



第 271 回つくば分子生命科学セミナー

TSUKUBA MOLECULAR LIFE SCIENCE SEMINAR

演題 : Simultaneous AFM/fluorescence analysis of single molecule interactions in DNA

演者 :

Dr. Humberto Sanchez

Department of Cell Biology & Genetics,
Erasmus MC, P.O.Box, 2040, 3000 CA Rotterdam,
The Netherlands

日時 : 2008 年 11 月 13 日 (木) 17:15-18:15

会場 : 学群棟 4 階 4A411

要旨 : Imaging of processes at the single-molecule level has revealed information otherwise inaccessible by “bulk” experiments. Atomic force microscopy is a powerful technique for studying biomolecular structures with nanometer resolution without necessity of external contrast agents. However, complex genetics transactions require the orchestrated action of different proteins, which would be undistinguishable by conventional AFM. On the other hand, fluorescence techniques can successfully localize proteins *in vivo* and *in vitro*, also for dynamic studies. However, optical resolution limits the details of structural studies in single molecule approaches. We show here the localization with nanometer resolution of recombination proteins using a combined atomic force and fluorescence microscope. Our experiments reveal the versatility of the system in the recognition and simultaneous localization of different fluorescent-tagged proteins on DNA. Moreover, we present the use of fluorescence polystyrene micro-spheres for a reliable alignment of the optical and the topographical images, which together with the DirectOverlay™ system (JPK instruments) have allowed us to precisely localized different proteins with nanometer resolution. By using fluorescence versions of the human recombination proteins RAD51 and RAD54, we could distinguish its relative position on dsDNA. We will show images that precisely correlate topography with fluorescence signal of nucleoprotein filaments, and we will discuss the significance of the data, in relation to the roles played by these two proteins involved in the DNA repair.

連絡先 : 人間総合科学研究科 基礎医学系 大庭良介 (内線 3928)

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