様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Critically testing magnetoreception mechanisms at the cellular level

研究課題名（英文）Critically testing magnetoreception mechanisms at the cellular level

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研究成果の概要（和文）：本研究では，生体系の磁場感受能力におけるラジカル対の役割を探求し，磁場が細胞生化学に影響を及ぼすメカニズムを批判的に評価することを目的とした．成果の1つ目は，未処理の生細胞の自家蛍光に対する磁場効果の初の直接観測である．2つ目は，自動多波長測定を実現する時間分解光吸収磁場効果顕微鏡の開発である．これは，異なる波長の光の吸収特性に基づいた過渡的なラジカル対の同定を可能にする．3つ目は，短寿命のラジカル対を数十ナノ秒の時間分解能で直接検出できるパルスレーザーと磁場を用いた新しい蛍光イメージング技術の原理実証である．これは，生物学におけるラジカル対の研究に革新をもたらす可能性のある技術である．

研究成果の学術的意義や社会的意義
Since the discovery of magnetism, humans have been fascinated with the idea that magnetic fields might affect the human body. This research provided the first direct evidence of magnetic fields directly influencing chemical reactions occurring in living cells through the formation of radical pairs.

研究成果の概要（英文）：This work aimed to investigate the role of radical pairs in the magnetic sensing ability of living systems and to critically evaluate the mechanisms by which magnetic fields can affect cellular biochemistry. The first achievement was the first direct observation of the effect of a magnetic field on the autofluorescence of untreated living cells. Second was the development of new instrumentation to allow automated multiwavelength measurements in our unique time-resolved optical absorption magnetic field effect microscope. This allows transient radical pairs to be identified based on their characteristic absorption of light at different wavelengths. Third was the demonstration of proof-of-principle of a new pulsed laser and magnetic field based fluorescence imaging technique. This allows the direct detection and characterisation of short lived radical pairs on timescales of tens of nanoseconds and is a potentially transformative technique for the study of radical pair effects in biology.

研究分野：Spin Chemistry and Biology

キーワード：磁気受容　細胞の自家蛍光　ラジカル反応　磁場効果　発光分析　スピン化学

１．研究開始当初の背景

Spin correlated radical pairs are currently hypothesized as responsible for the remarkable ability of many animals to sense and respond to the earth's magnetic field. They have also been proposed as providers of a general interaction mechanism by which magnetic fields might influence cellular biochemistry. However, to date there have been no direct experimental observations of RP processes in cells and experimental magnetic field studies on the proposed sensor proteins for avian navigation, the cryptochromes, exist only *in vitro* for purified proteins and the observed effects are far from matching the responses required for the avian compass to function with its observed acuity.

２．研究の目的

The goal of this research was to enhance and extend the unique instrumental approaches developed in our laboratory and employ them to critically examine biological magnetoreception mechanisms in living cells. Specifically, the aim was to examine the possibility of cellular magnetic field responses in both untreated living cells and also in cells transfected with cryptochrome proteins. Such measurements provide a new means to address the unresolved question of how weak magnetic fields can have significant effects in biology.

３．研究の方法

The key approaches taken to undertake the research were development of microscope imaging-based instrumentation, cell biology techniques including cell transfection, imaging of photochemical reactions at the cellular level based on fluorescence and transient optical absorption microspectroscopic methods and theoretical analysis of spin dynamics of radical pairs using Liouville-space density matrix techniques.

４．研究成果

1)　*Multi-wavelength transient optical absorption detection microscopy*

In order to investigate magnetic field sensitivity at the level of cellular photochemistry, the direct detection of species involved in the photochemical reaction cycle is necessary. As part of this research project, we made advancements to two distinct microspectroscopic techniques: Transient Optical Absorption Detection (TOAD) microscopy and Fluorescence Microscopy. The advancements made to TOAD are described below while those made to the Magnetic Field Effect Fluorescence Microscope (MFEFM) are described in the later sections.

One of the main aims of this project was to add multi-wavelength detection capabilities to TOAD to allow spectroscopic identification of radical pair (RP) species. In the original implementation, photoexcitation is provided by a 450nm laser pulse and transient radicals are observed based on their absorption spectra at longer wavelengths. A single continuous wave laser operating at 532nm serves as the probe beam. The original proposal was to replace the probe beam with a supercontinuum (SC) light source along with a wavelength separator, functioning as a tunable pseudo-CW monochromatic light source. Three different SC sources were borrowed from manufacturers and tested; however it was soon discovered that while the

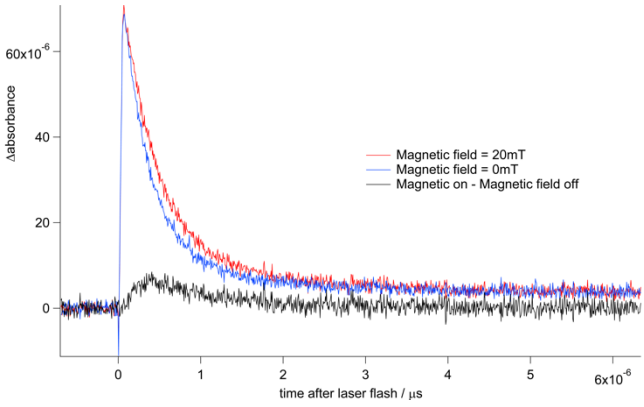

Figure 1. Transient absorption signals and the magnetic field induced absorption difference for a pH2.3 aqueous solution of 200 micromolar FAD using a path length of 2.5 μm recorded using a probe wavelength of 594nm.

current generation of these devices have a stable average power output over millisecond timescales, their shot-to-shot variability, and thus power output on short timescales is highly variable. As such, the SC sources proved incapable of providing a sufficiently stable probe beam to allow TOAD to function at an appropriate level of sensitivity (with the existing laser source, it can resolve magnetic field induced changes in absorption signals on the order of 10^{-7}). It should be noted that no manufacturers of SC systems provide public domain information about the shot-to-shot stability of their systems and so the only way to discover this issue was to test the systems in our instrument.

In order to address the situation, we abandoned the idea of using a SC source and instead elected to build an optical system capable of automatically switching between 5 different probe beam wavelengths (from a pool of 7) by employing a series of highly stable diode lasers. The wavelengths selected were 405nm, 488nm, 505nm, 532nm, 561nm, 594nm and 633nm, with the possibility to add additional longer wavelengths in the future (the selected wavelengths being particularly useful for flavin-based RP studies). This was the only approach using currently available light sources that could allow TOAD to operate at its current sensitivity. The system was constructed and optimised, including the use of a piezo-controlled rotation stage controlled variable beam splitter to allow auto-balancing of probe and detector beam intensities into the balanced detector along with auto switching of band pass filters for each wavelength to minimise early time fluorescence signals. New control software was written in Labview including new efficient waveform capture routines which reduce the signal capture time by around four times. A new laser triggering solution allowed us to reduce the minimum pulse laser width from 300ns to 50ns. Figure 1 shows example transient absorption signals (with and without magnetic field) and the magnetic field induced absorption difference for an acidic solution of flavin adenine dinucleotide (FAD) using a path length of 2.5 micrometers recorded using a probe wavelength of 594nm. The instrument is now ready for use with cellular samples.

2) *Magnetic Field Effects in living cells*

The most significant contribution made during this project was the publication of the very first observation of RP based magnetic field effects in living cells. One of the primary goals of this project was to study magnetic field sensitive RP chemistry at the cellular level and this was successfully demonstrated in the autofluorescence of untreated, unmodified HeLa cells. The work was originally reported in the Proceedings of the National Academy of Sciences in January 2021 and the publication received substantial scientific and media interest worldwide, being featured in a large number of news articles and interviews leading to editorials and feature articles, extensive twitter coverage, an interview with the PI on German National Radio inclusion in an episode of PBS Spacetime on US television and a highly read and commented sub-reddit.

In order to make these observations, the MFEFM was highly optimised to the autofluorescence signals produced by blue light excitation of HeLa cells. Most important was the unequivocal demonstration that the microscope system showed no artefacts (many published MFE

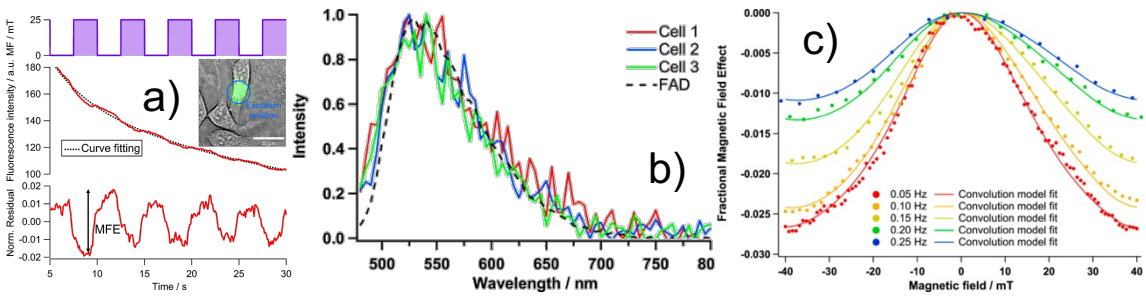

Figure 2. a) The effect of a 25 mT magnetic field on the autofluorescence from a single untreated HeLa cell (PBS buffer at pH 7.4). b) Fluorescence spectra of autofluorescence captured from 3 individual HeLa cells irradiated with 450nm light, with a FAD spectrum captures for control and comparison. c) Magnetic field dependence (MARY) curves captured for 5 different field seep frequencies. Analysis with a convolution model to compenstate for slow kinetic processes allowed extraction of a $B_{1/2}$ value of 18.0 mT and a saturation value of the magnetic field effect of 3.7%.

observations in biological systems have been the result of sample heating or unwanted influence of the magnetic field on the detection system (e.g. photomultiplier tubes are quite magnetic field sensitive)). By performing an extensive range of control measurements, we demonstrated that the observed cellular magnetic field responses could not arise from the instrumentation and must be a result of the cellular photochemistry. In addition, we added a monochromator based spectrometer system to the microscope to allow the fluorescence spectrum of the cells to be recorded which provided additional evidence that it was flavins in the cells whose fluorescence was modulated by the magnetic field.

We developed a linear magnetic field sweep method that could be applied to single cells to allow the cellular response at varying magnetic field strengths (the MARY curve) to be extracted. The variability of signal from cell to cell meant that recording different magnetic field strengths for different cells was unviable. In order to compensate for the slower kinetic steps in the reaction pathway, a convolution based analysis method was developed, that allowed the true magnetic field dependence to be cleanly extracted. Figure 2a shows the change in cellular autofluorescence for a square wave field modulation. Figure 2b shows the fluorescence spectra recorded from HeLa cells with FAD as a reference and figure 2c shows a series of MARY curves recorded at differing magnetic field sweep frequencies along with the convolution based fittings, allowing the true magnitude and $B_{1/2}$ values to be extracted.

The significance of this observation along with the extensive interest and manpower challenges associated with the Covid-19 pandemic led to the decision to shift the research focus from the proposed work involving transfection of cryptochromes into cells and employing ROS sensor proteins to developing new methods to provide detailed RP spectroscopic and dynamic information described below.

3) *Obtaining time-resolved dynamic information on RPs using fluorescence microscopy*

Having observed RP based magnetic field responses on the autofluorescence of untreated living HeLa cells, it became apparent that current existing fluorescence microscopic methods are incapable of providing structural or dynamic information about the RPs involved. In general, the magnetic field dependence can be used to extract some magnetic information about the radicals comprising the pair, but the information is obscured by the effects of incoherent electron spin relaxation when the RP lifetime is long. In addition as the excitation light is applied continuously and the fluorescence signal arises from the depopulated ground state, relying on the complete reaction cycle, no direct information about the kinetics of the RP can be obtained. In order to address this problem, we proposed a new technique involving pump and probe laser pulses of the same colour in combination with a rapidly switched (10s of nanoseconds) magnetic field to allow direct probing of RP lifetime and to allow separation of the coherent and incoherent contributions to the magnetic field dependence.

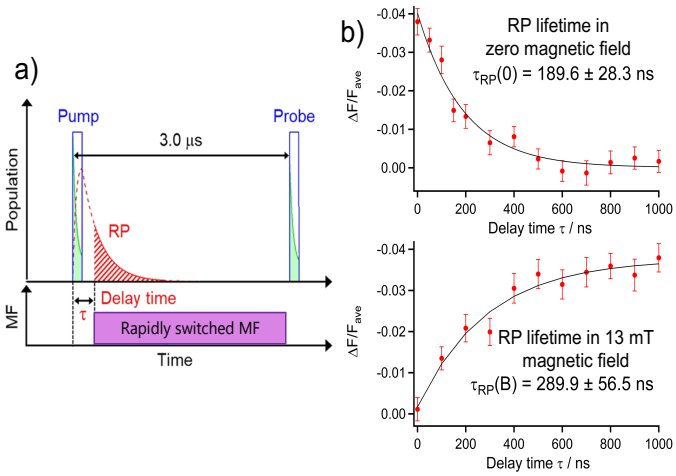

Figure 3. a) Excitation scheme involving single colour pump and probe laser pulses and a rapidly switched magnetic field with timing controlled relative to the laser pulses. b) Proof-of-principle measurements used to measure the lifetime of RPs generated from the photoexcitation of FAD (10 µM) with tryptophan (300 µM) in zero and applied magnetic fields obtained using field on-off and off-on switching schemes.

Figure 3a shows the excitation scheme used in our proof-of-principle experiment to test the viability of this approach. One laser pulse excites the system and generates RPs, simultaneously

depopulating the ground state of the precursor. The second pulse is a probe pulse which reports on the level of ground state depopulation based on the fluorescence signal. In addition, a magnetic field can be applied at any point during the photoreaction and the timing of both the probe laser pulse and magnetic field can be controlled relative to the photexcitation pulse. This allows detailed dynamic information on the RP to be measured directly. By repeating this process after all the photochemistry is complete and averaging the total observed fluorescence signal using the microscope's highly sensitive sCMOS camera allows for kinetic information on the timescale of 10s of nanoseconds to be extracted using imaging times of hundreds of milliseconds. The rapidly switched field is generated using custom circuitry developed previously by the PI.

Figure 3b shows the results obtained for a test system involving the photoexctiation of FAD in the presence of tryptophan. Switched field induced changes are observed in agreement with theoretical predictions and the RP lifetime can be extracted in both the presence and absence of a magnetic field by using on-off and off-on magnetic field switching schemes. This proof-of-principle investigation successfully demonstrates the efficacy of this technique which shows great promise for the study of RPs using fluorescence microscopy for cellular measurements and beyond. Work is now underway to improve the robustness, signal-to-noise, time resolution, flexibility and user interface of this technique and associated data analysis, and also to implement measurements and variable fields to allow the extraction of RP magnetic parameters. The proof-of-principle results have been presented at key international research conferences and a publication is currently in preparation.

4) *Theoretical studies on zero field triplet RP eigenstates*

A key aim of this work was to provide direct insight into the operation of the RP mechanism in cryptochrome and how it can explain the remarkable acuity of the avian magnetic compass. One idea for testing was that specific RP eigenstates might allow for particularly effective low field responses (through the so-called LFE - low field effect). To test this hypothesis, theoretical simulations were undertaken on cyclic RP reactions and the discovery was made that in zero field, some RPs generated in the triplet state never underwent coherent spin-state mixing to the singlet state. Application of even a very weak magnetic field allows these states to begin such mixing meaning that they can be trapped in zero field and then released when a weak magnetic field is introduced. Thus in a cyclic RP reaction they behave as a reservoir of states sensitive to weak fields. A simple pictorial model was introduced to explain these states and how they can be used to explain the LFE in a very straightforward manner. By counting the number of these states for a given RP system, the maximum possible field low and high field responses can be determined. To this end, an algorithm was determined to rapidly determine the number of these states for RPs with many nuclei. Work on the basic mechanism was published in the Journal of Magnetic Resonance and a second publication is currently in preparation.

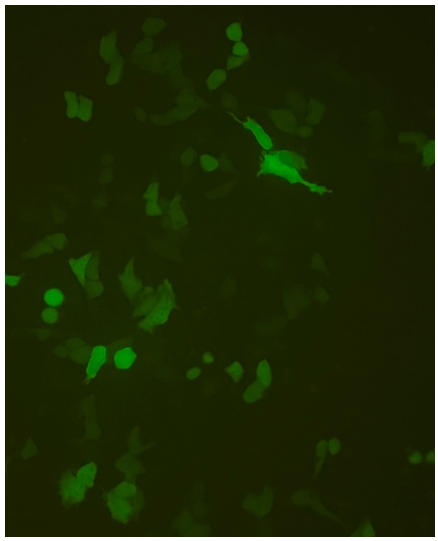

Figure 4. Epi-fluorescence image of HeLa cells transfected with the enhanced green fluorescence protein (EGFP) gene.

5) *Reinitiation of cell transfection experiments*

With a reduction in Covid-19 restrictions and the return of international students to Japan, work has recently recommenced on the originally proposed cell transfection experiments. Figure 4 shows HeLa cells transfected with a fluorescent probe (enhanced green fluorescent protein) demonstrating the reestablishment of functional cell / molecular biology protocols in the laboratory. With the upgraded TOAD microscope and new pulsed fluorescence imaging techniques, measurements will now be undertaken with cryptochrome transfected cells as originally proposed. The improvement of the techniques that has taken place during the project means that more sophisticated measurements will now be possible in these systems.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　2件）

１．著者名 Ikeya Noboru、Woodward Jonathan R.	４．巻 118
２．論文標題 Cellular autofluorescence is magnetic field sensitive	５．発行年 2021年
３．雑誌名 Proceedings of the National Academy of Sciences	６．最初と最後の頁 e2018043118
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.2018043118	査読の有無 有
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１．著者名 Woodward Jonathan R.	４．巻 54
２．論文標題 Triplet Born Radical Pairs and the Low Field Effect	５．発行年 2022年
３．雑誌名 Applied Magnetic Resonance	６．最初と最後の頁 47～58
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s00723-022-01485-9	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Woodward Jonathan R.、Ikeya Noboru	４．巻 -
２．論文標題 Radical pair based magnetic field effects in cells: the importance of photoexcitation conditions and single cell measurements	５．発行年 2022年
３．雑誌名 bioRxiv	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2022.11.09.515724	査読の有無 無
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 Woodward Jonathan R.	４．巻 8
２．論文標題 Animal magnetism: how magnetic fields can influence chemistry in living cells	５．発行年 2022年
３．雑誌名 TheScienceBreaker	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.25250/thescbr.brk635	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 ウッドワード ジョナサン，池谷 皐	4．巻 115
2．論文標題 生物と磁場とラジカル対機構	5．発行年 2023年
3．雑誌名 放射線化学	6．最初と最後の頁 11-23
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計19件（うち招待講演　6件／うち国際学会　4件）

1．発表者名 Jonathan R. Woodward
2．発表標題 Next generation magnetic field effect fluorescence microscopy: toward applications in nanoscience and life science
3．学会等名 ISMAR APNMR NMRSJ SEST 2021（国際学会）
4．発表年 2021年

1．発表者名 Jonathan R. Woodward
2．発表標題 Radical pair based magnetic field effects on cellular photochemistry
3．学会等名 Cell Bio Virtual 2021（招待講演）（国際学会）
4．発表年 2021年

1．発表者名 Noboru Ikeya
2．発表標題 生物の磁気感受性機構を細胞内で探る
3．学会等名 第94回日本生化学大会（招待講演）
4．発表年 2021年

１．発表者名
Jonathan R. Woodward

２．発表標題
Spin dynamics of cyclic photochemical radical pair reactions

３．学会等名
Kanto Area Spin Chemistry (KASC) meeting 2021（招待講演）

４．発表年
2021年

１．発表者名
Jonathan R. Woodward

２．発表標題
Radical pair based magnetic field effects on the autofluorescence of living Cells

３．学会等名
BfS International Workshop : Impact of electric, magnetic and electromagnetic fields on oxidative stress（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Through a glass brightly: radical pairs under the microscope

３．学会等名
Big Quantum Biology Meetings, UK（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Radical Pair Based Magnetic Field Sensitivity Of Native Cell Autofluorescence

３．学会等名
電子スピンサイエンス学会2020

４．発表年
2020年

１．発表者名
Jonathan R. Woodward

２．発表標題
Magnetic field sensitivity of cellular photochemistry

３．学会等名
第58回日本生物物理学会年会BSJ2020（招待講演）

４．発表年
2020年

１．発表者名
Jonathan R. Woodward

２．発表標題
Magnetic field effects on the autofluorescence of living cells

３．学会等名
2020年光化学討論会

４．発表年
2020年

１．発表者名
Jonathan R. Woodward

２．発表標題
Fluorescence Microscopy of Cyclic Radical Pair Based Photochemical Reactions

３．学会等名
7th International Symposium on Spin and Magnetic Field Effects in Chemistry and Related Phenomena, Evanston, USA

４．発表年
2022年

１．発表者名
Noboru Ikeya

２．発表標題
Time-resolved magnetic field effect fluorescence microscopy

３．学会等名
Annual Meeting of the Japanese Photochemistry Association

４．発表年
2022年

１．発表者名
Noboru Ikeya

２．発表標題
時間分解磁場効果蛍光顕微鏡の開発

３．学会等名
量子生命科学先端フォーラム

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Revealing the potential of quantum mechanics hiding in biology

３．学会等名
2022 Daesung Haegang Microbes Forum, Seoul, Korea

４．発表年
2022年

１．発表者名
Noboru Ikeya

２．発表標題
Time-resolved magnetic field effect fluorescence microscopy for direct measurement of transient radical pairs

３．学会等名
第61回電子スピンサイエンス学会年会

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Zero magnetic field eigenstates of radical pairs and implications for magnetoreception

３．学会等名
第61回電子スピンサイエンス学会年会

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Visualizing the low field effect

３．学会等名
Kanto Area Spin Chemistry (KASC) meeting 2022

４．発表年
2022年

１．発表者名
Jonathan R. Woodward

２．発表標題
Microspectroscopic Studies of Photochemically Generated Radical Pairs:

３．学会等名
1st Gordon Research Conference on Quantum Biology

４．発表年
2023年

１．発表者名
Noboru Ikeya

２．発表標題
Next Generation Magnetic Field Effect Microscopy for Direct Observation of Spin-Correlated Radical Pairs in Living Systems

３．学会等名
1st Gordon Research Conference on Quantum Biology

４．発表年
2023年

１．発表者名
Jonathan R. Woodward

２．発表標題
Quantum Biology: radical pairs under the microscope

３．学会等名
Okinawa Institute of Science and Technology MiS seminar series

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

Clubhouse event for Quantum Photonics Club
https://www.clubhouse.com/club/quantum-photonics
Magnets dim natural glow of human cells
https://www.u-tokyo.ac.jp/focus/en/press/z0508_00158.html
生きた細胞内で生体分子の磁気感受性を直接観測 動物の磁気受容メカニズムの解明へ大きな前進
https://www.u-tokyo.ac.jp/focus/ja/articles/z0508_00093.html
Scientists Observe CellsRespondingToMagneticFields
https://www.forbes.com/sites/davidbressan/2021/01/08/scientists-observe-cells-responding-to-magnetic-fields-for-first-time/?sh=7b85cd4e4c87
Magnetic field can influence cells
https://www.express.co.uk/news/science/1380217/earth-magnetic-field-navigate-globe-animals-birds-chemical-reaction-evg
Scientists observe ‘quantum sense’
https://www.rt.com/news/511905-quantum-sense-observed-first-time/
2022 DAESUNGHAEGANG MICROBES FORUM https://www.youtube.com/live/_Rn2YerxD5s
Do Bird Migration and Childhood Leukemia Have Something in Common?
https://doi.org/10.1017/S155192952100064X

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