TITLE

New insights in the transcriptional role of NF-YA phosphorylation sites

ABSTRACT

The heterotrimeric transcription factor NF-Y is known to regulate transcription by specifically recognizing CCAAT-box elements and recruiting other TFs and cofactors to promoter and enhancer regions. While the knowledge of genomic locations and 3D structures is deep, little is known about NF-Y regulation by post-translational modifications.

NF-YA, the regulatory subunit of the complex, is phosphorylated in vivo, possibly by CDK2, at two serine residues (Ser320-Ser326) localized at the C-terminus, close by the conserved DNA-binding domain of the subunit.

We generated NF-YA Ser320-Ser326 single and double mutants to abolish (Ser to Ala) or mimick (Ser to Glu) the phosphorylated state of the protein. Off-rate DNA binding assays show a NF-Y/CCAAT complex destabilization for the Ser320 phosphomimicking mutant, suggesting a direct involvement of this modification in the regulation of DNA-binding.

We solved the crystal-structure of the NF-Y trimer bound to DNA using a complete C-terminal NF-YA construct, revealing a distinct spatial positioning for the two serines: Ser320 indeed is in close proximity to the DNA phosphate backbone, at the edge of NF-YA DNA-binding module; this position is consistent with the DNA-binding destabilization observed with the correspondent phosphomimicking mutant. Ser326, instead, is exposed to the solvent along the extended C-terminal tail, away from the DNA.

To evaluate the transcriptional outcome of these modifications, we performed luciferase assays in HeLa cells cotransfected with NF-Y subunits, using two CCAAT-dependent promoters (RHOB and MDR1). Unlike Ser326, NF-YA Ser320 phosphomimicking mutant shows a severe impairment in the activation of both target promoters, again consistent with the in vitro data.

In conclusion, our data suggest distinct molecular and functional roles for Ser320 and Ser326 phosphorylation events, with the former having a direct impact in the regulation of the DNA-binding stability of the trimer, therefore affecting CCAAT-dependent transcription.