学位論文の要約

題目

Chemical Biology Approaches for Investigating Nucleosome Structure and Accessibility
(ケミカルバイオロジー的アプローチによるヌクレオソーム構造とアクセシビリティの研究)

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序論

Nucleosome, the basic repeating unit of chromatin, consists of an octamer of four core histone proteins (H3, H4, H2A, H2B), with 145-147 base pairs segment of DNA. The core histones assemble into a spool-like structure onto which the core DNA is wrapped, in about 1.75 left-handed superhelical turns. These nucleosome core particles were connected by linker DNA and joined together into chromatin fibers. Over the past decades, many import breakthrough researches have been reported, which significantly improve our understanding about gene regulation and accelerate the progressing of epigenetic research. In this study, we mainly focused on investigating the influence of histone binding on nucleosomal DNA's accessibility with exploring chemical biology approaches. Besides, we identified a new non-nucleosome structure, termed as H3-H4 octasome. A new synthetic molecule, PIP-L conjugating with the activator of HAT, also was confirmed that highly associated with regulating nervous system development upon the potentiality of altering nucleosome dynamic.

1) In vitro evaluation of nucleosome's accessibility for duocarmycin B_2

To evaluate the reactivity of antitumor agents in a nucleosome architecture, we conducted in vitro studies to assess the alkylation level of duocarmycin B_2 on nucleosomes with core and linker DNA using sequencing gel electrophoresis. Our results suggested that the alkylating efficiencies of duocarmycin B_2 were significantly decreased in core DNA and increased at the histone-free linker DNA sites when compared with naked DNA condition. Our finding that nucleosome assembly alters the accessibility of duocarmycin B_2 to duplex DNA could advance their design as antitumor agents.

2) Investigating the accessibility of nucleosome for Fe(II)•peplomycin and MNase using capillary electrophoresis

We adopted capillary electrophoresis coupling with DNA 5' Texas Red labelling to investigate

the accessibility of nucleosome. Duocarmycin B_2, peplomycin • Fe(II) and MNase was adopted for the evaluation, and distinct accessibility patterns of nucleosome for these reactions were observed. Duocarmycin B_2 was still able to enter into core region though the alkylating efficiency was significantly decreased. Peplomycin's intercalation in nucleosomal core region also was highly suppressed, but the reaction sites locating in nucleosomal core end was still accessible, which implied the flexibility of core DNA end. MNase was completely shut off from approaching to nucleosome core, and exhibited a higher site-specificity for targeting DNA sites locating close to core region.

3) Octasome constructed by histone H3-H4 and DNA, possesses different characteristics comparing to nucleosome

H3-H4 tetramer was reported to be more important as orientating the nucleosome positioning, and regulating the nucleosome's structure by high frequency of histone modifications. Here under the in vitro reconstitution system, we confirmed that DNA can wrap with two H3-H4 tetramers to form a stable octasome structure. Based on AFM imaging technology, the clear difference between tetrasome, octasome and nucleosome were demonstrated. Tetrasome showed significant short core DNA region encapsulating with one H3-H4 tetramer, while octasome occupied similar core proteins as nucleosome, but relatively short core DNA length (around 110 bp). These results were further affirmed by investigating the accessibility, both MNase and peplomycin approached to some DNA sites locating in the nucleosome core region where was clearly inaccessible in nucleosome. The two tetramers dissociated from DNA simultaneously around 87 °C without strengthening octasome's thermal stability, which might indicate that two tetramers were simply packaged together into DNA without interconnection.

4) PIP conjugating with HAT activator regulates brain genes' expression

Brain is the critical organ that plays pivotal role in the nervous system by precisely governing other organs in the body. Therefore, malfunction in brain cell`s intricate gene network lead to wide-range of neurological disorders. Synthetic dual-functional ligands targeting specific DNA sequences and histone-modifying enzymes have the capability to achieve regulatory control over multi-gene networks in living cells. CTB-PIP conjugates are such new class of synthetic dual-functional genetic switches that can site-selectively induce active epigenetic marks. Using Microarray and q-PCR studies, here we report new artificial genetic switching molecule (CTB-PIP-L) that trigger upregulation of therapeutically important brain function-related genes in human foreskin fibroblast, including CNTNAP2 and FZD8. Development of such tailor made genetic switches have the potential to restore the complicated disorders in brain cells.