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博士論文の要旨

Actin filaments are involved in many kinds of cell motility and are found in association with the plasma membrane. In interphase eukaryotic cells, two types of well-developed actin filament bundles which contract by themselves in cooperation with myosin filaments are observed: stress fibers and circumferential bundles. They are associated with plasma membranes at the cell-to-substrate and cell-to-cell adherens junctions, respectively. On the other hand in mitotic phase cells, contractile actin bundles, so-called contractile ring is formed just beneath the plasma membrane at cleavage furrow. An obvious function of the association of actin filaments with plasma membrane may be that membranes serve as attachment sites for actin filaments in cell motility system and the maintenance of cell shape. At these attachment sites, actin filaments are associated with plasma membranes unidirectionally polarized, with the arrowheads of myosin heads pointing away from the plasma membrane. However, our knowledge of the proteins responsible for this end-to-membrane association is still fragmentary.

Radixin is a barbed end-capping actin-modulating protein which was first identified in isolated cell-to-cell adherens junctions from rat liver (Tsukita,Sa., Hieda,Y. and Tsukita,Sh. 1989. J. Cell Biol. 108:2369-2382). In the chapter I of my study, I have analyzed the distribution of radixin in dividing cells. For this purpose, a monoclonal antibody (CR-22) specific for radixin was obtained using chicken gizzard radixin as an antigen. By immunofluorescence microscopy with this monoclonal antibody and a polyclonal antibody obtained previously, it was clearly shown that radixin was highly concentrated at the cleavage furrow during cytokinesis in rat fibroblastic cells (3Y1 cells). To precisely pursue the dynamic behavior of radixin during a cell cycle, the 3Y1 cells were partially synchronized and these cells were doubly stained with anti-radixin monoclonal antibody (CR-22) and DAPI. Judging from the appearance of chromosomes, at metaphase the monoclonal antibody began to stain the surface of rounded-up cells in a characteristic manner. As the separation of chromosomes proceeded, the characteristic staining on the cell surface increased in intensity. At the onset of furrowing, radixin appeared to be rapidly concentrated at the equatorial cell surface. Through late anaphase and early telophase, radixin continued to be concentrated at the cleavage furrow, and finally distributed at the midbody at late telophase. In 3Y1 cells, the concentration of radixin at the cleavage furrow was prominent, but the cell surface at the polar region was also stained in a dotted manner, not so intensely. This concentration of radixin at the cleavage furrow was detected in cells bearing cell-to-cell adherens junctions and in cells lacking both cell-to-cell and cell-to-substrate adherens junctions. Furthermore, it became clear that the epitope for the monoclonal antibody was immunofluorescently masked in the cell-to-cell adherens junctions in MDBK cells by performing Ca2+ switch experiments. Together, these results lead me to conclude that radixin is present in the undercoat of the cell-to-cell adherens junctions and that of the cleavage furrow, although their respective molecular architectures are expected to be distinct. The possible roles of radixin at the cleavage furrow are discussed with special reference to the molecular mechanism of the actin filament-plasma membrane interaction at the furrow.

Recently, cDNA encoding mouse radixin was isolated by Funayama et al., showing that radixin is highly homologous to but distinct from ezrin which was first isolated from the microvilli of intestinal epithelial cells and identified as the good substrate of the protein-tyrosine kinase. In the chapter II of my study, I have succeeded in isolating and analyzing cDNA encoding another radixin-related protein. Sequence analysis has demonstrated that this protein is a mouse homologue of human moesin(98.3% identity) which was originally identified as an extracellular protein by Lankes and Furthmayr. The amino acid sequence of moesin shares 71.7% and 80.1% identity with that of ezrin and radixin, respectively. These data led me to conclude that there is a gene family consisting of ezrin, radixin and moesin, and this ezrin-radixin-moesin (ERM) family is included in the band 4.1 "superfamily". Translation experiments in vitro combined with immunoblot analyses showed that ezrin, radixin, and moesin were identical to

the bands of 85 kDa, 82 kDa and 75kDa in SDS-PAGE, respectively. Immunoblot analyses revealed that these members coexpressed in various types of cells. Then, by immunofluorescence microscopy, I closely analyzed their distribution inside cells using polyclonal antibody and monoclonal antibody, which could recognize all three members. In addition to cell-to-cell adherens junctions and cleavage furrows, it was shown that they were concentrated at microvilli and ruffling membranes in various types of cells. Furthermore, the cell-to-substrate adherens junctions (focal contacts) were clearly stained by anti-radixin polyclonal antibody only after the apical/lateral membranes and cytoplasm were removed with the zinc method. I concluded that at least one of the members of the ERM family is concentrated at specific regions where actin filaments are densely associated with plasma membranes. I believe that this study can give us a clue to understanding the molecular basis for the end-to-membrane association of actin filaments with plasma membranes in general.

論文の審査結果の要旨

佐藤成樹君は細胞内情報伝達における細胞骨格の役割に興味を持ち、当大学院大学において主要細胞骨格アクチンフィラメントの細胞膜との結合様式及びその機能的側面を解析してきた。

Adherens Junction （以下ＡＪ）は発達した裏打ち構造を介してアクチンフィラメントが密に細胞膜に結合している細胞接着装置である。ラディキシンはこの細胞間ＡＪにおいてアクチンフィラメントと細胞膜の結合に重要な役割を持つと考えられたタンパク質である。そこで佐藤君はアクチンフィラメントが細胞膜に結合している他の部域においてもラディキシン様タンパク質が存在するのではないかと考え検討してきた。そしてまずモノクローナル抗体を作成しラディキシンが分裂中の細胞の分裂溝にも濃縮していることを明らかにした。この研究成果は彼が第一執筆者として
The Journal of Cell Biology, 113, 1991 に報告している。次に佐藤君はラディキシン、エズリンと高い相同性を持つタンパク質モエシンのcDNAを単離し、その塩基配列を決定した。そしてこれらが一つのファミリー（ ERM ファミリー）を形成していることを明らかにし、さらにこのファミリーがバンド 4.1 スーパーファミリーに属することを示した。また間接蛍光抗体法によりこのファミリーの分子は細胞間ＡＪ、分裂溝だけでなく微絨毛、細胞基質間ＡＪ、ラッフル膜にも存在することを示した。これらのことより彼はＥＲＭファミリーが広くアクチンフィラメントと細胞膜を結合させるタンパク質群であることを明らかにした。この研究成果は彼が第一執筆者として Journal of Cell Science, 103, 1992に報告している。

以上のように佐藤君の研究は細胞生物学において最も重要な課題の一つである細胞間相互作用に深く関与する新たなタンパク質の同定と細胞分裂における存在様式を明らかにしており、優れている。また論文もよくまとまっており考察も深くなされている。英語もわかり易くかかれており彼の語学力の高さを表している。よって佐藤君の論文は充分博士（学術）の学位論文として値する。