Introduction of the FCS Analysis and Its Application to RCC1 Binding to Chromatin

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Abstract:

A super resolution microscopy, ELYRA, and a confocal laser scanning microscopy, ConfoCor3 LSM780-FCS, have been introduced at Prof. Tashiro's lab. within our project.

In the journal club, firstly, I'm going to introduce fluorescence correlation spectroscopy (FCS). FCS is an experimental technique using statistical analysis of the fluctuations of fluorescence in a system in order to find dynamic molecular events [1, 3, 4]. The autocorrelation analysis provides us with information about the diffusion constant of the fluorescence molecules and the average number of molecules in the laser focal region.

Then I will talk about a recent paper studying RCC1 binding to chromatin by means of FCS [2]. The abstract of the paper as follows: The formation of an activity gradient of the small G-protein Ran around chromatin depends on the differential partitioning of the opposing enzyme activities of the Ran guanine nucleotide exchange factor RCC1 that resides on chromatin, and the cytoplasmic Ran GTPase activating protein RanGAP. We studied the time-dependent interaction kinetics between RCC1 and chromatin and the mobility of the Ran-RCC1 complex in living cells by fluorescence correlation spectroscopy to investigate whether binding of RCC1 to chromatin regulates the exchange activity of RCC1, and whether the stability of the RCC1-chromatin interaction is regulated during the cell cycle. We found that RCC1 mobility is dominated by two states: a highly mobile state that is trapped within chromatin, and a transiently immobilized state that is stabilized during mitosis. We show that only the immobilized state of RCC1 interacts with Ran and conclude that its guanine nucleotide exchange activity is restricted to specific sites on chromatin.

References:

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