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Live Dynamics on Femtoinjection of GFP-Tagged Nucleosome Chaperones into HeLa Cell

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1. Introduction

Injection of foreign materials, *e.g.*, genes, enzymes, antibodies, and drugs, into individual cells has found significant applications as an important embodiment of bio-manipulation. Matsuoka *et al.* developed a robot and relevant devices that enabled efficient injection even into individual cells and the quantity of injected material can be controlled at femto gram level [1]. They have succeeded in evaluating the utility of decoy oligodeoxynucleotide delivery via femtoinjection in an embryonic stem cell in which Venus fluorescent protein was expressed under the control of the tet-off system [2].

On the other hand, live dynamics of chromatin can visualized with fluorescent transcription activator–like effectors [3]. We are interested in observing the live dynamics of chromatin by tracing the position of injected fluorescent proteins after the pulse injection into individual cells.

Here, we demonstrate the time sequence of the injected materials into HeLa cells. Femtoinjection with the pulse time of 10 ms enabled to trace where the injected molecules are going from and to. We will also demonstrate the diffusion and reaction constants from the analyses of the time-resolved images with diffusion equations at a cell as a closed system.

2. Results and Discussion

Figure 1 shows the time evolution fluorescent images of green fluorescent protein (GFP)-tagged high mobility group box 1 (HMGB1) [4,5] proteins in a HeLa cell. The injected proteins are immediately spread in the HeLa cell except for the nucleus region in three seconds. Then the proteins gradually penetrated into the nucleus region and most of the proteins are transferred in the nucleus after 50 minutes of the injection. The distribution of the proteins in the nucleus is almost homogeneous during the penetration of the proteins into the HeLa cell, which indicates the diffusion constant of the protein in the nucleus is larger than that through the nuclear membrane.

We will show the diffusion constants of the injected proteins based on the theoretical calculation to explain our results in the presentation.

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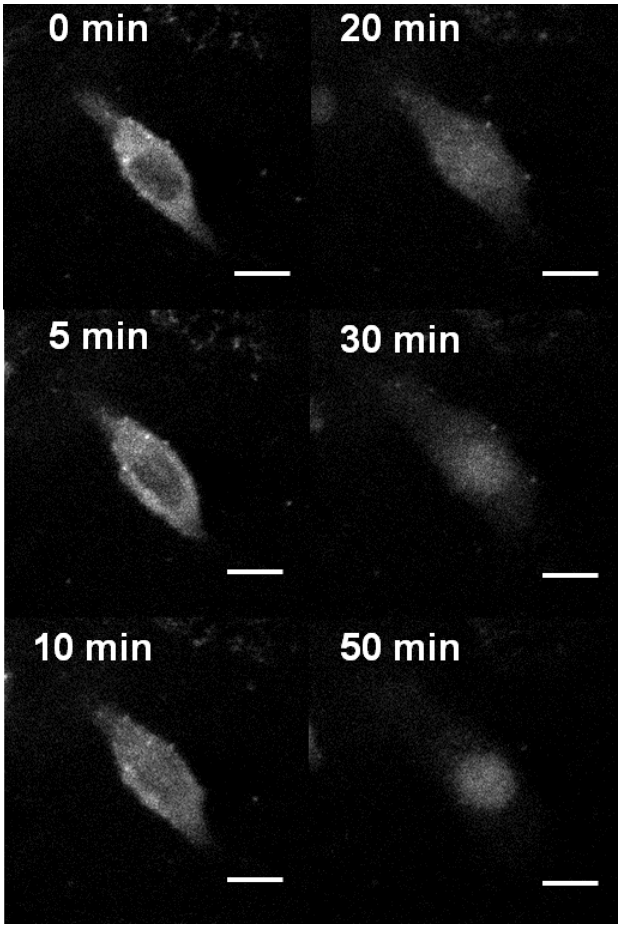

Fig. 1: Series of fluorescent image of GFP (mNeonGreen)-tagged nucleosome chaperones in HeLa cell. Scale bar: 20 μm.

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