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The SUMO system in *Caenorhabditis elegans* development

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ABSTRACT SUMO, a small ubiquitin-like modifier, is a highly conserved post translational modification and a central regulatory system in eukaryotes. Sumoylation modulates the activities of multiple proteins, mainly in the nucleus, such as transcription factors, chromatin modifiers, and proteins involved in DNA replication and repair. However, SUMO also modifies substrates in the cytoplasm, mitochondria, plasma and ER membrane. This review summarizes our current knowledge on the functions of sumoylation in *C. elegans* development. SUMO modification is highly reversible and several examples described here establish its function as a molecular switch during embryogenesis and postembryonic organogenesis.

KEY WORDS: *SUMO, post translational modifications, C. elegans*

Introduction

Sumoylation is a reversible post-translational modification that modulates the localization, stability, activity and the interactions of the target protein with other proteins and cellular components (Flotho and Melchior, 2013). Sumoylation is involved in the regulation of numerous cellular functions that participate in developmental processes such as cell division, cell fate decisions, differentiation and morphogenesis. Proteins in the nucleus, cytosol and the plasma membrane can be targets of SUMO. Higher eukaryotic genomes encode for several SUMO paralogs with different functions, while a single gene encodes for SUMO in yeast and invertebrate model organisms. Although this machinery resembles ubiquitination in many aspects, the effect of sumoylation is different from ubiquitination and usually, as described in this review, is substrate specific. One of the main characteristics of the SUMO machinery is its transient nature making it difficult for classical biochemical analysis but an excellent system to decode using model organisms. I will focus on the current main knowledge about the function of SUMO in *C. elegans* development.

The components of the SUMO system in *C. elegans*

SUMO. *C. elegans* encodes only a single SUMO protein called SMO-1 that closely resembles mammalian SUMO1. SMO-1 is essential for viability in *C. elegans* and knockdown caused sterility (due to a maternal product) and lethality (Broday *et al.*, 2004; Jones *et al.*, 2002). Overexpression of SUMO also impaired normal development, indicating that regulation of SUMO levels and probably the activity of the entire sumoylation system is critical for normal development (Rytinki *et al.*, 2011). The distinct enzymatic pathway that conjugates SUMO to target proteins is highly conserved (Flotho and Melchior, 2013).

E1. A heterodimeric E1 protein consisting of the UBA-2 and AOS-1 proteins and activates SUMO in a two-step reaction that involves ATP hydrolysis and forms a thioester bond with the Gly-Gly motif in the C-terminus of SUMO. The thioester-charged E1 enzyme interacts with the E2(UBC9) and SUMO is subsequently transferred to the E2 enzyme.

E2. The single E2 SUMO-conjugating enzyme, ubiquitin-like conjugating enzyme 9 (UBC-9), has a key function in the system. In addition to carrying the activated SUMO to the substrate, it recognizes SUMO acceptor site/s on the substrate and directly conjugates SUMO onto a target lysine. UBC-9 is essential for *C. elegans* viability (Jones *et al.*, 2002).

SUMO E3 ligase, GEI-17. Unlike the E3 ligases of the ubiquitin system, SUMO E3 ligases are not essential components of the sumoylation reaction *in vitro* and few proteins are known to be efficiently sumoylated in the absence of E3 ligases also *in vivo* (Flotho and Melchior, 2013). Proteins that are considered as SUMO E3 ligases have been shown to enhance specificity and accelerate the sumoylation process *in vivo* (mainly Siz/PIAS and RanBP2 (Gareau and Lima, 2010). GEI-17, a PIAS domain protein, has been shown in *C. elegans* to have multiple functions mainly during embryogenesis. It acts as an E3 ligase for MUS-101 sumoylation (Holway *et al.*, 2005), regulator of TBX-2 (Milton

Abbreviations used in this paper: DCC, dosage compensation complex; ER, endoplasmic reticulum; SUMO, small ubiquitin-like modifier.

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and Okkema, 2015; Roy Chowdhuri *et al*., 2006) and mediates sumoylation of POLH-1 to protect it from CRL4-Cdt2-mediated degradation in early embryos during DNA-damage response (Kim and Michael, 2008; Roerink *et al*., 2012). In addition, *gei-17* (as well as the SUMO protease *ulp-1)* was identified in genome-wide RNAi screen for genes sensitizing embryos to MMS (methyl methane sulphonate) and *gei-17(RNAi)* led to abundant RAD51 foci in embryos treated with MMS, indicative of replication stress (Roerink *et al*., 2012). GEI-17 was also shown to be required for telomere positioning in early embryos and *gei-17(RNAi)* resulted in delocalization of telomeres from the nuclear periphery (Ferreira *et al*., 2013). GEI-17 mediates accumulation of SUMO conjugates on the metaphase plate during the first embryonic cell division and enhance the sumoylation of AIR-2 (Aurora B orthologue) *in vitro* (Pelisch *et al*., 2014).

SUMO proteases. Sumoylation is a highly dynamic and reversible modification. Deconjugation of SUMO from target proteins is regulated by SUMO proteases (Mukhopadhyay and Dasso, 2007). Ulp/SENP is a family of cysteine proteases that cleave the isopeptide bond between SUMO and its target protein. These enzymes are also responsible for maturation of newly synthesized SUMO prior to conjugation in order to expose a C-terminal Gly-Gly motif (removing one amino acid, phenylalanine, in *C. elegans* SMO-1). There are four SUMO proteases in *C. elegans:* ULP-1, ULP-2, ULP-4 and ULP-5 (ULP-3 is the putative Nedd8 protease ortholog) (Sapir *et al*., 2014), their specific functions have been started to be elucidated recently (Pelisch *et al*., 2014; Sapir *et al*., 2014; Tsur *et al*., 2015). ULP-4 was shown to have a role during the first mitotic division in embryos, *ulp-4(RNAi)* led to multiple defects in chromosome alignment, spindle rotation and a delay in mitotic exit, however kinetochore proteins were normally recruited (Pelisch *et al*., 2014). During larval development and adult aging, ULP-4 is localized to the cytosol and mitochondria and regulates the mevalonate pathway by desumoylation of HMG-CoA synthase (HMGS-1), the first enzyme of this pathway. In accordance, *ulp-4* mutant worms exhibited phenotypes associated with impaired metabolism such as reduced locomotion and pharyngeal pumping rate and loss of mitochondrial homeostasis (Sapir *et al*., 2014).

SUMO-interacting motif (SIM). SIMs regulate noncovalent interactions with SUMO and are typically composed of multiple hydrophobic residues and an acidic residue. SIM-containing proteins can be recruited to sumoylated proteins and this may stimulate their own covalent sumoylation. SIMs can also regulate the recruitment of UBC9 that is loaded with SUMO to the protein, resulting a in its covalent sumoylation (Gareau and Lima, 2010). There is no yet functional evidence for SIMs in *C. elegans* or to an ortholog of the SUMO-targeted ubiquitin E3 ligase, RNF4 (Sun *et al*., 2007).

SUMO functions in *C. elegans*

Initial observations

The first genome wide RNAi screen in *C. elegans* for embryonic lethality (chromosome I) identified the *C. elegans* SUMO as required for viability (Fraser *et al*., 2000). Initial analysis of the SUMO system was done in Candido lab in 2002 (Jones *et al*., 2002). RNAi to *ubc-9* or *smo-1* resulted in embryonic arrest while less affected animals showed abnormal everted vulva and tail defects. Analysis of microarray data showed high expression of *smo-1* during periods of increased cell divisions i.e. embryogenesis and the fourth larval stage and young adults (germline development)(Jones *et al*., 2002). One of the early studies that demonstrated the conservation of the SUMO system in *C. elegans* was on SOP-2 sumoylation (Zhang *et al*., 2004). SOP-2 encodes a SAM domain-containing protein localized to nuclear bodies (Zhang *et al*., 2003) and required for maintaining a restricted pattern of Hox gene expression. A yeast two-hybrid (Y2H) screen revealed its interaction with UBC-9, mediated through the SAM domain. Sumoylation is required for localization of SOP-2 to distinct nuclear bodies. In *ubc-9(RNAi)* or *smo-1(RNAi)* animals an ectopic expression of the HOX genes *egl-5* and *mab-5* was detected as was observed in *sop-2* mutant animals (Zhang *et al*., 2003; Zhang *et al*., 2004) thus the SUMO machinery is required for the controlled repression of HOX genes during development.

Characterization of homozygous *smo-1* deletion mutant, *smo-1(ok359),* revealed that due to maternal contribution of the *smo-1* gene product these mutant animals develop into sterile adults with abnormal somatic gonad, germ line, and vulva (Broday *et al*., 2004). A specific substrate for SUMO highlighted in this study was the LIM homeodomain transcription factor LIN-11. Mutant *smo-1* animals lack a vulval-uterine connection (UTSE) as a result of impaired ventral uterine π-cell differentiation and anchor cell fusion. This phenotype highly resembles *lin-11* mutant phenotype and suggests that LIN-11 function is regulated by sumoylation. LIN-11 expression levels and nuclear localization are impaired in *smo-1* mutant worms. Expression of a SUMO-modified LIN-11 (linear fusion that mimics sumoylation (Ross *et al*., 2002)) in the *smo-1* mutant background partially rescued π-cell differentiation and retained LIN-11 in nuclear bodies. The SUMO acceptor sites on LIN-11 were mapped to Lysine 17 and 18. Expression of transgenes harboring mutations on these SUMO acceptor sites of LIN-11 showed rescue of the vulval defects of *lin-11* mutants, however, the UTSE was not normally formed. Thus sumoylation of LIN-11 has a tissue specific function that is required for its activity in the uterine π-cell for the proper formation of the UTSE. This tissue specific function of SUMO in the regulation of a single transcription factor, LIN-11, highlights the potential complexity of this system.

Germline development

Germline development is abnormal and lead to complete sterility in homozygous animals to *smo-1* deletion (Broday *et al*., 2004). However, probably due to the residual maternal product, germ cells are formed and pachytene-stage meiotic germ cell can be detected. Staining of *smo-1* mutant gonads with the spermatogenic-specific SP56 antibody revealed that the structure of these cells is abnormal and that sperm localization in adults is not restricted to the proximal gonad as in WT (Broday *et al*., 2004).

A requirement for sumoylation during meiosis is conserved across species (Egydio de Carvalho and Colaiácovo, 2006; Nottke *et al.,* In Press). In Saccharomyces cerevisiae, Zip3 is required for the initiation of the synaptonemal complex (SC) assembly and was suggested to act as an E3 SUMO ligase that sumoylates chromosome axis proteins, thus promoting SC polymerization (Cheng *et al*., 2006) and to bind to double-strand break (DSB) sites that are being repaired with a crossover (Serrentino *et al*., 2013). Analysis of *smo-1* mutant meiotic germ cells revealed impaired SC disassembly and bivalent organization that is elevated in double *smo-1; zhp-3* mutants (zhp-3 is the Zip3 ortholog) (Jantsch *et al*., 2004; Bhalla *et al*., 2008). DNA DSB repair is essential for crossover

events. RHINO is one of the proteins required for DSB repair and DNA damage-induced apoptosis (Cotta-Ramusino *et al*., 2011). Sumoylation of ZTF-8/RHINO is required for its function in DSB repair and DNA damage response also in *C. elegans* (Kim and Colaiácovo, 2014, 2015). Expression of a non-sumoylated ZTF-8 transgene resulted in the same phenotype as *ztf-8* null animals i.e. reduced fertility and impaired DNA damage repair.

Pharynx development

The *C. elegans* pharynx is formed during embryogenesis from the ABa and MS blastomeres (Mango, 2007). The T-box transcription factor TBX-2 is specifically required for the development of ABa-derived pharyngeal muscles (Roy Chowdhuri *et al*., 2006). A Y2H screen identified UBC-9 and GEI-17 as specific interactors of TBX-2. While *gei-17(RNAi)* resulted in mild pharyngeal defects, arrested *ubc-9(RNAi)* embryos and larvae exhibited major anterior pharyngeal defects similar to *tbx-2* mutants as analyzed by DIC and the ceh-22::gfp marker of pharyngeal muscle differentiation. Most pharyngeal muscles that could still be detected in *ubc-9(RNAi)* animals were the posterior MS-derived muscles which suggest that the SUMO system is specifically critical in the development of Aba-derived pharyngeal muscles and that this regulation is through modification of TBX-2 and possibly additional factors. Additional support for the direct regulation of TBX-2 by sumoylation is the abnormal accumulation of TBX-2::GFP in nuclear bodies in *ubc-9(RNAi)* embryos in contrast to uniform expression in the nucleoplasm in WT embryos (Roy Chowdhuri *et al*., 2006). TBX-2 is sumoylated on two sites located in the DNA binding domain and the C-terminal of the protein. Ectopic expression of a downstream target of TBX-2 was detected in *ubc-9(RNAi)* embryos similarly to *tbx-2* mutants, suggesting that sumoylation of TBX-2 is required for its transcriptional repression activity *in vivo* (Huber *et al*., 2013). Sumoylation of TBX-2 is also required for its negative autoregulation mechanism (Milton and Okkema, 2015).

Vulval development and chromatin regulation

Initial analysis of *smo-1* mutant worms showed abnormal vulval structures, ectopic vulva induction (multivulva, Muv) and a protruding -vulva phenotype (Pvl), demonstrating that SUMO is required for vulval morphogenesis (for review on vulval development (Sternberg, 2005)). Although vulval invagination appears normal (probably due to the maternal SUMO product), later vulval cell migrations and cell fusions were abnormal leading to abnormal final structure of the vulva. Ectopic vulva was detected in high percentage of animals and interestingly these pseudo-vulva structures were identified at the posterior side, originating from the P8.p cell, which normally remains epidermal and does not form a vulval tissue (Broday *et al*., 2004).

LIN-1. LIN-1 is an ETS DNA-binding domain protein required for the inhibition of the 1° vulval cell fate (form the inner parts of the developing vulva) and loss of function mutations of *lin-1* cause a Muv phenotype (Ferguson *et al*., 1987; Miley *et al*., 2004). LIN-1 is sumoylated on two acceptor sites and sumoylation was shown to mediate its transcriptional repression activity (Leight *et al*., 2005). Sumoylated LIN-1 interacted with MEP-1, a component of the NuRD transcriptional repression complex, suggesting that this interaction mediates LIN-1 transcriptional repression (Leight *et al*., 2005). A recent study showed that the SUMO acceptor sites of LIN-1 at its amino-terminus were required for the binding of LIN-1 to two nucleosome-remodeling enzymes chromatin regulatory factors: SET-6 and RAD-26. The binding was also detected using a translation fusion of SUMO-1::LIN-1 (non-sumoylated mutant) and confirmed that sumoylated LIN-1 recruited these chromatin factors. Genetic analysis identified functional interaction with RAD-26 in vulval development; SET-6 may act with sumoylated LIN-1 transiently or in other cells (Leight *et al*., 2015).

NHR-25. NHR-25 is a nuclear hormone receptor (NHR) and the ortholog of vertebrate SF-1 and LRH-1. As other targets described here, its interaction with SUMO was discovered using the Y2H system. NHR-25 and SMO-1 were shown to genetically interact during vulval development. Sumoylation decreases the transcriptional activity of NHR-25 and its levels in vulval cells to establish a gradient of active NHR-25 required for the correct determination of vulval cell fates (Ward *et al*., 2013). Importantly, the sumoylation sites on the *C. elegans* NHR-25 are conserved in the vertebrate proteins SF-1 and LRH-1.

Chromatin regulation. The *C. elegans* vulva serves as a model to study chromatin regulation. Three groups of genes called synthetic multivulval (synMuv) genes (class A,B, C), encode for chromatin regulators and inhibit vulval development by antagonizing Ras signaling through inhibition of the expression and secretion of LIN-3/EGF ligand in the hyp7 hypodermal syncytium that is in contact with the vulval precursor cells (VPCs)(Cui *et al*., 2006; Fay and Han, 2000). Animals mutant for a single gene of any class have a normal vulva, but animals doubly mutant in genes of two classes adopt the wrong cell fate: one or more of the VPCs that should have a 3° fate which is competent to form vulval tissue but remains epidermal under wild-type conditions, are induced to have a vulval fate, leading to the multivulval (Muv) phenotype. Some of the synMuv genes function through transcriptional repression. A genome-wide RNAi screen to identify synMuv genes highlighted SUMO as a regulator of synMuv function (Poulin *et al*., 2005). RNAi of either *smo-1*, *ubc-9* or *uba-2* (SUMO E1 subunit) induced the Muv phenotype in the three synMuv backgrounds (classes A,B,C). This result suggests that one or more proteins from each of the A,B,C classes are regulated by sumoylation. Along this study the authors found additional function of SUMO in the vulva: animals treated with *smo-1(RNAi)* in a background of reduced Ras function, surprisingly increased Muv levels compared to *smo-1(RNAi)* in WT background suggesting that SUMO is involved in the regulation of additional pathway in the vulva that is dependent on Ras. Indeed, additional genetic analysis showed that SUMO negatively regulates one or more components of LIN-12/Notch pathway (Poulin *et al*., 2005). A recent study highlighted a new role for SUMO in preventing misexpression of germline-specific P granules in somatic cells through the Mec complex (synMuv B class chromatin factors)(Wu *et al*., 2012). In *Drosophila*, the MEC complex recognizes SUMO modifications on transcription factors, which enables its recruitment to promoters and subsequent transcriptional repression (Stielow *et al*., 2008).

BET1. BET-1 is a conserved double bromodomain protein shown to recognize acetyl-lysines on histone tails and to maintain cell fate (Shibata *et al*., 2010). BET-1 was identified in an RNAi screen for Muv phenotype performed in the background of a balanced *smo-1* mutant strain (Gee *et al*., 2013). BET-1 interacts with SMO-1 through its second bromodomain and can be sumoylated *in vitro* (Gee *et al*., 2013). Adults harboring a double mutation *bet-1;smo-1* exhibit impaired muscle integrity and decrease in muscle myosin

levels (Fisher *et al.*, 2013). These studies emphasize the direct link between the SUMO system and chromatin complexes in the regulation of LET-60 (RAS)-mediated signaling.

The dosage compensation complex - SUMO modification of a protein complex

The dosage compensation complex (DCC) in *C. elegans* binds to both the X chromosomes of hermaphrodites to reduce transcription by half (Meyer, 2010). RNAi-mediated knockdown of *smo-1* disrupted DCC association with the X chromosome and microarray analysis showed increase in expression levels of X-linked genes in *smo-1* depleted embryos. Binding of several DCC condensin subunits was severely reduced along the X chromosome in *smo-1(RNAi)* embryos and these subunits were shown to be modified by SUMO in WT embryos. The authors demonstrated that sumoylation of DCC components is essential for proper dosage compensation assembly but it is not required for initial targeting of this complex to the X chromosome (Pferdehirt and Meyer, 2013). This study emphasizes the principle that multiple proteins of the same complex are sumoylated in order to enhance complex assembly and function, as was shown for DNA repair proteins in response to DNA DSB (Psakhye and Jentsch, 2012)

Epidermal morphogenesis and cytoskeleton regulation

Intermediate filament assembly

In a proteomics screen for SUMO targets, a group of both cytosolic and nuclear intermediate filament proteins was identified (Kaminsky *et al.*). Analysis of the cytoplasmic intermediate filament (cIF) protein named IFB-1 revealed that IFB-1 is sumoylated both *in vitro* and in vivo. Epidermal cIF are assembled at embryonic elongation, the process by which the embryo extends from a bean shape to a long thin worm (Zhang and Labouesse, 2010) and it is possible to follow their assembly in live embryos. Such analysis revealed that sumoylation of IFB-1 is required in order to retain a soluble pool of non-polymerized IFB-1, essential for its subsequent assembly into the filament and its proper function in embryonic elongation and in the maintenance of muscle attachment to the cuticle. In the absence of SUMO, IFB-1 formed ectopic filaments and protein aggregates. Soluble IFB-1 could be detected in WT embryos at early elongation while in *smo-1(RNAi)* embryos abnormal thick and short filaments were accumulated (Kaminsky *et al.*). Another study showed that knockdown of *ubc-9* gave the same abnormal IF assembly phenotypes in embryos (Lim *et al.*, 2014). It is possible that in addition to SUMO function as a sequestering factor, sumoylated IFB-1 binds accessory, regulatory and polymerization factors that are required for normal filament assembly. When SUMO is depleted this pool is eliminated which leads to aberrant polymerization of IFB-1 immediately following their translation. Fluorescence recovery after photobleaching (FRAP) showed that depletion of SUMO or mutation of the SUMO acceptor site on IFB-1 resulted in a reduction of IFB-1 cytoplasmic soluble pool. This mutation on the sumoylation site impairs IFB-1 activity in embryonic elongation (Kaminsky *et al.*). A regulated desumoylation process may be responsible for the ability of the free IFB-1 subunits to polymerize into the mature filaments.

The cadherin-catenin complex

In order to determine if transient sumoylation contributes to the regulation of dynamic morphogenetic events, we are currently dissecting the functions of the four *C. elegans* SUMO proteases. It is still unknown what are their targets, functional differences and mode of regulation of these enzymes. Knockdown of the SUMO protease ULP-2 caused embryonic arrest during epidermal morphogenesis. The *C. elegans* epidermal adherens junctions (AJs) were found to be the key target for ULP-2 activity at this developmental stage. AJs consist of E-cadherin transmembrane adhesion molecules linked to cytoplasmic structural and regulatory proteins forming stable adhesive contacts between cells. AJs mediate both the rigidity and plasticity of cell-cell contacts required during morphogenesis. HMR-1/E-cadherin is sumoylated and is a substrate for ULP-2 SUMO protease activity. In addition the results showed that (1) sumoylation of HMR-1/E-cadherin cytoplasmic tail impairs its binding to HMP-2/β-catenin and therefore its interaction with the actin cytoskeleton, and (2) continuous sumoylation and desumoylation of HMR-1/E-cadherin regulates its recruitment and function at the apical AJs. These observation highlight the SUMO system as a molecular switch that modulates the binding of E-cadherin to the actin cytoskeleton (Tsur *et al.*, 2015).

Cilia

Another evidence for the role of SUMO in cytoskeleton organization is the sumoylation of the small GTPase ARL-13, the worm orthologue of ARL13B (mutated in ciliopathy Joubert syndrome) (Li *et al.*, 2012). ARL-13 plays a role in ciliogenesis and its sumoylation is required for its function in ciliary targeting of sensory receptors but not for its targeting to cilia. Expression of ARL-13 chimera protein with SMO-1 (mimics constitutively sumoylated ARL-13) could rescue the ciliogenesis defects of *arl-13* mutant worms. The authors also found that UBC-9, in addition to its known nuclear localization is localized to the middle segments of cilia, similarly to ARL-13 and that the human ortholog ARL13B is also sumoylated, proving functional conservation of sumoylation of these ciliary protein (Li *et al.*, 2012).

Additional substrates for SUMO and possible cross talk with other post translational modifications

Two components of the SUMO system, UBA-2 and GEI-17 were identified in an RNAi -based genome-wide screen for synthetic lethality performed with conditional mutants of the PAR-1 and PAR-4 polarity proteins (Morton *et al.*, 2012). This suggests that sumoylation may be important for polarity or PAR protein function in *C. elegans*. However, since strong polarity defects were not observed in early embryos, the authors suggested that the role of the SUMO system in polarity could be later in development following the two-cell stage through regulation of cytoskeletal proteins (Morton *et al.*, 2012).

A few more examples of identified SUMO targets in *C. elegans* are the ER stress responsive transcription factor XBP-1 (Lim *et al.*, 2014), the nuclear AAA ATPase FIGL-1/Fidgetin (Onitake *et al.*, 2012) and BRD-1, the ortholog of BARD1 (BRCA1-associated RING domain 1) (Boulton *et al.*, 2004). As detailed above, many targets of SUMO were identified using the Y2H system. In a proteomics screen for SUMO targets, we identified ~250 putative targets in *C. elegans* (Kaminsky *et al.*). In addition to the expected high fraction of nuclear proteins we identified a large group of cytosolic, membrane, and other subcellular organelle proteins. An additional group included cytoskeleton components: actin-binding proteins, myosins, α- and β-tubulin and intermediate filament proteins. An

interesting group of putative targets were proteins involved in post-translational modifications such as ubiquitination, phosphorylation, glycosylation and myristoylation. This highlights possible cross-talk between sumoylation and various post-translational modification pathways (the cross-talk with ubiquitin is well established; Gareau and Lima, 2010). Advanced mass spectrometry methods have been developed in the last few years that allow identification of SUMO substrates as well as the sumoylation site/s on each target (Hendriks and Vertegaal, 2016).

Summary

Sumoylation is involved in multiple cellular processes. Here I summarized the main findings about SUMO functions in *C. elegans*. Proteomics and transcriptomic data suggest that SUMO has additional functions and targets during development yet to be elucidated. Sumoylation affects each substrate in a unique way by changing interactions with cellular macromolecules. Sumoylation introduce a major alteration to the substrate that changes its activity or localization dramatically. It is still unknown how this system is regulated. The advanced tools developed for *C. elegans* research together with the extensive knowledge gained on the biology of this multicellular organism will allow us to better decode how this reversible modification regulates different cell types and tissues during animal development from embryogenesis to adults.

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