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Biophysics of Molecular Motors

○ Ishiwata Shin'ichi (Department of Physics, School of Science and Engineering, Waseda University, Japan)

Molecular motors are classified into two categories, i.e., processive and non-processive motors. In the present talk, I would like to summarize our recent studies on 1) the mechanochemical properties of processive motors, i.e., kinesin and myosin V (Oguchi, Y. et al., Poster at this 43rd BSJ Annual Meeting), especially, focusing on the unbinding force (the relationship between the binding lifetime and the externally imposed force) in several nucleotide states, and 2) those of non-processive motors, i.e., myosin VI (Mikhailenko, S. V. et al., *ibid.*) and muscle myosin II, from a single-molecular level to an assembly of motors such as a myofibril composed of an array of myofilaments and a muscle fiber. Recent single-molecular studies have demonstrated that the molecular motors function stochastically on a single-molecular level, whereas cooperatively in an assembly of molecules. As a typical example for the latter, I will describe the spontaneous oscillatory contraction of the contractile apparatus (SPOC) observed in skeletal (Shimamoto, Y. et al., *ibid.*) and cardiac (Sasaki, D. et al., *ibid.*) muscles, especially, focusing on the sarcomere length oscillation synchronized with external mechanical impulse. I will emphasize that the intra- and the inter-molecular synchronizations, respectively, play an essential role in the processive and non-processive motors, through mechanochemical coupling (feedback reaction through the modulation of enzymatic activity by force generation), which is common to both types of synchronization.

S. Ishiwata : Biophysics of Molecular Motors

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位相差顕微鏡の拓く新しい生物イメージング

○永山 國昭 (自然科学機構・岡崎統合バイオ)

In light microscopy, phase contrast method was innovated by a German scientist, Zernike, in such early days as the 1930s. As the quintessence of the German science, the development of electron microscopy was also initiated in the same age and the idea of the combination of both innovations was proposed at the moment not so far from the advent of the first EM machine. Thirty years efforts to develop the idea had deadlocked with a disappointment of no chance to have such an ideal EM and 25 years silence then lasted till our 2001 report. The devastating hurdle to break down our morale has been the issue of charging, which is rather common in electron optics. It takes 6 years and 3 postdocs to find the complete solution to get over the obstacle. With non-charging phase plates, which are made of carbon films, we have developed two kinds of phase contrast EM methods, Zernike phase contrast and Hilbert differential contrast. The latter is the equivalent to the DIC (differential interference contrast) mode in light microscopy. They have been applied to imaging of protein molecules, various organelles, viruses, bacteria and cells. The high contrast imaging for non-stained biological samples with a spatial resolution of 2 to 3 nm is delineating novel aspects of nanometer-sized biological structures. There is, however, a decisive and future threatening issue remained. That is the electron loss due to the beam interception with the phase plate. Our efforts to materialize the non-loss phase contrast EM is also to be reported.

K. Nagayama : Novel Bioimaging Opened up with Phase Contrast Electron Microscopy

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IGF BINDING PROTEINS: FLEXIBILITY AND FUNCTION

○ Norton Raymond¹, Kuang Zhihe^{1,2}, Yao Shenggen¹, Keizer David¹, Wang Chunxiao¹, McNeil Kerrie², Forbes Briony², Wallace John², Bach Leon³ (¹Walter Eliza Hall Inst. Med. Res., ²School Mol. Biomed. Sci., Univ. Adelaide, ³Dept. Endocrinology Diabetes, Alfred Hosp.)

The insulin-like growth binding proteins belong to a family of six binding proteins (IGFBPs 1-6) that act as carriers and regulators of insulin-like growth factor-I and -II (IGF-I/-II). All IGFBPs have three domains of approximately equal size, made up of the conserved Cys-rich N- and C-terminal domains joined by a variable linker domain. The C-domains are not only essential for high IGF binding affinity, but also appear to confer IGF-I vs IGF-II binding specificity and to mediate IGF-independent functions. We have determined the structures of the C-domains of IGFBP-2 and -6. They consist of a thyroglobulin type-1 fold comprising an α -helix, a three-stranded anti-parallel β -sheet, and a disulfide-bonded flexible loop. C-BP-2 contains a longer unstructured loop I and longer flexible extension at the C-terminus. The IGF-II binding site on C-BP-6 was identified by examining NMR spectral changes upon complex formation. ¹⁵N relaxation measurements in both the laboratory and rotating frames for C-BP-6 reveal a good correlation between residues exhibiting motion at sub-ms to ms timescales and those involved in IGF-II binding. This is consistent with emerging evidence that protein dynamics in solution on the μ s-ms timescale may correlate with protein-protein interactions. The possible functional roles of flexibility in both the IGF binding proteins and their ligands will be discussed.

R.S.Norton, Z.Kuang, S. Yao, D.W.Keizer, C.C.Wang, K.A.McNeil, B.E.Forbes, J.C.Wallace, L.A.Bach : IGF BINDING PROTEINS: FLEXIBILITY AND FUNCTION

1SB01

細胞膜所における情報蛋白質 Ras と Raf1 の相互作用の 1 分子反応計測

日比野 佳代、○佐甲 靖志 (阪大院・生命機能・ナノ生体科学)

我々は、細胞内で分子スイッチとして働くといわれる低分子量GTPase Ras と、その相互作用相手 Raf1 の相互作用ダイナミクスとキネティクスを 1 分子解析している。細胞膜に存在する不活性型 (GDP 結合型) の Ras が細胞刺激により GTP 結合型に変化すると、Raf1 との結合性を獲得し、細胞質の Raf1 を細胞膜へ呼び寄せて膜上の未知のリン酸化酵素による Raf1 の活性化を促進するというのが現在主流の考え方である。Raf1 の細胞内局在変化を可視化するために、GFP と Raf1 の融合遺伝子を細胞に発現させた。細胞を上皮成長因子 (EGF) で刺激すると、数分後に細胞膜の広い範囲で GFP-Raf1 の密度上昇が起こる。その後 Raf1 の集積部位の分布は不均一になり、差し渡し数ミクロンの範囲で数カ所、さらに顕著な集積が見られるのに対し、他の細胞膜部位の Raf1 密度は減少していく。Raf1 の膜集積は 20-30 分持続し、やがて反応前と同じく細胞質に戻る。1 分子計測によって、Raf1 の膜集積は動的な準定常状態として維持されており、個々の Raf1 分子は細胞膜と細胞質を行き来していることが分かっている。Raf1 分子の細胞膜滞在時間計測から、結合反応の中間状態が検出された。Raf1 には RBD と CRD と呼ばれる 2 つの Ras 結合ドメインが存在する。Raf1 断片や点突然変異体の結合時間解析から、中間状態は Raf1 の構造変化に伴う Ras と CRD の相互作用変化を反映していると考えられる。また、Raf1 の Ras-GTP 認識には、Raf1 の別の構造変化が必要であるらしい。本講演では 1 分子計測から予想される Ras/Raf1 相互作用と Raf1 の活性化サイクルモデルを提唱し、さらに、Raf1 の局所的集積と Ras/Raf1 相互作用動態の関係について議論したい。

K.Hibino and Y.Sako : Single-molecule kinetics of Ras/Raf1 interactions in limited areas in the plasma membrane