1. Introduction

1-1. How is DNA packed into the cell nucleus?

In eukaryotes, the genomic DNA forms chromatin in the nucleus. There are several folding steps. First, DNA wraps around histone octamer to form a nucleosome. Second, linker histones play a significant role in the higher-order packaging of the “beads-on-a-string” fiber to form the 30 nm fiber. 80-100 nm structures are also identified as an one-step higher unit. In metaphase, chromosome exhibits typical X-shape structure. However, the folding mechanisms are still unknown.

1-2. Nucleosome, a fundamental structural unit of chromosome

(A) DNA of 146 bp wraps around histone octamer (two of each H2A, H2B, H3 and H4) in 1.65 turns to form a nucleosome. This histone octamer has a disk-like shape with the diameter of 6.5 nm and the thickness of 6.0 nm. (B) Possible arrangements of the core (orange) and linker (cyan) histones (LH) are schematically drawn. The linker histone binds to the dyad formed by the linker DNAs that enter into and exit out of the nucleosome. Reconstituted di-nucleosome was imaged by AFM in the absence (C) and the presence (D) of the linker histone.

2. Results

2-1. Effects of high-salt treatment on the chromatin composition and structures of S. pombe.

(A) Procedure for isolating chromatin from yeast cells. (B) CBB-staining on nuclear and chromatin fractions. (C) Time- and concentration-dependent dissociation profiles of the core histone from the chromatin were analyzed by immunoblot analyses using anti-H3 antibody. (D) AFM images of chromatin before the high-salt treatment (a), after 10 min incubation with 1 M NaCl (b), and after 30 min incubation with 1 M NaCl (c).

2-2. Salt-concentration dependency of the chromatin structures of S. pombe.

The chromatin structures were analyzed by AFM after high-salt treatment for 10 min. NaCl concentrations (mM) are indicated in each panel. 40 nm beads were observed in range of 0-300 mM NaCl. By increasing the NaCl concentration beyond 400 mM, beads with ~115 nm diameter were observed in range of 0-300 mM NaCl. By increasing the NaCl concentration beyond 400 mM, beads with ~115 nm in diameter were stably formed (E-K). Scale bars indicate 200 nm. The diameters of the beads were plotted against the concentration of NaCl (L).


(A) Supernatant (S) and pellet (P) were separated after the treatment with different concentrations of NaCl for 30 min (B-E). On-substrate lysis of the nucleus by 400 mM NaCl. The “beads-on-a-string” fibers spread out of the nucleus were visualized. A section profile of the spread fibers (X-Y line in E). The beads diameter was 12.8 ± 2.9 nm (n = 65).

2-4. Schematic presentation of the unfolding-refolding processes of chromatin.

Heat and chicken erythrocyte chromatin commonly possess the 80-100 nm beads and the 30-40 nm beads in the chromatin. Increasing NaCl concentrations affect a folding state of the chromatin. In yeast, ~10 min incubation of chromatin in high NaCl concentrations (400-1000 mM) facilitates the 115 nm chromatin. In yeast, a ~10 min incubation of chromatin in high NaCl concentrations (400-1000 mM) facilitates the 115 nm chromatin. The core histones were released from the chromatin, which might allow the refolding of the chromatin. Indeed, a nucleosome is nearly constant regardless of differences in the nucleosome repeat length (NRL) and the presence of LH. Our and the other groups show that the width of the higher-order chromatin is nearly constant regardless of differences in the nucleosome repeat length (NRL) and the presence of LH. On the other hand, when NLR becomes shorter, LH loses its accessibility to the chromatin, although the higher-order chromatin still can be formed. Thus, LH seems to be required only when NRL is long. In this context, a fundamental issue for the chromatin folding is what controls the nucleosome repeat length. We consider that the core histones would play a role in the length control for the following two reasons. (1) nucleosomes from different organisms exhibit subtle differences in the DNA path around the nucleosome (i) the intra-nucleosomal interactions, and (ii) the configurations of the histone tails. (2) the histone tails themselves may be responsible. The histone tails associate with the linker DNA and contribute to the inter-nucleosomal interactions. Indeed, a major difference in the structural characteristics between yeast and chicken chromatin can be found in their charge density. At pH 7.0, DNA has negative charge of ~2e per base pair, and thus, a yeast nucleosome has negative charge of ~18e, which is larger than that of a chicken nucleosome (~27e). This results in a lower charge density in the yeast chromatin, which might allow the refolding of the chromatin that had been unfolded by the salt, and in turn, the linker DNA length would significantly contribute to the higher-order folding of the chromatin.

3. Discussion

Our and the other groups show that the width of the higher-order chromatin is nearly constant regardless of differences in the nucleosome repeat length and the linker and core histones.