＜2021年度（９月期）　生命機能研究科博士論文公聴会について＞

生命機能研究科では、下記日程で博士論文公聴会を開催いたします。
ご興味のある方は、ご参加ください。

開催日程：２０２１年８月２７日（金）１３時～
開催場所：システム棟２階セミナー室
【必ずマスク着用してください。】

プログラムは以下のとおりです。
【プログラム及び要旨集について、当日、印刷したものは用意いたしませんので、
参加される場合は、各自、必要に応じて印刷したものをご持参のうえ、
ご参加ください。】

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２０２１年度（９月期）生命機能研究科博士論文公聴会プログラム
The schedule of Dissetation Defense 2021 (Sep.)

（実施会場：生命システム棟２階セミナー室）
(Place: The seminar rooms (2F) at the BioSystems Building)

セッション Session	日程 Date	時間 Time	氏名 Name	論文題目 Title of the Dissertation	主査 Chair	副査１ Vice Chair 1	副査2 Vice Chair 2	副査3 Vice Chair 3	座長 Chairman
1	8月27日	13:00-13:20	ｼﾝ ｷｺﾞｳ SHEN YIGANG	Microfluidic platforms for thermal convection-based manipulation and microcasting system (熱対流ベースの操作およびマイクロ鋳造システム用のマイクロ流体プラットフォーム)	上田　昌宏	石島　秋彦	深川　竜郎	田中　陽	上田　昌宏 長澤　丘司
		13:20-13:40	ｶｷﾂﾞｶ ﾀｲｼ 垣塚　太志	社会性アメーバにおける時空間自己組織化過程の定量トランススケール解析 （Quantitative trans-scale analysis of spatiotemporal self-organization in social amoeba）	柳田　敏雄	近藤　滋	上田　昌宏	平岡　泰	
		13:40-14:00	ﾌﾞｰ ｺﾝ ｸｧﾝ VU CONG QUANG	Development of genetically encoded temperature indicators for intracellular thermometry at the subcellular level (細胞以下レベルの温度計測のための遺伝子的にコードされた温度指示薬の開発)	永井　健治	上田　昌宏	平岡　泰	石井　優	
		14:00-14:20	ｻﾝﾁｪﾝｺﾜ ｸｾﾆｱ SANCHENKOVA KSENIA	miR-215 regulates the homeostasis of small intestine （ miR-215は小腸の恒常性維持に重要な役割をはたす)	岸本　忠三	長澤　丘司	山﨑　晶	鈴木　一博	
		14:20-14:40	ｷﾞｬﾇ ﾊﾟﾗｼﾞｭﾘ PARAJULI GYANU	Arid5a augments tryptophan metabolism and chemokine expression to promote the immune evasion of mesenchymal tumor subtypes. （Arid5aはトリプトファン代謝とケモカインの発現を増幅することによって腫瘍の免疫からの回避を促進する）	岸本　忠三	長澤　丘司	石井　優	山本　雅裕	
		14:40-15:00	**審　査　会　Board of Examiners**						

Form 3

Abstract of Thesis

Name (SHEN YIGANG)	
Title	Microfluidic platforms for thermal convection-based manipulation and microcasting system (熱対流ベースの操作およびマイクロ鋳造システム用のマイクロ流体プラットフォーム)

Abstract of Thesis

This thesis describes the applications of particle manipulation based on thermal convection in microfluidics and a microfluidic system based on microcasting technology in biological field. Microfluidics refers to the science and methods that control, manipulate, process, and analyze small amounts of fluids. This technique is revolutionizing the traditional methods and equipment with the great advantages of the miniaturization ability, saving time, space, reagent, and cost, together with accelerating reaction rate and enhancing the sensitivity. The main proposal of this thesis is to explore this new manipulation method by thermal convection in microfluidics, develop integrated microfluidic and microcasting systems, and show the new opportunities in the biological field. Therefore, this research is both of an exploratory nature and practical application. In the initial part of this research, I investigate thermal convection in a static fluid and develop a contactless manipulation platform based on thermal convection. The platform combines microheaters and an area cooling system to precisely steer sedimentary particles or cells through thermal convection. Results of a cell viability test confirmed the method's biocompatibility. In the second part, I explore thermal convection in a continuous fluid and present an integrated microfluidic system based on thermal convection to realize 2D/3D manipulation. By increasing the temperature of the microheater, the direction of particle motion changes from the horizontal direction to the vertical direction. Based on the principle, a polydimethylsiloxane (PDMS) microchannel with multi-outlets in different height is developed to demonstrate the application for 2D/3D manipulation. Third, I develop a microcasting system in microfluidics for cell patterning. The microcasting system is based on a degassed PDMS microchannel without any pumps to control the flow. Through experiments and simulations, the pressure change, geometry parameters, and viscosity effect are clarified. Long-time cell patterning is archived by using the cross-linked albumin as the casting material for cell culture. Overall, I develop the microfluidic platform based on thermal convection for particles/cells manipulation and microcasting system for cell culture, which extends the way of manipulation in microfluidics and showcases successful application in biological process.

様式３

論　文　内　容　の　要　旨

氏　　名　（　　垣塚　太志　　）	
論文題名	社会性アメーバにおける時空間自己組織化過程の定量トランススケール解析

論文内容の要旨

自然界には多数の要素が相互作用した結果として、集団レベルでの動的なパターンを自発的に形成する例が普遍的に観察される。回転螺旋はその代表的な例であり、興奮子を構成要素とした集団において生じる信号波伝播の時空間自己組織化パターンである。信号波伝播の回転螺旋化は、心疾患である頻脈や心室/心房細動などのヒトの生命にかかわる疾患や、癲癇などの重篤な脳疾患とも密接に関連していることから、その形成メカニズムに対する研究が盛んにおこなわれてきた。一般的に、回転螺旋は「伝播波が断片化される」ことによって引き起こされることが、実験的・理論的に示されている。一方で、「どのように自発的な断片化を引き起こしているのか」に関しては、多細胞システムに内在する多様性との関連が示唆されるものの、その関係性は明らかになっておらず、自発的な回転螺旋形成メカニズムは不透明なままである。

そこで本研究では、伝播波の自発的な回転螺旋化を実験的に再現できる多細胞システムのモデルとして、社会性アメーバである細胞性粘菌集団のcAMP波伝播を選択して、回転螺旋の自発形成メカニズムの解明に取り組んだ。細胞性粘菌がどのように波を断片化しているのかという問題に取り組もうと思うと、cAMP波が作り出す数ミリから１センチメートル程度の空間スケールの動態と、その構成要素となっている単一細胞レベルの挙動を同時に観察することが不可欠であった。本研究では、私も共同研究者として開発に携わったトランススケールスコープであるAMATERAS1.0を用いることで、1cm^{2}を超える視野を、単一細胞レベルの空間分解能で、定量的にcAMP発火動態解析することを実現した。この定量トランススケール解析の結果、細胞集団が場の興奮性のマクロスケールな空間構造を自発的に形成していく様子を可視化することに成功した。さらに、この興奮性のマクロ構造が足場になることで、単に波を断片化するだけでなく、波の伝播経路形成と整流を行うことで、効果的に回転螺旋を自己組織化していることを明らかにした。

興味深いことに、この興奮性のマクロ構造を自発形成する過程では、自発的に発火を繰り返すことができる１％以下の細胞群だけではなく、まだ集団としては発火応答しない時間から自発発火細胞に応答できる少数のフォロワー細胞が、重要な役割を担っていることを見出した。そこでは、フォロワー細胞が発火強度を上げることで、その方向に存在する細胞集団の応答を誘導する事が分かり、それは単一細胞レベルでも引き起こせることが分かった。そして、ある局所の細胞集団が一度発火応答を開始すると、発火回数依存的に、その発火感度と発火強度を増幅していくことがわかり、このフィードバックによって興奮性のマクロ構造が形成されていくことを明らかにした。

これまでに、細胞の多様性とマクロスケールなcAMP波伝播動態がいかに連動しているのかという問に対して、様々な仮説が立てられてきたが、観察技術に課題があり、それらの実証は実現されていなかった。本研究によって、この問いに対する実験的な知見を提供することに初めて成功し、その結果、回転螺旋の自己組織化メカニズムに対して、従来議論されてきた仮説とは異なる、新しい解釈を提示することができたと言えるだろう。

Form 3

Abstract of Thesis

Name （VU CONG QUANG）	
Title	Development of genetically encoded temperature indicators for intracellular thermometry at the subcellular level (細胞以下レベルの温度計測のための遺伝子的にコードされた温度指示薬の開発)

Abstract of Thesis

Genetically encoded temperature indicators (GETIs) allow the measurement of temperature dynamics at a subcellular resolution in live cells. However, GETIs have suffered from low temperature sensitivity when comparing to other nanothermometers, e.g., chemical-based fluorescent nanothermometers. To clearly visualize heat production from a biological process, it is highly desirable to develop GETIs that exhibit high temperature sensitivity for accurate temperature measurement as well as high specificity to temperature. In this thesis, I present two GETIs: (1) **E**lastin-**L**ike **P**olypeptide based **TEMP**erature indicator (**ELP-TEMP**) with the highest ever temperature sensitivity among the existing fluorescent nanothermometers, and (2) **B**lue-excited **g**enetically encoded **TEMP**erature indicator (**B-gTEMP**) that responded specifically to the temperature. ELP-TEMP is comprised of a temperature-responsive elastin-like polypeptide (ELP) fused with a cyan fluorescent protein (FP), mTurquoise2 (mT), and a yellow FP, mVenus (mV), as the donor and acceptor, respectively, of Förster resonance energy transfer (FRET). At elevated temperatures, the ELP moiety in ELP-TEMP undergoes a liquid-liquid phase transition leading to an increase in the FRET efficiency. In HeLa cells, ELP-TEMP responded to the temperature from 33 to 40 °C with a maximum temperature sensitivity of 45.1 ± 8.1 percent signal change per one degree Celsius (%/°C), which was the highest ever temperature sensitivity among the existing fluorescent nanothermometers. Although ELP-TEMP showed sensitivity not only to temperature but also to macromolecular crowding and self-concentration, I was able to correct the output of ELP-TEMP to achieve accurate temperature measurements at a subcellular resolution. I successfully applied ELP-TEMP to measure temperature changes in live cells induced by a local heat spot, even if the temperature difference was as small as <1°C, and to visualize heat production from Ca^{2+} influx induced by a chemical stimulation. Furthermore, I investigated temperatures in the nucleus and cytoplasm of live HeLa cells and found that their temperatures were almost the same within the temperature resolution of the measurement. This result would provide important information to shed light on a controversy about a discrepancy between a theoretical calculation and experimental measurements of intracellular temperature changes in single cells. On the other hand, B-gTEMP is a chimera of a green FP, mNeonGreen (mNG), showing low temperature sensitivity of −0.7%/°C, and a red FP, tdTomato (tdT), showing the highest temperature sensitivity of −2.9%/°C among the examined FPs. B-gTEMP is the improved version of gTEMP, a GETI composed of a blue FP, Sirius, and a green FP, mT-Sapphire (mT-Sap). B-gTEMP showed a response in a wide temperature range of 15–50 °C with an average temperature sensitivity of 2.2 ± 1.2%/°C, comparable to that of gTEMP (2.6%/°C). Because B-gTEMP utilized a visible light excitation whereas gTEMP utilized ultraviolet excitation, temperature imaging with B-gTEMP showed lower phototoxicity and autofluorescence background than that of gTEMP. Additionally, B-gTEMP showed higher temperature resolution than gTEMP. Furthermore, temperature measurement with B-gTEMP was not affected by macromolecular crowding and self-concentration, which are the advantages over ELP-TEMP. Using B-gTEMP, I successfully monitored quick temperature rises induced by a local heat spot with a temporal resolution of 10 ms. In addition, I demonstrated the functionality of B-gTEMP in conventional temperature imaging to measure heat production in mitochondria induced by a chemical stimulation, and investigated temperature in the nucleus and cytoplasm of live HeLa cells, and found that the temperature was almost the same between them within the temperature resolution of the measurement, consistent with the result of ELP-TEMP. Altogether, ELP-TEMP and B-gTEMP are useful GETIs for future investigation of cell thermobiology.

Abstract of Thesis

Name （Sanchenkova Ksenia）	
Title	miR-215 regulates the homeostasis of small intestine (miR-215は小腸の恒常性維持に重要な役割をはたす)

MicroRNAs are a class of non-coding short-chained RNAs, which control many functions of a cell by down-regulating their target genes. Recent works indicate that miRNAs play a role in the maintenance of a gut homeostasis. miR-215 was found to be highly expressed in the epithelial cells of the small intestine, however the involvement of miR-215 in gut immunity was until now unknown. Here, we show that miR-215 downregulates CXCL12, which leads to elevated recruitment of Th17 cells into small intestine, resulting in a strong inflammation. Mice lacking miR-215 showed increased number of Th17 cells in the lamina propria of the small intestine with the enhanced CXCL12 expression in the epithelial cells. The mutant mice further showed the high susceptibility to an inflammation induced by indomethacin. Our finding opens a promising perspective of targeting miR-215 to treat inflammatory conditions in small intestine.

Form 3

Abstract of Thesis

Name （Parajuli Gyanu）	
Title	Arid5a augments tryptophan metabolism and chemokine expression to promote the immune evasion of mesenchymal tumor subtypes. （Arid5a はトリプトファン代謝とケモカインの発現を増幅することによって腫瘍の免疫からの回避を促進する）

Abstract of Thesis

The acquisition of mesenchymal traits in immunologically cold tumors leads to immune evasion. However, the underlying molecular mechanisms that link tumor immune evasiveness and mesenchymal phenotypes remain unclear. In this study, I found that the expression levels of AT-rich interaction domain-containing protein 5a (Arid5a), an RNA-binding protein, is substantially increased in mesenchymal tumor subtypes. The deletion of Arid5a in tumor cell lines enhanced antitumor immunity in immunocompetent mice but not in immunodeficient mice, highlighting the role of Arid5a in immune evasion. Furthermore, an Arid5a-deficient tumor microenvironment was shown to have robust antitumor immunity, as manifested by the suppressed infiltration of granulocytic myeloid-derived suppressor cells and regulatory T-cells, whereas infiltrated T-lymphocytes were more cytotoxic and less exhausted. Mechanistically, Arid5a stabilized *Ido1* and *Ccl2* mRNAs and augmented their expression, resulting in enhanced tryptophan catabolism and an immunosuppressive tumor microenvironment. Furthermore, Arid5a expression was substantially increased in mesenchymal subtypes of pancreatic ductal adenocarcinoma (PDAC) and colorectal cancers (CRC), and Arid5a promoted TGF-β-induced and IL-6-induced epithelial-mesenchymal transition and acquisition of invasiveness in PDAC. Thus, my findings unraveled a novel role of Arid5a as a genetic driver of the immune evasion of mesenchymal tumors. My data expands the role of Arid5a beyond inflammatory diseases, and suggest Arid5a as a promising target for the treatment of immunotolerant malignant tumors.