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研究課題名（和文）Modelling of myosin V motor dynamics to understand its ATP-less walking along actin filaments under interactive high-speed AFM

研究課題名（英文）Modelling of myosin V motor dynamics to understand its ATP-less walking along actin filaments under interactive high-speed AFM

研究代表者
ＦＬＥＣＨＳＩＧ　ＨＯＬＧＥＲ（Flechsig, Holger）

金沢大学・ナノ生命科学研究所・特任助教

研究者番号：００７５８９６４

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研究成果の概要（和文）：本研究ではモーターの後ろ側のヘッドがアクチンフィラメントから機械的に切り離されると、ATP分子による化学エネルギーの供給無しにアクチンフィラメントに沿ってミオシンVが歩行するというインタラクティブ高速AFMでの観察の理論的説明に成功した。数理モデル、エネルギー解析、数値シミュレーションを実験結果と統合し、ミオシンVの動作にはATPによる構造変化は必須ではないと結論した。これは現在受入れられているモーターの動作の描像を変えるものである。本研究の結果はナノスケールの分子モーターのエネルギー伝達の一般的な機構について、力学的仕事を生み出すプロセスが化学エネルギーの入力無し進行し得ることを示している。

研究成果の学術的意義や社会的意義
The activity of nanoscale motors has also been linked to processes related to disease and functional disorder. Therefore, the detailed understanding of their operation as obtained in this project for myosin V is potentially important for the development of therapeutic strategies and drug design.

研究成果の概要（英文）：Results obtained within this project successfully explained experimental interactive high-speed AFM observations demonstrating walking the of the myosin V motor along actin filaments in the absence of chemical energy supply by ATP molecules, when the motor trailing head is mechanically detached from the actin filament. By integrating mathematical modelling, analysis of motor energetics, and numerical simulations with experimental observations, this study provides a definite conclusion that ATP-driven conformational motions are not essential for myosin V operation, thus changing the currently accepted picture of motor operation. The results obtained in this project address the general mechanism of energy transduction in nanoscale molecular motors, concluding that the process of generating mechanical work may not involve chemical energy input.

研究分野：Biophysics

キーワード：molecular motors　myosin V　high-speed AFM　molecular dynamics　modelling　energetics

１．研究開始当初の背景

The common understanding of molecular motors asserts that chemical energy from ATP molecules is absolutely essential to generate mechanical work. I.e., the free energy liberated during ATP hydrolysis and inorganic phosphate release drives mechano-chemical motions and generates work which allow unidirectional transport of cargo in cells. However, interactive high-speed atomic force microscopy (HS-AFM) experiments directly visualized successive stepping of the cytoskeletal two-headed molecular myosin V (M5) motor along actin filaments in the absence of any ATP molecules and related conformational motions when the trailing head is mechanically detached from actin by the AFM tip. Such unique experimental observations challenge the currently accepted mechanism of energy transduction in molecular motors.

２．研究の目的

The purpose of this project was to explain new experimental results by performing systematic theoretical investigations and computational modelling simulations of myosin V motor dynamics. Eventually, the project aims were to formulate a unified picture of myosin V mechanism. In a general context, this work is relevant to further understand the fundamental mechanistic principles of force generation in cytoskeletal molecular motors, and, furthermore, will open novel perspectives for future applications of designing biology-inspired artificial molecular motors.

３．研究の方法

(1) A simplified mathematical model of the myosin V motor has been constructed with all model parameters extracted from HS-AFM data.
(2) Analysis of this model allowed to reveal the energetics of individual conformational steps within the myosin V walking cycle.
(3) The dynamics of motor stepping was investigated in extensive numerical Brownian dynamics simulations of the model, and statistical data analysis was performed to provide comparison to experimental data.

４．研究成果

(1) Comparison of ATP-driven and ATP-less myosin stepping

Illustrations are provided in Figure 1. In the ATP-driven case a forward step proceeds through the following sequence: (a) ATP-binding to the trailing head (TH) leads to its detachment from actin, (b) strain release in the leading head (LH) results in the forward lever swing, (c) ATP-hydrolysis in the free head induces the conformational recovery-stroke re-orienting its shape for rebinding to actin, (d) rebinding of the free head to actin takes place, (e) release of inorganic phosphate in the new LH induces the conformational power-stroke and strain accumulation to power the next forward step.
In the ATP-less case a forward step proceeds through the following sequence: (a) the TH is detached by a mechanical perturbation, (b) strain release in the LH results in the forward lever swing, (c) energetically uphill Brownian search brings the unbound head into the shape for rebinding to actin and strain becomes accumulated, (d) rebinding to actin leads to locking of strain in the new LH to power the next forward step.

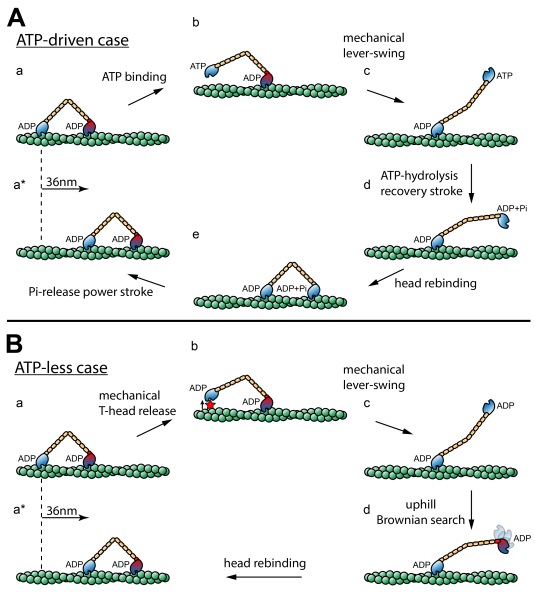

Figure 1: Comparison of ATP-driven with ATP-less stepping of the myosin V motor along the actin filament.

(2) Explanation of experimentally observed ATP-less walking

A forward step of myosin V in the absence of ATP molecules and ATP-related conformational dynamics is explained as follows: (a) Instead of ATP-binding to the TH, the energy input to trigger its release from the actin filament is mechanically applied by strong localized mechanical perturbations by the AFM scanning tip. (b) the TH release allows dissipation of strain energy loaded in the LH into lever swing motions quickly moving the myosin V dimer by about ~36nm towards the forward site. (c) slow Brownian search to bring the free myosin head into an orientation under which it can rebind to actin is compensating the missing ATP-hydrolysis related recovery stroke and leads to accumulation of strain energy. (d) storing this strain energy by binding of the new LH to actin powers the next forward step.

Since for a myosin V step to proceed under ATP-less conditions the external mechanically supplied energy is always larger than the energy temporarily borrowed from the thermal bath during Brownian search, the energetics of ATP-less stepping is perfectly consistent with the second law of thermodynamics.

Results obtained from modelling and numerical simulations are in excellent agreement with experimental HS-AFM data and provide detailed quantitative interpretation.

(a) In the HS-AFM experiments recording only a few hundred on stepping events, a strong preference of myosin V making a forward step was observed after mechanical TH release, while only occasionally no step was observed (step to failure-step ratio 0.925/0.075). By analyzing thousands of stepping events in numerical simulations, modelling results provided a statistically solid confirmation of stepping dynamics (step to failure-step ratio 0.926/0.074) and quantitative interpretation in terms of energy barriers for the stepping events.

(b) Stepping under ATP-less conditions is significantly slower compared to the ATP-driven natural conditions, with the characteristic step time estimated from HS-AFM experiments to be ~133ms. Statistical analysis of results from numerical simulations of our model shows remarkable agreement for the characteristic step time (~134ms). The explanation is that slow Brownian search to bring the free myosin head into an orientation under which it can rebind to actin has to compensate the missing ATP-hydrolysis related recovery stroke.

(3) Conclusions of performed research

Results obtained within this project successfully explained experimental interactive high-speed AFM observations demonstrating walking the of the myosin V motor along actin filaments in the absence of chemical energy supply by ATP molecules, when the motor trailing head is mechanically detached from the actin filament. By integrating mathematical modelling, analysis of motor energetics, and numerical simulations with experimental observations, this study provides a definite conclusion that ATP-driven conformational motions are not essential for myosin V operation, thus changing the currently accepted picture of motor operation. The results obtained in this project address the general mechanism of energy transduction in nanoscale molecular motors, concluding that the process of generating mechanical work may not involve chemical energy input.

(4) Impact and future prospects

The HS-AFM technology developed by Toshio Ando (Kanazawa University, Japan) allows to directly observe conformational dynamics of proteins under near-physiological conditions. Employing this technique and extending it further to actively manipulate proteins during their function lead to unprecedented insights into the operation of the myosin V molecular motor, which could not have been obtained by other single-molecule experimental methods. Complementing experimental observation by mathematical modelling and computational simulations a new view on energy transduction in nanoscale molecular motors has emerged within this project. This project results may therefore facilitate new research projects aiming to deeper understand fundamental aspects of the working mechanism of biological nanomotors or inspire the design of artificial molecular motors.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　5件）

１．著者名 Romain Amyot、Noriyuki Kodera、Holger Flechsig	４．巻 7
２．論文標題 BioAFMviewer software for simulation atomic force microscopy of molecular structures and conformational dynamics	５．発行年 2023年
３．雑誌名 Journal of Structural Biology: X	６．最初と最後の頁 100086～100086
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.yjsbx.2023.100086	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Romain Amyot, Arin Marchesi, Clemens M Franz, Igancio Casuso, Holger Flechsig	４．巻 18
２．論文標題 Simulation atomic force microscopy for atomic reconstruction of biomolecular structures from resolution-limited experimental images	５．発行年 2022年
３．雑誌名 PLoS Computational Biology	６．最初と最後の頁 e1009970
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pcbi.1009970	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Flechsig Holger、Toshio Ando	４．巻 80
２．論文標題 Protein dynamics by the combination of high-speed AFM and computational modeling	５．発行年 2023年
３．雑誌名 Current Opinion in Structural Biology	６．最初と最後の頁 102591
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.sbi.2023.102591	査読の有無 有
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１．著者名 Toshio Ando、Shingo Fukuda、Kien X Ngo、Holger Flechsig	４．巻 53
２．論文標題 High-Speed Atomic Force Microscopy for Filming Protein Molecules in Dynamic Action	５．発行年 2023年
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1．著者名 Romain Amyot、Kaho Nakamoto、Noriyuki Kodera、Holger Flechsig	4．巻 10
2．論文標題 Predicting the placement of biomolecular structures on AFM substrates based on electrostatic interactions	5．発行年 2023年
3．雑誌名 Frontiers in Molecular Biosciences	6．最初と最後の頁 1264161
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fmolb.2023.1264161	査読の有無 有
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〔学会発表〕　計10件（うち招待講演　10件／うち国際学会　0件）

1．発表者名 Holger Flechsig
2．発表標題 HFSP progress report
3．学会等名 HFSP Meeting: Self-organization and biomechanical properties of the endosomal membrane（招待講演）
4．発表年 2022年

1．発表者名 Holger Flechsig
2．発表標題 Molecular modeling of protein machinery and simulation atomic force microscopy for interpretation of limited-resolution Bio-AFM imaging
3．学会等名 1st Workshop on Computational Biophysics of Atomic Force Microscopy（招待講演）
4．発表年 2022年

1．発表者名 Holger Flechsig
2．発表標題 Simulation atomic force microscopy for interpretation and quantitative validation of resolution-limited Bio-AFM imaging
3．学会等名 AFM BioMed 2022 Nagoya/Okazaki（招待講演）
4．発表年 2022年

１．発表者名
Holger Flechsig

２．発表標題
Simulation atomic force microscopy for analysis of imaging

３．学会等名
Bio-SPM Summer School 2022, Kanazawa University（招待講演）

４．発表年
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１．発表者名
Holger Flechsig

２．発表標題
Simulation atomic force microscopy

３．学会等名
5th NanoLSI Symposium - Understanding nanoscale biological processes in the cells（招待講演）

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Holger Flechsig

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１．発表者名
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２．発表標題
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１．発表者名
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３．学会等名
Bio-SPM Summer School 2023, Kanazawa University（招待講演）

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関