（様式４－１）

二国間交流事業 共同研究報告書

平成　２３年　４　月　１３日

独立行政法人日本学術振興会理事長　殿

共同研究代表者所属・部局　東京医科歯科大学・大学院疾患生命科学研究部

（ふりがな）　　（くろやなぎ　ひでひと）
職・氏 名　准教授・黒柳秀人

1. 事　業　名　相手国（日仏交流促進事業〈SAKURA〉）との共同研究　　振興会対応機関（仏外務省）

2. 研 究 課 題 名　二色発現プロファイラによる選択的スプライシング制御機構の解析

3. 全 採 用 期 間

平成　２１　年　４　月　１　日 ～ 平成　２３　年　３　月　３１　日 （　２　年　０　ヶ月）

4. 経 費 総 額

(1) 本事業により執行した研究経費総額　1,800 千　円

初年度経費　1,000 千　円、　２年度経費　800 千　円、　３年度経費　０　円

(2) 本事業経費以外の国内における研究経費総額　300 千　円

5. 研究組織

(1) 日本側参加者（代表者は除く）

氏名（ふりがな）	所　属・職　名	研　究　協　力　テ　ー　マ
大野　源太 （おおのげんた）	東京医科歯科大学・大学院生	*let-2*遺伝子の選択的スプライシングの制御因子の解明
都甲　麻理奈 （とごうまりな）	東京医科歯科大学・大学院生	選択的スプライシング制御因子の標的遺伝子の網羅的探索
ZHAO Chenxi （ちゃおちぇんしー）	東京医科歯科大学・大学院生	レポーターミニ遺伝子の作製法の改良

(2) 相手国側研究代表者

所属・職名・氏名　European Institute of Chemistry and Biology・Group Leader・Denis Dupuy

(3) 相手国参加者（代表者は除く）

氏　　名	所属・職名（国名）	研　究　協　力　テ　ー　マ
Karine Rebora	European Institute of Chemistry and Biology・ポスドク（フランス）	ロボットとプロファイラを用いたRNAiスクリーニング系の開発
Ilyass Zniber	European Institute of Chemistry and Biology・大学院生（フランス）	プロファイラを用いた *let-2* レポーター発現の変異体のスクリーニング

6．研究実績概要（全期間を通じた研究の目的・研究計画の実施状況・成果等の概要を簡潔に記載してください。）

In this project, we utilized a newly developed bi-chromatic expression profiler for i) a genome-wide screening for novel regulators of the *let-2* alternative splicing reporter and ii) for characterizing splicing regulator mutants and mutant reporter mini-genes.

The France team modified the *in-vivo* spatiotemporal profiling system so that we can simultaneously monitor differential expression of two fluorescent proteins, GFP and RFP. The France team also set up a robotic system to facilitate genome-wide RNAi screening for modulators of the spatiotemporal expression profiles of GFP and RFP. Karine Rebora of the France team utilized the *let-2* alternative splicing reporter worm provided by the Japan team to optimize parameters of the profiler, and then screened the RNAi library for candidate regulators or modifiers of the developmental switching of the *let-2* alternative splicing. She identified hundreds of candidates in the first screening and one strong candidate in the second screening. The Japan team is now analyzing the effect of disruption of the gene on the alternative splicing of the *let-2* alternative splicing reporter. Ilyass Zniber of the France team utilized the profiler to screen chemically mutagenized worms for mutants defective in the developmental switching of the *let-2* alternative splicing reporter. He successfully identified several mutant strains and about half of them were new alleles of *asd-2*. He is now mapping the other loci.

The Japan team is searching for *cis*-elements required for the developmental switching of the *let-2* alternative splicing reporter by disrupting conserved elements in the reporter mini-genes. Genta Ohno of the Japan team has disrupted conserved UGCAUG element in intron 10 and found that the element is required for switching to the adult isoform. Kuroyanagi has just disrupted conserved GUGUGUG element in intron 10 and is now generating transgenic worms. The Japan team is also testing the effect of mutations in *trans*-acting alternative splicing regulators they have characterized in their previous and current studies. We noticed that mutations in the *asd-1* gene or in the *sup-12* gene slightly affected the developmental switching of the *let-2* reporter in the body wall muscles, which is consistent with our previous studies that ASD-1 and SUP-12 can co-ordinately regulate alternative splicing of the *egl-15* gene in a muscle-specific manner. We are now analyzing the effect of mutations in these genes on the alternative splicing of the endogenous *let-2* gene.

Kuroyanagi has recently taken the *let-2* alternative splicing reporter worms in several mutant backgrounds to the Dupuy Lab and analyzed the expression profiles with the profiler. The France team and Kuroyanagi co-ordinately modified Chronogram Maker, a software that analyzes the profiles of the worms and make up chronograms, to better demonstrate the developmental switching of the *let-2* alternative splicing reporter. We are going to run further worm strains in various mutant backgrounds or with mutation(s) in putative *cis*-elements to analyze the effect of the mutations on the developmental switching of the *let-2* reporter. The Japan team will finally show that these *trans*-factors can directly and specifically bind to the *cis*-elements in *let-2* pre-mRNA.