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# Regulation of Nur77 protein turnover through acetylation and deacetylation induced by p300 and HDAC1

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ABSTRACT

Although the roles of Nur77, an orphan member of the nuclear hormone receptor superfamily, in the control of cellular proliferation, apoptosis, inflammation, and glucose metabolism, are well recognized, the molecular mechanism regulating the activity and expression of Nur77 is not fully understood. Acetylation of transcription factors has emerged recently as a major post-translational modification that regulates protein stability and transcriptional activity. Here, we examined whether Nur77 is acetylated, and we characterized potential associated factors. First, Nur77 was found to be an acetylated protein when examined by immunoprecipitation and western blotting using acetyl protein-specific antibodies. Second, expression of p300, which possesses histone acetyltransferase activity, enhanced the acetylation and protein stability of Nur77. Treatment with a histone deacetylase (HDAC) inhibitor, trichostatin A, also increased Nur77 acetylation. Among the several types of HDACs, HDAC1 was found as the major enzyme affecting protein level of Nur77. HDAC1 decreased the acetylation level, protein level, and transcriptional activity of Nur77. Interestingly, overexpression of Nur77 induced expression of both p300 and HDAC1. Finally, the expression of Nur77 increased along with that of p300, but decreased with induction of HDAC1 after treatment with epithelial growth factor, nerve growth factor, or 6-mercaptopurine, suggesting that the self-control of the acetylation status contributes to the transient induction of Nur77 protein. Taken together, these results demonstrate that acetylation of Nur77 is modulated by p300 and HDAC1, and suggest that acetylation is an important post-translational modification for the rapid turnover of Nur77 protein.



## 1. Introduction

Nur77 (also called NR4A1, TR3, NAK-1, and NGFI-B) plays important roles in the regulation of cellular proliferation, apoptosis, and energy metabolism. It belongs to the NR4A (nuclear receptor group 4A) subfamily of nuclear hormone receptors and its DNA-binding domain (DBD) shows 90% homology with other subfamily members, Nurr1 and Nor-1 [1,2]. All of NR4A subfamilies are encoded by immediate early genes whose expression is induced in response to a variety of signals including mitogens and cellular stress [3–5]. Interestingly, Nur77 is activated by ligand independently, which may be explained by conserved, tightly packed, and bulky hydrophobic residues occupying the ligand-binding domain (LBD) of NR4A groups [6,7]. Both Nur77 mRNA and protein appear to be unstable *in vivo*, and thereby their expression is transiently induced by stimulation [3,5,8]. Because Nur77 acts independently to a ligand and is unstable *in vivo*, the regulation of its expression and post-translational modification are thought to be important to its function.

Post-translational modification is an important process in the regulation of nuclear hormone receptors. Phosphorylation, methylation, acetylation, ADP ribosylation, glycosylation, ubiquitination, sumoylation, and neddylation affect a broad spectrum of protein structures and functions [9]. Acetylation has emerged recently as a central step in the regulatory modification processes, and more than 100 proteins have been found to be acetylated. Acetylation commonly occurs at a lysine residue, and the functional consequences of acetylation are diverse. Acetylation affects the nuclear localization, stability, transcriptional activity, DNA binding, and interactions with other cofactors and proteins [10,11]. Protein acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which have opposite roles [12–14]. p300/CBP possess strong acetylase function on both histone and non-histone proteins. HDACs are classified

*Abbreviations:* HAT, histone acetyltransferase; HDAC, histone deacetylase; IP, immunoprecipitation; TSA, trichostatin A; DBD, DNA-binding domain; LBD, ligand-binding domain; NGF, nerve growth factor; EGF, epithelial growth factor; PMA, phorbol myristate acetate; CHX, cycloheximide; 6-MP, 6-mercaptopurin.

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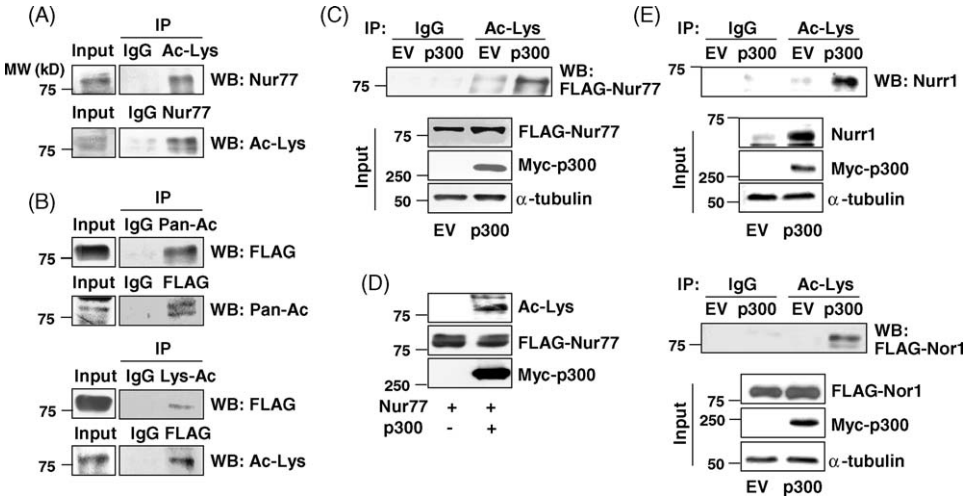


**Fig. 1.** Nur77 is acetylated in the presence of p300. (A) 2 mg of HepG2 cell lysates was immunoprecipitated (IP) using anti-Ac-lysine (Lys) or anti-Nur77 antibody, and probed by western blotting (WB) using anti-Nur77 or anti-Ac-lysine antibody, respectively. Immunoprecipitation with normal IgG was used as control. The expression of Nur77 in cell lysates was analyzed by western blotting as input. (B) NIH3T3 cells were transfected with FLAG-Nur77. 700 μg of whole-cell lysates were immunoprecipitated using anti-pan-Ac, anti-Ac-lysine, or anti-FLAG antibody and then probed by the indicated antibodies. (C) HEK293 cells were transfected with FLAG-Nur77 together with Myc-p300 or empty vector (EV). 500 μg of whole-cell lysates were immunoprecipitated using anti-Ac-lysine or normal IgG antibodies, and then probed by anti-FLAG antibody. (D) *In vitro* acetylation assay was performed as described in Section 2. Purified FLAG-Nur77 and Myc-p300 were incubated in the presence of acetyl-CoA for 1 h at 37 °C. The levels of acetylated and total recombinant Nur77 proteins and p300 were shown by western blot analysis. (E) HEK293 cells were transfected with pCMX-Nurr1 (upper) or FLAG-Nor-1 (lower) together with Myc-p300 or empty vector (EV) as indicated. 500 μg of whole-cell lysates were immunoprecipitated using anti-Ac-lysine or normal IgG antibodies, and then probed by anti-Nurr1 or anti-FLAG antibody.

into four classes and two families: classical (classes I, II, and IV) and silent information regulator 2 (Sir2)-related protein (sirtuin) families (class III) [14]. Because HATs and HDACs have been linked to diverse cellular factors of biological importance, many strategies have been used in therapeutic intervention.

Nur77 is also a target of post-translational modification [15]. Phosphorylation of Nur77 is induced by natural growth factors such as nerve growth factor (NGF) and epithelial growth factor (EGF), and chemicals such as phorbol myristate acetate (PMA) [16–19]. Several kinases, such as Jun N-terminal kinase, p90 ribosomal S6 kinase, and protein kinase B are known to phosphorylate Nur77 [18–20]. The fact that Nur77 interacts directly with coactivators that have HAT activity [21–23], suggests that Nur77 is acetylated by these cofactors. Here we examined whether Nur77 is an acetylated protein and we characterized potential associated coregulators. We also aimed to identify the role of acetylation in the regulation of Nur77 function.

## 2. Materials and methods

### 2.1. Cell and cell culture

Human hepatocellular carcinoma cell line, HepG2 (ATCC HB-8065), human cervical carcinoma cell line, HeLa (ATCC CCL-2) and mouse fibroblast cell line, NIH3T3 (ATCC CRL-1658) were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS). Human embryonic kidney cell line, HEK293 (ATCC CRL-1573) was maintained in Iscove's Modified Dulbecco's Medium containing 10% FBS. Rat pheochromocytoma cell line PC12 (ATCC CRL-1721) was maintained in Dulbecco's modified Eagle's medium containing 10% horse serum and 5% FBS. Cells were incubated at 37 °C in 5% $CO_2$/95% air. HeLa cells were subjected to serum deprivation for 20 h and PC12 cells had serum starvation for 4 h before treating growth factors. EGF and NGF were purchased from Sigma (St. Louis, MO, USA) and Invitrogen Corporation (Carlsbad, CA, USA), respectively. Cycloheximide (CHX), MG132, and 6-mercaptopurin (6-MP) were purchased from Sigma, Calbiochem (Darmstadt, Germany), and Fluka Ltd. (Buchs, Switzerland), respectively.

### 2.2. Plasmids, siRNA and transient transfection

The reporter NurRE-pomc-Luc and eukaryotic expression vectors encoding FLAG-Nur77, pCMX-Nurr1, FLAG-HDAC1, -2, -4, and -7, and Myc-p300 were described previously [23–26]. FLAG-Nor-1 was constructed by inserting a PCR-amplified full-length

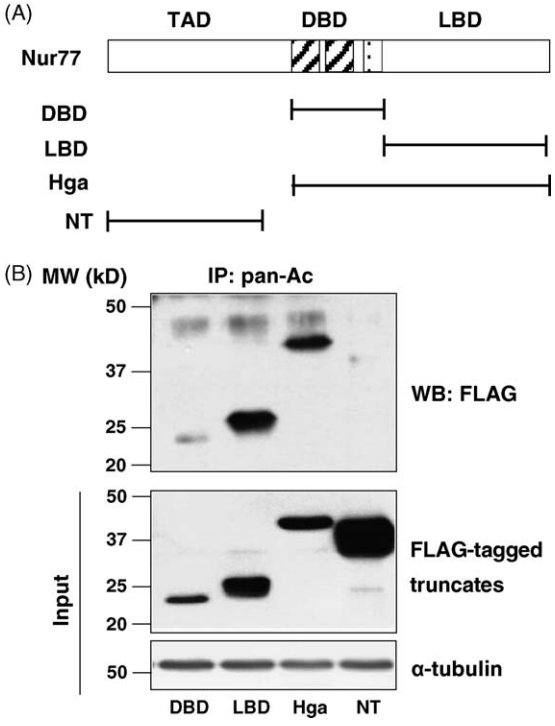


**Fig. 2.** Identification of acetylated domains of Nur77. (A) Schematic representation of the truncated mutants of Nur77. (B) Plasmids encoding the FLAG-tagged Nur77 truncates were transfected into NIH3T3 cells. 24 h after transfection, cell lysates were obtained, immunoprecipitated by anti-pan-Ac antibody and analyzed by western blotting with anti-FLAG antibody. The expression of FLAG-tagged Nur77 truncates was analyzed by western blotting as input.