

平成 30 年 1 月 30 日

## 海外特別研究員最終報告書

独立行政法人 日本学術振興会 理事長 殿

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受付番号 71

氏 名 申 本 文 雄  
(氏名は必ず自署すること)

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。

なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地： バルセロナ （国名： スペイン ）

2. 研究課題名（和文）※研究課題名は申請時のものと変わらないように記載すること。

哺乳類 Hox 遺伝子による四肢の組織アイデンティティ確立機構の統合的解析

3. 派遣期間：平成 29 年 4 月 1 日 ～ 平成 29 年 12 月 31 日

4. 受入機関名及び部局名

欧州分子生物学研究所（EMBL） 多細胞システム生物学グループ

5. 所期の目的の遂行状況及び成果

(研究・調査実施状況及びその成果の発表・関係学会への参加状況等)

(注)「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

- I shortened the period in which I received the fellowship to 9 months from Apr. 1, 2017 to Dec. 31, 2017, and continue to work on the project in the same laboratory after finishing this fellowship.
- The institute where the host laboratory belongs has changed from Center for Genomic Regulation (CRG) (Barcelona, Spain) to European Molecular Biology Laboratory (EMBL) (Barcelona, Spain) on Oct. 1, 2017.

## **Research Report**

### **I. Setting up an experimental system for a single-cell transcriptome analysis**

Because the results yielded by a single-cell transcriptome analysis are expected to become a basis for further analyses, I put a priority on it in my project. Comparing several methods for a single cell transcriptome analysis regarding the throughput, sensitivity, accuracy, and availability of reagents and equipment, I decided to adopt the method utilizing a microfluidic system and started to set up the experimental system closely collaborating with the core facilities of genomics in Centre for Genomic Regulation (CRG).

### **II. Exploring in vitro model for the analysis of tissue patterning**

To test the roles of Hox genes in tissue patterning, I have explored the potential of *in vitro* model systems particularly the micromass culture system.

The micromass culture system is one of the primary culture methods of limb bud mesenchyme cells in which system we isolate mesenchymal cells in limb buds from

Sox9-EGFP mouse embryos at embryonic day 11.5 and seed them as a disc-like small cluster on a glass slide. After 24 hours of culture, this small disc exhibits a characteristic Sox9 spatial expression pattern, resembling the one by the reaction-diffusion system. Interestingly, this patterning also happens when we seed only a SOX9-positive or negative cell population after sorting by a flow cytometer, which indicates this patterning is not a simple result of a cell-sorting effect by Sox9 positivity.

The condition required for this patterning, however, has been poorly defined. Therefore, I have tried to identify the conditions by quantifying discs and analyzing subpopulations of limb bud cells. This work will be linked to the single-cell analysis above and expected to be a basic system to understand the molecular mechanism of the patterning of mesenchymal cells in mouse limb buds.

### **III. Collaborating with core facilities in the host institute.**

Since the host laboratory is mainly performing imaging and computational biology, it is indispensable to collaborate with core facilities in the institute to promote experiments of molecular biology and histology for this project, including setting up the microfluidic system for a single-cell transcriptome analysis above. Indeed, I started collaborations with many core facilities such as flow cytometry, advanced light microscopy, genomics, tissue engineering, and histology and received their technical assistance.

### **Academic conferences**

During the period, I joined the following two international academic conferences. Since I had changed my research field on the starting of this fellowship, they were good opportunities to acquaint myself with the new field and communicate with other people in the field.

i) 14th International Conference on Limb Development and Regeneration

Jul. 23-Jul. 26, 2017. Edinburgh, UK

ii) Barcelona BioMed Conference: Morphogenetic Engineering

Nov. 27-Nov.29, 2017. Barcelona, Spain