMO and interacts non-covalently with it [Zhang et al., 2008]. Interaction with the SUMO machinery has also been seen with Bim1p, the EB1 homologue in yeast, but it is not known whether this interaction occurs through conjugation or non-covalent interactions [Meednu et al., 2008]. This growing list leads us to speculate that sumoylation may control multiple facets of microtubule biology via regulation of its MAPs.

Kar9p

Kar9p is required for correct orientation of the mitotic spindle and is important for nuclear migration in both mating and mitotic cells [Kurihara et al., 1994; Miller and Rose, 1998]. *KAR9* was discovered in a screen for bilateral karyogamy mutants, [Kurihara et al., 1994] and is thought to be analogous to the mammalian adenomatous polyposis coli protein (APC) [Bienz, 2001], which is mutated in a large percentage of human colorectal cancers [Grodin et al., 1991; Markowitz and Bertagnolli, 2009]. APC and Kar9p share a number of functional similarities, albeit they have limited homology at the amino acid level [Bienz, 2001; Gundersen, 2002]. At the protein level, Kar9p consists of an N-terminal acidic domain, a central coil-coil domain, and a C-terminal basic domain [Miller and Rose, 1998].

Kar9p plays a key role in positioning the mitotic spindle by orienting the cytoplasmic microtubule into the bud [Miller and Rose, 1998]. Kar9p links the actin and microtubule networks through a bridging complex that contains Bim1p-Kar9p-Myo2p [Beach et al., 2000; Hwang et al., 2003]. Bim1p is a microtubule-binding protein and the yeast homologue of EB1. Myo2p is a type V myosin. The EB1-like C-terminus of Bim1p binds the C-terminal domain of Kar9p [Miller et al., 2000; Moore and Miller, 2007]. Kar9p binds to the tail of Myo2p in a region that overlaps with other cargo-binding sites [Eves et al., 2012]. When this connection is formed, the myosin walks up the actin cable. The resulting pulling-force guides the end of the cytoplasmic microtubule into the yeast bud, thus orienting the mitotic spindle. The myosin motor then pulls the spindle up to the bud neck [Beach et al., 2000; Korinek et al., 2000; Miller et al., 2000; Yin et al., 2000].

Table I. Sumoylation Targets of the Cytoskeleton

Polymer system	Type of modification	Site of modification	Evidence	Reference
Septins				
Cdc3p	Covalent	K4, K11, K30, and K63	Pulldown	Johnson and Blobel [1999]
Cdc11p	Covalent	K412	Pulldown	Johnson and Blobel [1999]
Shslp	Covalent	K426, K437	Pulldown	Johnson and Blobel [1999]
Microtubules				
Alpha-beta tubulin	Covalent	N/A	Proteomic screens, pulldown	Panse et al. [2004]; Wohlschlegel et al. [2004]; Rosas-Acosta et al. [2005]
CENP-E	Covalent and non-covalent	N/A	Pulldown	Zhang et al. [2008]
Ndc80	Covalent	K231	Proteomic screens, pulldown	Panse et al. [2004]; Zhou et al. [2004]; Wykoff and O'Shea [2005]; Montpetit et al. [2006]
Tau	Covalent	K340	Pulldown	Dorval and Fraser, [2012, 2006]; Takahashi et al. [2008]
Pac1p	Covalent	N/A	Pulldown, Y2H	Alonso et al. [2012]
Kar9p	Covalent	K301, K333, K381, and K529	Pulldown, Y2H	Leisner et al. [2008]; Meednu et al. [2008]
Bik1p	Covalent	N/A	In vitro, Y2H	Alonso et al. [2012]
Bim1p	N/A	N/A	Y2H	Meednu et al. [2008]
La	Covalent	K41	Pulldown	van Niekerk et al. [2007]
Microfilaments				
Actin	Covalent	K68, and K284	Proteomic screens, pulldown	Panse et al. [2004]; Vertegaal et al. [2004]; Wohlschlegel et al. [2004]; Rosas-Acosta et al. [2005]; Hofmann et al. [2009]
Rac1	Covalent	K188, K183, K184, and K186	Pulldown	Castillo-Lluva et al. [2010]
RhoGDI	Covalent	K138	Pulldown	Liu et al. [2011]; Yu et al. [2012]
Arc35p	Covalent	N/A	Proteomic screen, pulldown	Wohlschlegel et al. [2004]; Sung et al. [2013]
Arc40p	Covalent	N/A	Proteomic screen	Wohlschlegel et al. [2004]
Arc19p	Covalent	N/A	Proteomic screen	Nie et al. [2012]
Arc15p	Covalent	N/A	Proteomic screen	Nie et al. [2012]
Intermediate filaments				
Vimentin	Covalent	N/A	In vitro, pulldown	Wang et al. [2010], Snider et al. [2011]
Keratin 8	Covalent	K285, K364	In vitro, pulldown	Snider et al. [2011]
Keratin 18	Covalent	K207, K373	In vitro, pulldown	Snider et al. [2011]
Keratin 19	Covalent	K208	In vitro, pulldown	Snider et al. [2011]
Lamin A	Covalent	K201, K420, and K486	Pulldown, Y2H	Zhang et al. [2008], Galisson et al. [2011], Simon et al. [2013]

In orienting the mitotic spindle, it is important that Kar9p is localized on just one of the poles of the spindle. In other words, its localization on the two poles needs to be asymmetric. Otherwise, both poles of the spindle would be pulled into the bud. Kar9p binds to the “old” or original spindle pole body that will be transferred to the daughter cell, whereas the “new” SPB lacking Kar9p is retained in the mother yeast cell [Liakopoulos et al., 2003; Moore et al., 2006; Moore and Miller, 2007].

Kar9p and Sumoylation

Several lines of evidence suggest that Kar9p is sumoylated. Kar9p interacts with SUMO by two-hybrid analysis. It also interacts with the E2 enzyme Ubc9p and the E3 Nfi1p [Meednu et al., 2008]. Kar9p has been shown to be sumoylated both in vitro and in vivo [Leisner et al., 2008; Meednu et al., 2008]. Four lysines are required for the sumoylation shift of Kar9p, lysines 301, 333, 381, and 529 [Leisner et al., 2008].

Sumoylation is important for multiple aspects of Kar9p function. It is important for the asymmetric localization of Kar9p on SPBs. Mutation of lysines 301, 333, 381, and 529 to arginine (4K→R) results in the mis-localization of Kar9p on both SPBs, rather than it being restricted to just the old SPB [Leisner et al., 2008]. Similar results were observed with Kar9p mutations at lysine 304, which resides within the sumoylation consensus site of K301 [Meednu et al., 2008]. Functionally, sumoylation is also important for spindle positioning [Leisner et al., 2008; Meednu et al., 2008]. Inhibition of SUMO with a temperature-sensitive SUMO allele, *smt3-331*, results in mispositioning of the mitotic spindle [Leisner et al., 2008; Meednu et al., 2008]. Both the Liakopoulos and Miller labs showed that *kar9* mutants lacking the ability to be sumoylated display defects in the position of the mitotic spindle [Leisner et al., 2008; Meednu et al., 2008]. The Kar9-L340P mutant results in a short-bipolar spindle that is positioned farther away from the mother-bud neck compared to wild type [Meednu et al., 2008]. Similarly, the Kar9-4K→R mutant also shows spindle-positioning defects, displaying increases in both the angle of spindle alignment and the distance to the bud neck. It is interesting to note, however, that the defect seen in the Kar9-4K→R mutant is not as severe as that seen in the *smt3-331* mutant of SUMO itself [Leisner et al., 2008]. This suggests that other components required for spindle positioning are also regulated by SUMO. Alonso et al. [2012] posit that at least one of these other components resides within the dynein pathway [Alonso et al., 2012]. Alternatively, the difference could be attributed to activation of the spindle assembly checkpoint (SAC) by the *smt3-331* mutant [Leisner et al., 2008].

The interaction between Kar9p and Bim1p is regulated by both sumoylation and phosphorylation [Huls et al., 2012]. Sumoylation of Kar9p promotes the interaction, with lysine 381 having the most prominent effect. In con-

trast, phosphorylation of Bim1p by the Ipl1p kinase impedes the interaction [Huls et al., 2012].

Phosphorylation of substrates is one mechanism by which sumoylation can be regulated. This can be either a positive influence or a negative one [Yang et al., 2003; Hietakangas et al., 2006]. Kar9p is one example that illustrates this type of regulation. Cdc28p phosphorylates Kar9p at serine 197 and 496 [Liakopoulos et al., 2003]. Disruption of these phosphorylation sites causes Kar9p to mislocalize to both old and new spindle pole bodies [Liakopoulos et al., 2003; Moore et al., 2006; Moore and Miller, 2007]. Phosphomimetic mutations at one of these sites, Kar9p-A196E S197E, does not interact with SUMO by two-hybrid analysis, suggesting that phosphorylation at serine 197 blocks the interaction of Kar9p with Smt3p [Meednu et al., 2008]. Consistent with this idea, the Liakopoulos lab showed that the phospho-inhibited Kar9p-S197A S496A mutant was still able to be sumoylated [Leisner et al., 2008].

In addition to phosphorylation and sumoylation, Kar9p is also regulated by ubiquitination [Maekawa et al., 2003; Moore et al., 2006; Moore and Miller, 2007; Leisner et al., 2008; Meednu et al. 2008; Kammerer et al., 2010]. Ubiquitination of Kar9p regulates the interaction of astral microtubules with the bud neck, appearing to be involved in the proteasomal degradation of the subset of Kar9p molecules interacting with the bud neck [Kammerer et al., 2010]. The relationship between sumoylation and ubiquitination in this context remains unexplored. However, Kar9p interacts with the STUbL, Uls1p-Nis1p, and Wss1p by two-hybrid analysis [Meednu et al., 2008].

Dynein

Dynein is the major motor protein that walks toward the minus-end of microtubules. 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 as silencing the spindle assembly checkpoint [Howell et al., 2001; Bader and Vaughan, 2010; 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]. Errors in any of these processes can lead to increases in abnormally segregated chromosomes, a condition known as aneuploidy. When dynein is anchored at the cell surface, it can participate in spindle orientation or nuclear migration by pulling on microtubules that are attached to the MTOC [Lee et al., 2005; Collins et al., 2012; Kotak and Gonczy, 2013; Kotak et al., 2014]. 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; LaMonte et al., 2002; Watson et al., 2005; Johansson et al., 2007; Rocha

et al., 2009; Scherer and Vallee, 2011; Tan et al., 2011; Moughamian and Holzbaur, 2012]. However, regulation of the attachment of cargo to dynein is still poorly understood.

There are two forms of cytoplasmic dynein DHC1a (dynein 1) and DHC1b (dynein 2), both of which are distinct from flagellar dynein [Paschal et al., 1987; Gibbons, 1995]. 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 [Pazour et al., 1999; Signor et al., 1999; Grissom et al., 2002; Helfand et al., 2002; Mikami et al., 2002]. Mutations in the dynein 2 complex result in a number of ciliopathies [Schmidts et al., 2013a, 2013b]. Dynein 2 associates with different intermediate and light chains than those associated with dynein 1. It also does not interact with other known regulators of dynein, including dynactin, LIS1, and BICD2 [Asante et al., 2014].

Crystallography work from the Vale lab provides detailed insight into how the structure of the dynein 1 motor couples ATP hydrolysis within the main AAA ATPase domain to allosteric changes that result in movement [Carter et al., 2008, 2011; Bhabha et al., 2014].

The dynein heavy chain is complexed with several accessory proteins. These are the intermediate chains, the light chains, and the light intermediate chains [Vaughan and Vallee, 1995; Waterman-Storer et al., 1995; Ma et al., 1999; Lo et al., 2001; Mok et al., 2001]. Two adaptors for dynein are Lis1/Pac1p and the dynein-activating complex, better known as the dynactin complex [Vaughan et al., 1999; Faulkner et al., 2000; Tai et al., 2002; Schroer, 2004; Levy and Holzbaur, 2006]. 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]. To date, no evidence suggests that the accessory chains, the dynactin complex, or dynein itself are SUMO substrates. However, two reports currently connect SUMO to dynein. One report investigates the dynein cargo, La; and the other examines the adaptor, Pac1p [van Niekerk et al., 2007; Alonso et al., 2012]. Alonso et al. postulate that sumoylation of adaptors could be a new mode of regulation for dynein [Alonso et al., 2012].

La

La is an 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 it can enhance mRNA translation as well as viral replication [Trotta et al., 2003; Kumar et al., 2013].

The Twiss lab demonstrated that La is sumoylated at a unique site, K41 [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 suggests that sumoylation of La promotes its interaction with dynein and is required for its 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?

Lis1/Pac1p

Pac1p is the yeast homologue of the Lis1 protein, occasionally referred to as PFAFH1B1 [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 [Sapir et al., 1999; Kato and Dobyns, 2003; Reiner et al., 2006; Liu, 2011]. The hallmark of the disease is a drastic decrease in convolutions of the cerebral cortex [Reiner and Sapir, 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 and Lechler, 2011; Sumigray et al., 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].

The structure of Pac1p/Lis1 provides clues as to how it serves as a critical regulator of the dynein motor protein. Pac1p is composed of three regions: a LisH domain, a coiled-coil domain, and a series of highly conserved WD40 repeats. Alone, none of the domains are sufficient for microtubule binding or tracking the plus-end of the microtubule in vivo [Markus et al., 2011]. In contrast, the WD40 repeats of Pac1p/Lis1 are thought to bind across the intersection of the AAA3 and AAA4 ATPase motifs of dynein [Faulkner et al., 2000; Vallee et al., 2001; McKenney et al., 2011; Huang et al., 2012; Wang et al., 2013; Toropova et al., 2014]. 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 [McKenney et al., 2010; Markus et al., 2011; Torisawa et al., 2011; Huang et al., 2012; 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 to 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.

In yeast, Pac1p functions in the dynein pathway by working with Bik1p to recruit dynein to the plus-end of the microtubule before dynein is off-loaded to the bud cortex [Sheeman et al., 2003; Lee et al., 2005; Li et al., 2005; Markus et al., 2011]. Bik1p is the yeast homologue of mammalian CLIP-170. 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].

Several approaches were employed to show that SUMO is linked to Lis1/Pac1p [Alonso et al., 2012]. 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. Second, inhibition of the SUMO protease Ulp1p resulted in multiple higher molecular weight forms of Pac1p, suggesting that Ulp1p removes SUMO from Pac1p [Alonso et al., 2012]. Third, the co-immunoprecipitation of Pac1p with SUMO strongly suggested that Pac1p is a SUMO substrate. Fourth and also consistent with Pac1p being sumoylated, Pac1p interacted with both components of the STUbL enzyme, Uls1p-Nis1p, by two-hybrid analysis [Alonso et al., 2012]. Pac1p shift was increased in strains deleted for the STUbL Uls1p, and in strains where the proteasome was inhibited with the drug MG132 [Alonso et al., 2012]. 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.

As Pac1p is one of the few examples known for substrates of the STUbL, Uls1p-Nis1p, many questions remain about its sumoylation. Additional work is needed to see if this modification is conserved in the 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. Work is currently in progress in the Miller lab to identify the sites of modification and determine the function of this modification.

Bik1p/CLIP-170

Bik1p is the yeast homologue of CLIP-170, a family of CAP-Gly proteins that track microtubule plus-ends

[reviewed in Miller et al., 2006; Gupta et al., 2014]. 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 et al., 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” [Miller et al., 2006; Gupta et al., 2010]. 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 [Vaughan et al., 1999; Tai et al., 2002; Goodson et al., 2003; Sheeman et al., 2003; Caudron et al., 2008]. Bik1p, together with Lis1/Pac1p and Ndl1p, the yeast homologue of nuclear distribution factor E, recruits dynein to the plus-end of the microtubule, prior to dynein’s off-loading to the cortex [Sheeman et al., 2003; Lee et al., 2005; Markus et al., 2011]. Bik1p also interacts with Kar9p, providing a link between the Kar9p and dynein spindle positioning pathways [Moore et al., 2006].

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 [Alonso et al., 2012]. 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 modification by SUMO [Alonso et al., 2012]. Bik1p can also be sumoylated using an in vitro assay, resulting in two and possibly three shifted bands. It is not known whether Pac1p might enhance this in vitro sumoylation, which would be consistent with the two-hybrid data. Sumoylated forms of Bik1p have also been observed in vivo when overexpressed

Bik1p and overexpressed SUMO were employed [Alonso et al., 2012]. Ulp1p is one of the major SUMO proteases in the cell that cleaves SUMO from target proteins. In a somewhat surprising finding, inactivation of Ulp1p with a temperature-sensitive allele did not reveal SUMO-shifted forms of Bik1p [Alonso et al., 2012]. Thus, identification of a sumoylated form of Bik1p at endogenous levels has remained elusive.

What hypotheses could reconcile this apparent discrepancy? 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. Perhaps 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.

Various +TIPs interact with each other to form a web of interactions at the plus-end of the microtubule [Akhmanova and Steinmetz, 2008]. However, the function of these interactions has remained a mystery [Gupta et al., 2014]. Considering that a growing list of +TIPs are seen to interact with SUMO, we postulate that sumoylation may help in the assembly of higher order molecular structures of +TIP assemblies. This may involve the SIMs of one MAP binding the sumoylated form of an adjacent MAP.

Tau

Tau, tubulin-associated unit, is a microtubule-associated protein that helps stabilize microtubules and is highly conserved in higher eukaryotes [Goedert et al., 1989a, 1989b, 1996; Maccioni et al., 1995]. Tau is found mainly in neurons, where it stabilizes microtubules and promotes their polymerization [Cleveland et al., 1977; Binder et al., 1985; Drubin and Kirschner, 1986; Drechsel et al., 1992]. 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 consisting of four repeats of conserved residues, and a basic C-terminal region. The extreme variation in charge between the N-terminus and the C-terminus region of tau can be modulated by various post-translational modifications. Tau shares homology with other 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 neu-

rofibrillary tangles are intracellular aggregates containing abnormally phosphorylated tau, whereas senile plaques are extracellular deposits of amyloid β -peptides [Grundke-Iqbal et al., 1986; Ihara et al., 1986; Delacourte et al., 1999]. In models for tau's role in Alzheimer's, tau first dissociates from microtubules in a phosphorylation-dependent manner, leading to destabilization of the microtubules. Subsequently, unbound tau oligomerizes 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 tagged by numerous post-translational modifications, including phosphorylation, glycosylation, glycation, prolyl-isomerization, nitration, polyamination, ubiquitination, oxidation, and sumoylation [Grundke-Iqbal et al., 1986; Schweers et al., 1995; Wang et al., 1996; Nacharaju et al., 1997; Murthy et al., 1998; Takahashi et al., 1999; Zhou et al., 2000; David et al., 2002; Horiguchi et al., 2003; Landino et al., 2004; Necula and Kuret, 2004; Zhang et al., 2005; Dorval and Fraser, 2006, 2007; Kuhla et al., 2007; Takahashi et al., 2008; Wang et al., 2008; Arnaud et al., 2009; Bulbarelli et al., 2009; Liu et al., 2009]. Tau has as many as thirty phosphorylation sites that can alter its structure, function, and localization [Grundke-Iqbal et al., 1986; Litersky et al., 1996; Fischer et al., 2009]. In general, an increase in tau phosphorylation reduces its affinity for microtubules and thus its ability to stabilize microtubules [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 can be ubiquitinated both in vitro and in vivo [David et al., 2002; Petrucelli et al., 2004; Zhang et al., 2005; Arnaud et al., 2009; Liu et al., 2009]. Tau is sumoylated mainly by SUMO1, but in some cases by SUMO2 and SUMO3 [Dorval and Fraser, 2006, 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 [Baner 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 upregulated, 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 is consistent with the diminished proteasome function that is commonly

found in many neurodegenerative diseases [Pountney et al., 2003; Dorval and Fraser, 2006].

Tau sumoylation is also partly dependent on phosphorylation. Treatment of cells with the phosphatase inhibitor, okadaic acid, promotes tau sumoylation [Dorval and Fraser, 2006, 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.

Kinetochores MAPs

Numerous proteins of the kinetochore are sumoylated [Mukhopadhyay and Dasso, 2010; Cubenas-Potts et al., 2013]. 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 an emerging field of interest, this section focuses on the kinetochore proteins that are also bona fide microtubule-binding proteins.

Ndc80p

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 [Panse et al., 2004; Zhou et al., 2004; Wykoff and O’Shea, 2005]. 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. This suggests that Ndc80p is regulated differently than these proteins [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 [Yen et al., 1991; Liu et al., 2007]. 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].

In summary, two classes of microtubule motors are linked to SUMO, but by different mechanisms. The kinetochore kinesin, CENP-E, both binds to and is conjugated by SUMO [Zhang et al., 2008]. The dynein motor is speculated to be regulated by SUMO, but indirectly, through conjugation of its adaptor, Pac1p [Alonso et al., 2012].

Actin

A third major cytoskeletal system is comprised of actin, also known as microfilaments [Chesarone et al., 2010; Ydenberg et al., 2011]. Actin is highly abundant, and can constitute as much as 5% of total cellular protein in some cell types. Actin is found in both the cytoplasm and in the nucleus, and actively shuttles between the two compartments [Dopie et al., 2012; Belin and Mullins, 2013]. Many of the cytoplasmic functions of actin are well characterized. In addition to serving as the cellular tracks on which myosin transports its cargo, the many roles of actin include maintaining cellular shape, formation of the cytokinesis furrow, cellular locomotion, scaffolding sites for signaling proteins, and roles in endocytosis and exocytosis [Pollard and Cooper, 2009; Gardel et al., 2010; Pollard, 2010; Mishra et al., 2014]. The nuclear functions of actin however are less well understood [Henzel, 2014], but include roles in transcription and chromatin remodeling [Louvet and Percipalle, 2009; Kapoor et al., 2013; Percipalle, 2013]. Nuclear actin also interacts with each of the RNA polymerases, as well as nuclear export and import factors [Hofmann et al., 2004; Hu et al., 2004; Philimonenko et al., 2004; Dopie et al., 2012].

Recent reviews of actin and actin binding proteins have discussed their various post-translational modifications including acetylation, methylation, phosphorylation, and ubiquitination [reviewed in dos Remedios et al., 2003; Terman and Kashina, 2013]. This review focuses on the effect that the SUMO modification exerts on actin.

Four proteomic studies identified actin as a likely target for SUMO conjugation [Panse et al., 2004; Vertegaal et al., 2004; Wohlschlegel et al., 2004; Rosas-Acosta et al., 2005]. Hofmann et al. [2009] confirmed these studies, showing that SUMO 2 and 3 are the preferential isoforms of SUMO that modify actin. Lysine to arginine mutagenesis established that two lysines, one at position 68 and another at position 284, are required for actin's sumoylation. However computer modeling predicts that only K284 is conjugated by SUMO and that salt bridges between lysine 68 and SUMO help to stabilize the actin-SUMO interaction, allowing K284 to be sumoylated.

Cellular fractionation experiments showed that it was predominately the actin in the nuclear fraction that is modified by SUMO. The current model suggests that sumoylation on K284 blocks access to a nuclear export sequence, NES-1, resulting in sumoylated actin being retained in the nucleus. This idea is supported by the finding that non-sumoylatable actin mutants are rapidly exported out of the nucleus back to the cytoplasm through an CRM1/exportin-1 dependent pathway. This export was blocked by leptomycin B, a compound that modifies CRM1, inhibiting its function and nuclear export [Kudo et al., 1998, 1999; Hofmann et al., 2009]. Recent reports also implicate Exp6 in the nuclear export of actin [Dopie et al., 2012].

The import of actin into the nucleus was previously linked to the actin binding protein, cofilin, which contains a nuclear localization signal motif. Early models suggested that actin could “piggy-back” on cofilin to gain entry into the nucleus [Nishida et al., 1987]. However, Dopie et al. [2012] recently showed that the import factor Ipo-9 is also critical for actin transport into the nucleus. Informing both models, Hoffman et al.'s finding that non-sumoylatable actin can easily enter the nucleus suggests that this modification may not be required for either of these import-dependent interactions [Hofmann et al., 2009].

Structurally, the position of SUMO on actin at lysine K284 suggests that sumoylation would physically block the formation of classical actin filaments. This provides a plausible explanation for the absence of classical actin filaments in the nucleus [Hofmann et al., 2009]. It is also possible that sumoylation provides a mechanism by which actin could adopt alternative structures within the nucleus [Schoenenberger et al., 2005; Jockusch et al., 2006]. This hypothesis is especially intriguing considering that SUMO expression is strongly influenced by stress, and cellular stresses like heat shock and DMSO treatment induce the formation of a type of actin bundle known as actin rods within the nucleus of *Xenopus* oocytes [Welch and Suhan, 1985; Iida and Yahara, 1986; Iida et al., 1986]. Additional work is warranted to determine the exact role that sumoylation plays in governing the functions of nuclear actin and the types of structures formed, as little is known on this topic [Belin and Mullins, 2013].

Actin Regulatory Proteins and SUMO

The function, dynamics, and interactions of actin in both the cytoplasm and the nucleus are regulated by numerous actin-binding proteins [Higgs and Pollard, 2001]. In addition to nuclear forms of myosin [Vreugde et al., 2006], several actin-binding proteins have been shown to be present in the nucleus, such as filamin A, members of the Arp2/3 complex, and thymosin β 4 [Vartiainen, 2008]. Their role in the regulation of nuclear actin is less clear [Dopie et al., 2012]. While it is known that actin-binding proteins undergo several types of post-translational modification including phosphorylation [Arber et al., 1998; Yang et al., 1998] and ubiquitination [Hao et al., 2013], actin-binding proteins and actin regulatory proteins are now emerging as new categories of SUMO substrates.

RhoA, and Rac1 are two members of the Rho family of GTPases that play significant regulatory roles for the actin cytoskeleton, and have also been linked to SUMO. They regulate the formation of stress fibers, membrane ruffles, and filopodia [Nobes and Hall, 1995]. In the cell, Rho family GTPases function as molecular switches that toggle between GDP-bound (inactive) and GTP-bound (active) forms. This switching is regulated by two other groups of proteins, GAPs (GTPase-activating proteins) and GEFs

(guanine-nucleotide exchange factors). GAPs facilitate the hydrolysis of GTP to GDP, returning the GTPase to its “inactive” form, whereas GEFs help facilitate the exchange of GDP for GTP, returning the GTPase to an “active” state [reviewed in Cherfils and Zeghouf, 2013].

Ran is another small GTPase, which is central to the regulation of nuclear transport. It also interacts with the nuclear pore protein Ran binding protein (RanBP2), which is a SUMO E3 ligase [Azuma and Dasso, 2002]. The GAP for Ran, RanGAP1, is conjugated by SUMO1 [Joseph et al., 2002]. Ran influences the interaction between microtubules and the kinetochore [Joseph et al., 2004] and this aspect of its function has been expertly reviewed elsewhere [Dasso, 2008; Flotho and Werner, 2012].

In 2010, Rac1 was shown to co-purify with the SUMO E3 ligase PIAS3, prompting further investigation. Castillo-Lluva et al. [2010] showed that the Rac1 is sumoylated and that this SUMOylation event promotes cellular migration. These researchers identified four lysine residues in Rac1 to which SUMO-1 could conjugate. These lysines were identified by using in vitro sumoylated Rac1 and mass spectrometry to reveal the branched “gly-gly stubs” that are left behind after trypsinolysis. This approach is based on the assumption that the fidelity of in vitro sumoylation is quite high. Indeed, they found that mutation of these four lysines to arginine resulted in the loss of the shifted SUMO bands in vitro and in vivo. The four sumoylated lysines reside in the C-terminal polybasic region of Rac1, a domain that is important for the binding of several effectors of Rac1. Surprisingly however, the non-sumoylatable Rac1 did not display altered binding to several known effectors. Instead, sumoylation appeared to be important for optimal GTP binding to Rac1. Defects were also observed in lamellipodia-membrane ruffling. Further, the E3-SUMO ligase PIAS3 preferentially sumoylated the GTP-bound activated form of Rac1 over the GDP-bound form of Rac1. Castillo-Lluva et al. [2010] postulate that while not all active Rac1 is SUMOylated, the percentage that is could be enough to boost Rac1 activity over a certain threshold that is required for lamellipodia formation and cellular migration.

RhoGDI

Rho family GTPases are regulated in part by RhoGDIs (Rho GDP-dissociation inhibitors). RhoGDI can both remove and prevent the binding of Rho-GTPases to cell membranes [Isomura et al., 1991; Dovas and Couchman, 2005], thereby controlling their cytosol-membrane cycling. Thus, RhoGDI regulates the activation state of Rho-GTPases from an active state that is membrane bound to an inactive state in the cytoplasm [Olofsson, 1999]. Rho-GTPases are known to regulate actin and a variety of cellular events including cellular morphology, cellular adhesion and aggregation, cellular motility, and ruffling of the

plasma membrane, as well as formation of stress fibers and focal adhesions [Paterson et al., 1990; Ridley and Hall, 1992; Tominaga et al., 1993; Nishiyama et al., 1994; Takaishi et al., 1994]. Thus, the regulation of RhoGDI has the potential to control many downstream effects.

The RhoGDI can be regulated by multiple mechanisms. The RhoGDI-RhoGTPase complex can be post-translationally regulated by the phosphorylation of RhoA and Cdc42 [Forget et al., 2002; Tu et al., 2003]. RhoGDI can itself be post-translationally modified by phosphorylation, causing the RhoGDI-RhoGTPase complex to dissociate [Price et al., 2003; DerMardirossian et al., 2006]. RhoGDI can also be modified by sumoylation at lysine 138 [Liu et al., 2011; Yu et al., 2012]. This acts as a switch to activate RhoGDI activity [Yu et al., 2012]. The active sumoylated form of RhoGDI inhibits Rho-GTPase activity, resulting in the down regulation of actin polymerization and cell motility by decreasing the recruitment of Arp2/3 complex to the cytoskeleton [Yu et al., 2012].

The sumoylation of RhoGDI can be regulated by the RING domain of X-linked inhibitor of apoptosis protein (XIAP) [Liu et al., 2011; Yu et al., 2012]. The RING domain of XIAP binds RhoGDI and blocks RhoGDI sumoylation [Yu et al., 2012]. By blocking the sumoylation site, XIAP reduces the sumoylation levels of RhoGDI, therefore increasing the recruitment of Arp2/3 to the cytoplasm causing an increase in actin polymerization and cell motility [Yu et al., 2012]. XIAP overexpression has been associated with malignant cancer progression in various types of cancer [Yamazaki et al., 1999; Nemoto et al., 2004; Akyurek et al., 2006; Kleinberg et al., 2007; Kluger et al., 2007; Nagi et al., 2007; Burstein et al., 2008]. However the molecular mechanism for how this occurs remains unknown. Thus, this finding suggests a possible molecular mechanism for how overexpression of XIAP can down regulate RhoGDI, leading to increased actin polymerization and cell motility of cancer cells.

Arp2/3 Complex

Another group of actin binding proteins is the Arp2/3 complex. The principal function of the Arp2/3 complex is to create branches in the elongating actin network near the protruding edge of the plasma membrane [dos Remedios et al., 2003; Firat-Karalar and Welch, 2011; Rotty et al., 2013]. This complex is conserved from yeast to mammals and consists of seven proteins: Arp2, Arp3, and five smaller proteins (Arcs) [Goley and Welch, 2006].

At least three proteomic studies have identified components of the Arp2/3 complex as potential SUMO targets. Arc35p and Arc40p were identified in a proteomics screen that combined nickel purification of his6-Smt3p with mass spectrometry [Wohlschlegel et al., 2004]. A second proteomic study using *Schizosaccharomyces pombe* also identified several Arcs as potential SUMO targets. These included

Arc34p, which is the *S. pombe* ortholog to *S. cerevisiae* Arc35p; Arc5p, which is an ortholog to *S. cerevisiae* Arc15p; and Arc4p which is an ortholog to *S. cerevisiae* Arc19p [Nie et al., 2012]. Recently, Sung et al. [2013] also identified Arc35p as a sumoylation candidate by using a bimolecular fluorescence complementation assay. This assay is based on the principle that a fluorescent complex will form when two proteins fused to fragments of a fluorescent protein interact with each other. This allows for direct visualization within the cell of the location of a protein-protein interaction. Arc35p was one of several proteins chosen to validate this approach. In a pull-down assay, Arc35p cofractionated with Smt3p, and anti-Smt3p reactive bands matched the shifted forms of Arc35p [Sung et al., 2013]. While proteomic studies have consistently identified Arc35p of the Arp2/3 complex as a likely sumoylated protein, follow-up studies providing more detail are currently lacking. For instance, it is not known whether Arc sumoylation activates or inactivates Arp2/3 for its ability to form actin branches.

Intermediate Filaments (IF)

Intermediate filaments (IF) are the fourth polymeric network of the cytoskeleton, and include six classes of proteins [Eriksson et al., 2009]. The pattern of expression for the various classes of IF is cell-type specific. For example, the type I and type II IF are the acidic and basic keratins. These are coexpressed in tissues of epithelial origin [Fuchs, 1995]. Type III IF are found in cells of mesenchymal origin, which include cells such as fibroblasts [Franke et al., 1978]. Vimentin is perhaps the best characterized type III IF. The type IV class of IF include synemin, nestin, and the neurofilament proteins of which there are the high, medium, and low molecular weight (H, M, L) forms [Jing et al., 2007; Lepinoux-Chambaud and Eyer, 2013]. Lamins are type V intermediate filament proteins that line the periphery of the inner membrane of the nuclear envelope [Eriksson et al., 2009]. Members of the type VI IF family include filensin and phakinin, which are present in the fiber cells of the lens [Oka et al., 2008]. Mutations in these proteins result in cataracts [Szeverenyi et al., 2008]. Certain cell types can express more than one class of IF and expression patterns can also be controlled developmentally. Several excellent reviews have been written recently on intermediate filaments [Eriksson et al., 2009; Goldman et al., 2012; Snider and Omary, 2014].

General Structure of IF

Intermediate filaments are 10 nm in diameter, thus giving them the name “intermediate” because they are intermediate in size between the 25 nm microtubules and 7 nm microfilaments [Ishikawa et al., 1968]. IF have three major domains. At the N-terminus, there is a non-alpha-helical head domain. The central part of the protein is composed

of an alpha-helical rod domain containing heptad repeats. These allow the formation of the dimeric coiled-coil architecture characteristic of IF. The coiled nature of the rod domain is disrupted by three conserved short non-helical linker regions. The C-terminus in lamins, also known as the tail domain, forms a β -fold similar to that seen in immunoglobulins [Herrmann and Aebi, 2004]. Several types of IF organize into homodimers, whereas other types can form heterodimers [Parry et al., 1985; Herrmann and Aebi, 2004; Goldman et al., 2008].

As discussed in more detail below, four of the six classes of IF are modified by SUMO in some capacity. These are the type I and II keratins, type III vimentin, and type V lamins [Zhang et al., 2008; Wang et al., 2010; Snider et al., 2011]. The *C. elegans* IF protein, IFB-1, which displays several structural and functional similarities to keratin but has a lamin-like tail, is also sumoylated [Carberry et al., 2009; Kaminsky et al., 2009]. This raises the question of whether sumoylation of IF is a conserved modification. Will members of the other classes of IF someday be found to be sumoylated?

Keratin

Approximately 30 different keratins have been catalogued and these are classified as either type I or type II IF based on their isoelectric points and sequence homologies. Type I keratins have an acidic isoelectric point, whereas type II keratins are neutral-basic. As obligate heteropolymers, keratin filaments can only form when a type I keratin forms a heterodimer with a type II keratin.

Keratin IF are specifically expressed in epithelial cells, where they play several vital roles. First, keratins confer structural support and mechanical durability to epithelial cells [Fuchs and Cleveland, 1998]. A second role of keratins is to modulate cell signaling processes through a variety of mechanisms including the recruitment of multiple kinases, phosphatases, and 14-3-3 proteins [Eriksson et al., 2009]. Keratins also play a role in the function of organelles and cell migration [Kim and Coulombe, 2007]. Mutations that disrupt the filament forming ability of keratins result in several diseases, including the blistering skin diseases, epidermal bullosa simplex (EBS) and epidermolytic hyperkeratosis (EHS) [Bonifas et al., 1991; Coulombe et al., 1991; Vassar et al., 1991; Coulombe and Fuchs, 1993; Letai et al., 1993; Chipev et al., 1994; Yang et al., 1994, 1996; Fuchs and Cleveland, 1998; Arin et al., 1999, 2000). Mutations in keratin 8 and 18 can predispose patients and mice to liver disease [Ku et al., 2005; Ku and Omary, 2006; Strnad et al., 2012]. Keratin expression can also modulate the invasive nature of some cancers [Chung et al., 2013; Seltsmann et al., 2013].

Keratins K8, K18, and K19, which are found in simple epithelia, were recently shown by the Omary laboratory to be sumoylated [Snider et al., 2011]. SUMO 2/3 is used

preferentially over SUMO1 [Snider et al., 2011]. Four sites were identified for K8, three for K18, and one for K19. The sites of sumoylation appear to lie within the coiled-coil alpha-helical rod domain, similar to the study from the Sarge lab for lamins A (see below) [Zhang et al., 2008]. Due to the geometry of packing of dimers and tetramers, it seems improbable that these sites would be available for sumoylation in the fully formed filament [Snider et al., 2011]. In contrast, an IF from *C. elegans*, IFB-1, was found to be sumoylated in the C-terminal tail domain [Kaminsky et al., 2009].

Keratin sumoylation is regulated by oxidative and other stresses. In cells treated with hydrogen peroxide, as well as compounds used in liver injury models, the sumoylation levels on K8, K18, and K19 increased dramatically [Snider et al., 2011]. Concomitantly, the levels of SUMO colocalizing with the keratin network also increased significantly [Snider et al., 2011]. It will be useful to know from future studies how keratin sumoylation transduces signals to downstream stress response pathways. It will be equally important to determine whether sumoylation is necessary for the “stress protection” conferred by keratins against various liver diseases.

Studies from two systems, human cells and *C. elegans*, show that sumoylation regulates the solubility of keratins. Using human keratins 8 and 18, mono-sumoylation was found to increase the solubility of keratins. In contrast, hyper-sumoylation decreases the solubility [Snider et al., 2011]. These findings have significant implications for the regulation of IF dynamics [Kaminsky et al., 2009; Snider et al., 2011]. In comparison to other cytoskeletal networks, keratins and other IF have small cytosolic pools of subunits, which are represented by a small number of biochemically soluble subunits [Soellner et al., 1985]. This contributed to an early but inaccurate viewpoint that keratin networks were static, rigid structures [reviewed in Goldman et al., 2012]. Work then began to emerge showing that keratin subunits did in fact exchange with the keratin polymer, albeit the rates for dynamic exchange of keratin were slower than for vimentin, microtubules, or microfilaments [Soellner et al., 1985; Miller et al., 1991, 1993; Yoon et al., 1998, 2001].

These findings are consistent with those made with the *C. elegans* IF protein, IFB-1A, which forms epidermal attachment structures. A sumoylation-deficient mutant of IFB-1 displayed decreased cytoplasmic staining and disrupted IF formation in vivo in comparison to wild type. Similarly, inhibition of the *C. elegans* SUMO gene itself with RNAi-feeding also showed decreased cytoplasmic staining of the IFB-1 and thicker and shorter filament bundles. These findings support the notion that sumoylation mediates the amount of keratin subunits available for incorporation into newly assembled bundles [Kaminsky et al., 2009]. To investigate the extent to which SUMO-mediated solubility correlates with IF dynamics, fluorescence recovery

after photobleaching (FRAP) experiments were carried out. Both non-sumolatable IFB-1 and SUMO mutants exhibited much slower rates of recovery of photobleached IF filaments, suggesting that subunit exchange within the filaments is impaired by the lack of sumoylation in vivo [Kaminsky et al., 2009]. Overexpression of SUMO increased the amount of IF at apparent IF nucleation sites. Together, these findings are consistent with the hypothesis that sumoylation mediates the cytosolic pool of keratin subunits [Kaminsky et al., 2009]. In the future, it will be interesting to find out whether extra sumoylation can induce the disassembly of pre-formed filaments and/or modulate their interaction with desmosomes and hemi-desmosomes. If so, what is the extracellular stimuli to which sumoylation of IF responds? This information may provide insight into the function of sumoylation on mechanisms of cell motility.

In polymer science, it is commonly accepted that only very low concentrations of non-functional subunits need be present to terminate the polymerization of polymer, resulting in much shorter chain lengths [Oadian, 1991]. This principle of polymer science leads one to speculate whether a SUMO-modified IF subunit may serve as a chain-terminating element for this family of biopolymers. Considering that the vast majority of cytoskeletal protein resides in the polymeric state, sumoylation in this capacity would also be consistent with the low level of sumo-modified subunits found within the entire population of molecules for a particular cytoskeletal network.

Phosphorylation of the target protein is a common paradigm by which sumoylation can be regulated [Hietakangas et al., 2003]. Phosphorylation of a serine or threonine can create a negatively charged residue that is functionally equivalent to the aspartic (D) or glutamic acid (E) within a canonical consensus site for sumoylation [Hietakangas et al., 2003; Yang et al., 2006; Blomster et al., 2009]. Such a phosphorylation dependent sumoylation motif (PDSM) paradigm is also seen with keratin. Several lines of evidence suggest that keratin 8 sumoylation is regulated in part by phosphorylation [Snider et al., 2011]. Inhibition of phosphatases with okadaic acid results in an increase in keratin 8 sumoylation seen by western blotting and an increase in the amount of SUMO-2/3 colocalizing with the keratin network seen by immunofluorescence [Snider et al., 2011]. Liver-injury agents such as porphyrinogenic compound DCC are known to increase keratin phosphorylation. These also result in increased sumoylation of keratin [Snider et al., 2011]. In cells transfected with the phospho-inhibitory keratin 8-S74A mutation, there is a moderate decrease in sumoylation of keratin 8 [Snider et al., 2011]. While the decrease was not dramatic, it leaves open the possibility that other uncharacterized phosphorylation sites could be regulating keratin sumoylation. Together these data support the hypothesis that phosphorylation regulates the sumoylation of keratin. Additional knowledge on this topic has the potential to be hugely important in understanding the

molecular mechanisms of diseases involving keratins, such as liver disease.

Vimentin

Vimentin is the intermediate filament protein that typifies mesenchymal tissue and its expression characterizes the epithelial to mesenchymal transition in development [Mendez et al., 2010]. In several epithelial cancers, vimentin expression correlates with an increase in cell migration and poorer cancer prognosis [Hendrix et al., 1997; Lepekhn et al., 2001; Mendez et al., 2010; Liang et al., 2014; Niwa et al., 2014]. However in astrocytomas, the correlation of vimentin expression and survival is less clear [Skalli et al., 2013]. The regulation of vimentin disassembly is an important step in the formation of lamellipodia, a key cellular structure at the leading edge of the cell that is needed for cell migration [Helfand et al., 2011]. Adding to its complexity, vimentin can co-polymerize with several other types of type III subunits to form IF co-polymers [Eliasson et al., 1999]. One use for this co-polymerization is to help assemble the glial fibrillary acidic protein (GFAP) network within astrocytes [Galou et al., 1996]. In addition, vimentin plays a role in anchoring mitochondria and thus modulating their intracellular migration [Nekrasova et al., 2011].

Recently SUMO has been linked to a mutant form of vimentin in an aggressive form of brain cancer, glioblastoma multiforme. In a model system for this, U373 cells displayed inhibited cell migration with overexpression of PIAS, a SUMO ligase [Wang et al., 2010]. In an effort to identify potential targets of SUMO that might play a role in the inhibition of cell migration, a pull down experiment of SUMO1 was carried out. A truncated version of vimentin was co-isolated from a nuclear fraction and identified by mass spectrometry and western blotting as a candidate [Wang et al., 2010]. Yet, many questions remain. Can full-length vimentin be sumoylated? Is cytoplasmic vimentin modified by SUMO? Do the other isoforms of SUMO modify vimentin? What is the fate of sumoylated vimentin?

Lamins

Lamins are the major element of a meshwork that provides structural support and shape for the nucleus [Aebi et al., 1986; Belmont et al., 1993; Houben et al., 2007; Dechat et al., 2008]. A fraction of the lamins A/C population is also present in the interior of the nucleus as “speckles” [Jagatheesan et al., 1999; Kumaran et al., 2002; Adhikari et al., 2004]. Lamins participate in a variety of processes, including DNA replication, DNA repair, and transcriptional regulation owing to their ability to segregate heterochromatic domains to the inner edge of the nuclear envelope [Kumaran and Spector, 2008; Shimi et al., 2010]. Lamins also have functions in cell signaling, cell proliferation, development, and differentiation [reviewed in Dechat et al., 2008; Eriksson et al., 2009]. Mutations in the genes

encoding lamins result in a class of devastating diseases called laminopathies, which include the premature aging disorder Hutchinson-Gilford Progeria, Emery-Dreifuss muscular dystrophy, and cardiomyopathies [Sullivan et al., 1999; Sylvius and Tesson, 2006; Eriksson et al., 2009; Schreiber and Kennedy, 2013; Burke and Stewart, 2014].

There are two types of nuclear lamins, A-type and B-type. The A-type lamins are encoded by a single gene in mammals, LMNA. Alternative splicing of this gene leads to expression of different proteins including lamin A and lamin C [Broers et al., 2006]. Mammals express three different B-type lamins, which are encoded by two different genes, LNMB1 and LNMB2 [Burke and Stewart, 2014].

The first hint that lamins were sumoylated came from two-hybrid experiments showing that lamin A interacted with the SUMO E2 conjugating enzyme, Ubc9p [Zhang et al., 2008]. Subsequent studies have since confirmed this finding, but with several noteworthy differences between them [Zhang et al., 2008; Boudreau et al., 2012; Simon et al., 2013].

The first study by Zhang and Sarge suggests that the lamin A conjugation occurs predominately with SUMO2, but not SUMO1. These authors identified lysine 201, which is located in the rod domain of lamin A as a sumoylation site [Zhang and Sarge, 2008a, 2008b]. Residing near this SUMO site are two mutations that are associated with familial dilated cardiomyopathy, E203G and E203K. Considering that the acidic residue in the canonical sumoylation consensus sequence (ψ KX D/E) is important for the efficiency of sumoylation, E203G and E203K were tested for their effect on sumoylation. Both mutants exhibited significantly decreased levels of sumoylation. GFP fusions with both mutants also displayed abnormal sub-cellular localization patterns, which were consistent with the K201R mutant. These findings are consistent with those of Boudreau et al. [2012], who also found that several lamin mutants associated with dilated cardiac myopathy were modified by SUMO1, whereas wild type lamin A or C were not. The lamin A of these myopathies was mislocalized into aggregates that also sequestered SUMO1 [Boudreau et al., 2012]. Combined, these results suggest that sumoylation plays an important role in lamin A function and implicate sumoylation in the pathology of cardiomyopathies associated with lamin malfunction.

In the second study from the Hoffman and Wilson labs, Simon et al. [2013] also demonstrated that lamin A is modified by SUMO, but the modification they observed employed SUMO1 preferentially over SUMO2. In a second point of contrast to the Sarge study, these authors showed that sumoylation of lamin A occurred on K420 and K486 in the IgG globular tail domain. Independent mass spectrometry confirmed modification at lysine 420 [Galison et al., 2011]. These findings are in contrast to the Zhang study in which sumoylation was found in the coiled-coil domain. The position of such a mutation in the tail

domain is noteworthy because as many as 21 different lamin A binding partners interact with lamin A through its tail domain [Simon et al., 2013]. These findings led Simon et al. to propose a model where modification in the tail by SUMO1 regulates the binding of lamin A to its known partners to control the assembly of lamin A filaments.

Why the differences in these two studies? The differences in the types of SUMO used and in the sites of conjugation could be attributed to the origin of cells studied. The Sarge lab used HeLa cells and myocytes, whereas the Wilson and Hoffmann labs used Cos-7 cells, a SV40 transformed kidney cell line derived from African green monkey. It has also been noted previously that the pattern and number of SUMO modifications can change with cell activation [Galissou et al., 2011], so the growth state of the cells used in these lamin studies could easily play a role in these differences.

In the Simon et al. study, the second site of sumoylation, K486, in the tail was somewhat unexpected because it did not lie within a canonical consensus site. After further examination of the three-dimensional structure of the IgG fold of the lamin A tail, it was apparent that two acidic residues, E460 and D461, are positioned directly below K486 [Krimm et al., 2002]. Two other acidic residues E536 and E537 were close to K420, but on a different side. Mutation of E460 and D461, or the double mutant E460 D461, diminished sumoylation by 65–80% compared to wild type. Mutations of E536 and E537 reduced sumoylation by 30–50% showing they contribute to sumoylation of K486, but are less important than E460 and D461. These findings suggest the hypothesis that canonical consensus sites can be “conformational” in nature. These results highlight the need for the development of algorithms that identify sumoylation sites not only by linear amino acid sequences, but also by the three-dimensional structure of the folded protein.

Laminopathies and Sumoylation

It is significant to note that several studies report abnormalities in the sumoylation patterns of patients afflicted with a range of different laminopathies. Lamin A mutations of G465 and K486 are known to cause familial partial lipodystrophy (FPLD), an adipose tissue disease characterized by decreased levels of adipose tissue. As mentioned above, the sumoylation of these mutants is decreased [Simon et al., 2013], leading these investigators to postulate that FPLD in patients with G465 or K486 mutations could arise from deficiencies in sumoylation levels. Lamin A mutations are also frequently seen in patients with dilated cardiomyopathy [Sylvius and Tesson, 2006]. In dilated cardiomyopathy disease, patients carrying a lamin C-D192G mutation displayed a number of aberrant nuclear phenotypes and greatly reduced SUMO1 patterns [Sylvius and Tesson, 2006]. As noted above, two lamin A mutations E203G and E203K

are linked with familial dilated cardiomyopathy. These mutant lamins display altered subcellular localization that match those seen in the sumoylation-deficient lamin A mutants [Zhang et al., 2008]. Consistent with this pattern, patients with the premature aging disease, Progeria, have a defective nuclear rim staining and also display a disrupted pattern of Ubc9 localization [Kelly et al., 2011]. However, not all laminopathy mutations result in decreased levels of sumoylation. In mouse myoblasts cell lines and mouse muscle tissue, laminopathy mutations associated with dilated cardiac myopathy and Emery-Dreifuss muscular dystrophy resulted in intracellular lamin aggregates that had higher levels of sumoylation, as well as increased Ubc9 colocalization [Boudreau et al., 2012].

Together, these findings are consistent with sumoylation playing a vital role in the physiology of various cell types important for human health. The differences in these studies highlight the need for additional research into this transformative area of cell biology, while providing critical insight into the cause of human diseases associated with mutations in lamin A. Many laminopathies are known [Eriksson et al., 2009; Boudreau et al., 2012; Burke and Stewart, 2014], but the sumoylation status in most of these diseases remains unknown. This represents an important avenue for future investigations that will likely lead to a better understanding of these diseases. For instance in these various laminopathies, does sumoylation modulate the solubility of lamins as it does for keratin? Does sumoylation promote the removal of damaged lamins by a sumo-mediated proteasome pathway? Could such a system be harnessed for the treatment of these diseases? [Liu and Zhou, 2008].

Conclusions and Perspectives

Many Questions Remain

A plethora of questions remain about the relationship between sumoylation and the cytoskeleton. How extensive is SUMO's control of cytoskeletal function? Are other cytoskeletal elements controlled by sumoylation? Could sumoylation regulate desmosomes, adherens junctions, tight junctions, or focal adhesions? Microtubule motors CENP-E and dynein have been linked to SUMO. Are the myosin motors walking along microfilaments also controlled by sumoylation? How wide spread is the altered sumoylation status of cytoskeletal proteins in disease?

An emerging theme is the role that sumoylation plays in controlling the structure of cytoskeletal systems. Sumoylation of actin is found on just the nuclear fraction of actin. It has been speculated that this could lead to a different configuration for actin assembly since the site of sumoylation would obstruct the formation of a classical actin filament. Sumoylation also influences intermediate filament solubility. Yet, a significant gap exists in knowing how this translates into control of other aspects of filament

dynamics. Can sumoylation influence the disassembly of pre-assembled intermediate filament networks? To what extent is the assembly/disassembly or solubility of other cytoskeletal polymers regulated by sumoylation?

Network Connectors

Much of this review discusses the four polymer networks of the cytoskeleton as separate entities, but in fact, several connections are known between these networks. These connections allow for inter-network communication that could, in theory, be modulated by sumoylation. The sumoylation of Kar9p, a linker between the actin and microtubule networks, supports this contention. The possibility of other linkers being regulated by sumoylation is just now beginning to emerge. For instance, plectin is a very large protein of the plakin family that plays a major role in cytoskeletal organization by providing linkages between the three major cytoskeletal networks; actin, microtubules and intermediate filaments [Svitkina et al., 1996; Wiche, 1998; Sonnenberg and Liem, 2007; Winter and Wiche, 2013; Bouameur et al., 2014]. In skin cells, plectin is an essential part of the hemidesmosome, a junction that plays a major role in anchoring the outer epithelial layer of the skin to the underlying dermal layer [Andra et al., 2003]. It does this in part by attaching keratin intermediate filaments to the basal membrane of cells in the basal cell layer [Wiche, 1998]. Plectin is also a component of desmosomes, which form junctions between neighboring cells [Huber, 2003]. It is intriguing that a recent sumoylation proteomics screen recently identified plectin as a potential substrate for SUMO2 [Wen et al., 2014]. Although further research is needed to confirm this interaction, this opens up a new research avenue that could impact multiple cytoskeletal systems simultaneously. This would provide needed insight into how signaling between the cytoskeletal networks might be coordinated.

Crosstalk With Other Signal Transduction Systems

While many unknowns exist about the relationship of sumoylation with the cytoskeleton, perhaps the biggest unknown is how cytoskeletal sumoylation is integrated with other signal transduction pathways. Can sumoylation be a mechanism of transmitting information between different cell cycle checkpoints, DNA repair, stress response pathways, and transcription to the cytoskeleton?

Sumoylation of many targets can be either increased or decreased with a variety of cellular stresses [Golebiowski et al., 2009; Ren et al., 2014]. Does cellular stresses alter the sumoylation of the entire cytoskeleton? And if so, which stresses? Some work on cellular stress affecting cytoskeletal sumoylation has been done for the intermediate filaments, but little has been done in this regard for the other networks. Does cytoskeletal sumoylation generate crosstalk

with other signaling cascades? What other signals are responsible for changes in the sumoylation of the cytoskeleton? A clear understanding these questions is still in its infancy, and sumoylation of the cytoskeleton will certainly be an exciting chapter of new research for years to come.

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