（ 続紙 1 ）

京都大学	博士 （工学）	氏名	OKEYO KENNEDY OMONDI
論文題目	Mechanical Regulatory Mechanism of Actin Cytoskeletal Structure Dynamics in Migrating Cells		

（論文内容の要旨）

The present thesis describes the results of quantitative experimental studies focused on understanding the regulatory mechanism of the actin cytoskeletal structure dynamics during cell migration. The actin cytoskeletal structure is a mechanical system whose dynamics is critical for protrusive force generation, as well as cell-substrate interactions during cell migration; a process fundamental to various physiological phenomena, including cancer metastasis, immune response, and tissue and organ morphogenesis. The main body of the thesis consists of 6 chapters as detailed below.

Chapter 1 presents a general and broad background introduction to cell motility, and outlines both the physiological and engineering importance of the process. In addition, the chapter introduces the major cellular components involved in cell migration, and the fundamental steps of the process.

Chapter 2 describes the actin cytoskeleton system with focus on the mechanical aspects of the system that govern its structure-function dynamics during cell migration. The structural features of the system and the biochemical factors that regulate its dynamic reorganization are highlighted. Moreover, molecular forces involved in cell migration are reviewed and correlated with network dynamics. Furthermore, cell-substrate interactions are discussed with emphasis on the relationship between actin network dynamics and traction force development. Finally, the molecular clutch mechanism proposed for the coupling interactions between actin cytoskeleton and the substrate is reviewed, and its importance for the regulation of actin network flow dynamics is discussed.

Chapter 3 focuses on the dynamic coupling between actin network flow and turnover. It describes a unique reductionist approach of using fragments derived from fish keratocytes as simple but elegant motility systems for the quantitative analysis of the actin network dynamics. The chapter highlights the procedures for extracting fragments from fish keratocytes, and also for labeling the actin network with quantum dots for fluorescence speckle microscopy (FSM). In addition, quantitative flow maps of the actin network obtained by particle imaging velocimentry (PIV) based on FSM images are presented. The flow maps reveal the centripetal organization of actin network flow consisting of retrograde flow at the anterior periphery of the lamella, and anterograde flow at the posterior region. Moreover, the flow maps demonstrate that the two flows converge at the middle of the lamella to create a convergence zone of reduced flow intensity, and increased network deformation. Furthermore, the results of network turnover analysis elegantly

reveal that polymerization, which drives protrusion, is predominant in the anterior periphery of the lamella while depolymerization, which recycles monomers, is confined to the posterior region. Thus, it is demonstrated that network flow and turnover are dynamically coupled.

Chapter 4 examines the mechanical significance of actin network deformation for the reorganization of the actin cytoskeleton in the lamellipodia of fish keratocytes. A novel approach is presented that utilizes continuum mechanics to quantitatively evaluate network strain analysis based on actin network flow maps obtained by the procedures described in Chapter 3. The results of this analysis highlight the existence in the lamellipodia of a negative (compressive) strain whose direction is parallel to that of cell movement. Moreover, the distribution of this strain is shown to be closely correlated with that of actin network density in the lamellipodia, suggesting that tension release may stimulate network depolymerization. Based on this finding, a model is proposed for the involvement of negative strain in actin network depolymerization. The model suggests that by coupling with biochemical factors such as cofilin, negative strain, which implies tension release in the actin network, could promote selective depolymerization of actin filaments oriented in the direction of the deformation, since such filaments experience relatively higher levels of the strain. Thus, using the model, the observed reduction in filament density from the leading edge toward the rear of the lamellipodia, as well as the realignment of actin filaments in the lamellipodia of migrating fish keratocytes can be explained.

Chapter 5 investigates the role of mechanical forces generated from the interactions between the actin network and myosin II motors (actomyosin interactions) in modulating actin network dynamics and deformation discussed in Chapters 3 and 4. To do this, the actomyosin system is perturbed by treating keratocytes with calyculin to activate actomyosin interactions, and also with blebbistatin to partially inhibit the interactions. Then, using the techniques outlined in Chapters 3 and 4, it is demonstrated that network flow and deformation directly depend on contractile forces generated by actomyosin contractility. Based on these results, it is proposed that the actin cytoskeletal structure is a spatiotemporally self-regulating mechanical system whose dynamics is modulated by the actomyosin contractility. This is a unique and fascinating concept that is bound to have tremendous impact on the study and modeling of cell migration. In addition, a new mechanism for the mechanical regulation of cell migration is proposed that integrates the different mechanochemical processes of cell migration described in Chapters 3, 4 and 5.

Chapter 6 presents the major conclusions and future prospects of the study. Here, the future directions of mechanobiology as a new field that is expected to discern the complex structure-function relationships undelying many biological processes are addressed.

（続紙 ２ ）

氏　名	OKEYO KENNEDY OMONDI

（論文審査の結果の要旨）

本論文は，様々なタンパク質分子の複雑な連携機構により実現される細胞運動について，その駆動力を生み出すアクチン細胞骨格構造を動的な力学構造体として捉えることにより，同構造の自己組織化過程，および，構造・機能ダイナミクスに及ぼすひずみ等の力学的因子の影響を定量的に調べ，工学的な観点から，細胞運動の力学的制御機構の解明を試みたものである．得られた主な成果は，以下の通りである．

1. まず，細胞運動の単純な系として，細胞の葉状仮足部分を分離した細胞フラグメントを作製し，その主な構成要素であるアクチン細胞骨格構造を quantum dot を用いて蛍光ラベリングすることで可視化した．次に，蛍光スペックル顕微鏡法，および，粒子画像流速測定法を組み合わせて適用することにより，細胞運動に伴うアクチンネットワーク構造の微細な流れ場を定量的に評価した．さらに，流れ場を構成する逆行性流れと順向性流れの時空間的ふるまいが，アクチン細胞骨格構造の重合・脱重合による再構築過程において支配的であることを示した．
2. 移動性細胞の葉状仮足におけるアクチン細胞骨格構造を quantum dot により蛍光ラベリングし，前述の手法を適用して同構造の変位場を求めた．また，同構造のネットワークひずみを定量的に評価し，細胞運動方向に負のひずみ分布が存在することを示した．さらに，負のひずみ分布とネットワーク構造密度の分布との高い相似性により，負のひずみが，アクチンフィラメントの切断・脱重合因子としてアクチン細胞骨格構造の再構築過程に関与する可能性を示した．これらの結果に基づき，アクチンネットワーク構造の再構築過程，および，配向過程における負のひずみの役割を説明できるモデルとして，選択的脱重合モデルを新たに提案した．
3. アクチンストレスファイバー，および，ミオシン II モータータンパク質により構成される移動性細胞の収縮機構に対して，活性・抑制の摂動を与え，それらがアクチン細胞骨格構造ダイナミクスに及ぼす影響を調べた．その結果，アクチンフィラメントとミオシン II の相互作用により生じる収縮力が，アクチンネットワーク構造の流れ，および，ひずみの支配的な力であることを示した．さらに，これらの結果に基づいて，細胞運動過程において，収縮力，アクチンネットワークひずみ等の力学的因子が果たす役割を理解するため，細胞運動の力学的制御機構を系統的にまとめた．

以上のように，本論文は，定量的な実験手法を導入し，工学的なアプローチに基づいてアクチン細胞骨格構造ダイナミクスを評価することにより，細胞運動における同構造のダイナミクスの力学的制御機構を明らかにした．得られた成果は，細胞運動メカニズムの解明に重要な知見を与えるものであり，学術上，当該分野の発展に寄与するところが多大である．よって，本論文は，博士（工学）の学位論文として価値あるものと認める． また，平成 21 年 12 月 24 日，論文内容とそれに関連した事項について試問を行った結果，合格と認めた．