【Grant-in-Aid for Scientific Research (S)】

Structural basis of transcription in chromatin

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Purpose and Background of the Research

●Outline of the Research

In eukaryotes, genomic DNA is stored in the cell nucleus as chromatin. The basic unit of chromatin is the nucleosome, consisting of eight core histones wrapped twice by DNA. An array of multiple nucleosomes forms a beads-on-a-string chromatin fiber (Figure 1). All genetic processes occur on this chromatinized DNA, and are regulated by the chromatin structure (*i.e.* epigenetic regulation). RNA polymerase II (RNAP II) synthesizes mRNA, as it passes through such a chromatinized DNA region (Figure 1). The mechanism by which RNAP II transcribes a gene while maintaining chromatin structure and how the chromatin structure and transcription influence each other remain obscure. In this study, we will visualize the structure of higher-order complexes formed during transcription, and elucidate the mechanisms of transcription and regulation in chromatin.

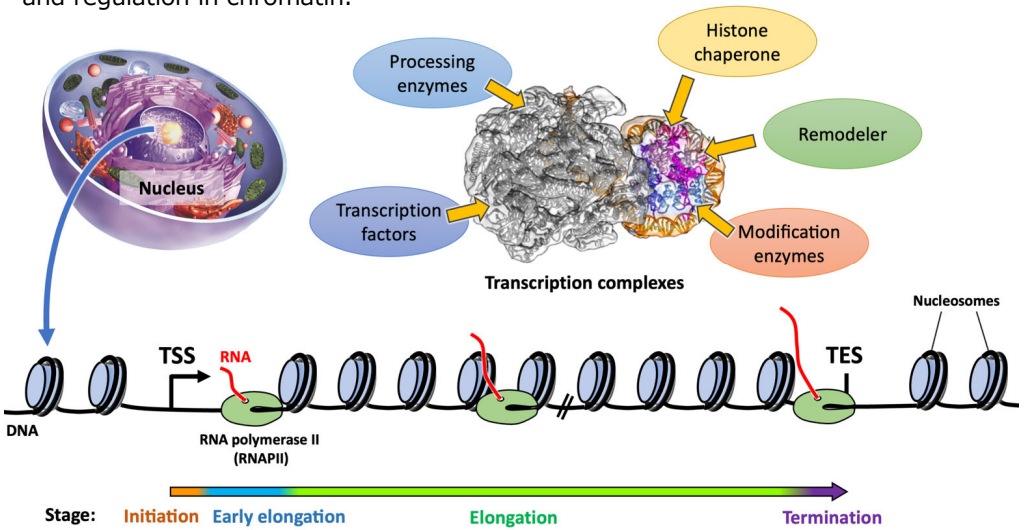

Figure 1. Transcription in chromatin

●Background

Transcription is a highly regulated process consisting of three stages: transcription initiation, elongation, and termination. At each stage, RNAP II interacts with nucleosomes and various protein factors to form huge transcription complexes (Figure 1). For example, the elongation complex of RNAP II is able to transcribe DNA without disrupting the chromatin structure. Transcription is also accompanied by chromatin activation and mRNA processing. However, the molecular basis of such higher-order complexes and the interplay between RNAP II and chromatin in those processes remain poorly understood.

●Methods

We have developed a method to analyze functional transcription complexes by cryo-EM. Transcription is performed on a nucleosomal template DNA by RNAP II. The complex species formed during transcription are analyzed by cryo-EM single particle analysis (Figure 2). We will analyze transcription complexes formed at different stages of transcription to elucidate their molecular mechanisms at high resolution.

●Goals

Through high-resolution structural analysis of functional transcription complexes, we will provide molecular basis of transcription and epigenetic regulation in chromatin.

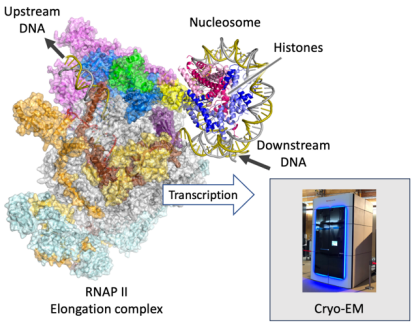

Figure 2. Cryo-EM structure of an RNAP II elongation complex transcribing a nucleosome

Expected Research Achievements

●Structural basis of transcription in chromatin

During gene transcription, RNAP II advances along the chromatinized DNA to read the nucleotide sequence, while maintaining the chromatin structure (Figure 3). Multiple factors, including transcription elongation factors, histone chaperones, remodelers, modification enzymes, RNA processing enzymes, etc. associate with the RNAP II and nucleosomes, to support chromatin transcription, modify chromatin, and process mRNA. We will prepare functional complexes involved in these processes and perform structural and functional analysis to reveal the detailed molecular structures and interactions. This will lead to a comprehensive understanding of the mechanisms of transcription and its regulation in the chromatin environment. Specifically, we will focus on the following points.

(1) Maintenance of chromatin integrity during transcription.
(2) Chromatin activation during transcription.
(3) Checkpoint regulation and mRNA quality control.
(4) Intracellular transcription complexes.

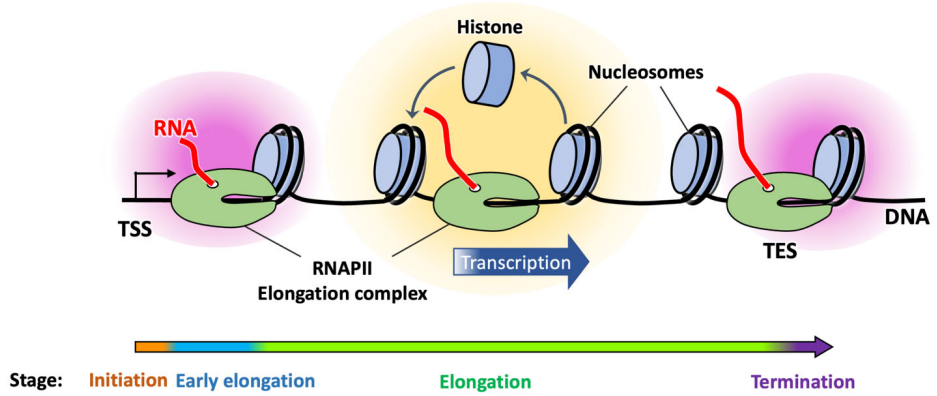

Figure 3. Various transcription complexes formed during chromatin transcription

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