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Effects of spermidine and ATP on stabilities of chromatosomes and histone H1-depleted chromatosomes

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ABSTRACT

It is shown in our FRET studies that both chromatosomes and histone H1-depleted chromatosomes exist in their arm-closed forms in the absence of spermidine. In the presence of spermidine, however, these two types of structural assemblies are converted into their arm-open forms. In addition, ATP as polyanion is capable of suppressing the polycationic effect of spermidine, thus facilitating re-formation of arm-closed forms of these two types of structural assemblies. Our studies therefore illustrate that conversion between arm-closed and arm-open forms of chromatosomes and histone H1-depleted chromatosomes can be manipulated by varying concentrations of polycationic spermidine and polyanionic ATP.

Histones H2A, H2B, H3 and H4 belong to core histone proteins that emerge in equal amount in the eukaryotic cells.[1–3] Two copies of each of these four types of core histones are capable of forming histone octamers that display cylinder-like shapes on the whole.[4–6] Under physiological conditions, DNA is able to wrap itself around this cylindrical structure to form a nucleosome core particle, in which 146 base-pair DNA segments are in close physical contacts with histone complexes.[6–9] Further binding of histone H1 to these nucleosome core particles results in the formation of histone H1-bound nucleosomes,[9–15] which act as the fundamental repeating units of chromatin structures of eukaryotic cells.[16–19] Earlier studies revealed that when chromatins were digested using micrococcal nuclease, stable complexes of (i) ~166 base pairs of DNA, (ii) histone octamers, and (iii) histone H1 were resulted,[11,20–24] assemblies of which are defined nowadays as chromatosomes.[24–28] Spermidine and adenosine triphosphate (ATP), on the other hand, (i) are polycations and polyanions respectively, both of which exists abundantly in the mammalian cells.[29,30] With the aim of understanding how polycationic spermidine and polyanionic ATP affect the structural assemblies of chromatosomes (Structure 2 in Fig. 1) and histone H1-depleted chromatosomes (Structure 1 in Fig. 1), new studies were carried out in our lab recently. Here we report that structural entities of both chromatosomes and histone H1-depleted chromatosomes are susceptible to the presence of spermidine. In addition, ATP could suppress the effects of spermidine to facilitate the re-formation of arm-closed forms of chromatosomes and histone H1-depleted chromatosomes from their arm-open forms. It is also shown in our studies that distances between two termini of DNA in the arm-closed forms of chromatosomes and H1-depleted chromatosomes are ~4.6 nm, which indicates that they reside in a close proximity on the surfaces of histone octamers.

Fig. 1 depicts schematic representations of our preparation of chromatosomes (Structure 2) starting with Sequence 1, which is a 166 base-pair DNA sequence that contains Cy3 and Cy5 fluorophores at its 5′ and 3′ ends separately. This 166 base-pair DNA possesses 146 base-pair "601 element" in the middle of its sequence (Fig. S1), which is known to bind to histone octamers more preferentially than many other 146 base-pair DNA sequences.[31] As shown in Fig. 2a, when Sequence 1 in a solution alone was excited at 500 nm, there was no emergence of signal of fluorescence resonance energy transfer (FRET) at 670 nm, which is the sign that Cy3 and Cy5 reside too far apart to transfer energy to each other in free Sequence 1. Structure 2, on the other hand, is a structural assembly of chromatosome that is composed of Sequence 1, histone octamer and histone H1. Previous studies showed that within chromatosomes, two terminal arms of 166 base-pair DNA segment were in close physical contact with histone octamers, to which histone H1 bound to prevent DNA from hydrolysis by nucleases.[11,20–24] As a result, the two ends of 166 base-pair DNA segment position at an adjacent range on the surfaces of histone octamers within chromatosomes.[11,27] In order to know whether the previously observed compact structures of chromatosomes[24–28] could be measureable by using FRET

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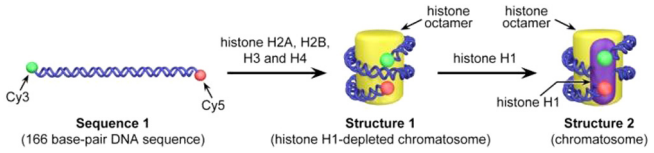

Fig. 1. Schematic illustrations of our preparation of chromatosomes (Structure 2) through formation of histone H1-depleted chromatosomes (Structure 1) as an intermediate (detailed procedures for preparation of Structure 1 and Structure 2 are given in Figs. S1 and S2 in Supplementary Material).

spectroscopy, Structure 2 was excited at 500 nm. As shown in Fig. 2b, intensity of FRET with its maximum at 670 nm was observed from the chromatosomes, which demonstrates that the close distance between the two ends of 166 base-pair DNA in chromatosomes[11,27] is determinable on the basis of fluorescence spectroscopy. Based on (i) the R_0 value for Cy3 and Cy5 pairs reported by Fang et al. in their studies of nucleosome structures (R_0 = 5.4 nm),[32] and (ii) the FRET efficiency shown in Fig. 2b, our calculated distance between Cy3 and Cy5 in Structure 2 is ~4.6 nm (Fig. 2b) according to Förster equation[33–35] (Fig. S2). In addition, with the intention of further knowing how chromatosome (Structure 2) could differ from histone H1-depleted chromatosome (Structure 1) in their FRET spectra, Structure 1 was examined during our investigations as well. As shown in Fig. 2c, FRET spectrum of Structure 1 is nearly identical to the one of Structure 2 (Fig. 2b). This observation indicates that similar to Structure 2, the Cy3 and Cy5 are in close proximity (~4.6 nm) as well on the surface of histone octamers in Structure 1.

With the purpose of knowing how spermidine affects stability of Structure 2, this Sequence 1-containing chromatosome (Fig. 3a) was incubated next with different concentrations of spermidine during our investigations followed by fluorescence spectroscopic examination. As seen in Fig. 3b, FRET intensities of Structure 2 decreased at 670 nm with increase of concentrations of spermidine from 0 mM to 30 mM. These decreases of FRET intensity occurred most likely because polycationic spermidine molecules were in competition (i) with histone H1 for their binding to DNA and (ii) with terminal arms of DNA for their binding to histone octamers, which led to the detachment of two terminal arms of DNA from histone octamers. Once the two terminal arms of DNA were detached from histone octamer and oriented away from each other, the distances between them were beyond the range for energy transfers between Cy3 and Cy5. As a consequence, FRET intensity decreases when the ratio of Structure 2 (arm-closed form) to Structure 3 (arm-open form) decreased (Fig. 3b).

Besides our studies shown in Fig. 3a, effects of spermidine on Structure 1 (histone H1-depleted chromatosomes) were examined as well in our studies. As shown in Fig. 3d, FRET intensities of Structure 1 decreased with the increase of concentrations of spermidine as well. Different from Structure 2 whose FRET intensity at 670 nm vanished at 30 mM spermidine (Fig. 3a), on the other hand, disappearance of FRET intensity of Structure 1 at 670 nm occurred at 8 mM spermidine. The observations shown in Fig. 3b and Fig. 3d

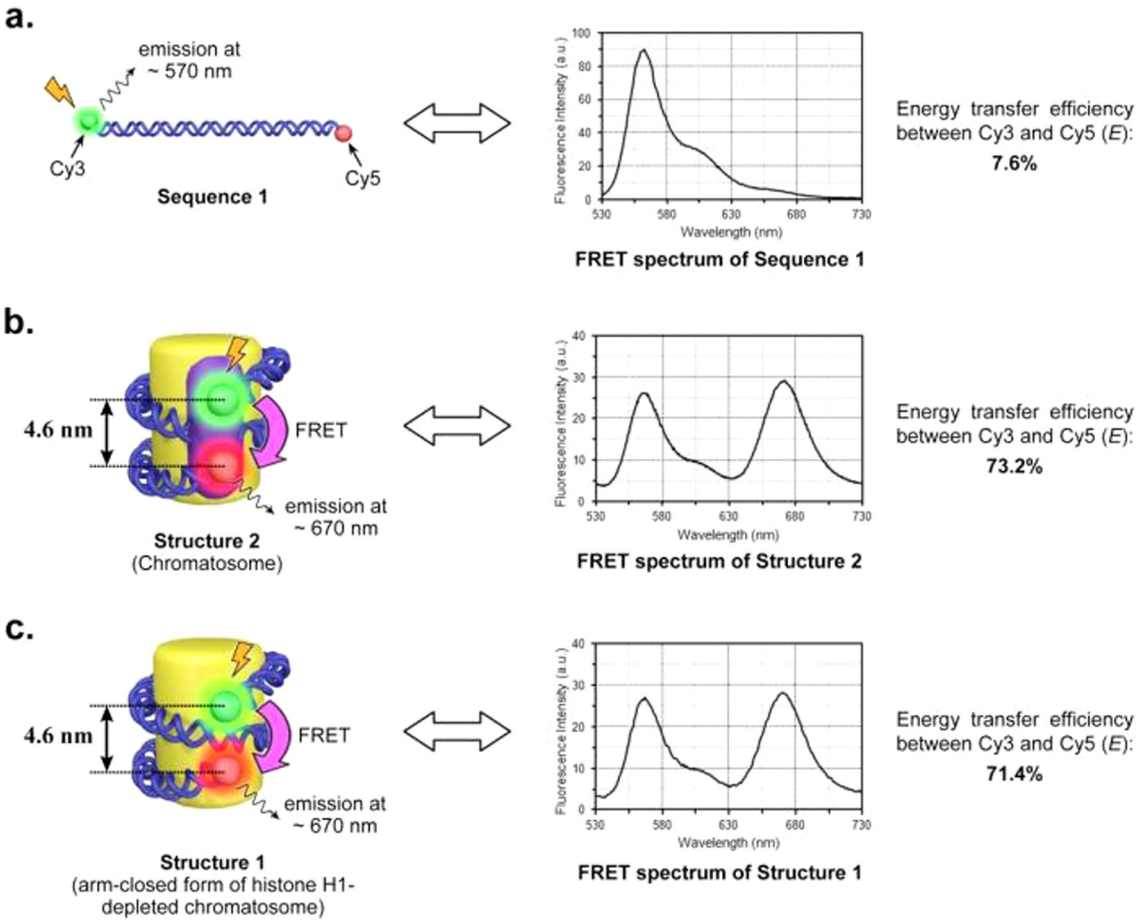

Fig. 2. FRET spectra of Sequence 1 (a), Structure 2 (Sequence 1-containing chromatosomes) (b), and Structure 1 (Sequence 1-containing histone H1-depleted chromatosomes) (c) (detailed procedures for sample preparation and fluorescence spectroscopic examination of the three structural entities are given in Fig. S2 in Supplementary Material).