SUMO and ubiquitin modifications during steroid hormone synthesis and function

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Abstract

Steroid hormones control many aspects of animal physiology and behaviour. They are highly regulated, among other mechanisms, by post-translational modifications of the transcription factors involved in their synthesis and response. In the present review, we will focus on the influence of SUMO (small ubiquitin-related modifier) and ubiquitin modifications on the function of transcription factors involved in adrenal cortex formation, steroidogenesis and the hormonal response.

Introduction

Steroid hormones (glucocorticoids, mineralocorticoids, androgens and oestrogens) regulate many aspects of mammalian physiology and metabolism and are responsible for sexual differentiation and manifestation of secondary sex characteristics. The main steroidogenic tissues in mammals are the adrenal cortex and the gonads. All steroid hormones are derived from cholesterol through a cascade of steroidogenic enzymes, the cytochrome P450 haem-containing proteins and the hydroxysteroid dehydrogenases [1]. Cytochrome P450 enzymes are either associated with the mitochondrial membranes (CYP11A, CYP11B1 and CYP11B2) or with the endoplasmic reticulum (CYP17, CYP21 and CYP19) and catalyse the hydroxylation and cleavage of the steroidogenic substrates in the adrenal cortex (Figure 1). The rate-limiting step in steroid biosynthesis is the delivery of cholesterol from the outer to the inner mitochondrial membrane and relies on the StAR (steroidogenic acute regulatory protein). Mutations in the above-mentioned proteins cause congenital adrenal hyperplasia, one of the most common inherited metabolic disorders, which includes adrenal insufficiency, genital ambiguity and effects on sexual characteristics.

Key words: adrenal cortex, small ubiquitin-related modifier (SUMO), steroidogenesis, steroid receptor, SUMOylation, ubiquitylation.

Abbreviations used: ACD, adrenocortical dysplasia; ATF-1, activating transcription factor 1; AP-1, activator protein 1; AR, androgen receptor; CoREST, co-repressor element 1-silencing transcription factor; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CREB, cAMP-response-element-binding protein; CYP, cytochrome P450; DAX-1, dosage-sensitive sex reversal-adrenal hypoplasia congenital-critical region on the X chromosome; EGR-1, early growth-response gene product 1; ER, oestrogen receptor; FOXD2, forkhead box D2; GATA, GATA-binding protein; GLI3, GLI Krüppel family member 3; HDAC, histone deacetylase; HH, Hedgehog; HSD3β, 3β-hydroxysteroid dehydrogenase/isomerase; NR4A, nuclear receptor 4A subfamily; NUR77, nuclear receptor 77; NURR1, nuclear receptor related 1; PIAS, protein inhibitor of activated STAT (signal transducer and activator of transcription); PR, progesterone receptor; RNF31, RING finger protein 31; SALL1, Spalt-like 1; SC, synergy control; SF-1, steroidogenic factor 1; Sp, specificity protein; SREBP, sterol-regulatory-element-binding protein; StAR, steroidogenic acute regulatory protein; SUMO, small ubiquitin-related modifier; PBX1, pre-B-cell-leukaemia transcription factor 1; UBC9, ubiquitin-conjugating enzyme 9; UPS, ubiquitin–proteasome system. WT1, Wilms' tumour 1.

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A number of transcription factors important for steroidogenesis have been identified, some of them being involved in adrenal gland and/or reproductive development and others in regulating the steroidogenic pathway. In addition, the receptors for the steroid hormones are well characterized. These factors can undergo a number of post-translational modifications, such as phosphorylation, acetylation, SUMOylation or ubiquitylation, the last two being our focus here.

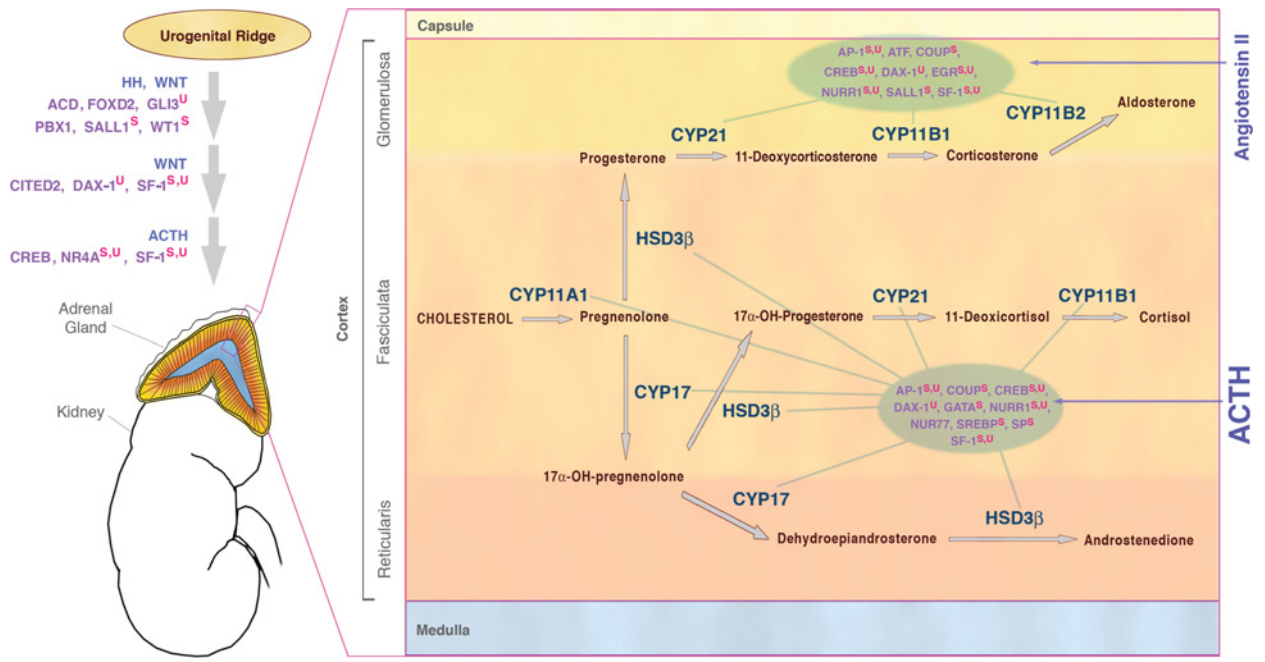
Ubiquitin and SUMO (small ubiquitin-related modifier) belong to the ubiquitin family of protein modifiers, which function through their covalent conjugation to lysine residues in target proteins [2]. Ubiquitylation and SUMOylation can modulate various properties of the substrate, such as stability, localization and protein–protein interactions. The most well-known effect of ubiquitylation is, however, to mediate degradation through the UPS (ubiquitin–proteasome system). The effect of the modification frequently relies on recognition of the conjugate by a specific receptor, containing domains or motifs binding the modified protein. As opposed to ubiquitin, where a plethora of ubiquitin-binding domains exists, only one SUMO-interacting motif has been identified to date. Interestingly, recent advances suggest that both SUMO and ubiquitin might have critical roles in the pathogenesis of adrenal insufficiencies. In the following sections, we summarize the existing data on SUMOylation and ubiquitylation of factors involved in adrenal cortex development and steroidogenesis, as well as of steroid receptors.

Adrenal cortex development

In the adult, the outer cortex of the adrenal glands, located above the kidneys, consists of three histologically differentiated zones, namely the zona glomerulosa, the zona fasciculata and the zona reticularis, and these synthesize and secrete mineralocorticoids, glucocorticoids and androgens respectively. The adrenal cortex, together with the gonads and kidneys, arise from the adrenogonadal primordium derived

Figure 1 | SUMOylation and ubiquitylation of transcription factors involved in adrenal gland development and steroidogenesis

Left: schematic representation of the transcription factors involved in the formation of the adrenal gland and kidney from the urogenital ridge (see text for details). Right: schematic representation of the process of steroidogenesis. Steroid hormones aldosterone, cortisol and androstenedione are synthesized in the cortex of the adrenal gland by the enzymes CYP11A1 (P450scc), CYP11B1 (P450c11b1), CYP11B2 (P450c11b2), CYP17 (P450c17), CYP21 (P450c21) and HSD3β. The synthesis of these enzymes is under the control of angiotensin II in the zona glomerulosa and of corticotropin in the zona reticularis and zona fasciculata. These hormones exert their action through the indicated transcription factors, which activate or repress the expression of the enzymes. S and U indicate respectively the SUMOylation or ubiquitylation of each transcription factor, although no relation of these modifications with processes of development or steroidogenesis is implied. Abbreviation: EGF, early growth response.



from the mesonephric mesenchyme and/or overlying the coelomic epithelium of the urogenital ridge (Figure 1) [3–5]. This early differentiation step depends on the HH (Hedgehog) and Wnt signalling pathways, together with a number of transcription factors such as WT1 (Wilms' tumour 1), GLI3 (GLI Krüppel family member 3), SALL1 (Spalt-like 1), FOXD2 (forkhead box D2), PBX1 (pre-B-cell-leukaemia transcription factor 1) and ACD (adrenocortical dysplasia). As development proceeds, the adrenogonadal primordium separates and differentiates, leading to adrenocortical and gonadal primordium individualization, involving Wnt signalling, SF-1 (steroidogenic factor 1; also known as NR5A1), DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenital-critical region on the X chromosome; also known as NR0B1) and CITED2 {CBP [CREB (cAMP-response-element-binding protein)-binding protein]/p300-interacting transactivator with glutamic acid/aspartic acid-rich C-terminal domain}. Finally, subsequent specific adrenal differentiation programmes and maintenance occur, which depend on corticotrophin (ACTH) stimulation and signalling. The transcription factors that mediate corticotropin include CREB, SF-1, NR4A (nuclear receptor 4A subfamily) and the GATA (GATA-binding protein) family.

Despite the functional importance of these transcription factors, the molecular and biological consequences of their post-translational modifications are mostly unknown, as is the case with the zinc finger transcription factors WT1, GLI3 and SALL1. WT1 and SALL1 have important roles in renal, gonad and adrenal development [6–8] and are substrates for SUMO conjugation [9,10]. For WT1, its localization to nuclear speckles requires the SUMOylation machinery, although probably in an indirect way. For SALL1, which mediates the role of angiotensin II in the regulation of steroidogenic enzymes [11], the function of SUMO modification has not been explored.

Ubiquitylation of GLI3 does not lead to its proteasomal degradation, but regulates its phosphorylation-dependent processing, necessary for proper Sonic HH signalling [12]. The full-length protein acts as a transcriptional activator during adrenal gland development [13]. However, in the absence of signalling, GLI3 is proteolytically processed into a truncated repressor protein. A similar C-terminally truncated protein is found in patients with the Pallister–Hall syndrome that present with adrenal malformations [14].

SF-1 and DAX-1 are the best characterized of these transcription factors, owing to their dual role in adrenogonadal

development and in steroidogenesis. They are considered the main transcriptional regulators of the hypothalamic–pituitary–adrenal–gonadal axis during development and in adult tissues. Both have similar expression patterns, mainly restricted to tissues involved in steroid hormone biosynthesis and in reproduction. They are expressed in the urogenital ridge, in the adrenal and gonadal primordium during development and in the adrenal cortex in the adult [15]. Gene disruption of *Sf-1* in mice leads to adrenal and gonadal agenesis and in humans heterozygous loss-of-function mutations cause impaired adrenal function and XY reversal [16]. Gene mutations in human *DAX-1* leads to congenital adrenal hypoplasia with associated hypogonadism, and dosage sex reversal syndrome when the gene is duplicated [17,18]. Furthermore, SF-1 is required to activate the expression of DAX-1 and might work as a DAX-1 co-activator, whereas DAX-1 inhibits the transcriptional activity of SF-1 on downstream genes [19,20]. SF-1 and DAX-1 can both be modified by ubiquitin and SF-1 by SUMO. The implications of these modifications on their roles will be discussed in the next section.

Steroidogenesis

Nuclear receptor superfamily

A number of transcription factors involved in steroidogenesis, such as SF-1, DAX-1, NUR77 (nuclear receptor 77; also known as NR4A1), NURR1 (nuclear receptor related 1; also known as NR4A2), NOR1 (neuron-derived orphan receptor 1; also known as NR4A3) and the COUP-TFs (chicken ovalbumin upstream promoter-transcription factors), belong to the nuclear receptor superfamily. SF-1, besides its role in adrenal gland and gonadal development, plays a central part in the regulation of steroidogenic enzymes (CYP11A1, CYP21, CYP11B1, CYP11B2, CYP19 and CYP17) and hydroxysteroid dehydrogenases [HSD3β (3β-hydroxysteroid dehydrogenase/isomerase)], as well as other proteins crucial for steroidogenesis. These include the intracellular cholesterol transporters SCP2 (sterol carrier protein 2) and StAR, the scavenger B-I receptor (mediator of the lipid uptake required for steroidogenesis) and MC2R (melanocortin 2 receptor; the receptor for corticotropin). SF-1 activates the basal expression of the above-mentioned target genes (except for CYP11B2, which is repressed) and also mediates hormonal stimulation via cAMP signalling [21].

SF-1 interacts with UBC9 (ubiquitin-conjugating enzyme 9) and the SUMO ligases PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] 1 and PIAS3, and is modified by SUMO at Lys-119 and Lys-194. As reported for other transcription factors, its SUMOylation has been associated with inhibition of transcription [22,23]. Interestingly, Lys-194 overlaps with an SC (synergy control) motif, identified within the negative regulatory regions of multiple transcription factors, which controls synergistic transcription driven by multiple but not single response elements. Furthermore, the transcriptional synergy through this motif is regulated by SUMOylation [24,25]. SUMOylation of SF-1 is involved in its synergy with Sox9, but not with WT1 [23,26]. It remains to be analysed whether SUMOylation regulates the known SF-1 synergy with other transcription factors such as PBX1, GATA-4, GATA-6, PITX1 and TReP-132. In addition, although the sequence containing Lys-194 interacts with the DEAD-box-containing protein DP103, which represses the activity of SF-1, SUMOylation is not involved in this interaction. Nevertheless, SUMO modification could be implicated in the recruitment of other co-repressors. These studies have also shown that SUMOylation of SF-1 does not affect its DNA-binding activity. In spite of this, SUMOylation of SF-1 at Lys-119 resulted in the loss of DNA binding to non-canonical sites [27]. Interestingly, a recent study has shown that non-SUMOylated SF-1 leads to enhanced phosphorylation and transcriptional activity, suggesting that phosphorylation of SF-1 is altered by its SUMOylation status [26]. Overall, these studies show that SUMOylation is an important mechanism to control SF-1 activity. Moreover, ubiquitylation of SF-1 by an SCF (Skp1/cullin/F-box) ubiquitin ligase, induced by HDAC (histone deacetylase) inhibitors, leads to its degradation, resulting in decreased expression of SF-1 target genes and steroidogenesis [28]. However, this result is in contrast with previous studies showing that HDAC inhibitors increased SF-1 half-life or activity [29,30].

Conversely, DAX-1 is an important negative regulator of the genes involved in steroid hormone synthesis and metabolism such as CYP11A, CYP17, CYP19, HSD3β and StAR. In fact, it is considered the main negative regulator of SF-1-mediated transcriptional activation in steroidogenic tissues. In addition, DAX-1 inhibits the transcriptional activity of other nuclear receptors, such as the ER (oestrogen receptor), AR (androgen receptor), PR (progesterone receptor), NUR77 and LRH1 [31]. DAX-1 interacts with RNF31 (RING finger protein 31), a member of the ring-between-ring family of ubiquitin ligases [32]. Interestingly, RNF31 association triggers DAX-1 mono-ubiquitylation, leading to its stabilization. DAX-1 stabilization could have important consequences for the SF-1–DAX-1 interaction, as the cellular balance of both transcription factors is crucial for the activation or repression of target genes. Interestingly, RNF31 and DAX-1, together with SMRT (silencing mediator of retinoid and thyroid receptors), co-operate within a chromatin-bound co-repressor complex to repress the expression of the steroidogenic genes StAR and CYP19. Therefore this study shows that the ubiquitin pathway, through RNF31, is involved in the transcriptional repression of steroidogenesis.

The NR4A subfamily of orphan nuclear receptors consists of NUR77, NURR1 and NOR1, all of which are involved in steroid hormone biosynthesis. In fact, NURR1 plays a central role in aldosterone production through the regulation of CYP11B2 expression in the zona glomerulosa of the adrenal cortex and is a target for angiotensin II and K^+ signalling. NUR77 might have a similar function in the expression of HSD3β and is a target gene for both angiotensin II

and corticotropin signalling. These transcription factors also activate the expression of CYP21 [33–35]. NURR1 is regulated by ubiquitylation and SUMOylation [36,37]. Although ubiquitylation appears to control NURR1 stability, SUMOylation is required for NURR1 transrepression of the inflammatory *iNOS* (inducible nitric oxide synthase) and *TNF-α* (tumour necrosis factor α) genes, mediated by the CoREST (co-repressor element 1-silencing transcription factor) repressor complex. Interestingly, CoREST has been reported to have a non-consensus SUMO interacting motif, which is required for gene-specific repression [38]. Furthermore, the conjugation of SUMO to NURR1 has been suggested to induce its monomerization [37]. It is at present unknown whether the SUMOylation of NURR1 influences its control of steroidogenic gene expression.

COUP-TFs are involved in the transcriptional regulation of CYP17 and CYP11B2 [39,40]. Both UBC9 and PIAS1 have been identified as co-activators of COUP-TFI for the transcription of the human CYP11B2 gene. Surprisingly, the function of UBC9 and PIAS1 seems to be independent of their SUMOylation activity [40,41]. Further studies are required to understand the role of SUMOylation in COUP-TFI and the contribution of this pathway to aldosterone biosynthesis in the adrenal cortex.

Other transcription factors

Other transcription factors not belonging to the nuclear receptor superfamily, such as GATA, SREBP (sterol regulatory element binding protein), Sp (specificity protein), CREB, ATF-1 (activating transcription factor 1), EGR (early growth-response gene product) or AP-1 (activator protein 1) among others, are also involved in steroidogenesis, although little is understood about the contribution of SUMOylation or ubiquitylation to their function. In this section, we revise what is currently known for some of them.

Of the six members of the GATA family of zinc finger transcription factors, GATA-4 and GATA-6 are expressed in the adrenal gland. GATA-4 is expressed during fetal adrenocortical development, whereas GATA-6 is expressed mainly in the zona reticularis of the adult cortex [42]. As previously indicated, both transcription factors act in synergy with SF-1 to enhance the activation of genes encoding proteins such as StAR, CYP11A1 and CYP17. GATA-1, -2 and -4 are modified by SUMO [43]. SUMOylation of GATA-4 promotes its nuclear localization and transcriptional activity at cardiac-specific genes [44], although it is currently unknown whether it can also modulate the control of genes involved in steroidogenesis.

SREBPs, transcription factors containing a basic helix–loop–helix leucine zipper motif, activate genes involved in cholesterol and fatty acid biosynthesis and also regulate CYP17 and StAR [45]. SUMOylation of SREBP-1 and SREBP-2 reduces their transactivation activities [46]. In addition, these factors are degraded via the UPS [47].

Sp1 and Sp3, members of the Sp family of transcription factors, have also been implicated in the transcriptional regulation of CYP11A1 and CYP17 [45,48]. Both Sp1 and Sp3, which can act as activators or repressors, are modified by SUMO. SUMO modification of Sp3 represses transcription by leading to the establishment of local heterochromatin-like structures, whereas Sp1 modification alters its localization and increases its ubiquitylation, leading to degradation [49–51]. Both Sp1 and Sp3 interact with GATA proteins and nuclear factor 1 to control the expression of CYP17, although it is unknown whether their post-translational modifications regulate the interaction with these proteins.

Increased synthesis of steroidogenic enzymes relies also on corticotropin hormonal stimulation via the cAMP/PKA (protein kinase A) signalling pathway that leads to phosphorylation of CREB. CREB activity is regulated by ubiquitylation and SUMOylation, which stabilizes CREB and enhances its activity in some cellular processes [52,53]. However, it is unknown how these modifications modulate the transcription of CYP17, CYP11B2, CYP11A1 and other steroidogenic enzymes.

Other factors binding CREs (cAMP-response elements) are ATF-1 and ATF-2, as shown in the CYP11B2 promoter, or AP-1, which activates synergistically with SF-1 the CYP11A1 promoter and also increases CYP11B1 expression [54]. The adrenal induction of AP-1, composed of c-Jun and c-Fos, is regulated by angiotensin II and corticotropin. Both components are degraded by the proteasome, UPS-mediated for c-Jun and independently of ubiquitylation for c-Fos [55,56]. In addition, SUMOylation of c-Fos/c-Jun reduces AP-1 transcriptional activity [57].

Angiotensin II also increased the expression of the EGR transcription factor family in adrenocortical cells, with CYP11B1 and CYP11B2 expression being activated by EGR1 and EGR2 [58]. Although both EGR1 and EGR2 are degraded by the UPS [59], only EGR1 is known to be modified by SUMO [60].

Steroid receptors

Steroid hormones operate through binding to members of the nuclear receptor superfamily, which regulate gene expression by binding to short steroid response elements located upstream of target genes. Whereas binding of the steroid to the receptor results in recruitment of co-activator proteins and activation of transcription, non-ligated receptors interact with co-repressors impairing gene transcription [61].

A common feature is that steroid receptors, the exception being the ER, are ligand-dependent targets for SUMOylation in their N-terminal SC motifs. In these cases, SUMOylation suppresses the transcriptional activity of the receptors by impairing co-operativity at complex promoters. Hence, mutations at the SUMOylation sites of the AR, PR, glucocorticoid and mineralocorticoid receptors increase their transcriptional activity, specifically on complex, multimerized response elements, but has no effect on single-site-containing promoters [24,25,62–65]. Furthermore, in some cases, SUMOylation is also implicated in the interaction of the receptors with different co-activator and co-repressor proteins, such as Daxx with AR and the

SRC1 (steroid receptor co-activator-1) with PR [63,66]. Conversely, mutations of the two SUMOylation sites of the ERα, located within the hinge region, impair rather than activate transcription of target genes [67].

All steroid receptors are also targeted for ubiquitylation. In all cases, their ubiquitylation influences hormone response by priming the receptor for degradation by the UPS, a feature that has been demonstrated as necessary for maintaining transcription. For example, proteasome activity, which is necessary for rapid exchange of glucocorticoid and ERα receptors on target promoters, is crucial for the transcriptional activity of both receptors [68,69].

Concluding remarks

SUMOylation and ubiquitylation play essential roles in fine-tuning the regulation by steroid hormones. For example, in the case of steroid receptors, SUMOylation could co-ordinate their action on complex promoters. Additional studies are required to clarify the role of SUMOylation or ubiquitylation of WT1, GLI3, SALL1, SF-1, DAX-1 and other factors, and to correlate these modifications with adrenal and gonadal abnormalities. Further research on SUMO and ubiquitin modifications is necessary to clarify their role in the highly regulated process of steroid hormone signalling.

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