Identification of noncoding RNAs that are essential for epigenetic modifications for cancer treatment

鑑定對表觀遺傳修飾至關重要并有癌症治療潛力的非編碼 RNA

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Recent studies have suggested that non-coding RNAs are involved in controlling epigenetic mechanisms, either directly interacting and regulating the communications between promotor and enhancer or recruiting chromatin modifying complex to change the state of chromatin. Identifying the ncRNA physically associated with specific chromatin modification would help to predicate their potential regulatory functions. Here, we're developing a method to identify chromatin modification-associated lncRNAs.

Specifically, we devised a bifunctional recombinant protein by fusing the peroxidase APEX2 with readers of four different chromatin modification that binds to DNA methylation or histone tri-methylation at H3K4, H3K9, and H3K27 residues. The recombinant proteins were expressed in *E.coli* in a soluble form. As shown in Figure 1A, for example, the recombinant protein TAF3 was extracted, and its size is approximate 55 kD. We also validate the APEX2 peroxidase and reader-recognition activities of the purified protein in vitro (Figure 1B, 1C). To snapshot and profile lncRNA architecture near epigenetic marks of the cell under specific physiological conditions, we treat the cell with formaldehyde and permeabilize the samples with 70% ethanol (EtOH). Subsequently, the formaldehyde/EtOH-treated 293T cells were hybridized with the purified recombinant protein against specific chromatin modification, washed unbound recombinant protein, and exposed to APEX2 substrates, Biotin-phenol or Biotin-aniline, and hydrogen peroxide. Proteins and RNAs that are physically proximal to the chromatin modification would be biotinylated *in situ*. Then, the tagged RNAs could be captured by streptavidin beads and analyzed with RNA sequencing. This method is expected to detect lncRNA physically near specific epigenetic marks. Our approach does not require transfection of genetic perturbation, extending its application to a broader range of cell types and organisms compared with live proximity-labeling methods.

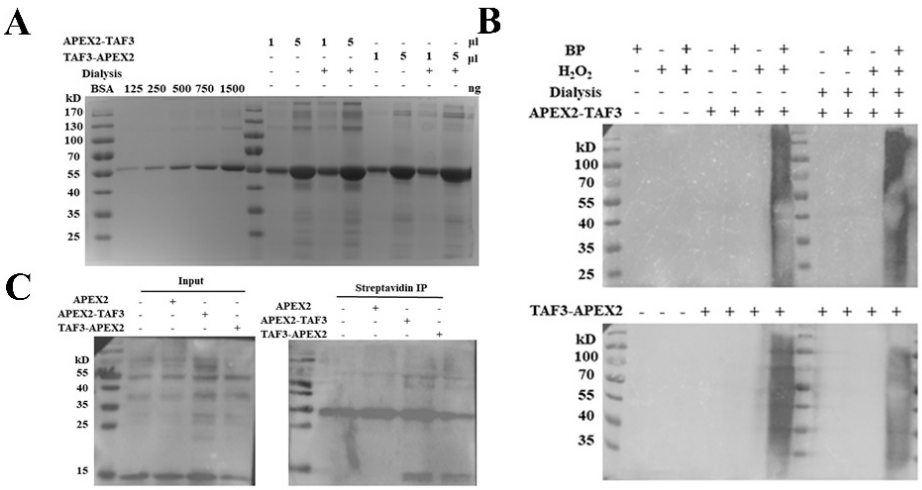

Figure 1(A) Inducible expression of soluble recombinant protein in E.coli bacteria. (B) Western Blot of peroxidase activity of purified recombinant. (C) Proximity labeling analysis by immunoprecipitation via streptavidin beads capture.