Transient binding and non-rotational coupled motion of p53 revealed by sub-millisecond resolved single-molecule fluorescence tracking

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In living cells, DNA binding proteins (DBP) are involved in various cellular processes and are crucial in maintaining and regulating various cellular functions including cell replication and protein biosynthesis. In order to function, these proteins are required to search for and bind to their target DNA sequences quickly and accurately. Failure of the proteins to find and bind their targets may cause diseases, such as cancers, Alzheimer and Parkinson diseases. One example of DBP is tumor suppressor p53 that is responsible in conserving the stability of genomes by preventing genome mutations. It has been proposed that p53 can search for its target efficiently by utilizing the various dynamics along DNA such as 1D sliding, 3D diffusion, and intersegmental transfer. These search mechanisms are collectively known as facilitated diffusion (1).

Single-molecule Fluorescence Microscope (SMFM) is a powerful tool that allow us to directly visualize and analyze the target search dynamics, in particular the 1D sliding and intersegmental transfer, of p53 along DNA. However, many more events postulated in the target search are unobserved due to limited time resolution of the previous SMFM, several tens of milliseconds. For example, many DBPs should be bound to DNA in cells and might interfere with the target search of p53. These DBP might be considered as obstacles for the target search dynamics of p53. p53 might use novel dynamics for the avoidance or bypass the obstacles; however, such dynamics are expected to occur within sub-ms timescale. To resolve this issue, I developed a new SMFM having the time resolution of 0.5 ms, which is 60 times improvement over the conventional SMFM.

Using the new SMFM, I discovered new dynamics of p53 including the transient binding intermediate and the jumps along DNA. The residence time distribution of p53 on DNA obtained under different KCl concentrations in the range from 25 mM to 150 mM showed that more than 88% of p53 once associated to DNA dissociated within a few milliseconds after the association without demonstrating the 1D diffusion. The fraction was independent of the salt concentration. The remaining 12% of p53 bound to DNA for longer periods and exhibited the jumps and the 1D diffusion along DNA. At 150 mM, near the physiological condition, p53 showed the average jump frequency (f) of 6 jump/second and the averaged 1D diffusion coefficient (D) of 1.4 $\mu m^2/s$.

The f and D values showed clear dependency on the salt concentration. Both of the f and D values at 150 mM KCl were roughly 4 times higher than those obtained at 25 mM KCl. The plot of f against D at various salt concentrations showed a strong correlation of 0.85. Considering the salt-dependent 1D diffusion along DNA and the correlation between f and D, we propose that p53 searches for the target DNA with non-rotational coupled motion. These findings suggest that the multiple conformations of p53 caused the observed dynamics and facilitated the target search. This discovery also may serve as an important step in the understanding of how DNA binding proteins search and functions in a living cell, providing the foundation for protein and drug design.

1. Kamagata, K., Y. Itoh, and D.R.G. Subekti. 2020. How p53 molecules solve the target DNA search problem: A review. Int. J. Mol. Sci. 21.