Probing the role of histone ageing by semi-synthetic 'designer' chromatin

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Over the lifetime of proteins, asparagine (Asn) and aspartate (Asp) residues can spontaneously isomerize into isoAspartate (isoAsp) which features an unusual β-peptidic linkage [1]. IsoAsp formation is usually considered as protein damage and associated with several pathologies. To counteract negative effects of isoAsp formation, Nature has evolved a 'repair' enzyme, protein carboxyl methyltransferase (PIMT) to partially restore isoAsp back to Asp [2]. It was found that histone H4 can be methylated at Asp24 (H4D24me) in mouse and human by PIMT [3], which indicates that during H4 protein ageing, aspartate 24 of histone H4 can become gradually and spontaneously converted to isoaspartate.

We hypothesize isoAsp 24, the product of histone H4 aging, will impact molecular recognition events between the H4 tail and its binding partners. We therefore aimed to test whether isoAsp formation affects chromatin compaction (through interactions between the H4 *N*-terminal tail and the acidic patch of neighbouring nucleosomes [4]), H4K20 methyltransferase activity (Set8 & Suv4-20) and chromatin remodelling. To enable direct measurement of such effects, we prepared isoAsp24-containing histone H4 by convergent synthesis where recombinant and synthetic fragments were joined by sequential native chemical ligation and desulfurization. This convergent approach significantly improved the purity of final products by precluding aspartimide formation during the synthesis, a common problem during solid-phase peptide synthesis. The designer H4 and its unmodified counterpart were assembled into histone octamers, nucleosomes and nucleosome arrays, enabling direct comparison between the isoforms.

The consequences of isoAsp in H4 tail on chromatin conformation were examined by a Mg^{2+} precipitation assay; while crosstalk with adjacent methylation marks on the peptide and nucleosomal level were explored by kinetics assays by means of mass spectrometry and radioactive SAM, respectively. Finally, since the H4-binding pocket is conserved in the chromatin-remodeling ATPase ISWI family [5], the potential effect of H4isoAsp24 to chromatin remodelling was explored with remodelling factor CHD1 by a nucleosome repositioning electrophoretic mobility shift assay. Overall, the variants exhibited broadly similar properties and further experiments will be required to tease apart potential subtle differences.

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