



**Karolinska  
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# Role of Posttranslational Modifications in Regulation of Notch and Transcriptional Coactivator Mastermind-Like 1

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## ABSTRACT

The Notch signaling is an evolutionary conserved pathway enabling short range cell-cell interactions, crucial for diverse developmental and physiological processes during embryonic and adult life. The Mastermind-like (MAML) family of transcriptional coactivator proteins has been shown to play an essential role in regulation of Notch-mediated transcription. Formation of DNA-bound ternary CSL-Notch ICD-MAML complex is a crucial event in transcriptional regulation of Notch target genes. More recent studies highlight a broader role of MAML1 by showing that MAML1 coactivates MEF2C, p53,  $\beta$ -catenin and NF- $\kappa$ B. Data presented in this thesis studies demonstrate that MAML1 enhances autoacetylation and HAT activity of p300 acetyltransferase, which coincidences with increased acetylation of histones H3/H4. We further show that p300 acetylates Notch1 ICD, and MAML1 strongly enhances Notch acetylation, presumably by potentiating p300 autoacetylation. MAML1-dependent acetylation of Notch1 ICD by p300 decreases the ubiquitination of Notch1 ICD in cell culture, which might be a mechanism to regulate Notch activity in the nucleus by interfering with ubiquitin dependent pathways. MAML1 has been show to recruit CDK8 kinase, which phosphorylates Notch1 ICD and targets Notch for proteasome-mediated degradation. We found that CDK8 inhibits p300 acetylation of Notch1 ICD and Notch1 ICD-p300 mediated transcription. These findings underscore MAML1 function as coregulator of Notch that, depending on signaling time frame and interacting partner, can modulate the strength of Notch responses in cells.

Considering the importance of MAML1 for Notch and other signaling pathways we investigated the molecular mechanisms of how MAML activity is regulated. Data presented in this thesis reveal that MAML1 transcriptional activity can be modulated by two mechanisms. First, we found that MAML1 is phosphorylated and inhibited by GSK3 $\beta$  kinase. Active and inactive GSK3 $\beta$  interacts with N-terminal MAML1, and GSK3 $\beta$  subcellular localization is changed to nuclear bodies in the presence of MAML1, where they both colocalize. Only active GSK3 $\beta$  is capable of inhibiting MAML1 activity, moreover GSK3 inhibitor SB41 significantly increases the levels of acetylated histones H3 in cells stably expressing MAML1. Although GSK3 $\beta$  interacts and phosphorylates N-terminal MAML1, Notch ICD-MAML1 binding remains unaffected regardless of the phosphorylation status. Second, we found that MAML1 is a target of SUMOylation at two highly conserved lysines residues (K217 and 299), and that MAML1 SUMOylation deficient mutant has significantly higher transcriptional activity. Furthermore, SUMOylation of MAML1 potentiates interaction with HDAC7, which decreases MAML1 activity, and thus might serve as an additional mechanism to control MAML1 function as a coactivator.