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研究課題名（和文）ATP-dependent coupling of pre- and post-synaptic organelles movements at central synapses and its implication in synaptic plasticity and transmission

研究課題名（英文）ATP-dependent coupling of pre- and post-synaptic organelles movements at central synapses and its implication in synaptic plasticity and transmission

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研究成果の概要（和文）：マウスとヒトのニューロンの活性ミトコンドリアによって生成される細胞内濃度ATPは、液相分離（LPS）を介してシナプス前放出機構と軸索タンパク質の溶解性を維持するために極めて重要です。 光退色実験後の蛍光回復は、ミトコンドリア阻害後の細胞内ATPの減少が、軸索末端サイトゾル、シナプス小胞、およびアクティブゾーンコンポーネントの凝縮につながり、機能組織シナプスを制御することを示しています。 PD、AD、ALSに関与する精製タンパク質は、ATP濃度依存性LPSを示します。 PDおよびALS患者からのヒトニューロンは、細胞内ATPの有意な減少と相関する軸索細胞質ゾル液相の一貫した減少を示します。

研究成果の学術的意義や社会的意義
Future research to improve mitochondria activity and ATP production might contribute to the development of novel therapeutics to prevent or alleviate protein aggregation before the apparition of severe synaptic defects and pathological symptoms in PD, AD or ALS.

研究成果の概要（英文）：Here, I present compelling evidences showing that intracellular concentration ATP produced by active mitochondria in mouse and human neurons is pivotal to maintain the presynaptic release machinery and axonal proteins solubility via liquid phase separation (LPS). Fluorescence recovery after photobleaching experiments demonstrate that decrease in intracellular ATP after mitochondria inhibition, leads to condensation of axo-terminal cytosol, synaptic vesicles, and active zone components and control the functional organization of mouse calyceal synapses. In vitro experiments on purified proteins involved in PD, AD and ALS show they all undergo ATP concentration-dependent LPS at different ATP concentration. Human iPSC-derived neurons from PD and ALS patients show consistent reduction in axonal cytosol fluid phase correlated with significant reduction in intracellular ATP.

研究分野：Neuroscience

キーワード：liquid phase separation　ATP　synapse　neurodegeneration

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Biological functions are highly regulated by liquid phase separation of proteins and molecules within cells and in extracellular environment. Neurodegenerative diseases, such as AD, PD, or ALS, involve synaptic dysfunctions and protein aggregation (Tau, α-synuclein, TDP-43), where these proteins switch from soluble form to insoluble aggregates and perturbate normal cell functions. Deciphering the mechanisms underlying soluble/insoluble phase transition in physiological and pathological conditions will thus improve our ability to understand and potentially develop treatment for these diseases often associated with aging. Recently it has been shown that ATP hydrotropic activity play critical roles in the regulation of biocondensate phase changes *in vitro*. In addition, mitochondrial activity, and thus ATP production, are significantly altered in aged brain or in mouse model of PD. Thus, I proposed to analyze how mitochondrial activity and ATP concentration can modify intracellular biocondensate phase changes during neuronal development and how these transitions in molecules liquid/solid phases play critical roles in synaptic organization and in the physiopathology of neurological disorders such as PD or ALS. These findings could potentially lead to the development of new approaches and treatments for PD and ALS, in which protein aggregations could be reversed by promoting mitochondrial activity or increasing ATP concentration.

２．研究の目的

ATP has long been thought to act as the energy source for cell functions, however its hydrotropic property of has only been discovered recently and essentially assessed by *in vitro* experiments. To the best of my knowledge, my research proposal and results have demonstrated that the hydrotropic activity of ATP in cultured neurons is a key regulator of synaptic protein liquid phase transition, and that biocondensate phase separation is critically dependent on mitochondrial activity in healthy as well as in diseased neurons. Thus, the main purposes of my project were: 1) To correlate mitochondria activity, ATP production and liquid phase separation in presynaptic terminals. 2) To show that components of the presynaptic release machinery undergo ATP-dependent liquid phase separation. 3) To link the ATP-dependent reorganization of the presynaptic release machinery with post-synaptic relocation of AMPARs. 3) To determine whether ATP-dependent liquid phase separation is affected or perturbated in human iPSC-derived neurons from healthy individuals or PD and ALS patients. 4) To estimate the concentration of ATP necessary to solubilize protein condensates *in vitro*. The completion of this project has established a new paradigm regarding the dual functions (energetic and hydrotropic) of ATP in neuronal biology and during neurodegeneration.

３．研究の方法

My research strategy and methods essentially relied on fluorescence live cell imaging of neuronal protein liquid phase separation in mouse cultured neurons and in human iPSC-derived neurons with real time observation of mitochondrial activity and local ATP concentration. Briefly, fluorescently tagged proteins of interest were overexpressed in mouse

or human neurons using electroporation or AAV and their fluidic state was monitored by fluorescence recovery after photobleaching (FRAP) analysis. Mitochondrial activity was monitored in real time using fluorescent membrane potential sensor TMRE and local ATP concentration measured using ATP/ADP fluorescent sensor PercevalHR and by *in vitro* bioluminescence assay. Condensation/decondensation of proteins in solution was also observed and analyzed by confocal microscopy in absence or presence of different concentration of ATP.

４．研究成果

The main research outcomes of this proposal are as followed:

A) Functions of ATP-dependent LPS in synapse organization

1) Mitochondria activity and ATP production in mouse giant presynaptic terminals

I showed that mitochondria activity (monitored with fluorescent dye TMRE) in presynaptic area is transient both in resting conditions and during synaptic stimulations, and the presence of active mitochondria in presynaptic regions correlated with higher solubility of cytosolic GFP (measured by FRAP cGFP).

2) Mitochondria activity regulates liquid phase separation of presynaptic release machinery

Next, I showed that blocking mitochondria activity by FCCP bath application in mouse cultured neurons drastically impaired normal liquid phase separations of peri-active zone cytosol (cGFP), active zone protein CFP-RIM1 and synaptic vesicle pool VNS-VGluT2, as observed by significant reduction in fluorescence recovery and decrease in their mobile fraction during FRAP experiments.

3) ATP rescues abnormal liquid phase separation in FCCP treated terminals

I also observed that mitochondria activity (monitored by TMRE) regulates local ATP concentration (monitored by PercevalHR fluorescent ATP/ADP sensor) in presynaptic terminals and that blocking mitochondria activity with FCCP drastically decreased presynaptic ATP concentration locally.

I further demonstrated that increasing the concentration of ATP in FCCP-treated synapses culture, gradually restored normal liquid phase separation of cGFP in presynaptic terminals.

4) ATP-dependent LPS promotes relocalization of active release sites during synaptic stimulations

High synaptic activity is often reported to be associated with significant reduction in ATP and this localized loss of mitochondria activity and ATP near release sites (monitored by TMRE or PercevalHR) was also confirmed in our synapse cultures.

I further observed that repetitive electrical stimulations promoted the translocation of synaptic vesicles (SVs) as well as post-synaptic receptors (AMPARs) from their initial release sites toward new release sites with higher mitochondria activity.

It is important to note that energy-dependent long-distance trafficking of vesicles was not

abolished by the diminution in ATP concentration observed during FCCP treatment, suggesting that ATP energetic processes were preserved while ATP hydrotropic activity was significantly reduced.

Taken together this data showed that the reduction in mitochondrial activity and the concomitant local decrease in ATP promoted the condensation/solidification of the components of the presynaptic release machinery. I proposed that ATP-dependent LPS might be biological mechanism by which release sites can be switched ON and OFF.

B) Role of ATP-dependent LPS in neurodegeneration

1) Neuronal protein condensation/decondensation sensitivity to ATP in vitro

Next, I observed that neuronal proteins such as synapsin-1 as well as numerous proteins involved in neurodegenerative diseases (synuclein, Tau, etc…) also formed phase-separated condensates in solution.

I demonstrated that the addition of ATP to the protein solution prevented the initial formation of these bio-condensates and promoted the decondensation of existing protein aggregates. I also showed that the condensation/decondensation of these proteins is ATP-concentration dependent and that the sensitivity to ATP varied from protein to protein.

This *in vitro* data strongly supported the results obtained in mouse calyceal synapses and also suggested the potential and critical implication of ATP-dependent LPS in neurodegenerative diseases.

2) LPS and ATP production are impaired in human iPSC-derived neurons from PD and ALS patients

Finally, I showed that the cytosolic fluid phase in axons and the intracellular concentration of ATP are significantly reduced in cultured neurons derived from iPSCs obtained from patients with PD compared to healthy individuals. The reduction of ATP and cytosolic fluidity observed in human cultured neurons from PD patients was similar to the decrease observed in mouse calyceal synapses, indicating a conserved mechanism among mammals.

Interestingly similar observations were obtained in cultured neurons from ALS patients, suggesting that ATP-dependent liquid phase separation is a common molecular mechanism regulating protein aggregation observed in various neurodegenerative diseases.

C) Conclusions

Taken together, the results of this research project provide compelling evidence that the intracellular concentration of ATP plays a critical role in the regulation of neuronal protein LPS both in physiological and pathological conditions. This molecular mechanism appears to be conserved in mammalian neurons and among major neuropathologies such as PD and ALS. In addition, ATP-dependent liquid phase separation of presynaptic machinery and postsynaptic density might be a key regulator of trans-synaptic organization, synaptic homeostasis, and function. Finally, mitochondrial dysfunctions observed in neurodegenerative diseases such as PD and ALS might promote proteins aggregation via perturbation in their ATP-dependent LPS. Thus, protein LPS driven by mitochondria activity and ATP may be an essential regulatory mechanism to maintain neuronal functions.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　5件）

１．著者名 Guillaud Laurent、El-Agamy Sara Emad、Otsuki Miki、Terenzio Marco	４．巻 13
２．論文標題 Anterograde Axonal Transport in Neuronal Homeostasis and Disease	５．発行年 2020年
３．雑誌名 Frontiers in Molecular Neuroscience	６．最初と最後の頁 NA
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fnmol.2020.556175	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 Mohamed Mahmoud Shaaban、Hazawa Masaharu、Kobayashi Akiko、Guillaud Laurent、Watanabe-Nakayama Takahiro、Nakayama Mizuho、Wang Hanbo、Kodera Noriyuki、Oshima Masanobu、Ando Toshio、Wong Richard W.	４．巻 256
２．論文標題 Spatiotemporally tracking of nano-biofilaments inside the nuclear pore complex core	５．発行年 2020年
３．雑誌名 Biomaterials	６．最初と最後の頁 120198～120198
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１．著者名 Piriya Ananda Babu Lashmi、Wang Han-Ying、Eguchi Kohgaku、Guillaud Laurent、Takahashi Tomoyuki	４．巻 40
２．論文標題 Microtubule and Actin Differentially Regulate Synaptic Vesicle Cycling to Maintain High-Frequency Neurotransmission	５．発行年 2019年
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１．著者名 Dimitar Dimitrov, Laurent Guillaud, Kohgaku Eguchi and Tomoyuki Takahashi	４．巻 1727
２．論文標題 Culture of mouse giant central nervous system synapses and application for imaging and electrophysiological analyses	５．発行年 2018年
３．雑誌名 Methods in Molecular Biology	６．最初と最後の頁 201-215
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-1-4939-7571-6_15	査読の有無 有
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１．著者名 Lokesh Agrawal, Menouer Saidani, Laurent Guillaud, Marco Terenzioa	４．巻 131
２．論文標題 Development of 3D culture scaffolds for directional neuronal growth using 2-photon lithography	５．発行年 2021年
３．雑誌名 Materials Science and Engineering: C	６．最初と最後の頁 NA
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.msec.2021.112502	査読の有無 有
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２．論文標題 Microtubule assembly by tau impairs endocytosis and neurotransmission via dynamin sequestration in Alzheimer’s disease synapse model	５．発行年 2022年
３．雑誌名 eLife	６．最初と最後の頁 NA
掲載論文のDOI（デジタルオブジェクト識別子） 10.7554/eLife.73542	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

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　1件）

１．発表者名 Laurent Guillaud
２．発表標題 ATP-dependent liquid phase transitions in synapse organization and neurodegeneration
３．学会等名 2nd Osaka University/OIST Symposium
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３．学会等名 Gordon Research Conference "Cell Biology of neuron"
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１．発表者名 Laurent Guillaud
２．発表標題 ATP-dependent phase transitions of peri-active zone and active zone bio-condensates regulate synapse organization and function
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〔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