Abstract
SUMOylation, a reversible process used as a ‘fine-tuning’ mechanism to regulate the role of multiple proteins, is conserved throughout evolution. This post-translational modification affects several cellular processes by the modulation of subcellular localization, activity or stability of a variety of substrates. A growing number of proteins have been identified as targets for SUMOylation, although, for many of them, the role of SUMO conjugation on their function is unknown. The use of model systems might facilitate the study of SUMOylation implications in vivo. In the present paper, we have compiled what is known about SUMOylation in Drosophila melanogaster, where the use of genetics provides new insights on SUMOylation’s biological roles.

Introduction
SUMOylation is an essential cellular process conserved in all eukaryotic organisms analysed to date. In Drosophila, all of the components of the SUMOylation pathway have been identified, and their function has been studied (Table 1). The in vivo analysis of SUMOylation in the fruitfly presents a number of advantages. On one hand, gene redundancy is lower in Drosophila when compared with vertebrate models, which simplifies functional analysis. On the other hand, and related to the genetic accessibility of this organism, the various components of the pathway have been implicated in multiple cellular and physiological processes by means of genome-wide genetic screens performed in vivo. The information derived from these screenings will be, in many instances, translatable to vertebrate models. In the present review, we will show the components of the SUMOylation pathway identified in Drosophila and summarize the cellular and developmental roles in which these components have been involved.

The SUMOylation machinery in Drosophila
Unique orthologues for SUMO (small ubiquitin-related modifier) and for the isopeptidase, activating and conjugating enzymes have been identified in the fruitfly (Table 1). Drosophila Smt3 is closely related to the vertebrate homologue SUMO-3 and is expressed throughout development, being more prominent during embryogenesis and in adult females [1–4]. High levels of smt3 transcript and protein are maternally inherited and accumulate in the preblastoderm embryos, appearing uniformly distributed throughout the embryo at cellular blastoderm and gastrulation stages. The protein accumulates in the cytoplasm initially and rapidly goes to the nuclei where it localizes to dots. Later, the transcript accumulates preferentially in the CNS (central nervous system) and in the gonads [3,5]. In interphase nuclei, Smt3 can accumulate on the chromosomes [6] and, during mitosis, it is redistributed in the cytoplasm and localizes to the midbody during cytokinesis [3,7].

Other orthologous of the vertebrate SUMOylation machinery, such as Ulp1, Aos1, Uba2 and Lesswright (Lwr), are also enriched during embryogenesis, preferentially in the CNS and gonads [3,5,7–12]. They are also preferentially expressed in females [13] and in undifferentiated tissues [14]. Ulp1 localizes to the nuleoplasmic face of the nuclear pore complex in S2 cells, which is related to its role in nuclear transport [15].

The identification of E3 ligases is based on sequence homology with their vertebrate homologues. Tonalli (Tna) is the orthologue of Zimp7 and Zimp10, two novel human PIAS (protein inhibitor of activated STAT [signal transducer and activator of transcription])-like proteins that contain a SP-RING (Siz-PIAS really interesting new gene) zinc-finger domain characteristic of this family of proteins [16]. Genetic analysis shows that lack of Tna is lethal at post-embryonic stages [17], whereas expression analysis shows that it is down-regulated in the reproductive tissues of females after mating [18], it is induced at late stages of embryonic cellularization [19] and it is differentially expressed in embryonic head [20]. Functional analysis in Drosophila revealed that Tna interacts genetically with the SWI/SNF chromatin-remodelling complex [21] and is involved in the regulation of homeotic gene expression during development. The lack of Tna promotes a partial transformation of halteres to wings and other homeoetric transformations [21].

Another putative E3 ligase described in Drosophila is Su(var)2–10 (suppressor of variegation 2–10), also known as dPIas, which is required to complete embryogenesis [22]. Position–effect variegation, a phenomenon well studied in, but not exclusive to, Drosophila causes the inactivation of genes by juxtaposition to heterochromatin regions, indicating a role for Su(var)2–10 in normal heterochromatic functions [23]. Su(var)2–10 is involved in the maintenance
of the proper chromosomal structure and chromosomal inheritance [24,25]. Some combinations of su(var)2–10 mutations die as late larvae or early pupa and show melanotic tumours. Similarly to its vertebrate homologues, Su(var)2–10 is a negative regulator of the JAK (Janus kinase)/STAT pathway [26] and participates in the biological processes where this pathway is active, such as in the antiviral response [27], border cell migration during oogenesis [28] or blood cell and eye development, probably through the negative regulation of the transcription factor Stat92E [29].

**Cellular roles for SUMOylation**

During the last 10 years, a large number of proteins have been identified as SUMO substrates in vertebrates, as well as in *Drosophila* (Table 2). However, it is difficult to predict the impact that SUMOylation has on their biological roles. In fact, for some of the Smt3 target proteins, such as CaMKII (Ca²⁺/calmodulin-dependent protein kinase II), glutamyl-prolyl-tRNA synthetase, methionyl-tRNA synthetase, heat-shock proteins, Septin, Seven in absentia, Stumps or Tramtrack (Ttk), the role of SUMOylation remains unknown (Table 2). However, as discussed below, Smt3 conjugation to other factors links SUMOylation with various cellular processes such as cell survival and proliferation, nuclear import, intracellular trafficking, transcriptional regulation and maintenance of genomic and nuclear integrity.

Two lines of evidences associate SUMOylation with cell survival and proliferation. First, the SUMOylation components are expressed in proliferative tissues, such as the undifferentiated cells of imaginal discs or the gonads [5,12–14]. Secondly, mutations in *smt3* or *lwr* compromise cell proliferation and cell viability in imaginal discs and CNS [30,31].

The role of SUMOylation in cellular trafficking has been reported in various examples. Mutations in *lwr* impede the entrance of the embryonic morphogen Bicoid into the nucleus, and down-regulation of other components of the pathway causes accumulation of SUMOylated proteins in the cytoplasm [15,32]. Nuclear transport of the Rel transcription factor Dorsal (Dl) seems to be also influenced by SUMOylation, although further research is necessary to clarify the consequences of this modification [33,34]. *smt3* and *lwr* are also related to intracellular trafficking and autophagy, interacting genetically with blue cheese, an autophagic-linked gene in which mutations lead to reduced lifespan, neuronal death and CNS degeneration [35].

SUMOylation has been associated with the enhancement of transcriptional activation or transcriptional repression. SUMOylation of Dl and the cofactor Vestigial (Vg) activate transcription, whereas the modification of SoxNeuro (SoxN) and Stat92E involves transcriptional activity repression (Table 2). In the case of SoxN, SUMOylation does not influence the subcellular localization of SoxN, although in other cases, it might affect the subcellular or nuclear distribution of the target transcription factor. A relationship between SUMO-dependent transcriptional regulation and subnuclear localization has been suggested, but the link between these two processes remains obscure.

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**Table 1 | SUMOylation pathway components in *Drosophila***

<table>
<thead>
<tr>
<th>Gene</th>
<th>CG accession number</th>
<th>Homologues</th>
<th>Function</th>
<th>Biological processes</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>smt3</em></td>
<td>CG4494</td>
<td>SUMO-3</td>
<td>SUMO moiety</td>
<td>Cell proliferation or survival; chromatin modification; embryogenesis; EGFR signalling; immune response; lysosomal transport; oogenesis; wing morphogenesis</td>
<td>[1,2,6,31,33,35,53,54,56,57]</td>
</tr>
<tr>
<td><em>Ulp1</em></td>
<td>CG12359</td>
<td>Sentrin/SUMO-specific protease 1</td>
<td>Isopeptidase</td>
<td>Smt3-conjugates nuclear-cytoplasmic shuttling</td>
<td>[15,33]</td>
</tr>
<tr>
<td><em>Aos1</em></td>
<td>CG12276</td>
<td>Activating enzyme subunit 1</td>
<td>E1A-activating</td>
<td>–</td>
<td>[2,7,52]</td>
</tr>
<tr>
<td><em>Ubo2</em></td>
<td>CG7528</td>
<td>Activating enzyme subunit 2</td>
<td>E1B-activating</td>
<td>–</td>
<td>[2,7,52]</td>
</tr>
<tr>
<td><em>lesswright</em></td>
<td>CG3018</td>
<td>UBC9; ubiquitin-conjugating enzyme E2</td>
<td>E2-conjugating</td>
<td>Cell proliferation; chromatin modification; embryogenesis; immune response; wing morphogenesis</td>
<td>[6,11,30–34,36,37,52,55]</td>
</tr>
<tr>
<td><em>tonalli</em></td>
<td>CG7958</td>
<td>Zimp7 and Zimp10; retinoic acid-induced 17</td>
<td>E3 ligase</td>
<td>Chromatin modification</td>
<td>[16,21]</td>
</tr>
<tr>
<td>Suppressor of vanegation 2–10</td>
<td>CG8068</td>
<td>PIAS3</td>
<td>E3 ligase</td>
<td>Chromatin modification and chromosomal inheritance; negative regulation of JAK/STAT signalling; wing morphogenesis</td>
<td>[22–25,27–29,31,58]</td>
</tr>
</tbody>
</table>
### Table 2 | SUMOylation substrates in *Drosophila*

<table>
<thead>
<tr>
<th>SUMOylation substrates</th>
<th>CG accession number</th>
<th>Type of protein</th>
<th>Homologues</th>
<th>Interaction assay</th>
<th>Role of SUMOylation</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺/calmodulin-dependent protein kinase II</td>
<td>CG18069</td>
<td>Serine/threonine kinase</td>
<td>CAMK2D</td>
<td><em>in vivo</em></td>
<td>Unknown</td>
<td>[2]</td>
</tr>
<tr>
<td>Centrosomal protein 190 kDa</td>
<td>CG6634</td>
<td>Microtubule binding</td>
<td>ZBTB</td>
<td><em>in vivo</em></td>
<td>Disrupts nuclear clustering</td>
<td>[6]</td>
</tr>
<tr>
<td>Dorsal</td>
<td>CG6667</td>
<td>Transcription factor</td>
<td>NFKB1</td>
<td>S2 cells/<em>in vitro/in vivo</em></td>
<td>Enhance the transcriptional activity, nuclear localization</td>
<td>[33,52,55]</td>
</tr>
<tr>
<td>Glutamyl-prolyl-tRNA synthetase</td>
<td>CG5394</td>
<td>Aminoacyl-tRNA synthetase</td>
<td>EPRS</td>
<td>S2 cells</td>
<td>Unknown</td>
<td>[15]</td>
</tr>
<tr>
<td>Groucho</td>
<td>CG8384</td>
<td>Transcription cofactor</td>
<td>TLE4</td>
<td>Yeast two-hybrid</td>
<td>Unknown</td>
<td>[4]</td>
</tr>
<tr>
<td>Methionyl-tRNA synthetase</td>
<td>CG3122</td>
<td>Aminoacyl-tRNA synthetase</td>
<td>MARS2</td>
<td>S2 cells</td>
<td>Unknown</td>
<td>[15]</td>
</tr>
<tr>
<td>Modifier of mdg4</td>
<td>CG32491</td>
<td>Chromatin binding</td>
<td>ZBTB</td>
<td><em>in vivo</em></td>
<td>Disrupts nuclear clustering</td>
<td>[6]</td>
</tr>
<tr>
<td>Septin-1</td>
<td>CG1403</td>
<td>GTPase</td>
<td>SEPT2</td>
<td>Yeast two-hybrid</td>
<td>Unknown</td>
<td>[7]</td>
</tr>
<tr>
<td>Seven in absentia</td>
<td>CG6949</td>
<td>E3 ubiquitin ligase</td>
<td>SIAH1</td>
<td>Yeast two-hybrid</td>
<td>Unknown</td>
<td>[59]</td>
</tr>
<tr>
<td>SoxNeuro</td>
<td>CG18024</td>
<td>Transcription factor</td>
<td>SOX1</td>
<td>S2 cells</td>
<td>Transcriptional activity repression</td>
<td>[42]</td>
</tr>
<tr>
<td>Stat92E</td>
<td>CG4257</td>
<td>Transcription factor</td>
<td>STAT5B</td>
<td><em>in vitro</em></td>
<td>Transcriptional activity repression</td>
<td>[29]</td>
</tr>
<tr>
<td>Stumps</td>
<td>CG31317</td>
<td>Adaptor protein</td>
<td>PIK3AP1</td>
<td>Yeast two-hybrid</td>
<td>Unknown</td>
<td>[60]</td>
</tr>
<tr>
<td>Tramtrack</td>
<td>CG1856</td>
<td>Transcription factor</td>
<td>ZBTB</td>
<td>S2 cells</td>
<td>Unknown</td>
<td>[3]</td>
</tr>
<tr>
<td>Vestigial</td>
<td>CG3380</td>
<td>Transcription co-factor</td>
<td>VGLL2</td>
<td>S2 cells</td>
<td>Enhances transcriptional activity</td>
<td>[31]</td>
</tr>
</tbody>
</table>

A role for SUMOylation in chromatin regulation is reflected in the suppression of cytological defects shown in female meiotic mutations by *lwr* mutations. *Lwr* mediates the dissociation of heterochromatic regions at the end of the meiotic prophase I [36]. In addition, SUMOylation negatively regulates the activity of the gypsy chromatin insulator by inhibiting the long-range interaction of insulator-binding proteins [6,37]. Su(var)2–10 is also involved in chromosomal stabilization and maintenance [25]. This protein associates with telomeres and is closely associated with the nuclear periphery during interphase [25]. In its absence, telomere clustering is aberrant, as well as the association of telomeres with the nuclear lamina.

If SUMOylation is involved in various cellular processes, it is not surprising that it has been involved in various developmental contexts, exemplified in the next section.

### Developmental roles for SUMOylation in *Drosophila*

In *Drosophila*, the components of the pathway are expressed throughout development [2–4]. A number of processes seem to be influenced by SUMOylation, such as embryogenesis, wing morphogenesis and CNS development, as well as neurodegeneration and immune response.

### Embryogenesis

Smt3 and Lwr are expressed at high levels during embryogenesis, and their absence produces embryonic lethality [3,7,11]. The analysis of mutations in *lwr* allowed elucidation of the biological role of the SUMOylation pathway in embryonic patterning. The mutation *semushi*, caused by an insertion in the 3′-UTR (untranslated region) regulatory region of *lwr*, results in late embryonic or first instar larvae lethality with defects in embryonic patterning. The anterior segmentation abnormalities in these homozygous mutant embryos are caused by defects in the nuclear import of Bicoid [32]. In addition, the defects in meiotic chromosome segregation founded in *lwr* mutations could also explain the relevance of this pathway during embryogenesis [36].

### Wing morphogenesis

Vg, a selector gene necessary for wing morphogenesis, can be modified by Smt3 in S2 cells and interacts genetically with *smt3*, *lwr* and *su(var)2–10* [31]. Expressed in the wing blade of the wing imaginal disc, Vg plays an essential role in the regulation of wing cell proliferation and differentiation [38]. Together with Scalloped, Vg forms a functional transcription factor that is required for wing development [39]. Interestingly, SUMOylation is required for the
transcriptional activation of Vg during wing morphogenesis, although there are no data on how SUMOylation affects the Vg–Scalloped interaction [31].

**Nervous system development and neurodegeneration**

Some of the transcription factors modified by Smt3 are involved in the development of the CNS (Table 2). This is the case for SoxN, an SRY (sex-determining region Y) high-mobility-group box transcription factor, expressed from the earlier stages of neurogenesis and involved in neuroblast formation [40,41]. Overexpression of a SoxN-SUMO-deficient mutated form produced several defects including fusion or absence of neural commissures and reduction or absence of longitudinal axon tracts that lead to disruption of CNS development and embryonic lethality. Therefore the SUMO-mediated transcriptional repression of SoxN seems to be important for proper CNS development [42].

The zinc-finger protein Ttk, which is another in vivo substrate of Smt3 [3], represses neural stem cell–specific genes and maintain glial differentiation in embryonic CNS [43], acting as a repressor of neural fate determination in the peripheral nervous system [44]. As Smt3 is expressed at high levels in sensory organ cells such as sensory bristles, it could play a role in the fate determination in the peripheral nervous system. However, the biological role of Smt3 conjugation of Ttk in neuronal differentiation repression is unclear [3].

The components of SUMOylation machinery are highly expressed in neurons. The reported Ttk conjugation in vivo of one isoform of the neuronal CaMKII, with important roles in synaptic plasticity, learning and memory, implicates SUMOylation in the regulation of differentiated neurons [2]. In fact, the overexpression of wild-type Uba2 or a putative Uba2 dominant-negative mutant in the nervous system leads to pupal lethality. This suggests the requirement of a normal SUMOylation pathway for Drosophila CNS differentiation [2].

SUMO has been implicated in several neurodegenerative diseases (reviewed in [45]), based on co-localizing SUMO with neuronal inclusions associated with some of these diseases. In addition, several proteins implicated in these disorders such as huntingtin (HTT), Ataxin-1, tau and α-synuclein are modified by SUMO. Drosophila, as a model for human diseases, has been used to investigate the role of SUMOylation in neurodegenerative pathologies [46,47]. The best examples are HD (Huntington’s disease) and SBMA (spinal and bulbar muscular atrophy), both included in the group of human polyglutamine neurodegenerative diseases associated with the expansion of CAG triplet repeats. Studies in Drosophila showed the SUMOylation of the N-terminal fragment of human HTT, the pathogenic protein accumulated in HD [48]. Although SUMOylation of HTT reduces aggregate formation, it enhanced the ability of HTT to induce neurodegeneration, probably by increasing the levels of toxic soluble oligomers. The mechanism of SUMO action in this neurodegenerative disorder is not completely clear. HTT can also be ubiquitinated, thus SUMOylation could antagonize the ubiquitin–proteasome degradation pathway or both post-translational modification systems could co-ordinate co-operate and contribute to HD pathogenesis. In addition, SUMO modification increased the transcriptional repression mediated by HTT [48].

SBMA, caused by the expansion of a polyglutamine repeat within the androgen receptor protein [49], is another neurodegenerative disease modelled in Drosophila [50,51]. There, the overexpression of a mutated form of Uba2 enhanced neurodegeneration, showing again the important role of SUMOylation in the modulation of polyglutamine pathogenesis [50].

**Immune response**

Several studies have also implicated SUMOylation in the immune response. As mentioned above, the transcription factor Dl, with a role in innate immunity, is an Smt3 target protein [33,52]. In addition, the Smt3 conjugation machinery seems to be required for lipopolysaccharide-induced expression of the antimicrobial peptides cecropinA1 and drosomycin and for phagocytosis and intracellular growth of pathogens [33,53,54]. Mutations in lwr lead to overproliferation of haemocytes and differentiation defects. Lwr seems to play an important role in the regulation of larval haemopoiesis by the negative regulation of the Rel-related proteins Dl and Dorsal-related immunity factor [34,55,56]. However, further studies are required, as divergent results have been obtained related to the Lwr-mediated nuclear localization of Dl and Dorsal-related immunity factor [33,55].

**Concluding remarks**

A growing body of evidence relates SUMOylation to crucial cellular and developmental processes. In development, where transcription, translation and cellular localization are critically regulated, the versatility and reversibility of SUMOylation makes it a plausible candidate to participate in ‘fine-tuning’ regulation of signalling pathways and downstream effectors. We predict that the genetic advantages of Drosophila will allow the study of SUMOylation to progress rapidly in the coming years, and that the fruitfly model will provide crucial correlations between biochemical and cellular observations and complex organismal phenotypes.

**Note added in proof (received 4 August 2008)**

While this article was in the press, our paper describing the role of Smt3 during metamorphosis was published, representing a new function for SUMOylation during development [61].

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References


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