Histone acetylation by ATAC complex regulates steroid hormone biosynthesis

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Steroidogenic factor 1 (*Sf-1*) is a nuclear receptor which plays an important role in adrenogonadal development and steroidogenic gene expression. The activity of *Sf-1* is controlled by posttranslational modifications. Phosphorylation at Ser203 and acetylation by GCN5 and p300 have been reported to enhance *Sf-1* function.

The *Sf-1* shows tissue specific expression being highly expressed in the adrenal cortex, testis, ovary, hypophysis, ventromedial hipothalamus, skin and spleen. Its mutation or disregulated expression can lead to tumor formation.

Recently we have reported that the lack-of-function mutations of the GCN5 histone acetyltransferase (HAT)-containing ATAC complex influence steroid biosynthesis. In contrast, the lack of the other GCN5-containing HAT complex, SAGA has only mild effect on steroid biosynthesis. The mechanism by which ATAC affects steroid synthesis, however, remains to be discerned. The two most probable scenarios could be that ATAC influences the transcription of genes involved in steroid hormones biosynthesis directly by histone acetylation at their promoters, or that it acetylates FTZ-F1/SF1 and by this regulates the transcription of steroid converting gene indirectly.

We studied whether ATAC- (H4K5ac) or SAGA- (H3K9ac) specific histone acetylation is present at the promoter, initiator and 3'UTR (untranslated) regions of the *Cyp11a1* gene which catalyzes the first regulatory step of the mammalian steroid conversion. We found that H4K5 acetylation can be detected at the regulatory regions (promoter and initiator) of *Cyp11a1* gene, while SAGA-specific H3K9 acetylation is present mostly at the initiator region of *Cyp11a1* gene. We also investigated whether the acetylation level is altered upon TSA (trichostatin A) histone deacetylase inhibitor treatment. We found that neither H4K5 acetylation level nor H3K9 acetylation level was significantly increased upon TSA treatment (**Figure1**).

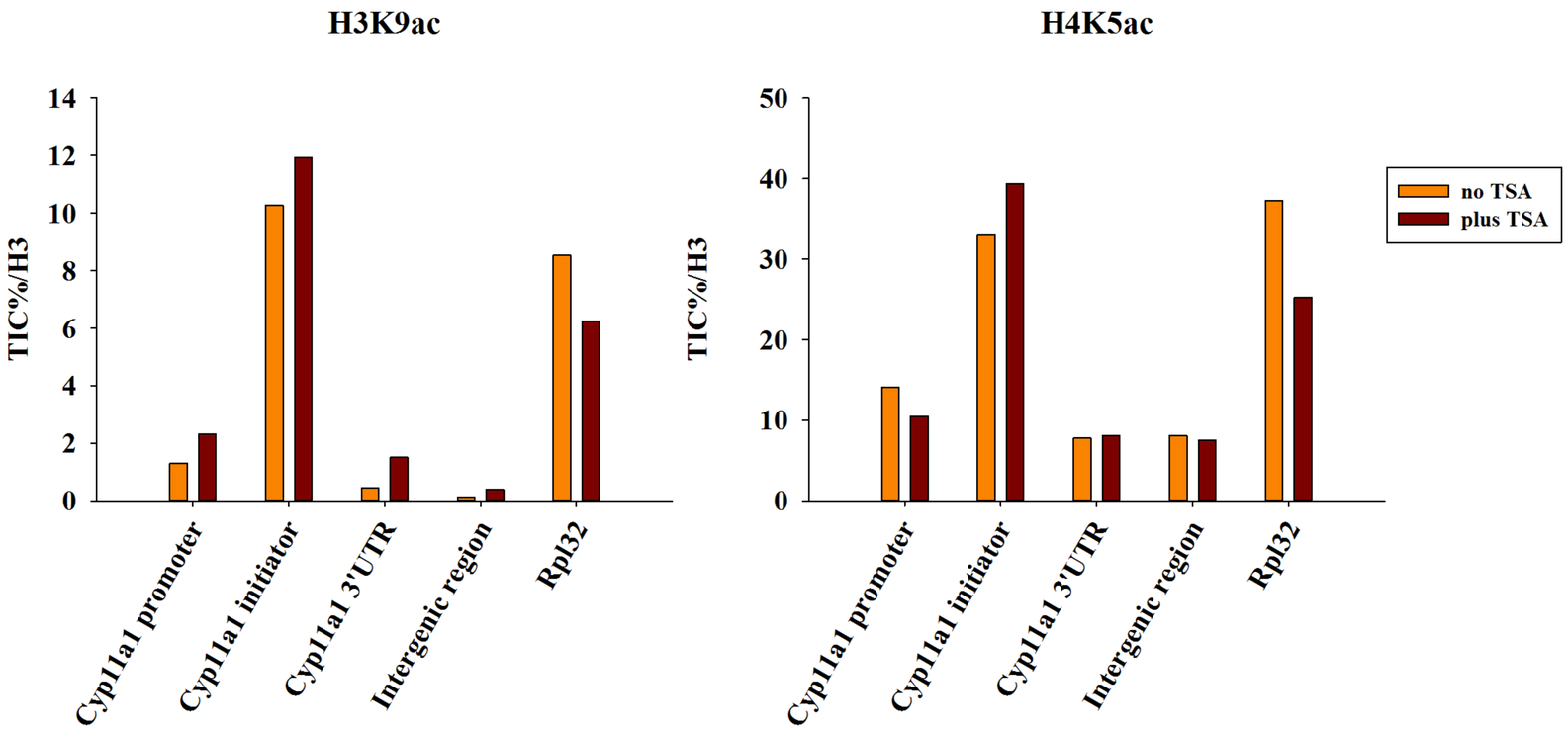

Figure 1. H3K9 and H4K5 acetylation level at the promoter, initiator and 3'UTR regions of *Cyp11a1* gene. ChIP experiments were done on chromatin samples which were prepared from Y1 canceorous adrenocortical mouse cell line treated with 400 ng/ml TSA and as well as on untreated control. Intergenic region was used as negative control, Rpl32 was used as positive control of ChIP. Acetylation levels were normalized to H3.

We investigeted whether ADA2a (ATAC-specific subunit) or ADA2b (SAGA-specific subunit) protein binds to the *Cyp11a1* gene promoter, initiator and 3'UTR regions and to the *Sf-1* gene initiator and 3'UTR regions. We detected no ADA2a binding at any studied regions, while ADA2a protein can bind to the initiator and 3'UTR regions of Sf-1 gene. This binding can also be present at the 3'UTR region of *Cyp11a1* gene upon TSA treatment. We also examined the changes in ADA2a and ADA2b binding upon TSA treatment and found that the TSA treatment did not cause significant alterations (**Figure2**).

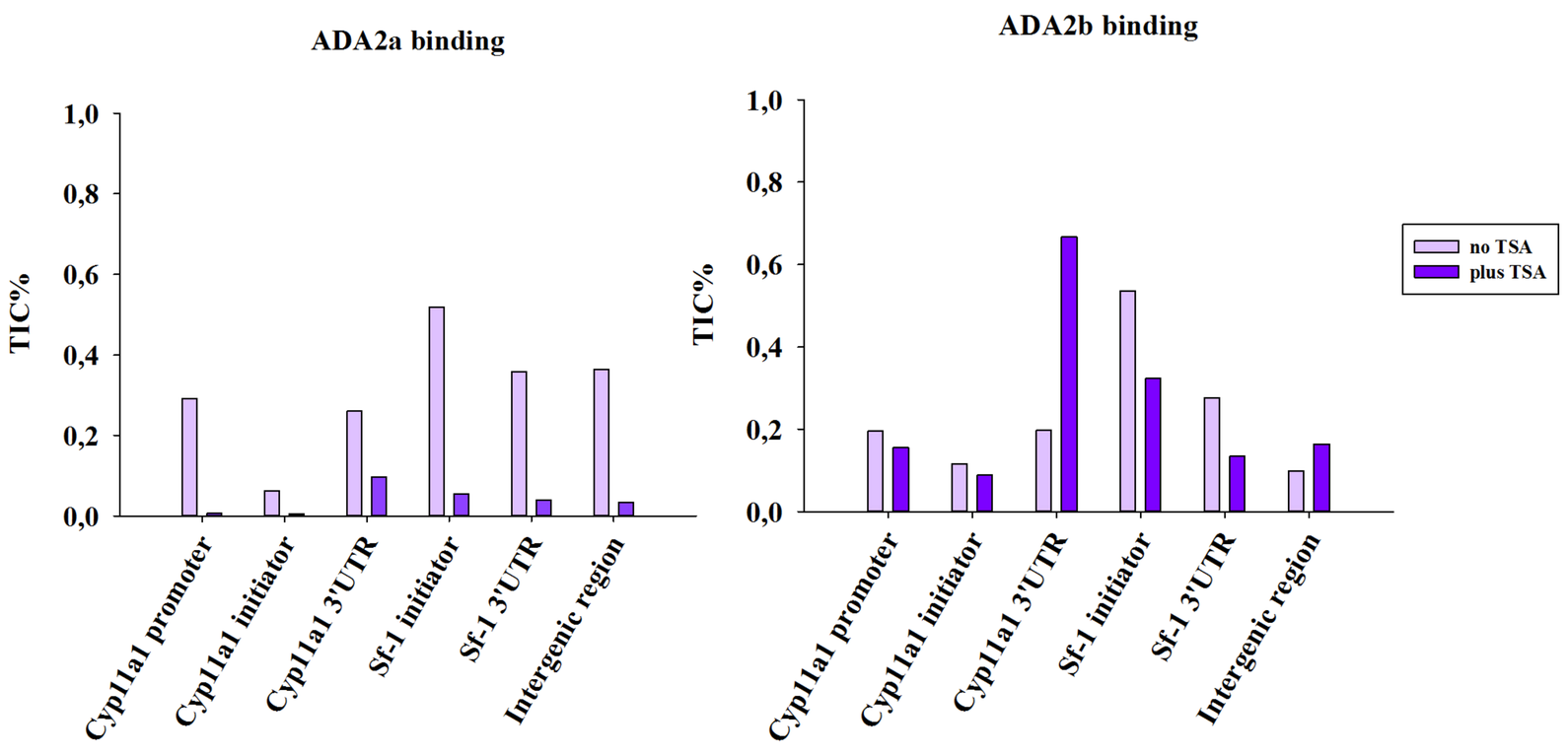

Figure 2. ADA2a and ADA2b binding at the promoter, initiator and 3'UTR regions of *Cyp11a1* gene and at the initiator and 3'UTR regions of *Sf-1* gene. ChIP experiments were done using chromatin prepared from untreated and 400 ng/ml TSA treated Y1 cells. Intergenic region was used as negative control of ChIP.

H3K9 acetylation can be observed at the initiator region of *Cyp11a1* gene, while H4K5 acetylation can be detected at the promoter and initiator regions. We have not been able to detect ADA2a binding at any regions of *Cyp11a1* or *Sf-1* gene. In contrast, we can observe weak ADA2b binding at the *Sf-1* initiator and *Sf-1* 3'UTR regions. ADA2b binding can be also detected at *Cyp11a1* 3'UTR region upon TSA treatment.

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