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Review Article

The role of SUMOylation during development

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During the development of multicellular organisms, transcriptional regulation plays an important role in the control of cell growth, differentiation and morphogenesis. SUMOylation is a reversible post-translational process involved in transcriptional regulation through the modification of transcription factors and through chromatin remodelling (either modifying chromatin remodelers or acting as a 'molecular glue' by promoting recruitment of chromatin regulators). SUMO modification results in changes in the activity, stability, interactions or localization of its substrates, which affects cellular processes such as cell cycle progression, DNA maintenance and repair or nucleocytoplasmic transport. This review focuses on the role of SUMO machinery and the modification of target proteins during embryonic development and organogenesis of animals, from invertebrates to mammals.

Introduction

SUMO belongs to the Ubiquitin-like modifier (UbL) family of proteins and attaches covalently to target proteins in a transient and reversible process termed SUMOylation. SUMO proteins are highly conserved in eukaryotes, but the number of paralogues varies among species. A single *SUMO* gene has been identified in *S. cerevisiae* (*smt3*), *C. elegans* (*smo-1*) and the insect *Drosophila melanogaster* (*smt3*), whereas three *SUMO* paralogues are found in mammals and eight in plants. There are three *SUMO* genes in the human genome, *SUMO1* to *3*. Human SUMO2 and SUMO3 share 97% identity at amino acid level (referred as SUMO2/3), and they share 47% sequence identity with SUMO1. SUMO4 shares 87% identity with SUMO2, and its expression is limited to some tissues [1].

SUMO proteins are synthesized as precursors that need to be matured by SUMO isopeptidases to expose the C-terminal di-glycine motif. The matured SUMO is activated by the heterodimeric E1 enzyme, comprised by SUMO-activating enzyme subunit 1 (SEA1, Aos1) and 2 (SAE2, Uba2). E1 forms a thioester bond between its catalytic cysteine and the SUMO C-terminal glycine. Once activated, SUMO is passed to the catalytic cysteine of the only E2 conjugating enzyme UBC9 (Ubiquitin Conjugating Enzyme E2 I). Finally, SUMO is transferred to the substrate either directly or through a SUMO E3 ligase (Figure 1 and Table 1). The transfer through the E3 ligases ensures a higher conjugation rate and the use of a particular E3 confers substrate specificity. The SUMO E3 ligases best characterized are Protein inhibitor of activated STAT 1 to 4 (PIAS1 to 4) and the Ran-binding protein 2 (RanBP2) Recently, the zinc finger protein ZNF451 was shown to have SUMO E3 ligase activity and to assemble efficiently SUMO2/3 chains (Table 1) [2,3]. The proteases involved in maturation and in the reverse de-conjugation are the ubiquitin-like protein-specific proteases (Ulps) in yeast and invertebrates and sentrin-specific proteases (SENPs) in mammals (SENP1–3 and SENP5–7). Moreover, two additional SUMO isopeptidases have been described in humans, deSUMOylating isopeptidase (DeSI), and the ubiquitin-specific protease-like 1 (USPL1) (Table 1) [4–6].

SUMOylation modulates the function of target proteins by changing their subcellular localization, modifying their DNA-binding or chromatin association ability, recruiting histone-deacetylases and other corepressors or interfering with other post-translational modifications. This review is focused on

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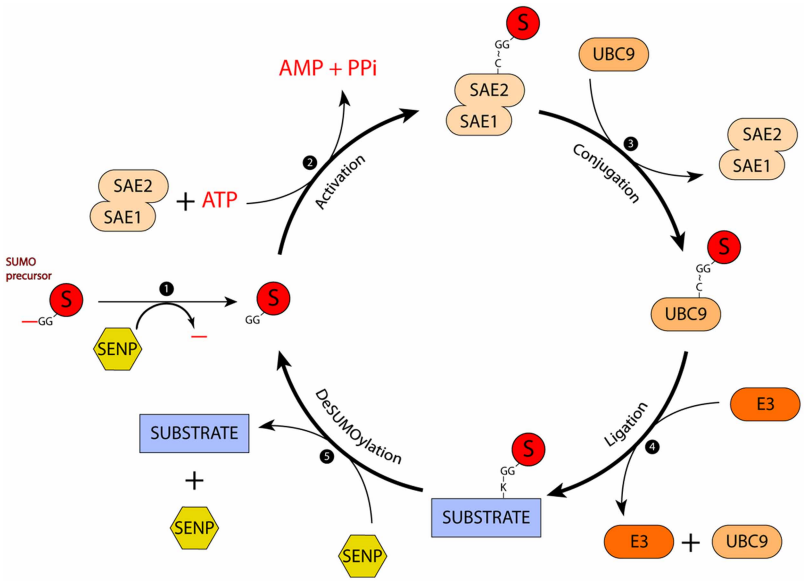

Figure 1. The SUMOylation/deSUMOylation cycle.

(**1**) First, SENPs process newly synthesized SUMO precursor into mature SUMO. (**2**) Then, SUMO's exposed di-glycine forms a thioester bond with the SAE2's catalytic cysteine in an ATP-dependent manner. (**3**) SUMO is then passed from the SAE1/SAE2 E1 activating heterodimer to the E2 conjugating enzyme UBC9, which also forms a thioester bond. (**4**) Substrates can be directly modified by E2-SUMO, but E3s might enhance conjugation rates by binding either E2-SUMO or substrates. (**5**) SENPs cleave the isopeptide bond and SUMO as well as the substrate are recycled. *S: SUMO. ~: thioester bond*.

the role of SUMO during embryonic development highlighting the most recent studies related to organogenesis (Table 2). In each section, we first review the expression and/or roles of SUMOs, E1, E2, ligases or proteases and then, we provide examples of SUMO target proteins and the effect of SUMOylation in their function during development.

SUMO in the germ cells

Primordial germ cells are a specialized population of cells that undergo meiosis to generate gametes, oocytes and spermatozoa. This involves several tightly coordinated processes such as pairing of homologous chromosomes, formation of the synaptonemal complex (SC) and the completion of meiotic recombination that leads to physical attachments between homologous chromosomes.

In yeast, both Ubc9 and Smt3 localize to synapsed regions of meiotic chromosomes. An *ubc9-t* mutant exhibited inefficient synapsis [7] and a meiotic *smt3* reduction-of-function strain displayed abnormal levels of crossover recombination and diminished SC assembly [8]. These studies show that SUMOylation regulates chromosome synapsis during meiosis in budding yeast [9]. In *Drosophila,* mutations of the *Drosophila* UBC9 homologue *lesswright (lwr*), associated with either insertions in the 5′unstranslate region (lwr^{5486}) or with a point mutation (G-to-A) in the coding region that leads to substitution of Arg_{104} by His (lwr^{5}), show defects in meiotic chromosome segregation [10]. In *C. elegans,* the homolog of SUMO1, SMO-1 and the E2 conjugation enzyme UBC9 localize to germline nuclei throughout prophase I [11]. Both, the *smo-1(ok359)* null mutant and *ubc9(tm2610)* mutant, with deletion of the sequences that encoded for the catalytic domain, were sterile. Although the germ cells enter the meiotic prophase, they have defects in meiotic progression and failed to form normal sperm and oocytes [12]. All these studies show the important roles for SUMOylation during meiosis that include the maintenance of meiotic centromeric heterochromatin, meiotic DNA double-strand break repair and homologous recombination, centromeric coupling and the assembly of the SC [13].

Spermatogenesis

The roles that SUMO plays during spermatogenesis include meiotic sex chromosome inactivation, centromeric heterochromatin organization, XY body formation, microtubule nucleation and nuclear restructuring [14–18].

Table 1 E3 SUMO ligases and deSUMOylases Part 1 of 2

Organism	Name	Tissue expression/Subcellular localization	Mammalian Ortholog	Biological/Cellular process	References
E3 Ligases					
S. cerevisiae	Ull1/Siz1	-	PIAS4	Sumoylation of septins and histone H3, Mitosis	[99,100]
	Nfi1/Siz2	-	PIAS4	Septin regulation	[101,102]
	Cst9/Zip3	-	RNF212	Synaptonemal complex formation, Meiosis	[103]
	Mms21	-	NSE2	DNA replication and repair	[104,105]
C. elegans	GEI-17	Germ cells, embryo, pharinx, neurons	PIAS2-4	Meiosis, Telomere positioning, DNA damage	[32,106–108]
Drosophila	tonalli	Salivary gland, Ring gland, imaginal discs, other tissues	ZMIZ1, ZMIZ2	Chromatin modification	[109–112]
	Suppressor of variegation 2-10	Nervous system, reproductive system, other tissues	PIAS1-4	Chromatin modification, JAK-STAT signaling	[113–115]
Mammals	RNF212	Ubiquitous, Germ cells	-	Meiotic recombination	[23]
	PIAS1	Ubiquitous, Germ cells	-	Embryogenesis, Neuronal differentiation, Cardiac development	[50,58,81]
	PIAS2	Testis, pancreas, others/ PML body	-	Post-Synaptic dendritic differentiation	[82]
	PIAS3	Ubiquitous/Nucleus	-	Neuronal differentiation, Steroidogenic tissue, Retinal differentiation	[66,79,87]
	PIAS4	Ubiquitous, enhanced in testis/ PML body	-	Early embryogenesis stage	[51]
	RanBP2	Ubiquitous/Nuclear membrane and vesicles	-	Macromolecular transport	[116]
	NSE2	Ubiquitous/Nucleus	-	Myogenic differentiation, DNA damage repair	[117]
	ZNF451	Ubiquitous/Nucleoplasm	-	SUMO chain formation	[2]
	Pc2/CBX4	Ubiquitous/Nucleoplasm and nuclear bodies	-	Heart development	[54]
	TOPORS	Ubiquitous/Nucleoplasm	-	Chromatin modification	[118]
	SLX4	Ubiquitous/Nucleoplasm, cytosol and cell junctions	-	Genome maintenance	[119]
	hDREF	Ubiquitous	-	Nucleosome remodeling and cell proliferation	[120]
	MAPL	Nucleoplasm, mitochondria and cytosol	-	Mitochondrial fission	[121]
	Krox20	Ubiquitous	-	Hindbrain development	[78]
	ZMIZ1	Ovary, prostate, spleen and testis/Nucleoplasm	-	Embryonic development, vascular development	[111,122,123]
	ZMIZ2	Gallbladder, testis and germ cells/Nuceloplasm and mitochondria	-	Embryonic development of neural tissue	[111,124]
	TRIM 1–19–22 —27–28–32–39	Subcellular proteinaceous bodies	-	Senescence, Apoptosis, Innate immunity, Antiviral defense, Gene silencing, Autophagy, Genomic stability	[125–130]
DeSUMOylases					
S. cerevisiae	Ulp1	Nuclear pore	SENP1, SENP2	Cell cycle progression, Telomeric silencing, DNA damage	[131,132]
	Ulp2	Nucleoplasm	SENP6	Cell cycle, DNA damage, DNA replication	[133,134]
C. elegans	ulp-1	Neurons, intestine, germ line, body wall muscle cell	SENP1	Meiosis, embryonic development	[135]
	ulp-2	Hypodermis and neuroblasts/ Citosol and nucleus	SENP7	Embryonic development	[136]
	ulp-4	Body wall muculature, hypodermis/Mitochondrial matrix	SENP7	Cholesterol metabolism cell cycle, Unfolded protein response	[137–139]
	ulp-5	n.s.		Predicted deSUMOylase	[138]
Drosophila	Ulp1	Early embryo, embryonic CNS, adult germ line, other tissues	SENP1, SENP2	Central nervous system projection neuron axonogenesis, negative regulation of Toll signaling pathway and inmflamatory response	[140–142]

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Table 1 E3 SUMO ligases and deSUMOylases Part 2 of 2

Organism	Name	Tissue expression/Subcellular localization	Mammalian Ortholog	Biological/Cellular process	References
	CG12717	n.s.	SENP6, SENP7	Predicted deSUMOylase	[141]
	Veloren	Early embryo, embryonic CNS	SENP7, SENP6	Axon targeting, negative regulation of cell death	[141]
Mammals	SENP1	Ubiquitous, enhanced in testis/ Nuclear pore and nuclear foci	-	Embryogenesis, Mitotic progression, Senescence, Hematopoiesis	[47,143–146]
	SENP2	Ubiquitous/Nuclear pore, nuclear foci, cytoplasm	-	Trophoblast development, cardiac development, Myogenesis	[48,54,147,148]
	SENP3	Ubiquitous/Nucleolus	-	Osteogenic differentiation, Sarcomere organization, Oocyte meiosis, Ribosome biogenesis	[29,60,149,150]
	SENP5	Nucleolus and mitochondria	-	Ribosome biogenesis, RNAPI-mediated transcription 47S rRNA, tmitochondrial fragmentation during mitosis	[151,152]
	SENP6	Ubiquitous/Nucleoplasm	-	Osteochondro-progenitor homeostasis, Hematopoiesis	[153,154]
	SENP7	Ubiquitous/Nucleoplasm	-	Neuronal differentiation,Chromatin remodeling	[76,155]
	DeSI1	Ubiquitous, enhanced in gastrontestinal tract, pancreas and muscle tissues/Cytoplasm and nucleus	-	Modulation transcriptional repressor activity	[5]
	DeSI2	Cytoplasm	-	Modulation transcriptional repressor activity	[5]
	USPL1	Ubiquitous, enhanced in gastrointestinal tract and kidney/ Cajal bodies	-	RNAPII-mediated snRNA transcription	[4,156]

The organism, tissue expression, subcellular localization, orthologs and biological and cellular processes of the E3 SUMO ligases and deSUMOylases are shown.
n.s.: not specified.

In mouse prophase I of meiosis, SUMO1 is localized to the XY body in spermatocytes, whereas only SUMO2/3 are detected near centromeres in metaphase I spermatocytes [14,15,19]. During human meiotic prophase, SUMO1 is associated with XY chromosome axes and also found in centromeric and pericentromeric heterochromatin [17,20].

The proteasome is involved in ensuring that homologous chromosomes pair each other during meiosis [21]. SUMO acts in coordination with ubiquitin-proteasome to regulate major transitions of meiotic recombination. Interestingly, in mouse, a SUMO-ubiquitin relay recruits proteasomes to the axes between homologous chromosomes to mediate chromosome pairing and recombination between homologs. The Ring Finger Protein 212 (RNF212), involved in SUMO conjugation, mediates the formation of axis-associated SUMO conjugates, while the ubiquitin ligase Cyclin B1 Interacting Protein 1 (CCNB1IP1 or HEI10) antagonizes RNF212 by promoting its turnover from synapses chromosomes [22,23]. Recently, novel proteins modified by SUMO during spermatogenesis have been identified in human and mouse: Cyclin Dependent Kinase 1 (CDK1), RNA polymerase II (RNAP II), Cell Division Cycle 5 Like (CDC5), Piwi Like RNA-Mediated Gene Silencing 2 (PIWIL2 or MILI), DEAD-Box Helicase 4 (DDX4), TAR DNA Binding Protein (TARDBP or TDP-43) and Serine/Threonine Kinase 31 (STK31); but the functional role of SUMOylation of these factors in spermatogenesis has not been reported [24,25].

Oogenesis

During mouse oocyte maturation and growth, different expression patterns and protein localizations have been described for SUMO1 and SUMO2/3 [18]. In transcriptionally active oocytes, both SUMO1 and SUMO2/3 are localized to the nucleoplasm and chromatin. In transcriptionally quiescent oocytes, SUMO1 is weakly detected with chromatin, while SUMO2/3 is localized throughout the nucleoplasm and on chromatin [26]. During oocyte maturation, SUMO1 is localized to the spindle poles in prometaphase I, metaphase I and II stages and around the separating homologues in anaphase I and telophase I stages of first meiosis. SUMO 2/3 is mainly

Table 2 SUMOylation components in developmental processes Part 1 of 2

Organ/Process	SUMO/ Ubc9	E3 Ligase	De SUMOylase	Target	Organism	Pathway/Function	Reference
Germ cells	Ubc9				Yeast	Chromosome synapsis during meiosis	[7]
	Smt3				Yeast	Chromosome synapsis during meiosis	[8]
	Lwr				Drosophila	Defects in meiotic chromosome segregation	[10]
	SMO-1				*C. elegans*	Sterility	[12]
	UBC9				*C. elegans*	Sterility	[12]
Spermatogenesis		RNF212			Mouse	Formation of axis-associated SUMO conjugates	[22]
				CDK1	Mouse	n.s.	[25]
				RNAPII	Mouse	n.s.	[25]
				CDC5	Mouse	n.s.	[25]
				PIWIL2 or MILI	Mouse	n.s.	[25]
				DDX4	Mouse	n.s.	[25]
				TARDBP or TDP-43	Mouse	n.s.	[25]
				STK31	Mouse	n.s.	[25]
Oogenesis	SUMO1			PLK1	Mouse	microtubule and spindle pole organization	[28]
	SUMO2/3			PLK2	Mouse	kinetochore	[28]
			SENP2		Mouse	metaphase II spindle organization	[27]
			SENP3		Mouse	G2-M transition and spindle assembly	[29]
			SENP7		Mouse	meiosis and egg maturation	[30]
		GEI-17		KLP-19	*C. elegans*	Recruitment to the Ring Complex	[31]
				BUB-1	*C. elegans*	Localization between segregating chromosomes during early anaphase I	[32]
				CLS-2	*C. elegans*	Localization to central spindle	[32]
	SUMO1			Septin2	Mouse	Chromosome congression and meiosis progression	[33]
Embryogenesis and ZGA	Ubc9				Mouse	nuclear organization and chromosome segregation at postimplantation stage	[36]
	Smt3, Lwr				Drosophila	early embryogenesis	[37–39]
	UBC9				C. elegans	embryogenesis	[40]
	ubc9				Zebrafish	embryogenesis	[41]
	SUMO2				Mouse	Embryogenesis, stage E10.5, growth, cell proliferation, cell survival	[42]
			SENP1	HIF1alpha, GATA1	Mouse	Mid-gestational embryogenesis; Erythropoiesis; placental development	[45–47]
			SENP2	p53/Mdm2	Mouse	Cell cycle progression during mouse trophoblast development, endoreduplication	[48]
		PIAS1			Mouse	Embryogenesis, stages E10.5 and E12.5, red blood cells, angiogenesis, capillary plexus and blood vessel formation, heart development	[50]
		PIAS4		DPPA2	Mouse	zygotic genome activation; chromosome segregation; heterochromoatine state	[51,52]

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Table 2 SUMOylation components in developmental processes Part 2 of 2

Organ/Process	SUMO/ Ubc9	E3 Ligase	De SUMOylase	Target	Organism	Pathway/Function	Reference
Heart			SENP2	cyclin and cyclin-dependent kinase inhibitors	Transgenic mice overexpressing human SENP2 Mouse	cardiomyocyte proliferation	[54,55]
			SENP5		Mouse	cell death	[56]
	SUMO1		SENP2	Pc2/CBX4	Mouse	SUMO1-conjugated CBX4 decreases cardiomyocyte proliferation/suppressing the expression of Gata4 and Gata6/ regulation of chromatin remodelling complexes	[54]
	SUMO1	PIAS1		GATA4	Mouse	enhanced transcriptional activity	[57,58]
	SUMO1	PIAS1		Nkx2.5	Mouse	enhanced transcriptional activity	[53,59]
				SRF	Mouse	enhanced transcriptional activity/ synergy with Nkx2.5 in activation of cardiac target genes	[59,157,158]
				MEF2	Mouse	reduced transcriptional activity	[159–162]
	SUMO1	PIAS1		myocardin	Mouse	enhanced transcriptional activity	[163]
				Prox1	Human	downregulation corepressor activity, increased transcriptional activity	[164,165]
				Tbx2/Tbx5	Human, mouse, *C. elegans*	pharyngeal muscle development	[108,166]
Osteogenic differentiation			SENP3	RbBP5	Human	activation of HOX gene DLX3	[60]
Adrenal Gland	SUMO1	PIAS1, PIAS3		SF1	Human, mouse	Attenuation of its transcriptional capacity	[64–66]
	Smt3			Ftz-f1	Drosophila	Sterol uptake. Attenuation of its transcriptional capacity	[69,70]
	SMO-1			NHR-25	*C. elegans*	cell fate of reproductive organs	[71]
Neuronal development and differentiation			SENP2	Drp1	mouse	neurodegeneration through the modulation of mitochondrial morphogenesis	[75]
			SENP7			neuronal differentiation	[76]
				Braf35	Mouse	repression of neuronal specific genes and inhibition of neuronal differentiation	[77]
		Krox20		Nab		negative regulation	[78]
	SUMO1, 2, 3	PIAS1, 3	SENP2	FOXP2	Human	modulates transcriptional activity on downstream target genes (DISC1, SRPX2, and MiR200c); Purkinje cell development, cerebellar motor function and vocal communication	[79–81]
		PIAS2		MEF2A	Human, rat	represses transcriptional activity; postsynaptic dendritic differentiation	[82]
Retinal proliferation and differentiation	ubc9			Sp1	*Xenopus laevis*	promotes retinal progenitor proliferation by repressing the cell cycle exit; suppresses p27Xic1 expression	[84]
	Smt3, Aos1/ Uba2, Lwr				Drosophila	proliferating cells in the developing eye	[85,86]
		PIAS3		Nr2e3	Mouse	specification of the rod subtype in the retina while preventing cone-like characteristics	[87]

Proteins modified by SUMO in Germ Cells, embryogenesis, ZGA, Heart, osteogenic differentiation, adrenal gland, Neuronal development and differentiation and retinal proliferation and differentiation. The organism, SUMO ligases, DeSUMOylases, their targets, and the pathway and function are shown.
n.s.: not specified.
ZGA: zygotic genome activation.

concentrated near centromeres [27]. Interestingly, the SUMOylation of the Polo-like kinase 1 (PLK1) by different SUMO paralogues correlates with its different functions and localizations: PLK1 modification by SUMO1 is related to its function in microtubule and spindle pole organization, whereas modification by SUMO2/3 regulates its function at the kinetochore [28].

Studies in mouse show the important roles of deSUMOylases during oogenesis. The overexpression of Senp2 led to defects in metaphase II spindle organization in mature eggs [27]. Other examples are the regulation of G2-M transition and spindle assembly by SENP3 [29] and the meiotic arrest and decrease of mature eggs in SENP7 deficient oocytes [30].

SUMO modification plays also a role in chromosome congression in oocyte meiosis in *C. elegans* by regulating the multi-protein ring complex (RC) assembly [31]. There, the SUMO E3 ligase GEI-17 modifies and recruits the kinesin KLP-19 to the RC. Recently, the same group showed that SUMO regulates the dynamic localization of the central spindle proteins Mitotic Checkpoint Serine/Threonine Kinase (BUB-1) and CLS-2 during female meiosis [32]. Few SUMO modified proteins have been identified in mouse oocyte. As an example, the GTP binding protein Septin2 is modified by SUMO1 and its inhibition showed that it plays an essential role in regulating chromosome congression and meiosis progression [33]. Thus, SUMO1 plays crucial roles during meiotic oocyte maturation by regulating spindle organization, chromosome congression and chromosome segregation [34]. In addition to the role in oogenesis, SUMOylation is also required for the communication of the oocyte with the ovarian somatic cells [35].

SUMO in embryogenesis and zygotic genome activation (ZGA)

SUMO plays important roles in embryogenesis, as revealed by embryonic lethality when the conjugating enzyme UBC9 is deleted or knocked-down. *Ubc9* deficient mouse embryos show severe defects in nuclear organization and chromosome segregation, and die at early post implantation stage [36]. In *Drosophila* embryogenesis, Smt3 coordinates multiple regulatory pathways and loss of function mutations in Ubc9 results in impaired embryogenesis [37–39]. Embryonic arrest is also observed in *Ubc9* knockdown in *C. elegans* and zebrafish [40,41].

In mouse, the SUMOs orthologues have non-overlapping roles during embryonic development. SUMO2 is expressed at higher levels than SUMO3 in early embryonic stages and is indispensable for embryonic development, as shown by the phenotype of the null mutant mice. *Sumo2*$^{-/-}$ embryos die at stage E10.5 and exhibit severe growth retardation with reduced cell proliferation and increased cell death. However, embryos deficient in SUMO3 are viable [42]. Similarly, SUMO1 deficient mice are viable, likely because its function can be compensated by SUMO2 or SUMO3 [43,44].

Consistently with a central role for SUMOylation in embryonic development, knockout mice of the SUMO proteases SENP1 or SENP2 are mid-gestational embryonic lethal [45,46]. Mutations of *SENP1* in mouse produce defects in erythropoiesis by impairing the physiological deSUMOylation of the hematopoietic factors Hypoxia Inducible Factor 1 Subunit Alpha (HIF1α) and GATA Binding Protein 1 (GATA1) [45,47]. *SENP2* mutations cause deficiencies in cell cycle progression during mouse trophoblast development: SENP2 ablation disturbs the p53/Mdm2 pathway, affecting the expansion of trophoblasts progenitors and their maturation [48]. Moreover, deficiency of SUMO E3 ligases, such as PIAS1/PIASy double-knockout mice, impairs embryonic development between E10.5 and E12.5 in mouse [49,50].

While early phases of embryonic development are driven by maternal determinants, development comes under the control of the zygotic genome activation (ZGA) after fertilization. Two recent studies have analyzed the function of SUMOylation in regulating the maternal to zygotic transition and ZGA. Overexpression of the E3 ligase PIAS4 in mouse zygotes inhibited ZGA and impaired early embryo development. PIAS4 effect is partially caused by the SUMOylation of Developmental Pluripotency Associated 2 (DPPA2), which converts this transcriptional activator to a potent inhibitor of zygotic transcriptional program [51]. In agreement, another study shows that overexpression of PIAS4 after fertilization led to a failure of chromosome segregation and impaired ZGA, due to the enhanced SUMO ligase activity. Overexpressed PIAS4 disturbed the demethylation of histone H3 lysine 9 trimethylation (H3K9me3), affecting the heterochromatin state [52].

SUMO in developing tissues and organs

To illustrate the role of SUMO in different developmental pathways, we selected several examples in which SUMO components and SUMOylation of transcription factors play fundamental roles in organ development.

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Heart development

The enrichment of SUMO1 and SUMO2 mRNAs in cardiac chamber regions undergoing proliferation and differentiation suggests a central role for SUMOylation in heart development [53]. A critical issue to achieve correct cardiac development is the balance between SUMOylation/deSUMOylation. Deletion of the deSUMOylating enzyme SENP2 in mice caused defects in cardiac development due to decreased cardiomyocyte proliferation: knockout of *SENP2* lead to accumulation of SUMO1-conjugated Chromobox 4 or Polycomb 2 Homolog (Pc2/CBX4), a subunit of the polycomb repressive complex 1 (PRC1). SUMOylation of Pc2/CBX4 facilitated its binding to H3K27me3, suppressing the expression of the cardiac transcription factors encoding genes *Gata4* and *6* [54], revealing a role for SUMOylation in the regulation of chromatin remodelling complexes during cardiogenesis. Moreover, SENP2 overexpression produced abnormal cardiomyocyte proliferation, with dysregulation of cyclin and cyclin-dependent kinase inhibitors, leading to cardiac defects [55]. Likewise, overexpression of SENP5 in mouse cardiomyocytes increased cell death and led to cardiomyopathy. Indeed, dysregulated levels of SENP5 and SUMO conjugation are observed in human failing hearts [56]. A role for PIAS1 has also been described for erythropoiesis and angiogenesis in the yolk sac. PIAS1 regulates proliferation in cells from the endoderm and mesoderm and its inactivation reduces the myocardium muscle mass, impairing cardiac development [50].

SUMO modifies a multitude of transcription factors that are important for normal cardiac development. These factors include NK2 homeobox 5 (Nkx2.5), GATA4 and 6, Serum Response Factor (SRF), myocyte enhancer factor-2 (MEF2), myocardin, T-box transcription factors-2 and -5 (TBX2 and 5) and prospero-related homeobox (Prox1) [57]. The zinc finger-containing transcription factor GATA4 is modified by SUMO1 in its transactivation domain, which results in enhanced transcriptional activity. The E3 ligase PIAS1 enhances the GATA4 SUMOylation efficiency via its RING finger domain [58]. Similarly, SUMO1 modification of the homeodomain transcription factor Nkx2.5 by PIAS1 increased its transcriptional activity by enhancing the physical association with its binding partners [53,59].

Osteogenic differentiation

Mesenchymal stem cells have the ability to differentiate into multiple cell types including adipocytes, chondrocytes and osteocytes. SUMOylation is required for the epigenetic control of gene expression during osteogenic differentiation of human stem cells. Notably, some studies show that SUMO affects the expression of *HOX* genes, which are evolutionary conserved master regulators that determine body plan in vertebrate development. For instance, the SUMO isopeptidase SENP3 associates with the Lysine Methyltransferase 2A and 2D (KMT2A/KMT2D or MLL1/MLL2) histone methyltransferase complexes and catalyzes the deSUMOylation of RB Binding Protein 5 (RbBP5), which is required for activation of HOX genes such as *Distal-Less Homeobox* (*DLX3*) [60]. A recent study by this group further showed that flightless-I-homolog (FLII), member of the gelsolin family of actin-remodelling proteins, determines the SENP3 recruitment and MILL1/2 complex assembly on the *DLX3* gene [61].

Adrenal gland development and hormone synthesis

SUMO function is necessary in cell fate determination during adrenal gland development. SUMOylation components are expressed in human adrenal cortex and SUMO modification of transcription factors Steroidogenic Factor 1 (SF-1 or NR5A1), Wilms Tumor Protein 1 (WT1), GLI Family Zinc Finger 3 (GLI3), Spalt Like Transcription Factor 1 (SALL1) and Nuclear Receptor Subfamily 0 Group B Member 1 (NR0B1 or DAX1) have been described [62,63]. SF-1, member of the NR5A subfamily of nuclear receptors, is crucial for the development of the adrenal gland and for the expression of steroidogenic genes. SF-1 interacts with UBC9, PIAS1 and PIAS3 and is modified by SUMO, which results in attenuation of its transcriptional capacity [64–66]. Interestingly, a knock-in mouse model expressing a non-SUMOylatable form of SF-1 exhibits endocrine abnormalities and changes in cell fate, due in part to the inappropriate activation of the Hedgehog signalling [67]. The resulting mutant adrenal glands in this model exhibit a persistent foetal tissue, suggesting that SUMOylation interferes as well with normal maturation. Indeed, another recent study shows that foetal adrenal cortex regression is controlled by the synergistic interaction between SF-1 SUMOylation and DAX1, a nuclear receptor corepressor that interacts with SF-1 and inhibits genes involved in adrenal development and steroidogenesis [68]. In *Drosophila*, SUMO is as well required in steroidogenic tissues for the synthesis of steroid hormones [69]. *Drosophila* SF-1 homolog Fushi Tarazu Transcription Factor 1 (Ftz-f1) is modified by SUMO and

is involved in sterol uptake, in part through the scavenger receptor member Snmp1 [70]. In addition, SUMOylation of the *C. elegans* homologue NHR-25 regulates it activity and maintains proper cell fate during development of the reproductive organs [71].

Neuronal development and differentiation

SUMOylation exerts a central role during embryonic brain development. Several studies have analyzed the spatiotemporal distribution of the SUMO moieties, UBC9, SAE1, SENP1 and SENP6 in the developing mouse and rat brains [72–74]. Total conjugation by SUMO1 and SUMO2/3 peaked at E12, whereas the highest levels of UBC9 expression were detected between E15 and E18.

Similarly to the heart, a controlled SUMOylation and deSUMOylation balance is important in the developing brain. A mouse model deficient for SENP2 in neural progenitors shows increased neuronal SUMOylation levels and produces neurodegeneration through the modulation of mitochondrial morphogenesis. This degeneration is a consequence of the hyper-SUMOylation of Dynamin-related protein 1 (Drp1), which promotes its association with mitochondria and neuronal apoptosis [75]. Recent studies showed that SENP7 is involved as well in proper neuronal differentiation [76].

SUMO regulates the function of several transcription factors during neuronal differentiation, including PHD Finger Protein 21A (PHF21A or Braf35), Early Growth Response 2 (EGR2 or Krox20), Myocyte Enhancer Factor 2A (MEF2A) and Forkhead Box P2 (Foxp2). In mouse developing brain, Braf35, a subunit of the LSD1-CoREST histone demethylase complex, is expressed in immature neurons. SUMOylation of Braf35 is required for the repression of neuronal specific genes and for the inhibition of neuronal differentiation [77]. An interesting case of cross-regulation is exemplified by the zinc finger transcription factor Krox20, which has essential roles in vertebrate hindbrain segmentation. Krox20 was described as a SUMO ligase for its coregulators, the NGFI-A Binding (Nab) proteins. As a consequence, the SUMOylation of Nab by Krox20 negatively modulates Krox20 activity and the extension of Krox20-positive territories [78]. During neuronal differentiation in the cerebellum, SUMOylation of the transcription factor FOXP2 increases, as a result of the function of PIAS1 and PIAS3 SUMO ligases and isopeptidase SENP2 [79,80]. This modification is required for the regulation of cerebellar motor function and vocal communication [81]. SUMOylation is also involved in the postsynaptic dendritic differentiation in the cerebellar cortex. The E3 ligase PIAS2 induces SUMO modification of the transcription factor Myocyte Enhancer Factor 2A (MEF2A), repressing the MEF2-dependent transcription in neurons [82]. Furthermore, the SUMOylation machinery participates in the synapsis plasticity and is associated with neurodegenerative diseases [83].

Retinal proliferation and differentiation

During embryonic development, the vertebrate retina originates from the central nervous system. In *Xenopus laevis*, ubc9 controls retinal progenitor proliferation by repressing the cell cycle exit in an high mobility group box 3 (hmgb3)-dependent manner. This function is partially mediated by the SUMOylation of the transcription factor Sp1, which suppressed p27Xic1 expression leading to the promotion of retinal progenitor proliferation [84]. In *Drosophila,* knockdown of Smt3 or E1 and E2 enzymes disrupts the proliferating cells in the developing eye, as well as in other imaginal tissues [85,86].

Two retina photoreceptors, rods and cones, arise from a common progenitor. Interestingly, SUMOylation promotes the specification of the rod subtype in the retina while preventing cone-like characteristics. This function involves the E3 SUMO ligase PIAS3 that SUMOylates the transcription factor Nuclear Receptor Subfamily 2 Group E Member 3 (Nr2e3) and converts it into a repressor of cone-specific gene expression [87]. In addition, PIAS3 has been involved in establishing dorsoventral patterning and visual response of cone photoreceptors in the mouse retina [88].

Conclusions

The development of organisms requires a fine-tune regulation of diverse signalling pathways. Several findings during the last years have unravelled the crucial role of SUMOylation for developmental and differentiation processes through the modification of relevant transcription factors. To these previous examples, we could add the role of SUMOylation during limb development. For instance, wing formation in *Drosophila* depends on the regulation of the SALL transcription factors by SUMO [89,90], or the role of SUMOylation in hedgehog signalling, a pathway that is relevant in limb formation [91]. In addition, SUMOylation of epigenetic regulators modifies their transcriptional activity, localization or stability. Additional complexity is driven by the interplay

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of SUMOylation with other post-translational modifications such as acetylation, phosphorylation or ubiquitylation. By the modification of transcription factor and chromatin remodelers, SUMO is involved in the regulation of cell division, cell lineage commitment, specification, and differentiation during the developmental processes.

A fine balance of SUMOylation/deSUMOylation is required during embryonic development and for normal cardiac and neuronal development. The disruption of the SUMO homeostasis led to inhibition of cell cycle progression, changes in gene expression through chromatin remodelling and to apoptosis. Thus, in normal physiological conditions, the isopeptidase activity is essential to maintain a stable fraction of SUMO modified proteins. Interestingly, changes in SENPs levels that disrupts SUMO equilibrium are observed also in several carcinomas. For example, in hepatocellular carcinoma, the complex between Cbx4 and PIASy mediates hypoxia-induced angiogenesis through enhancing HIF-1α sumoylation and increasing the transcriptional activity of HIF-1 [92].

A crucial step to understand the diverse cellular functions of this modification is the detection of targets of SUMOylation *in vivo*, due to the low abundance of the SUMOylated forms for any given target. For this reason, the technology to identify SUMO targets is crucial. Emergence of new techniques for the analysis of protein SUMOylation and characterization of the SUMO pathway across species and organs have been described [93–96]. Remarkably, as dysregulation of SUMO conjugation is associated to different human diseases it represents a potential therapeutic target. The study of *in vivo* role of SUMOylation in higher eukaryotes and also in more simple organisms with powerful genetic tools such as yeasts and invertebrates [97,98] will allow to elucidate more functions of SUMO targets during development.

Perspectives

1. SUMOylation has been proven crucial in the regulation of developmental processes.
2. Fine-tuning of key transcription factors function and signalling pathways components by SUMOylation contributes to modulate developmental processes.
3. Generation of new technologies to identify SUMOylation targets *in vivo* in model systems and in an organ specific manner will be crucial to achieve a more complete knowledge of the role of SUMO during development.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

CDC5, cell division cycle 5; CDK1, cyclin dependent kinase 1; DDX4, DEAD-box helicase 4; GATA1, GATA binding protein 1; MEF2, myocyte enhancer factor-2; PLK1, Polo-like kinase 1; RC, ring complex; RNF212, ring finger protein 212; RanBP2, Ran-binding protein 2; RbBP5, RB Binding Protein 5; SC, synaptonemal complex; SENPs, sentrin-specific proteases; SRF, serum response factor; STK31, Serine/Threonine Kinase 31; USPL1, ubiquitin-specific protease-like 1; ZGA, zygotic genome activation.

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