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SUMO Regulation of Cellular Processes

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Contents

Contributors

Dina B. Anderson MRC Centre for Synaptic Plasticity, Department of Anatomy, University Walk, University of Bristol, Bristol, BS8 1TD, UK, dina.anderson@bristol.ac.uk

Donald D. Anderson Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA, dda7@cornell.edu

Sung Hee Baek Department of Biological Sciences, Seoul National University, Seoul 151-742, South Korea, sbaek@snu.ac.kr

Mark Benson Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, USA, mdbenson@umich.edu

Hee June Choi Department of Biological Sciences, Seoul National University, Seoul 151-742, South Korea, ian2019@gmail.com

Helena Cimarosti MRC Centre for Synaptic Plasticity, Department of Anatomy, University Walk, University of Bristol, Bristol, BS8 1TD, UK, anhic@bristol.ac.uk

Monica P. Colaiácovo Department of Genetics, Harvard Medical School, Boston, MA 02115, USA, mcolaiacovo@genetics.med.harvard.edu

Mary Dasso Section on Cell Cycle Regulation, Laboratory of Gene Regulation and Development, National Institute of Child Health and Development, National Institutes of Health, Bethesda, MD 20892, USA, mdasso@helix.nih.gov

Adeline F. Deyrieux Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center, College Station, TX 77845-1113, USA, adeline_deyrieux@millipore.com

Feili Gong Department of Immunology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China, flgong@163.com

Sebastian Haferkamp Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead Hospital, Westmead NSW 2145, Australia, sebastian_haferkamp@wmi.usyd.edu.au

Kiyohiko Hatake Division of Clinical Chemotherapy and Olympus Bio-Imaging Labo, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo, Japan 135-8550, khatake@jfcr.or.jp

Jeremy M. Henley MRC Centre for Synaptic Plasticity, Department of Anatomy, University Walk, University of Bristol, Bristol, BS8 1TD, UK, j.m.henley@bristol.ac.uk

Jorge A. Iñiguez-Lluhí Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, USA, iniguez@umich.edu

Ryoko Kuniyoshi Division of Clinical Chemotherapy and Olympus Bio-Imaging Laboratory, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo, Japan 135-8550, rkuniyoshi@jfcr.or.jp

Jason S. Lee Department of Biological Sciences, Seoul National University, Seoul 151-742, South Korea, jasonlee@snu.ac.kr

James L. Manley Department of Biological sciences, Columbia University, New York, NY 10027, USA, jlm2@columbia.edu

Jeffrey Martens Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA, martensj@umich.edu

Jacqueline C. Merrill Department of Biochemistry and Molecular Genetics, and Center for Cell Signaling, University of Virginia, Charlottesville, VA 22908, USA, jcm9j@virginia.edu

Yuko Mishima Division of Clinical Chemotherapy and Olympus Bio-Imaging Laboratory, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo, Japan 135-8550, ymishima@jfcr.or.jp

Debaditya Mukhopadhyay Section on Cell Cycle Regulation, Laboratory of Gene Regulation and Development, National Institute of Child Health and Development, National Institutes of Health, Bethesda, MD 20892, USA, mukhopad@mail.nih.gov

Amanda C. Nottke Department of Genetics and Department of Pathology, Harvard Medical School, Boston, MA 02115, USA, anottke@fas.harvard.edu

Erwin Pauws Neural Development Unit, Institute of Child Health, University College London, WC1N 1EH, London, UK, e.pauws@ich.ucl.ac.uk

Christopher Ptak Department of Cell Biology, University of Alberta, Edmonton, AB T6G 2H7, Canada, chris.ptak@ualberta.ca

Helen Rizos Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead Hospital, Westmead NSW 2145, Australia, helen_rizos@wmi.usyd.edu.au

Lyndee L. Scurr Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead Hospital, Westmead NSW 2145, Australia, lyndee_scurr@wmi.usyd.edu.au

Philip Stanier Neural Development Unit, Institute of Child Health, University College London, WC1N 1EH, London, UK, p.stanier@ich.ucl.ac.uk

Patrick J. Stover Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA, pjs13@cornell.edu

Yasuhito Terui Division of Clinical Chemotherapy and Olympus Bio-Imaging Laboratory, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo, Japan 135-8550, yterui@jfcr.or.jp

Helle D. Ulrich Cancer Research UK London Research Institute, Clare Hall Laboratories, Blanche Lane, South Mimms, EN6 3LD, UK, helle.ulrich@cancer.org.uk

Vasupradha Vethantham Department of Biological sciences, Columbia University, New York, NY 10027, USA, vv2003@gmail.com

Cong-Yi Wang The Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, GA 30912, USA; The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China, cwang@.mcg.edu

Van G. Wilson Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center, College Station, TX 77845-1113, USA, wilson@medicine.tamhsc.edu

David Wotton Department of Biochemistry and Molecular Genetics, and Center for Cell Signaling, University of Virginia, Charlottesville, VA 22908, USA, dw2p@virginia.edu

Richard W. Wozniak Department of Cell Biology, University of Alberta, Edmonton, AB T6G 2H7, Canada, rick.wozniak@ualberta.ca

Ping Yang The Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, GA 30912, USA; The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China, pyang@mail.mcg.edu

Jixin Zhong The Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, GA 30912, USA; The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; Department of Immunology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China, jzhong@mcg.edu

Nicola Zilio Cancer Research UK London Research Institute, Clare Hall Laboratories, Blanche Lane, South Mimms, EN6 3LD, UK, nicola.zilio@cancer.org.uk

Chapter 1
Introduction to Sumoylation

Van G. Wilson

Abstract Reversible post-translational modification is a rapid and efficient system to control the activity of pre-existing proteins. Modifiers range from small chemical moieties, such as phosphate groups, to proteins themselves as the modifier. The patriarch of the protein modifiers is ubiquitin which plays a central role in protein degradation and protein targeting. Over the last 10 years, the ubiquitin family has expanded to include a variety of ubiquitin-related small modifier proteins that are all covalently attached to a lysine residue on target proteins via series of enzymatic reactions. Of these newly discovered ubiquitin-like proteins, the SUMO family has gained prominence as a major regulatory component that impacts numerous aspects of cell growth and differentiation. Unlike ubiquitinylation which often leads to protein turn over, sumoylation performs a variety of functions such as altering protein stability, protein trafficking, protein-protein interaction, and protein activity. This chapter will introduce the basic properties of SUMO proteins and the general tenets of sumoylation.

Keywords SUMO · Ubc9 · SAE1/2 · SENP

1.1 The SUMO Proteins

Over a decade ago, a small cellular protein of 12 kDa, with 18% homology to the well-known ubiquitin protein, was co-discovered and termed **S**mall **U**biquitin-like **MO**difier or SUMO. SUMO was independently identified by four groups in 1996: Freemont′s group found it as a small ubiquitin-like protein associated with PML in an interacting complex and called it PIC1 (Boddy et al., 1996), Chen′s group identified it in a two-yeast hybrid screen of proteins associated with cellular DNA repair proteins (Shen et al., 1996), Yeh′s group identified it as a small modifier

V.G. Wilson (✉)
Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center, College Station, TX 77845-1113, USA
e-mail: wilson@medicine.tamhsc.edu

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associated with Fas which they called sentrin (Okura et al., 1996), and Blobel's group discovered that RanGAP was modified by a small ubiquitin-like protein which they designated GMP1 (Matunis et al., 1996). These modifiers were all the same protein that is now commonly referred to as SUMO.

There are four different genes in the human genome coding for the different SUMO modifiers, SUMO1, 2, 3, and 4. SUMO2 and 3 share about 92% identity but they only related to SUMO1 at 48% identity (Kamitani et al., 1998a). While SUMO1, 2, and 3 are expressed in all tissues tested, SUMO4 is encoded by an intron on the TAB2 gene, and its expression is restricted primarily to the kidneys, dendritic cells, and macrophages. SUMO4 has been less studied than the others, but seems to play a role in diabetes (see Chapter 16). SUMO1 is a 12 kDa protein of 101 amino acids that is related in structure and in sequence to the 9 kDa ubiquitin protein, as both modifiers share ∼18% primary structure identity to each other and have 48% similarity in their three-dimensional structure (Bayer et al., 1998). Ubiquitin is only a 76 amino acid polypeptide, and the difference between those two modifiers mainly resides in the extended tail at the N-terminal structure of SUMO; this tail is not present in ubiquitin. SUMO is conserved from yeast to mammalian cells (Chen et al., 1998); the budding yeast Saccharomyces cerevisiae expresses only one SUMO gene, Smt3, which possesses 50% identity and 75% similarity with the mammalian SUMO (Huang et al., 2004). SUMO1 is the most prominent conjugate and almost no free SUMO1 can be detected in the cells. SUMO2, 3 and 4 can form poly- SUMO chains *in vivo*, and this property may be conferred in their extended N-terminal tail (Bohren et al., 2004; Tatham et al., 2001).

At the tertiary level, the basic structures have been solved for SUMO1 (Bayer et al., 1998), SUMO2 (Huang et al., 2004), and SUMO 3 (Ding et al., 2005). All three SUMOs share a central compact, globular domain with the characteristic ββαββαβ ubiquitin fold. The SUMOs also each have both N- and C-terminal extensions, with the N-terminal extension being much longer than for ubiquitin. The biological role and function(s) of the N-terminal extension are not well understood, but the C–terminal extension is important for direct contact with the SUMO activating enzyme, SAE1/2 (Lois and Lima, 2005).

One of the remaining unanswered questions about the SUMOs is the functional difference between the SUMO1 and SUMO2/3 families. Certain biological variations have already been identified, including different responses to environmental conditions (Saitoh and Hinchey, 2000; Manza et al., 2004; Deyrieux et al., 2007), different susceptibilities to various SUMO proteases (Gong and Yeh, 2006; Mikolajczyk et al., 2007), and differences in subcellular localization and abundance (Saitoh and Hinchey, 2000; Manza et al., 2004; Ayaydin and Dasso, 2004). The substrate pool for these two SUMO groups is also different with some substrates capable of being modified by either SUMO1 or SUMO2/3, and other substrates showing a clear preference for one or the other SUMO type (Saitoh and Hinchey, 2000; Rosas-Acosta et al., 2005; Vertegaal et al., 2006). For both the SUMO1 and SUMO2/3 modified proteins, the substrates were predominantly nuclear, were involved in regulation of nucleic acid structure and function, and there was no obvious difference in protein functional types that were modified by SUMO1 versus SUMO2/3.

However, just how biologically important is this demarcation in the substrate preference remains unclear as recent SUMO1 knockout mouse studies have suggested that SUMO2/3 can compensate for absent SUMO1 (Evdokimov et al., 2008; Zhang et al., 2008). Much additional work is needed to clarify the common and distinct roles of the various SUMO proteins. Interestingly, a recent study showed that SUMO3 can be phosphorylated at serine 2, while SUMO2 cannot be phosphorylated since it has an alanine at this position (Matic et al., 2008). This observation implies that there could even be functional, regulatory, and substrate preference differences between the highly identical SUMO2 and SUMO3 proteins.

1.2 The Enzymology of Sumoylation

Sumoylation is the enzymatic activity which results in the covalent attachment of SUMO to a large number of proteins, including cellular and viral proteins. This multi-step enzymatic process (Fig. 1.1) includes a heterodimeric activating enzyme, SAE1/2, a monomeric conjugating enzyme, Ubc9, and multiple ligases and isopeptidases (Wilson, 2004). SUMO is translated as a precursor form which is first processed by specific isopeptidases (SENPs) to remove C-terminal residues and generate a mature SUMO, terminating with a C-terminal diglycine (Johnson et al., 1997). The mature form of SUMO then interacts with the SUMO E1 activating enzyme, SAE1/2. SAE1 is a 346 amino acid polypeptide while SAE2 is 640 amino

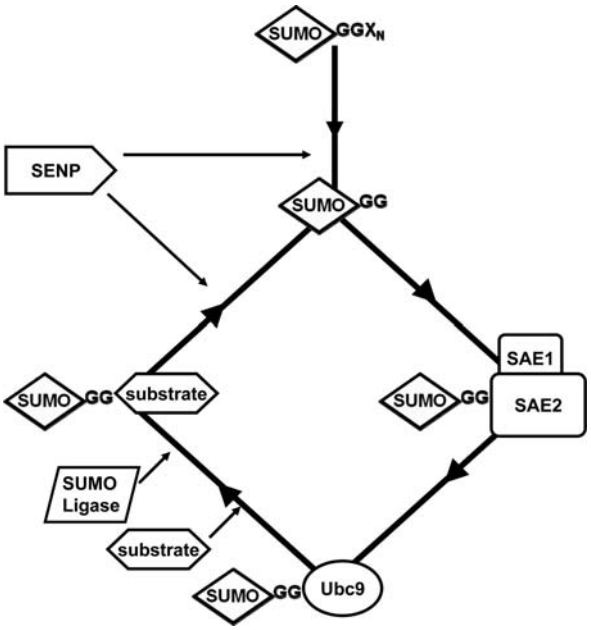

Fig. 1.1 Representation of the enzymatic cascades leading to the covalent attachment of SUMO to a substrate protein. The SUMO enzymes are the SENP isopeptidase, the SAE1/SAE2 activating enzyme, the Ubc9 conjugating enzyme, and the SUMO ligases

acids and contains the catalytic cysteine at residue 173; the SUMOs interact exclusively with the SAE2 subunit. The SAE2 subunit also contains a nuclear localization signal that may contribute to the enrichment of sumoylation components in the nucleus. Together, the SAE1 and 2 proteins form an U-shaped heterodimer complex with a large groove that has the ATP-binding motif at the base of the groove (Lois and Lima, 2005). Binding of SUMOs to SAE2 positions the SUMO diglycine motif for adenylation, then the activated SUMO can be covalently attached to the catalytic cysteine via a thioester linkage.

Subsequent to formation of the SAE1/2-SUMO complex, the activating enzyme transfers SUMO to SUMO E2 conjugating enzyme, Ubc9. Unlike the ubiquitin pathway that contains many E2 enzymes, Ubc9 is the sole conjugating enzyme for SUMO and functions with all 4 SUMOs. Once again, there is a conserved domain motif [αββββββ(ββ)ααα] common to all E2 enzymes known as the ubc superfold (Tong et al., 1997). Within this domain is the catalytic groove that contains the active site cysteine, amino acid 93. Binding of SAE1/2 to Ubc9 allows transfer of the SUMO C-terminus to cysteine 93, again through formation of a thioester linkage. Lastly, Ubc9 transfers SUMO to the substrate protein, where SUMO is covalently linked to a lysine residue through an isopeptide bond between the epsilon amino group of the lysine and the carboxyl group of the C-terminal glycine on SUMO.

The lysine residue utilized for sumoylation commonly falls in the ΨKxE/D motif, where Ψ is a hydrophobic residue (typically Val, Ile, Leu, Met, or Phe), K is the target lysine, x is any amino acid, and the fourth position is an acidic residue (Hay, 2005). However, early studies of sumoylated proteins found that not all were modified at lysines in sequence contexts that match the consensus motif, indicating that alternative sequence features could also specify a particular lysine for SUMO modification (Kamitani et al., 1998b; Rangasamy et al., 2000; Hoege et al., 2002). More recently, Zhou et al. used a proteomics approach and found that five of the ten sumoylation sites determined for yeast proteins were in non-canonical sequences (Zhou et al., 2004). Similarly, Chung et al. examined SUMO2 conjugation sites for *in vitro* sumoylated proteins and found that half the identified sumoylation sites (three of six) where in sequences which did not conform to the ΨKxE/D motif (Chung et al., 2004). Clearly these studies confirm that while the ΨKxE/D motif is often associated with SUMO addition, lysines in other sequence contexts can also be utilized for sumoylation.

Unlike ubiquitinylation, which absolutely requires an E3 ubiquitin ligase for transfer of ubiquitin to the substrate, sumoylation occurs readily *in vitro* without a ligase requirement (Melchior, 2000). Nonetheless, several SUMO ligases have now been identified [the PIAS family (Johnson and Gupta, 2001), RanBP2 (Pichler et al., 2002), Pc2 (Kagey et al., 2003), MMS21 (Potts and Yu, 2005), and TOPORS (Weger et al., 2005)], and these ligases enhance sumoylation both *in vitro* and *in vivo*. For instance, PIAS acts as a SUMO ligase, preferentially targeting the tumor suppressor p53, c-Jun, STAT1, and the nuclear androgen receptor AR (Schmidt and Muller, 2002; Ungureanu et al., 2003; Sachdev et al., 2001). RanBP2 stimulates sumoylation of the promyelocytic leukemia protein (PML), the nuclear body SP100 protein, and the histone deacetylase HDAC4 (Pichler et al., 2002), while Pc2 is

the unique E3 ligase for the transcriptional factor co-repressor CtBP (Kagey et al., 2003). *In vivo*, these ligases may also provide additional determinants of substrate specificity and/or preferential utilization of SUMO1 versus SUMO2/3, as well as simply enhancing the overall sumoylation reaction.

The SENPs, the SUMO isopeptidases, play a dual role; they are involved in the maturation of SUMO and in the de-conjugation of SUMO from its target proteins (Hang and Dasso, 2002; Gong et al., 2000). There are 6 SENPs that function with SUMO, 1-3 and 5-7 (there is no SENP 4, and SENP 8 is a Nedd 8 protease). In mammalian cells these enzymes are differentially located, with SENP1 located at the PML bodies, SENP6 in the cytoplasm, SENP3 in the nucleolus, and SENP2 at the nuclear pore complexes (Gong and Yeh, 2006). Therefore, it appears that de-sumoylation of conjugates is possible at different subcellular locations, and access of individual substrates to specific SENPs may provide an additional level of regulation. Overall, the diversity and specificity of SENP localization highlights the dynamics of sumoylation inside the cell and illustrates that this modification is a dynamic and reversible process. Additionally, deletion of the protease genes, like deletion of Ubc9 in yeast, stops cell cycle progression and further highlights that reversible sumoylation is an essential and critical function in the cell life cycle (Li and Hochstrasser, 1999).

1.3 Sumoylation Functions

Functionally, sumoylation is a more diverse modifier than ubiquitin. Unlike ubiquitinylation, which has a major role of targeting proteins for proteasome degradation, sumoylation does not directly target proteins to the proteasome. While there are examples of substrates where sumoylation blocks degradation by competing with ubiquitinylation for a common lysine residue (Desterro et al., 1998; Klenk et al., 2006) or promotes subsequent ubiquitinylation through SUMO-Targeted ubiquitin ligases (STUbls) (Xie et al., 2007; Prudden et al., 2007; Sun et al., 2007; Uzunova et al., 2007), our understanding of the interactions between these two modification systems is in its infancy. Though sumoylation appears to have only an occasional, substrate-specific role in protein stability, it is likely that further examples of cross-talk between the ubiquitin and SUMO pathways await discovery, and that these two systems will have much richer interplay than currently imagined.

In contrast to its limited role in protein stability, it is now clear that SUMO has a major role in transcriptional regulation (see Chapter 2), both through direct modification of individual transcription factors (Verger et al., 2003) and through DNA structural modification (Heun, 2007). For most transcription factors, sumoylation reduces their transactivation capacity, though enhanced transcriptional activity has also been demonstrated for a few substrates, including heat shock factors (Goodson et al., 2001; Hong et al., 2001), Oct4 (Wei et al., 2007), and Smad4 on some promoters (Long et al., 2004). The negative transcriptional effects can be due to changes in transcription factor stability and/or subcellular localization, particularly

the recruitment of sumoylated transcription factors into PML nuclear bodies as has been observed for HIPK2 (Kim et al., 1999) and Sp3 (Ross et al., 2002). Alternatively, SUMO modification can have effects directly at promoters by facilitating the recruitment of histone deacetylases (HDACs) which alter chromatin structure (see Chapter 4) to reduce transcriptional accessibility (Girdwood et al., 2003; Yang et al., 2003). Clearly, both of these mechanisms could be reversed by desumoylation of the transcription factors by the SENPs. Thus, sumoylation effects on transcriptional activity would reflect the dynamics of sumoylation/desumoylation that may vary with cell cycle, cell growth conditions, and disease state.

In addition to affecting transcriptional initiation, sumoylation has an important regulatory role for other nuclear functions, including RNA processing (see Chapter 3), chromatin remodeling (Chapter 4), genome maintenance (see Chapter 5), and nucleocytoplasmic transport (see Chapter 6). More recently, non-nuclear functions of sumoylation have been identified (Martin et al., 2007), and Chapters 7 and 8 will explore the role of SUMOs in regulating ion channel activity and metabolic pathways, respectively. Because of this pleiotropic ability to modify numerous proteins and affect transcriptional activity or cellular environment on a global scale, sumoylation in now recognized as a regulatory process involved in mitosis (Chapter 9), meiosis (Chapter 10), differentiation and development (Chapter 11), senescence (Chapter 12), and apoptosis (Chapter 13). For most of these processes there are many more questions than answers, and the next several years should bring exciting new insight into the role of sumoylation in fundamental cellular pathways.

Lastly, given the breadth of SUMO modified targets and the critical pathways involved, it is not surprising that misregulation of the SUMO system can contribute to disease states. Increasing evidence links over or under expression of various sumoylation components to diseases as diverse as neurodegeneration (Chapter 14), cancer (Chapter 15), diabetes (Chapter 16), and craniofacial disorders (Chapter 17). It is also now apparent that utilization and/or modulation of the host sumoylation system is an important aspect of many viral infections (Chapter 18). While much further understanding is required, this emerging recognition of a role for sumoylation in disease and infection is exciting as it may ultimately offer new insights for diagnosis, treatment, and prevention.

1.4 Conclusion

In the 12 years since its discovery, SUMO has gone from an obscure and functionally unknown protein to one that is recognized as a key regulator of multiple nuclear and cytoplasmic events. The principal components of this modification system have been identified, their basic structures elucidated, and the general features of their enzymology understood. Thanks to the combination of individual targeted protein studies and more global proteomics approaches, hundreds of sumoylation targets are now known, providing a rich resource for subsequent functional studies. The sumoylation system has been shown to be an important player in many biological

processes, such as cellular differentiation, transcriptional regulation, and cell growth (Deyrieux et al., 2007; Gill, 2005; Ihara et al., 2007). Perturbing this biological system changes cellular response to diverse signaling pathways (Sharrocks, 2006) and likely leads to disease. In the chapters that follow, the role of sumoylation in a variety of cellular processes will be explored. The focus will range from effects on molecular targets through cell processes to the organismal level. While many questions remain unanswered, by spanning from molecules to multicellular systems, the full impact and profound significance of the sumoylation system should become apparent. We hope that both newcomers to this field, as well as veterans, will find this comprehensive compilation of state-of-the-art reviews on current sumoylation topics useful and insightful.

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Chapter 2
SUMO Modification and Transcriptional Regulation

Jorge A. Iñiguez-Lluhí

Abstract Transcriptional regulation is a complex multistep process involving numerous protein-protein and protein nucleic acid interactions influenced by post-translational modifications. Both sequence specific DNA binding factors as well as coregulator proteins are targets of sumoylation, and many of the paradigms for SUMO-mediated effects have been identified in the context of transcriptional regulation. Although SUMO modification is most commonly associated with transcriptional repression, it is now evident that this modification is utilized in multiple contexts to regulate the assembly, function and disassembly of multiprotein and nucleic acid transcription complexes. By focusing on specific DNA binding factors and their coregulators, the goal of this chapter is to provide representative examples that illustrate the pervasive and dramatic influence of sumoylation on transcriptional regulation and provide insights on the underlying mechanisms and factors involved.

Keywords SUMO · SUMO Interacting Motif · Synergy Control Motif · Transcription

2.1 Introduction

In contrast to replication, where each and every single base of the genome is duplicated a single time each cell cycle, transcription of DNA into RNA is much more circumscribed and differential. Only a small fraction of the entire genome is *ever* transcribed, and while many transcripts are very rare and accumulate to less than one copy per cell, others are massively expressed. As epitomized in complex multicellular organisms, this differential expression permits a single genome to instruct the development of a remarkable variety of unique cellular types that in turn give rise to the vastly different and specialized tissues of the body. Even within a single

J.A. Iñiguez-Lluhi (✉)
Department of Pharmacology, University of Michigan Medical School,
Ann Arbor, MI 48109, USA
e-mail: iniguez@umich.edu

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