3P-338 蛍光相互相関分光法によるシロイヌナズナのオーキシン・シグナル伝達因子間の分子間相互作用の解析

MEASURING MOLECULAR INTERACTION AMONG AUXIN SIGNALING FACTORS OF ARABIDOPSIS BY FLUORESCENCE CROSS-CORRELATION SPECTROSCOPY

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A plant hormone, auxin, acts in many aspects of plant growth regulation such as development, differentiation, and tropic responses. Auxin can elicit different responses by the use of a combination of auxin response factors (ARFs) and Aux/IAA proteins, it is important to determine interaction between the two protein families in a quantitative manner. We previously expressed the C-terminal protein-protein interaction domains (CTDs) of Arabidopsis ARFs, MP/ARF5 and NPH4/ARF7, and MSG2/IAA19, fused to fluorescent proteins in HeLa cells, and determined their molecular interactions with fluorescence cross-correlation spectroscopy (FCCS)(Muto et al., 2006). We found almost complete association between IAA19 and either ARF-CTD and much smaller association for each homotypic dimerization and between ARF5- and ARF7-CTDs. These results showed that FCCS was a useful physical technique to determine molecular interactions that complemented the use of conventional biological and immunochemical approaches. Aux/IAA also interacts with TOPLESS protein, which represses ARF activity, and auxin signaling F-box (AFB), which promotes proteasome dependent degradation of Aux/IAA. Thus, Aux/IAA is a key factor in auxin signaling pathway. Using FCCS, we plan to measure interaction between Aux/IAA and the other proteins those constitute auxin signaling pathway, and to carry out chemical screening to identify specific inhibitors which may provide us with powerful tools for plant biology and useful agricultural chemicals such as herbicides.

3P-339 Visualization of intrinsically disordered regions of proteins by high-speed atomic force microscopy

Visualization of intrinsically disordered regions of proteins by high-speed atomic force microscopy

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It is known that intrinsically disordered (ID) regions of proteins are involved in various biological processes such as transcription, translation, and cellular signal transduction. Despite the important roles of ID regions, effective methods to directly observe their thin and flexible structures have not been available yet. In this study, we used high-speed atomic force microscopy (AFM) to observe the heterodimeric FACT (facilitates chromatin transcription) protein that is predicted to contain large ID regions in each subunit. In eukaryotic cells, FACT plays crucial roles in elongation of RNA polymerase II. In fact, the predicted ID region of the larger subunit is essential for mRNA transcriptional elongation. FACT also participates in the differential process through chromatin remodeling. Successive AFM images of FACT on a mica surface, captured at rates of 5-17 frames s⁻¹, clearly revealed two distinct tail-like segments that protrude from the main body of FACT and are fluctuating in position. Using deletion mutants of FACT, we identified these tail segments as the two major ID regions predicted from the amino acid sequences. Their mechanical properties estimated from the AFM images suggested that they have more relaxed structures than random coils. These observations demonstrate that this state-of-the-art microscopy method can be used to characterize unstructured protein segments that have been difficult to visualize using other experimental techniques.

2P-342 高圧ジャンプX線小角散乱法を用いた細胞骨格フィラメントの安定性とヌクレオチドキャップの研究

Nucleotide caps and the stability of cytoskeletal fibrous proteins:

A high-pressure small-angle x-ray scattering study

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In the cell, cytoskeletal fibrous proteins play an important role in various cellular processes such as cell division, motility or DNA segregation. Determining underlying physical parameters of filament stability is the first step to understand the molecular functions of these biopolymers *in vivo*. It has been proposed that the presence or absence of nucleotide caps determine the stability or dynamic instability of cytoskeletal biopolymers. We have systematically probed these predictions for four different cytoskeletal proteins: eukaryotic F-actin, microtubules as well as prokaryotic DNA segregation protein ParM and cell division protein FtsZ. Elevating pressure on these fibrous proteins influences nucleotide binding and induces conformational changes of monomers in the filament leading to depolymerization. Our high-pressure small-angle x-ray scattering (HP-SAXS) data indicates that the dynamic instability of both microtubules and ParM results from a loss of their protective nucleotide cap, whereas the properties of treadmilling F-actin or the high subunit turnover of the FtsZ polymer are not governed by ATP or GTP caps. HP-SAXS is also a powerful tool to probe the stabilizing or destabilizing effects of various cytoskeletal binding (CBP's) proteins. On the other hand, increasing osmotic pressures reverses the effect of high hydrostatic pressure, which leads to the formation of lateral bonds between filaments and the formation of complex supermolecular structures.