Article

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Using single-molecule approach to visualize the nucleosome assembly in yeast nucleoplasmic extracts

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Abstract

In eukaryotic cells, the smallest subunit of chromatin is the nucleosome, which consists of a segment of DNA wound on histone protein cores. Despite many years of effort, the process of nucleosome assembly and disassembly is still not very clear. Here, we present a convenient method to investigate the process of nucleosome assembly at the single molecule level. We invented a novel system derived from the yeast nucleoplasmic extracts (YNPE), and demonstrated that the YNPE supports the nucleosome assembly under physiological condition. By combining the total internal reflection fluorescence microscopy with microfluidic flow-cell technique, the dynamic process of nucleosome assembly in YNPE was visualized at single-molecule level. Our system provides a novel *in vitro* single-molecule tool to investigate the dynamics of nucleosome assembly under physiological conditions.

Keywords: Nucleosome assembly, Single-molecule, Yeast nucleoplasmic extracts, Total internal reflection fluorescence microscopy, Microfluidic flow-cell

1. Introduction

The Eukaryotic genome is packaged into chromatin for fitting in the nucleus. The fundamental unit of chromatin is nucleosome which consists of an octamer of four core histones (H2A, H2B, H3 and H4) wrapped by 146 base pairs of DNA[1]. The repeating nucleosomes are further compacted into higher-order structures by the linker histone H1[2]. It is well known that the dynamic regulation of nucleosome assembly is a key to controlling multiple biological processes that require access to nuclear DNA, such as transcription, DNA replication, and DNA damage repair [3, 4].