NMR Study of the Structural Organization of ING4 and Specific Recognition of Methylated Histone 3 Tails by its PHD Finger.

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The inhibitor of growth 4 (ING4) is a 29 kDa protein that belongs to the ING family of tumour suppressors [1]. It is thought that ING proteins direct histone acetylation and deacetylation complexes to specific regions of the chromatin to alter its structure and hence the transcriptional activity of the corresponding genes. Sequence homology and phylogenetic analysis indicate that the ING proteins have a similar arquitecture, with a conserved N-terminal region, a central non conserved region and a conserved C-terminal region homologous to the plant homeodomain PHD. To characterise experimentally the domain structure and organization of ING4, several constructs and the full protein have been obtained and analysed in solution. We show that ING4 contains three structurally and independent domains: a N-terminal domain rich in helical structure, a central flexible and unstructured domain, and a plant homeodomain finger at the C-terminus. The protein is a dimer in solution, and the dimerization site is at the N-terminal domain. The central region does not show evidence of p53 binding in ^{1}H-^{15}N chemical shift perturbation (CSP) measurements, in contrast with previously reported pull-down experiments. The PHD is the site for recognition of histone 3 trimethylated at lysine 4 (H3K4me3) [2, 3, 4], a post-translational modification of the histone N-terminal tail that is a hallmark of genes that are active in transcription. PHD binds with reduced affinity H3 and H3K9me3 tails, and does not bind to H4K20me3, all of them associated with gene silencing. The crystal structure of ING4-PHD bound to H3K4me3 [5] reveals a tight complex which is stabilized by cation-π interactions involving aromatic rings of the PHD and the charged trimethyl-amino group of histone Lys4. However, the discrimination between methylated and non-methylated H3 is entropy driven, due to the burial of the hydrophobic methyl groups. ^{15}N spin relaxation measurements shows a reduction in the backbone mobility of regions of the PHD that participate in peptide binding, and chemical shift perturbation measurements on complexes of PHD with peptides of different lengths, show that additional interactions as found in the crystal stabilize the complex with longer peptides. The molecular basis of H3K4me3 recognition by ING4 differs from that of ING2, suggesting a distinct role in transcriptional regulation for these two ING family members. These results illustrate the versatility of PHD fingers as readers of the histone code.

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