Human chromatin reconstitution by cell-free translation for epigenetic modifier enzyme assays

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Structural changes of histone proteins caused by a diverse array of epigenetic modifications. Their dynamic, temporal, and spatial regulation provides access to the genetic information, moreover, their role was found in carcinogenesis, mental and metabolic diseases. To date, a complex understanding of the epigenetic network is limited, partially due to a lack of methodology for functional enzyme screening. We established a novel, straightforward in vitro chromatin assembly assay that could serve as a universal tool for epigenetic studies. Firstly, we selected and cloned a set of human histone modifiers: writers (methyltransferases, acyltransferases, kinases, ubiquitin ligases) and erasers (demethylases, deacetylases, phosphatases, deubiquitinases). Secondly, the wheat germ based, cell-free translation system enabled us to reconstitute functional, unmodified human chromatin (composed of 4 core histones H2A, H2B, H3.1, H4, and pBSK plasmid), that is used as a substrate for modifier enzyme assays, which are also prepared by *in vitro* translation. Finally, we analyze the modified histone samples with state-of-art mass spectrometry (Q Exactive Plus Orbitrap) at Hokkaido University, Japan. We aim to demonstrate the proof of concept by confirming known modifications of histones, then to determine novel histone marks and histone modifiers. The effect of various histones chaperons and assembly factors (NapI, ISWI/Acf1, NLP) along with histone H1 was also tested. Recently, we focused on the lesser-known ubiquitination and phosphorylation event on the human chromatin by testing histone modifier ubiquitin ligases and kinases. Ultimately, our method can be automatized, and used for high-throughput screening to test epigenetic drugs.
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