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Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction

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Abstract

Post-translational protein modifications are integral components of signalling cascades that enable cells to efficiently, rapidly and reversibly respond to extracellular stimuli. These modifications have crucial roles in the CNS, where the communication between neurons is particularly complex. SUMOylation is a post-translational modification in which a member of the small ubiquitin-like modifier (SUMO) family of proteins is conjugated to lysine residues in target proteins. It is well established that SUMOylation controls many aspects of nuclear function, but it is now clear that it is also a key determinant in many extranuclear neuronal processes, and it has also been implicated in a wide range of neuropathological conditions.

The modification of proteins by the covalent attachment of chemical groups, lipids, sugars or other proteins is important for the spatial and temporal regulation of their function. Of the protein-based modifications, ubiquitylation has been most extensively studied. However, the importance of an analogous modification, SUMOylation, is becoming increasingly apparent. SUMOylation involves the reversible covalent attachment of a member of the SUMO family to lysine residues in target proteins. In the ten years since its discovery, SUMOylation has predominantly been regarded as a nuclear process; however, multiple cytosolic and plasma-membrane targets of SUMOylation have recently been reported, many of which are integral to neuronal function. Furthermore, there is accumulating evidence that perturbations in neuronal SUMOylation contribute to numerous pathological conditions. Here we give an overview of recent studies that demonstrate the growing number of extranuclear functions of neuronal SUMOylation and the implications of SUMOylation in neurological disorders.

The SUMO family

SUMOylation was first reported as a modification that affects the localization of the nuclear pore component Ran GTPase activating protein (RanGAP)^{1,2}. Since then, over 100 targets for SUMOylation have been reported. The fundamental importance of SUMOylation to eukaryotic cells has been established by the discovery that knocking down or deleting the SUMO genes or components of the SUMO pathway is fatal for mammalian cells³ and causes cell-cycle arrest in yeast^{4,5}.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

[parkin](#) | [PIAS1](#) | [PIASx](#) | [RanBP2](#) | [SAE1](#) | [SAE2](#) | [Smt3](#) | [SUMO1](#) | [SUMO2](#) | [SUMO3](#) | [SUMO4](#) | [UBC9](#)

OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>

[AD](#) | [autosomal recessive juvenile Parkinsonism](#) | [DLB](#) | [DRPLA](#) | [HD](#) | [NIID](#) | [PD](#) | [SBMA](#) | [SCA type 1](#)

FURTHER INFORMATION

Jeremy M. Henley's homepage: http://www.bristol.ac.uk/Depts/Synaptic/research/pi/JMH_res.html

Yeast contain only one SUMO protein, called Smt3 (REF. 6), whereas mammalian cells possess four SUMO isoforms, designated SUMO1 to SUMO4 (REF. 7). SUMO proteins are members of the family of ubiquitin-like proteins (Ulp), and are conjugated to substrate proteins by an enzymatic pathway that is analogous to the ubiquitylation pathway⁸. SUMO1 is an ~11.6 kDa, 101-amino-acid protein that has ~18% homology with ubiquitin. SUMO2 and SUMO3 differ from each other by only 3 amino (N)-terminal residues and have yet to be functionally distinguished, but together they share only ~47% homology with their paralogue SUMO1 (REF. 6). Despite the low sequence homologies, SUMO1, SUMO2/3 and ubiquitin share very similar three-dimensional structures⁹ (FIGS 1,2). SUMO4 is the least well characterized isoform. Expression of SUMO4 mRNA appears to be limited to kidney cells and spleen tissue⁷; however, expression of endogenous SUMO4 protein *in vivo* has yet to be established, and no native SUMO4-modified substrates have been reported¹⁰. In addition, *in vitro* studies have generally used an artificial mature form of SUMO4, which might not exist *in vivo* owing to an inability of the SUMO4 precursor to undergo maturation and subsequent conjugation¹¹. Thus, the physiological relevance of SUMO4 is still unclear. It remains possible that SUMO4 might be a pseudogene or might act through non-covalent interactions rather than by post-translational modification¹¹.

The SUMOylation pathway

SUMO1 and SUMO2/3 are conjugated by the same pathway¹² (FIG. 3) but can target different substrates¹³, although a subset of proteins can be modified by both SUMO1 and SUMO2/3 (REFS 14,15). SUMOylation is a reversible enzymatic process that occurs predominantly at a core consensus motif in substrate proteins (Ψ -K-X-[D/E], where Ψ is any large hydrophobic residue (such as I, V or L), K is the target lysine, X can be any residue and D/E is aspartate or glutamate)^{16,17}. It is important to emphasize that not all SUMO substrates are modified in this motif, and not all Ψ -K-X-[D/E] motifs are SUMOylated. Analysis of SUMOylated substrates suggests that the sequence flanking the core consensus site is important in determining whether a site can be SUMOylated and, if so, how SUMOylation is regulated. This information has led to the definition of several extended SUMOylation consensus motifs (TABLE 1).

SUMO isoforms are synthesized as inactive precursors that must be matured by a family of SUMO-specific proteases (SENPs) (FIG. 3). SUMO proteins are then activated by an ATP-dependent heterodimer of SUMO1 activating enzyme subunit 1 (SAE1) and SAE2 (REF. 18), which passes the activated SUMO protein onto the specific and unique 'conjugating' enzyme, ubiquitin conjugating enzyme 9 (UBC9), through a transesterification reaction^{8,19}. UBC9, usually acting in conjunction with an E3 'ligating' enzyme, then catalyses SUMO conjugation to the substrate. SUMOylation and ubiquitylation are mechanistically similar, but there are fundamental differences between the two pathways. Although an E3 enzyme is generally essential for ubiquitylation (for exceptions, see REF. 20), SUMOylation can proceed with just UBC9. UBC9 binds directly to the consensus SUMOylation sequence¹⁷, aligning its active site with the ϵ -amino group of the lysine residue to which the SUMO is to be conjugated²¹.

As UBC9 is capable of directly recognizing substrate proteins and conjugating SUMO to them, it was initially debated whether E3 enzymes were necessary. However, a number of proteins that have SUMO E3 activity have been discovered (TABLE 2). The yeast Siz proteins were the first SUMOylation-specific E3 enzymes to be discovered²²⁻²⁴. Deletion of the genes that encode these proteins almost totally abolishes SUMOylation in yeast²². The mammalian homologues of the Siz proteins are the protein inhibitor of activated STAT (PIAS) proteins^{25,26}. Other mammalian E3 enzymes include Ran binding protein 2 (RanBP2)²⁷ and the polycomb protein Pc2 (REF. 28). The mechanisms by which E3

enzymes enhance the kinetics, specificity and efficiency of UBC9-mediated SUMOylation remain unclear, but they might be particularly important for SUMO conjugation to substrate proteins that contain atypical consensus motifs (TABLE 1).

SUMOylation can be readily and rapidly reversed by the isopeptidase activity of the SENPs (TABLE 2). SENPs have preferences for particular SUMO paralogues and also show distinct subnuclear and subcellular localization patterns²⁹. They are responsible for both the maturation and the deconjugation of SUMO and they therefore regulate the available SUMO pool (for reviews, see REFS 29,30). Although the SUMOylation state of a protein is a delicate balance between conjugation and deconjugation, for most substrates little is known about how these processes are regulated. However, it is becoming increasingly apparent that SUMOylation can also be modulated by other post-translational modifications of the substrate protein (TABLE 3).

There is no clear consensus as to whether SUMO1 and SUMO2/3 conjugation have different roles. Interestingly, SUMO2 and SUMO3 (and Smt3 in yeast) can form polySUMO chains through internal Ψ-K-X-[D/E] motifs^{12,31,32}. The direct functional consequences of polySUMO chain addition are not well defined, although polySUMO3 modification of the tumour suppressor promyelocytic leukemia (PLM) appears to be required for retention of PLM in the nucleus³³. Whether SUMO1 is capable of chain formation remains open to debate, because SUMO1 lacks the lysine residues that are required for SUMO2/3 or Smt3 polymerization¹². Nonetheless, there have been reports of SUMO1 chain formation *in vitro*³⁴⁻³⁶, although no SUMO1 polymers have yet been detected *in vivo*¹².

Non-covalent SUMO binding

The effects of SUMOylation are often all-or-none, despite the fact that purified target proteins are only partially covalently SUMOylated. This has been termed the 'SUMO enigma'¹⁹. This conundrum might be explained, at least in part, by observations that both covalent and non-covalent SUMO interactions can contribute to SUMO-dependent functional changes. In some cases covalent modification is not required at all (for a review, see REF. 37). More specifically, it has recently been discovered that some proteins contain SUMO-interacting motifs (SIMs) (BOX 1). These motifs allow proteins to bind SUMO proteins non-covalently and add a further level of complexity to the regulation and consequences of protein SUMOylation.

There are multiple implications for non-covalent SUMO interactions. In the nucleus they are important for DNA repair, transcriptional activation, nuclear-body formation and protein turnover (for a review, see REF. 37). Binding of SUMO to SIMs can precede SUMO conjugation and persist after conjugation, occur after SUMO conjugation or occur in the absence of covalent modification. In each case, binding of SUMO to SIMs can potentially induce conformational changes in the substrate protein and thereby regulate its interactions with other proteins. Intriguingly, SIMs are present in many extranuclear proteins³⁸, but no non-covalent SUMO interactions have yet been reported for SIM-containing proteins outside the nucleus.

Non-covalent interactions with SUMO proteins that are independent of SIMs have also been described. For example, dynamin 1 does not appear to be SUMOylated, but it interacts non-covalently with SUMO1, UBC9 and *PIAS1* (REF. 39). All three proteins bind to the GTPase effector domain (GED) of dynamin, which does not contain a SIM. both SUMO1 and UBC9 inhibit lipid-dependent dynamin oligomerization, presumably by occluding the GED. As a result, overexpression of SUMO1 or UBC9 in mammalian cells downregulates dynamin-mediated endocytosis³⁹.

Another example of a SIM-independent SUMO interaction comes from the E3 ubiquitin ligase *parkin*. Mutations in the gene that encodes parkin have been linked to autosomal recessive juvenile Parkinsonism⁴⁰. Parkin binds selectively to SUMO1, resulting in a dramatic increase in the nuclear transport of parkin and its autoubiquitylation⁴⁰.

SUMOylation outside the nucleus

Whereas ubiquitin is generally involved in protein degradation pathways⁴¹, the functions of SUMOylation are diverse and have been best characterized for nuclear proteins^{42,43}.

However, important roles for SUMOylation in extranuclear signal transduction, trafficking and modification of cytosolic and integral membrane proteins are emerging (FIG. 4). Here we focus mainly on non-nuclear SUMOylation substrates that are present in neurons. For a number of excellent reviews on the nuclear roles of SUMOylation, see REFS 8,42-45.

Cytosolic roles for SUMOylation

Regulation of G-protein signalling

G-protein-coupled receptors (GPCRs) are integral membrane proteins that are characterized by the presence of seven trans-membrane domains. They constitute one of the largest and most diverse protein families in the mammalian genome. More than 90% of GPCRs are expressed in the brain, and they participate in all of the functions that are controlled by the nervous system⁴⁶. In their inactive state, GPCRs are bound to heterotrimeric G proteins⁴⁷. Following agonist stimulation of the GPCR, the G protein G_{α} subunit exchanges bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and dissociates from the $G_{\beta\gamma}$ complex, allowing both G_{α} and $G_{\beta\gamma}$ to activate various second messenger pathways⁴⁷. Recently a role for SUMOylation in the regulation of G-protein signalling has emerged, with reports that regulator of G-protein signalling (RGS) proteins and phosducin⁴⁸ are SUMOylated.

RGSZ1 and RGSZ2 are GTPase-activating proteins (GAPs) that regulate G_{α} G-protein subunits⁴⁹. They are almost exclusively expressed in the brain⁵⁰ and are important for μ -opioid-receptor desensitization⁵¹. In synaptosomes, RGSZ1 and RGSZ2 can be conjugated to SUMO1, SUMO2 and SUMO3 (REF. 52). SUMOylated RGSZ proteins interact strongly with μ -opioid receptors and, following receptor activation, there is a robust and sustained increase in G_{α} interaction with SUMOylated RGSZ2 that mediates receptor desensitization⁵².

Phosducin regulates G-protein signalling by binding to $G_{\beta\gamma}$ subunits and inhibiting their function⁴⁸. Phosducin is ubiquitously expressed, but is particularly abundant in the retina and the pineal gland⁵³. In retinal tissue, SUMOylation of phosducin increases its stability, by antagonizing the ubiquitin system and decreasing its capability to bind $G_{\beta\gamma}$ ⁵⁴. Although the overall effect of SUMOylation on this system is unclear, one possibility is that it provides a rapid, reversible means of negatively regulating phosducin activity. These examples indicate the previously unsuspected involvement of SUMOylation in the regulation of G-protein function and infer that SUMOylation might have much wider, as-yet undiscovered roles in GPCR signalling.

Regulation of kinase and phosphatase signalling

Phosphorylation cascades are integral to cell signalling, and important elements of these pathways can be modulated by SUMOylation. For example, focal adhesion kinase (FAK) and protein tyrosine phosphatase 1b (PTP1b) are both SUMO substrates. FAK regulates the maturation and turnover of contacts between the extracellular matrix and the cytoskeleton and is a key regulator of neuronal growth-cone motility and guidance⁵⁵. It is activated by

integrins and growth-factor receptors, and activation leads to the FAK-dependent phosphorylation of substrates involved in focal-adhesion dynamics and changes in the cytoskeleton. SUMO1 modification of FAK leads to increased autophosphorylation⁵⁶, which allows FAK to bind to Src. This in turn leads to further FAK phosphorylation and its full activation. SUMOylation of FAK might also be involved in the regulation of its nucleocytoplasmic shuttling⁵⁷.

PTP1b is involved in the downregulation of insulin and growth-factor signalling, through the dephosphorylation of multiple receptor tyrosine kinases⁵⁸. It is highly expressed in the brain⁵⁹ and is a critical negative regulator of leptin signalling in the hypothalamus⁵⁹⁻⁶¹ (leptin acts on the hypothalamus to decrease feeding behaviour and increase energy expenditure⁶²). In fibroblasts, SUMOylation of PTP1b is stimulated by insulin, causing a transient downregulation of both its catalytic activity and its expression⁶³. Although it has not yet been established whether this also occurs in hypothalamic neurons, SUMOylation of PTP1b might play an important part in the neuronal regulation of body mass.

A further role of SUMOylation has been demonstrated for the scaffolding protein axin. Functions of axin include the regulation of WNT signalling and the activation of c-jun N-terminal kinase (JNK) in neurons. Axin is SUMOylated at two sites in its extreme carboxyl (c) terminus. A SUMOylation-deficient form of axin is unable to interact with MAPK/ERK kinase kinase (MEKK) and activate the JNK pathway; however, its effect on WNT signalling is unaffected⁶⁴. These examples raise the intriguing possibility that SUMOylation might be a general regulatory mechanism of phosphorylation dynamics.

SUMOylation and axonal mRNA trafficking

Local axonal protein synthesis is important for growth-cone guidance in developing neurons⁶⁵ and axonal regeneration and synaptic plasticity in adult neurons⁶⁶. La is an mRNA-binding protein that aids translation by protecting particular mRNAs from exonucleases⁶⁷. Many of La's target mRNAs are present in axons, and La is transported into axonal processes from the cell body *in vivo* in both cultured dorsal root ganglion neurons and peripheral nerves⁶⁸. Fast axonal transport is mediated by the microtubule-associated motor proteins dynein and kinesin⁶⁹, which transport cargo retrogradely and anterogradely, respectively. SUMOylated La interacts efficiently with dynein, whereas non-SUMOylated La binds only to kinesin⁶⁸. A non-SUMOylatable mutant of La undergoes only anterograde transport due to its inability to bind dynein. Thus, by creating a new interface for protein binding, SUMOylation of La dictates the direction of its axonal transport. Although the overall effect of preventing SUMOylation of La on axonal protein synthesis has not yet been addressed, it seems likely that defects in SUMOylation could prevent the recycling of La back to the cell soma, with potentially serious effects on neuronal function.

SUMOylation in mitochondrial fission and apoptosis

In both cultured neurons and hippocampal slices, neuronal activity increases the number of mitochondria in dendritic protrusions. Inhibiting mitochondrial fission severely reduces the number of dendritic protrusions, indicating that mitochondrial fission is required for the maintenance of synapse number⁷⁰. Mitochondrial fission is dependent on the GTPase dynamin-related protein 1 (DRP1) (REF. 71). SUMOylation of DRP1 appears to protect it against degradation⁷². Interestingly, the SUMOylation of DRP1 appears to be controlled in part by nucleotide binding to DRP1. DRP1 SUMOylation is also promoted by the pro-apoptotic protein Bax, raising the possibility of a role for SUMOylation in the induction of apoptosis⁷³. Indeed, both caspase 7 (REF. 74) and caspase 8 (REF. 75) are SUMO targets, potentially providing a direct role for SUMOylation in apoptotic pathways. Further support for the involvement of SUMO1 in mitochondrial morphology and fission has come from the

report that SENP5 has a regulatory role in both of these processes⁷⁶, and from the observation that multiple as-yet unidentified mitochondrial proteins are substrates for SUMOylation^{72,77}.

Roles for SUMOylation at the plasma membrane

SUMOylation and glucose transport

The glucose transporters GLUT1 and GLUT4 were the first membrane proteins to be shown to be SUMOylated⁷⁸, and they are highly expressed in the brain^{79,80}. UBC9 interacts with GLUT1 and GLUT4 in yeast two-hybrid screens, and their SUMOylation was subsequently demonstrated by immunoprecipitation from rat adipocytes. Interestingly, overexpression of UBC9 invokes a 65% decrease in GLUT1 levels but causes an eightfold increase in GLUT4 expression. However, how SUMOylation differentially affects GLUT1 versus GLUT4 is unknown, as is whether SUMOylation of these proteins takes place at the membrane. It is also unclear how SUMOylation of these two transporters is regulated. However, recent evidence has suggested that the selective increase in GLUT4 versus GLUT1 stability can be mimicked with a catalytically inactive mutant of UBC9, suggesting that this phenomenon might be due to a scaffolding role of UBC9. These results imply that the UBC9 interaction, rather than SUMOylation, is the key regulator of glucose transport⁸¹. Although most research into these transporters has been carried out in adipocytes, their abundant expression in neurons suggests that their interaction with UBC9 and SUMO might be a major regulatory mechanism for glucose transport in the brain.

SUMOylation and neuronal excitability

SUMO conjugation has been reported to modulate the function of two neuronal K⁺ channels, K2P1 and Kv1.5, implicating SUMOylation in neuronal excitability. Together with the observation that kainate receptors are regulated by SUMOylation (see below), these data suggest that SUMOylation might be a general mechanism for regulating ion-channel function. K2P1 channels are background K⁺-selective plasma membrane leak channels. No native currents have been recorded from surface-expressed K2P1 channels, possibly because they are silenced in their basal state⁸². Similarly, no K2P1 activity was recorded when these channels were expressed in *Xenopus* oocytes. This was attributed to basal SUMOylation of the channels at residue K274 blocking the pore⁸³. Expression of a SUMOylation-deficient lysine point mutant (K274E) resulted in the appearance of an active, pH-sensitive, open-rectifying K⁺ channel in COS-7 cells⁸³. Consistent with this, expression of wild-type K2P1 together with the SUMO-specific protease SENP1 also resulted in active channels⁸³, suggesting an inhibitory role for SUMOylation. It must be noted, however, that a more recent report has questioned a role for SUMOylation in the regulation of K2P1 (REF. 84). These workers were unable to detect SUMOylation of K2P1, although they did not use the same experimental paradigms as the earlier group. The authors of the more recent study propose that the effects of the K274E mutation are caused by the exchange of a positively charged lysine for a negatively charged glutamate, rather than the inhibition of SUMOylation, because no effect was observed with the conservative charge mutation K274R, which should also prevent SUMOylation⁸⁴. The SUMOylation status of K2P1 therefore remains unclear, and further studies will be required to resolve these apparently contradictory findings.

The voltage-gated K⁺ channel Kv1.5 can be SUMOylated *in vivo* with all three SUMO paralogues. In the CNS, Kv1.5 channels are involved in microglial cell proliferation and the production and release of nitric oxide following brain injury⁸⁵. In a recent study, inhibition of Kv1.5 SUMOylation by disruption of the conjugation sites or expression of the SUMO protease SENP2 led to a selective hyperpolarizing shift in the voltage-dependence of steady-

state inactivation, indicating that SUMOylation has a role in fine-tuning channel function⁸⁶. Multiple Kv channel α -subunits are expressed in neurons^{87,88}, and Kv1.1 and Kv1.2 contain similarly located consensus SUMOylation sites, suggesting that similar SUMO regulation of these channels might also occur in the CNS.

SUMOylation of glutamate receptors

Metabotropic glutamate receptors (mGluRs) mediate the slow component of glutamatergic signalling. They can be subdivided into three groups on the basis of their sequence homology, their preferred signalling pathways and their pharmacological properties⁸⁹. Group III mGluRs act predominantly as autoreceptors, inhibiting presynaptic glutamate release through G-protein-mediated regulation of ion channels⁸⁹. Each of the group III mGluRs interacts with PIAS1 in yeast two-hybrid screens, and the intracellular C-terminal domain of mGluR8 is SUMOylated in HEK293 cells^{38,90}. Although the functional consequences of mGluR8 SUMOylation have not yet been established, multiple interactions with both scaffolding molecules and components of signalling pathways occur at the C termini of mGluRs⁹¹, and addition of a SUMO protein to this region would be expected to disrupt at least some of these interactions. Alternatively, SUMO might provide an interface for the binding of different subsets of interacting proteins. Furthermore, because of the central role of group III mGluRs in regulating presynaptic activity through G-protein modulation of ion channels and signal-transduction pathways, it seems highly likely that their SUMOylation will prove to regulate these biological functions.

Kainate receptors (KARs) are tetrameric glutamate-gated ion channels⁹². Presynaptic kainate receptors can modulate neurotransmitter release, whereas postsynaptic kainate receptors contribute to excitatory transmission (for reviews, see REFS 93-95). The GluR6a subunit undergoes activity-dependent SUMOylation *in vivo*⁷⁷. GluR6a is selectively and rapidly SUMOylated in response to agonist activation, leading to endocytosis of the receptor. Expression of SENP1 in hippocampal neurons prevents kainate-evoked KAR internalization. Moreover, a SUMOylation-deficient point mutant (K886R) does not internalize in response to kainate. Consistent with these results, KAR-mediated excitatory postsynaptic currents in hippocampal slices are decreased by SUMOylation and enhanced by de-SUMOylation. Thus, SUMOylation of GluR6a acts as a specific signal for agonist-induced KAR endocytosis⁷⁷.

These data suggest previously unsuspected roles for protein SUMOylation in the regulation of membrane-protein endocytosis and, more widely, in the control of synaptic function. Pertinent to this, anti-SUMO1 immunoblots of synaptic fractions from brain and cultured neurons reveal multiple as-yet unidentified SUMO substrates at synapses, in addition to GluR6a⁷⁷. The identification and functional characterization of these proteins will undoubtedly be of interest for future studies.

Neuronal development and synapse formation

Neuronal activity regulates the strength and number of synapses that are formed during neuronal development. For example, during cerebellar development, granule-neuron dendrites undergo differentiation of dendritic claws, onto which mossy-fibre terminals form synapses. The myocyte enhancer factor 2 (MEF2) family of transcription factors are critical for dendritic claw differentiation and fate (for a review, see REF. 96). PIASx is a SUMO E3 ligase that represses MEF2-dependent transcription in neurons and drives the differentiation of dendritic claws, suggesting a role for SUMO E3 ligases in brain development and plasticity⁹⁷. MEF2 also suppresses the number of excitatory synapses in a neuronal-activity- and calcineurin-dependent manner in developing hippocampal neurons⁹⁸. Calcineurin dephosphorylates and activates MEF2, leading to the transcription of a set of genes that

restrict synapse number — for example, those that encode activity-regulated cytoskeleton-associated protein (ARc) and synaptic Ras GTPase activating protein 1 homolog (synGAP)⁹⁸.

At early stages of differentiation, one of the MEF2 family, MEF2A, is repressed by SUMOylation (at residue K403), and this promotes postsynaptic granule-neuron differentiation⁹⁶. Activity-dependent calcium signalling induces a calcineurin-mediated dephosphorylation of MEF2A at S408, favouring de-SUMOylation at K403 and the subsequent acetylation of this residue. This leads to MEF2A activation and inhibition of dendritic-claw differentiation⁹⁹. Thus, SUMOylation of MEF2A can be influenced by both phosphorylation and acetylation in an activity-dependant manner, to alter transcriptional processes that control synapse formation and stabilization (TABLE 3). Furthermore, as this phosphorylation-dependent SUMO–acetyl switch is also found in a number of transcription factors⁹⁹, de-SUMOylation and consequent acetylation might have a role in the activity-dependent expression of multiple genes that are involved in neuronal development and differentiation.

SUMOylation and neurological disorders

Neurodegenerative diseases can result from inappropriate modifications to protein stability or from aberrant protein targeting and the subsequent accumulation of misfolded proteins in inclusions¹⁰⁰. It is becoming apparent that protein SUMOylation is implicated in the altered protein dynamics that are associated with various aspects of neurodegenerative disease¹⁰¹ (TABLE 4).

There are a large number of reports documenting the involvement of SUMO in diseases that are associated with the aggregation of intranuclear proteins (for a recent review, see REF. 101). Neuronal intranuclear inclusion disease (NIID) is a rare neurodegenerative disease of the central and peripheral nervous systems that in adults results in progressive ataxia and dementia (for a review, see REF. 102). Strong SUMO1 immunoreactivity has been associated with fibrils of the insoluble neuronal inclusions in three unrelated cases of familial NIID¹⁰³. SUMO1 immunoreactivity in intranuclear inclusions has also been reported in the sporadic form of the disease¹⁰⁴ and in juvenile NIID¹⁰⁵, leading to the proposal that nuclear de-SUMOylation or shuttling pathways are altered, in turn causing the accumulation of SUMOylated substrates in the nucleus.

PolyQ disorders

CAG-repeat disorders, also known as polyglutamine (polyQ) disorders, include several spinocerebellar ataxias (ScAs), spinobulbar muscular atrophy (SbMA), dentatorubro–pallidoluysian atrophy (DRPLA) and Huntington's disease (HD). They are characterized by the presence of a toxic stretch of polyglutamine repeats (coded by the disorder-specific genes) and feature neuronal inclusions composed of aggregates of polyQ. A striking feature of these neurodegenerative diseases is the inverse correlation between age-of-onset and disease severity with the number of polyQ repeats (for a review, see REF. 106).

HD causes severe motor dysfunction, psychiatric disturbances and progressive cognitive impairment. It results from the toxic gain-of-function of an expanded cAG repeat in the N-terminal domain of the protein Huntingtin (HTT), and the subsequent accumulation of this protein in affected neurons. A pathogenic fragment of HTT can be modified by both SUMO1 and ubiquitin at the same lysine residue¹⁰⁷. In neuronal cell lines, SUMOylation stabilizes the pathogenic fragment and reduces its ability to form aggregates, but probably also increases its intracellular concentration. In a *Drosophila melanogaster* model of HD, HTT-fragment SUMOylation increases neurodegeneration whereas ubiquitylation decreases

neurodegeneration¹⁰⁷. because mutations that prevent both post-translational modifications of HTT reduce the HD pathology in *D. melanogaster*, it is likely that there is a balance between SUMOylation and ubiquitylation that controls the stability and correct targeting of HTT in neurons, and that this balance is disrupted in HD.

Interplay between SUMOylation and ubiquitylation pathways has also been reported for SbMA. This inherited condition is caused by the expansion of a polyQ strand in the androgen receptor (AR). Expression in *D. melanogaster* of a pathogenic AR protein with an expanded polyQ repeat results in nuclear and cytoplasmic inclusions and neuronal degeneration¹⁰⁸. However, unlike with the HD model, disruption of the ubiquitylation or SUMOylation pathways leads to intensified degeneration, suggesting that both pathways have roles in diminishing the pathogenesis of SbMA¹⁰⁸.

Human brain tissue from patients with SbMA, HD¹⁰⁹ or DRPLA displays high levels of SUMO1 immunoreactivity in neuronal intranuclear inclusions¹¹⁰. DRPLA is an autosomal-dominant disease that is caused by polyQ expansions in atrophin 1, a protein of unknown function. co-expression of mutant polyQ atrophin 1 with a non-conjugatable form of SUMO1 in a neuronal cell line decreases the number of intranuclear inclusions and the amount of cell death. consistent with this, cotransfection with active SUMO1 leads to a significant increase in intranuclear inclusions and consequent cell death¹¹⁰, indicating that SUMOylation is involved in aggregate formation and cell death.

Strong SUMO1 immunoreactivity also occurs in neurons from ScA type 3 patients¹⁰³ and in a mouse model of ScA type 1 (REF. 109). The ScAs are dominantly inherited, progressive neurodegenerative diseases that result in the atrophy of cerebellar Purkinje cell layer. ScA1 is caused by an abnormally long polyQ strand in ataxin 1 (REF. 111). Ataxin 1 is SUMOylated on at least five lysine residues, and the level of SUMOylation is reduced by the presence of the polyQ expansion. Introduction of a non-phosphorylatable polyQ ataxin 1 mutant (S776A) restores the SUMOylation level to that of the wild-type protein, highlighting an antagonism of the SUMO system by phosphorylation in this case.

Parkinson's disease

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of neuronal inclusions known as lewy bodies, which are often positive for both ubiquitin and α -synuclein (for a review, see REF. 112). α -synuclein has been shown to be SUMOylated preferentially by SUMO1 (REF. 113), but neither its native SUMOylation status in neurons nor a function for this post-translational modification have yet been reported.

SUMO1 is a component of lewy bodies in neurons from the brain tissue of patients with dementia with lewy bodies (DLB), as well as a component of glial cytoplasmic inclusions in patients with multiple system atrophy (MSA). chemical inhibition of proteasomal protein degradation in glioma cells results in nuclear and perinuclear accumulation of SUMO1 aggregates¹¹⁴. Parkin, which is linked to autosomal-recessive juvenile Parkinsonism, non-covalently interacts with SUMO1 both *in vitro* and *in vivo*⁴⁰. Furthermore, RanBP2 is ubiquitylated in a parkin-dependent manner to promote its proteasomal degradation¹¹⁵. As SUMO1 interaction with parkin enhances its ubiquitin-ligase activity, this represents an interesting example of negative feedback, mediated by the ubiquitin system, on the SUMO system.

Another intriguing potential role for SUMOylation in PD involves the multifunctional protein DJ-1. DJ-1 participates in the transcriptional regulation of genes concerned with the cellular regulation of oxidative stress. Loss-of-function of DJ-1 results in the onset of PD.

DJ-1 is a SUMO substrate, and mutation to prevent SUMOylation abolishes all of its known functions¹¹⁶. Interestingly, a PD-associated DJ-1 mutation, 1166P, shows higher levels of SUMOylation, resulting in protein insolubility and increased protein degradation¹¹⁶.

Alzheimer's disease

Alzheimer's disease (AD) is a progressive, age-dependent neurodegenerative disorder that is characterized by amyloid- β (A β) plaque formation and the presence of neurofibrillary tangles composed of hyperphosphorylated tau (for a review, see REF. 117). Tau can be both SUMOylated and ubiquitinated. Inhibition of the proteasomal degradation pathway increases the level of tau ubiquitination and decreases its SUMOylation, suggesting that SUMO and ubiquitin might compete to regulate the stability of tau¹¹³. Strong SUMO3 immunoreactivity has been reported in AD neurons; however, the role of SUMO3 remains unclear, as exogenous expression of SUMO3 in a cell-culture model has been reported by different groups to either reduce¹¹⁸ or increase¹¹⁹ the production of neurotoxic A β . It has also been reported that expression of a non-conjugatable mutant of SUMO3 still leads to increased A β generation¹¹⁹, suggesting that the effect of SUMO3 is not a result of covalent protein modification.

Cellular stress, excitotoxicity and ischaemia

Neuronal death can arise from severe cellular stress. SUMO1 and SUMO2/3 differ in their conjugation dynamics in response to cell stress. Under resting conditions, little unconjugated SUMO1 is present, yet there is a large free pool of SUMO2/3 (REF. 13). In response to oxidative stress, osmotic stress or heat shock, there is an increase in SUMO2/3 conjugation, suggesting that under some conditions SUMO2/3 might act as a cellular SUMO reserve. by contrast, physiologically relevant levels of reactive oxygen species (ROS) cause the rapid loss of almost all SUMO conjugates¹²⁰, as a result of the direct and reversible inhibition of UBC9 (REF. 120).

Ischaemia causes extreme metabolic stress due to oxygen and glucose deprivation. One characteristic of brain ischaemia is massive glutamate release that leads to excitotoxic neuronal cell death. levels of SUMOylation are greatly increased in the brains of hibernating squirrels, where blood flow is severely reduced, and it has been proposed that this might provide a mechanism for protecting cells against low levels of oxygen and glucose¹²¹. Transient global cerebral ischemia induces a marked increase in SUMO2/3 conjugation in the hippocampus and the cerebral cortex in mice¹²². changes in protein SUMOylation by SUMO1 and SUMO2/3 also occur in a rat transient middle-cerebral-artery occlusion (MCAO) model of focal ischaemia with reperfusion and a mouse MCAO model without reperfusion¹²³. In rats, MCAO causes dramatically increased SUMO1 and SUMO2/3 conjugation in the striatal infarct area and in the non-ischemic hippocampus. In mice, MCAO results in selective cortical infarct, with increased SUMO1 conjugation in the infarct and the non-ischemic hippocampus. SUMOylation by SUMO2/3 occurs only outside the infarct area. Interestingly, levels of excitatory kainate and AMPA receptors were decreased by MCAO¹²³. It is therefore possible that one role of increased SUMOylation in ischaemia could be to downregulate the surface expression of certain proteins, including receptors, which in turn would reduce excitotoxicity and subsequent cell death.

Concluding remarks

There has been rapid progress towards understanding the mechanisms and functions of SUMOylation, ranging from its involvement in synaptic development and neurophysiology to its implication in neurodegenerative disorders. because SUMOylation regulates a wide range of essential cellular processes, disruption or dysregulation of this pathway will have

major consequences for neuronal function. For example, there are multiple SUMOylated proteins present at synapses⁷⁷, but the identity and functions of most of these proteins are unknown. Identification of these SUMO substrates and elucidation of their functional roles and regulation by SUMOylation will undoubtedly lead to new and important insights into synaptic regulation.

There remain many outstanding questions. For example, what is the relationship between SUMO1 and SUMO2/3? Given the common SUMOylation pathway, how is specificity achieved, and how does the fate of their substrate proteins differ? This is particularly interesting in cases where substrate proteins can be SUMOylated by either SUMO1 or SUMO2/3. The finding that SUMOylation of the transcription factor MEF2A is involved in synapse formation begs the wider question of the roles of nuclear and extranuclear SUMOylation in the developmental and activity-dependent regulation of neuronal architecture — such as, for example, spine dynamics. A related area of intense interest is the role of SUMOylation in the regulation of synaptic transmission and plasticity. The functional effect of kainate receptor SUMOylation that we recently reported⁷⁷ is mediated at the postsynaptic membrane. However, several proteins that have already been verified as SUMO substrates, including kainate receptors and mGluR8, as well as many other proteins that contain SUMOylation consensus motifs, have partly or wholly presynaptic distributions. Thus, we consider it highly likely that SUMOylation has key roles in neurotransmitter release and presynaptic vesicle cycling. It is also intriguing that late-phase long-term potentiation requires protein synthesis, and the regulation of La or other mRNA-transport proteins by SUMOylation might be an important factor in this process. Similarly, the potential for SUMOylation to antagonize ubiquitin-mediated protein degradation through the ubiquitin–proteasome system, lysosomal degradation or autophagy might extend the half-life of synaptic proteins and potentially be implicated in synaptic plasticity. Conversely, the link between SUMOylation and receptor endocytosis suggests that SUMOylation might also be involved in long-term depression. Furthermore, the direct modification of channel function and the regulation of neuronal excitability by SUMOylation, together with the modulation of G protein and phosphorylation-dependent intracellular signalling pathways, also strongly infer that the SUMOylation status of both pre- and postsynaptic proteins has profound effects on synaptic transmission and plasticity. These are exciting possibilities that are likely to be the focus of intense investigation by many groups interested in the mechanisms that underlie synaptic regulation and function in the normal and the diseased brain.

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Glossary

Ubiquitylation	The covalent attachment of the 76-amino-acid protein ubiquitin to lysine residues in target proteins. Ubiquitylation is typically involved in the sorting of proteins for degradation, although there are numerous exceptions.
Paralogue	Either of a pair of genes that derive from the same ancestral gene.
Consensus motif	A sequence that is conserved among proteins that undergo a particular modification within that sequence. Consensus motifs also exist for several non-covalent interactions.

Isopeptidase	An enzyme that specifically recognizes isopeptide bonds and cleaves them. SENPs have SUMO-specific isopeptidase activity, and cleave SUMO from SUMO-conjugated substrates.
Deconjugation	The removal of covalently attached SUMO from the substrate.
G protein	A heterotrimeric GTP-binding protein that interacts with cell-surface receptors, often stimulating or inhibiting the activity of a downstream enzyme. G proteins consist of three subunits: the α subunit, which contains the guanine-nucleotide-binding site, and the β and γ subunits, which function as a heterodimer.
Synaptosomes	Discrete structures formed from the synaptic terminals upon brain homogenization in which the main structural presynaptic features are preserved. These structures retain the ability take up, store and release neurotransmitters.
Nucleocytoplasmic shuttling	Bidirectional protein transport between the cytoplasm and the nuclear matrix through the nuclear pore complex.
Synaptic plasticity	A cellular process that results in lasting changes in the efficacy of neurotransmission.
Yeast two-hybrid screen	A system used to determine whether proteins directly interact. It involves the use of plasmids that encode two hybrid proteins, one of which is fused to the GAL4 DNA-binding domain and the other of which is fused to the GAL4 activation domain. The two proteins are expressed together in yeast and, if they interact, the resulting complex will drive the expression of a reporter gene, commonly β -galactosidase.
Metabotropic glutamate receptors	A family of eight GPCRs that are activated by glutamate. They are classified into three groups (I–III) on the basis of their pharmacological properties and their downstream effector cascades.
Dendritic claw	A postsynaptic differentiation of dendrites that is morphologically characteristic. In the cerebellum, granule cells develop dendritic claws with which they form synapses with mossy-fibre terminals.
α-synuclein	A neuronal protein of unknown function that is detected mainly in presynaptic terminals. It can aggregate to form insoluble fibrils known as Lewy bodies, which are observed in pathological conditions such as Parkinson's disease.
Proteasome	The protein complex that is responsible for degrading intracellular proteins that have been tagged for destruction by the conjugation of ubiquitin.
Oxidative stress	A disturbance in the oxidant–antioxidant balance in favour of the former, leading to potential cellular damage including the mutation of DNA bases, protein oxidation and the generation of lipid peroxidation products.
Amyloid-β (Aβ)	A peptide of size 39–43 amino acids that is the main constituent of amyloid plaques in the brain of Alzheimer's disease patients. These plaques are composed of a tangle of regularly ordered

Reactive oxygen species

fibrillar aggregates called amyloid fibres. Among these heterogeneous peptide molecules, A β 40 and A β 42 are the most common isoforms. A β 42 is the most fibrillogenic peptide and is thus associated with disease states.

Excitotoxicity

(ROS). These include free radicals, peroxides and oxygen ions. ROS form as a natural byproduct of oxidative phosphorylation in the mitochondria. Under pathological conditions, ROS levels can increase significantly, resulting in serious cellular damage. Cells normally defend themselves against ROS damage by the action of enzymes such as catalases and superoxide dismutase.

Long-term potentiation

A pathological process by which neurons are damaged and killed by the overactivation of glutamate receptors.

Long-term depression

(LTP). A form of synaptic plasticity that results in a longlasting increase in the strength of synaptic transmission.

(LTD). A form of synaptic plasticity that results in a longlasting decrease in the strength of synaptic transmission.

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SUMO-interacting motifs (SIMs)

Ubiquitin-dependent protein sorting relies on proteins that recognize ubiquitin, and the small ubiquitin-like modifier (SUMO) system is likely to act in a similar way. Currently, at least 16 ubiquitin-binding motifs¹²⁴ and 4 SUMO-interacting motifs (SIMs; see table) have been reported. SIMs contain a hydrophobic core flanked by acidic residues and/or phosphorylatable serines. In mammals, the motifs bind SUMO1 and SUMO2/3 but the affinity varies depending on the context of the sequence, and deletion of the acidic flanking regions favours binding to SUMO2/3 over SUMO1 (REF. 125). In addition, SIMs seem to be able to bind SUMO in both forward and reverse orientations, between the $\alpha 1$ helix and the $\beta 2$ strand¹²⁶. In contrast to most non-covalent interactions with ubiquitin, SIM binding is reasonably high affinity (2–10 μM ^{125,126}, compared with over 100 μM ¹²⁴). Numerous neuron-specific and synaptic proteins contain SIMs. Further investigation will shed light on how these proteins help to mediate the effects of SUMOylation by being recruited to modified proteins. A detailed dissection of SIMs is beyond the scope of this review, but see REF. 37 for further information.

Motif	Method of detection	Found in	Refs
[V/I]-[V/I]-S-X-[S/T]-[D/E]-[D/E]-[D/E]	Yeast two-hybrid	Mammals	127
[V/I]-X-[V/I]-[V/I]-[V/I]	NMR	Mammals	128
K-X ₍₃₋₅₎ -[I/L]-[I/L]-[I/L]-X ₃ -[D/E/Q/N]-[D/E]-[D/E]	Yeast two-hybrid	<i>Saccharomyces cerevisiae</i> (Smt3)	129
[V/I]-X-[V/I]-[V/I]-[V/I] flanked by acidic residues and/or phosphorylatable serines	Yeast two-hybrid	Mammals	125

Standard single-letter amino acid-abbreviations are used throughout; X = any residue.

Figure 1. Comparison of the three-dimensional structures of ubiquitin, SUMO1 and SUMO2/3 Ribbon diagrams representing NMR structures of human ubiquitin (UniProt identifier: 1D3Z), SUMO1 (UniProt identifier: 1A5R) and SUMO2 (UniProt identifier: 2AWT). Note that the structure of SUMO3 is identical to SUMO2, as they only differ by three residues in their active primary sequence. Despite their low sequence homology, ubiquitin and the SUMO paralogues share a highly conserved three-dimensional structure.

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Ubiquitin  -----MOTFVKLTGKTTITLEVPSDTIENVRAKIQDKEGPPDQQLIFAGKQLEDGRLLSDTNVQRESLHLVLRGG 76
SUMO1     NSDQEARPSTEDLGKKRG-EYIKLVIGQSSSTHFVKVMTTHLKKLESYCOROGVPPNNSLRELEGORTADNHFKELGHEEDVLEVYQDTCG 97
SUMO2     NSEKPKK-----GVKTEN-DHINLVAGDGEVVOFKIKRHPTLSKIMKAYCEROGLSHRQIKEREDGQINETDTPAOLEMEDEDITDVFOODTCG 92
SUMO3     NADKPKK-----GVKTENNNDHILNVAGDGEVVOFKIKRHPTLSKIMKAYCEROGLSHRQIKEREDGQINETDTPAOLEMEDEDITDVFOODTCG 93
  
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Figure 2. Comparison of the primary sequences of ubiquitin, SUMO1 and SUMO2/3

Sequence alignment of the active forms of human ubiquitin and the SUMO paralogues. Residues that are identical in all four proteins are shown in red and include the active carboxy-terminal diglycine conjugation motif. Residues that show only conservative changes between sequences are shown in green, and residues that are identical between the three SUMO paralogues are shown in blue. The alignment and determination of conservation was performed by the ClustalW program.

Figure 3. The SUMOylation pathway

All SUMO paralogues are synthesized as precursors and matured by the hydrolase activity of specific SUMO proteases (SENPs). The SUMO is then activated in an ATP-dependent process that results in the formation of a thioester bond (shown as a red ‘string’) with the activating enzyme subunit, SUMO activating enzyme 2 (SAE2). Activated SUMO is then passed to the active-site cysteine of ubiquitin conjugating enzyme 9 (UBC9). UBC9 can directly recognize substrate proteins and catalyse the transfer of SUMO to them (represented by the dashed line), or conjugation can occur in conjunction with an E3 enzyme. Both of these possibilities ultimately result in the formation of an isopeptide bond (shown as a blue ‘string’) between SUMO and the target lysine residue in the substrate, altering the function of the substrate. The target lysine residue often lies in a Ψ-K-X-[D/E] consensus motif, in which Ψ denotes any large hydrophobic residue (I, V or L) and X denotes any residue. SUMO can subsequently be removed from the substrate by the isopeptidase activity of the SENP family.

Figure 4. extranuclear functions of SUMOylation in neurons

Extranuclear functions of SUMOylation, along with example substrates. Unconfirmed or speculative functions are indicated with a question mark. DRP1, dynamin-related protein 1; FAK, focal adhesion kinase; GluR6a, glutamate receptor 6a; GLUT, glucose transporter; GPCR, G-protein-coupled receptor; PTP1B, protein tyrosine phosphatase 1B; RGSZ, regulator of G-protein signalling (RGS) proteins.

Table 1
Sites of SUMO conjugation and modulation

Motif	Consensus sequence	Example substrates	Notes
Core	Ψ -K-X-[D/E]	RanGAP, p53, GluR6a	Forms a core site that is directly bound by UBC9, allowing modification of the lysine residue
NDSM	Ψ -K-X-E + downstream cluster of [D/E].	ELK1 (REF. 130)	Based on the core motif, but the downstream acidic patch can bind a corresponding basic patch on UBC9 and enhance substrate binding
PDSM	Ψ -K-X-E-X-X-S-P	MEF2A, GATA1, HSF1 (REF. 131)	Phosphorylation of the serine residue creates a local negative charge, similar to the NDSM. For some targets, phosphorylation can therefore act as a SUMOylation 'on' switch
Synergy control	[P/G]-X ₍₀₋₄₎ - Ψ -K-X-[D/E]-X ₍₀₋₄₎ -[P/G]	Androgen receptor ¹³² , K _v 1.5 (REF. 86)	It is currently unknown how this motif enhances substrate SUMOylation
SUMO-acetyl switch	Ψ -K-X-E-P	HIC1 (REF. 133)	The local proline appears capable of directing acetylation to the same lysine that SUMOylation targets. Interestingly, in some cases the acetylation-to-SUMOylation switch can be controlled by phosphorylation of a nearby serine
Others	H-E-L-K-K-F-R (no acidic residue)	K2P1 (REF. 83)	Some SUMO substrates are modified at sites that are not based around the core SUMOylation motif; it is not known how they are recognised by UBC9. Potentially, these substrates might require greater input from E3 enzymes for their SUMOylation
	I-I-G-K-V-E-K-V-D (no large hydrophobic residue)	Axin ⁶⁴	
	M-I-T-K-E-T-I (no acidic residue, no large hydrophobic residue)	PCNA ¹³⁴	

Ψ denotes any large hydrophobic residue (I, V or L); X denotes any residue; all other letters represent standard single-letter amino-acid abbreviations. GATA1, GATA binding protein 1; GluR6a, glutamate receptor 6a; HIC1, hypermethylated in cancer 1; HSF1, heat shock transcription factor 1; MEF2A, myocyte enhancer factor 2A; NDSM, negative-charge dependent SUMOylation motif; PCNA, proliferating cell nuclear antigen; PDSM, phosphorylation-dependent SUMOylation motif, RanGAP, Ran GTPase activating protein; SUMO, small ubiquitin-like modifier.

Table 2
SUMOs and known enzymes of the SUMO pathway in yeast and mammals

Protein type	Identity in yeast (<i>Saccharomyces cerevisiae</i>)	Identity in mammals
SUMO	Smt3 (REF. 135)	SUMO1 (REFS 1,2)
		SUMO2 (REF. 6)
		SUMO3 (REF. 6)
SUMO E1 (activating enzyme)	Aos1 (REF. 135)	SAE1 (REF. 136)
	Uba2 (REF. 135)	SAE2 (REF. 136)
SUMO E2 (conjugating enzyme)	Ubc9 (REF. 4)	UBC9 (REF. 137)
SUMO E3 (ligase enzyme)	Siz1, Siz2 (REF. 22)	PIAS1 (REF. 25), PIAS3 (REF. 138), PIASx α ¹³⁹ , PIASx α ²⁶ , PIASy ¹⁴⁰
		RanBP2 (REF. 27)
		PC2 (REF. 28)
	Mms21 (REF. 141)	Mms21 (REF. 141)
		TOPORS ¹⁴²
		TRAF7 (REF. 143)
SUMO proteases (SUMO proteases and SUMO isopeptidases)	Ulp1 (REF. 144), Ulp2 (REF. 145)	SEN1 (REF. 146), SENP2 (REF. 147), SENP3 (REF. 148), SENP5 (REF. 147), SENP6 (REF. 149), SENP7 (REF. 147)

PIAS, protein inhibitor of activated STAT; RanBP2, Ran binding protein 2; SAE, SUMO activating enzyme; SENP, SUMO specific protease; SUMO, small ubiquitin-like modifier; TOPORS, topoisomerase I binding, arginine/serine-rich; TRAF7, TNF receptor-associated factor 7; Uba2, ubiquitin activating enzyme 2; Ubc9, ubiquitin conjugating enzyme 9; Ulp, ubiquitin-like protein.

Table 3
Regulation of the SUMOylation pathway by other post-translational modifications

Modification	Effect on SUMOylation	Example substrates	Notes
Phosphorylation	Positive or negative	MEF2A ¹³¹ (positive), c-Fos ¹⁵⁰ (negative), IκBα ¹⁵¹ (negative)	Phosphorylation of the SUMO substrate can enhance its SUMOylation by introducing a local negative charge which induces Ubc 9 binding to the substrate ¹³¹ . However, phosphorylation close to the target lysine has also been reported to inhibit the SUMOylation of a number of proteins, including c-Fos ¹⁵⁰ and IκBα ^{151,152}
Acetylation	Negative	MEF2A ⁹⁹ , SP3 (REF. 153)	SUMOylation and acetylation can target the same lysine residue, effectively antagonizing each other.
Ubiquitylation	Positive or negative	Huntingtin ¹⁰⁷ (negative), NEMO ¹⁵² , p53 (REF. 154) (positive)	As with acetylation, both modifications can target the same lysine residue, antagonizing each other (as is the case for Huntingtin ¹⁰⁷). However, the two modifications act sequentially in the activation of NEMO ¹⁵² , and ubiquitylation of p53 appears to enhance its SUMOylation at a different site, by recruiting SUMO E3 enzymes ¹⁵⁴ .
Oxidation	Positive or negative	SAE2, UBC9 (REFS 30,128)	Oxidation of the SUMOylation enzymes themselves can prevent further SUMOylation, shifting the equilibrium to favour local de-SUMOylation ^{30,128} . However, oxidative stress has also been reported to massively increase protein SUMOylation ¹³ .

IαBa, inhibitor of κBa: MEF2A, myocyte enhancer factor 2A; SAE2, SUMO activating enzyme subunit 2; SUMO, small ubiquitin-like modifier; UBC9, ubiquitin conjugating enzyme 9.

Table 4
SUMOylation and neurodegenerative disorders

Disease	Identified SUMO substrate protein	SUMO immunoreactivity of neuronal inclusion bodies	Effect of SUMO expression on neurodegeneration and/or cell death	Refs
Huntington's disease	Huntingtin	SUMO1 +++	Increased	107
SBMA	Androgen receptor	SUMO1 +++	Reduced	108
DRPLA	Atrophin 1	SUMO1 +++	Increased	110
SCA type 1	Ataxin 1	SUMO1 +++	Nd	109
NIID	Nd	SUMO1 +++	Increased	103
Alzheimer's disease	Amyloid- β (A β)	SUMO3 +	Reduced	118
	Tau	SUMO3 +	Increased	119
Parkinson's disease	α -synuclein	SUMO1 +	Nd	112
	Parkin (non-covalent)	SUMO1 +	Nd	40,115
	DJ-1	SUMO1 +	Nd	116
Ischaemia	Multiple substrates	SUMO1, SUMO2/3	Nd	122,123

DRPLA, dentatorubro-pallidoluysian atrophy; Nd, not determined; NIID, neuronal intranuclear inclusion disease; SBMA, spinobulbar muscular atrophy; SCA, spinocerebellar ataxia; SUMO, small ubiquitin-like modifier.