(Form4-2)

JOINT RESEARCH PROJECT

FINAL REPORT
For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics 2. Chemistry & Material Science ③. Biology 4. Informatics & Mechatronics 5. Geo-Science & Space Science 6. Medical Science 7. Humanities & Social Sciences

1. Research Title:

Epigenetic regulation in proliferation and differentiation of *Drosophila* intestinal stem cell and its modification by aging

2. Term of Research: From 2010/7/1 To 2012/6/30

3. Total Budget

a. Financial Support by JSPS: Total amount: 2,400,000 thousand yen

1st Year 600,000 thousand yen 2nd Year 1,200,000 thousand yen

3rd Year 600,000 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Masamitsu YAMAGUCHI
Institution / Department Position	Kyoto Institute of Technology Graduate Schoolo of Technology Department of Applied Biology Professor
b. Korean Principal Researcher	
Name	Mi-Ae YOO
Institution / Department Position	Pusan National University Department of Molecular Biology Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
INOUE Yoshihiro	Kyoto Institute of Technology/ Insect Biomedical Research Center	Associate Professor
YOSHIDA Hideki	Kyoto Institute of Technology/ Graduate School of Technology	Assistant Professor
FUJIWARA Shunsuke	Kyoto Institute of Technology/ Graduate School of Technology	Doctoral Student
USHIJIMA Yuta	Kyoto Institute of Technology/ Graduate School of Technology	Master Student
SAHASHI Ritsuko	Kyoto Institute of Technology/ Graduate School of Technology	Doctoral Student
Luong Linh Ly	Kyoto Institute of Technology/ Graduate School of Technology	Doctoral Student
KITAZAWA Taishi	Kyoto Institute of Technology/ Graduate School of Technology	Doctoral Student
KONISHI Takahiro	Kyoto Institute of Technology/ Graduate School of Technology	Master Student
SHIMAJI Kohie	Kyoto Institute of Technology/ Graduate School of Technology	Master Student
YAMADA Momoko	Kyoto Institute of Technology/ Graduate School of Technology	Master Student

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Jae-Hun Cheong	Pusan National University/ Department of Molecular Biology	Professor
Young-Shin Kim	Pusan National University/ Research Institute of Genetic Engineering	Researcher
Joung-Sun Park	Pusan National University/ Department of Molecular Biology	Post-doctoral fellow
Joong-Gook Kim	Pusan National University/ Department of Molecular Biology	Doctoral Student
Shin-Hae Lee	Pusan National University/ Department of Molecular Biology	Doctoral Student
Jung-Hoon Pyo	Pusan National University/ Department of Molecular Biology	Doctoral Student
Hyun-Jin Na	Pusan National University/ Department of Molecular Biology	Master Student
Ho-Jun Jeon	Pusan National University/ Department of Molecular Biology	Master Student

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea

*Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
YAMAGUCHI Masamitsu	Kyoto Institute of Technology	2012/6/17-6/19	Pusan National University
Luong Linh Ly	Kyoto Institute of Technology	2012/6/17-6/19	Pusan National University
SHIMAJI Kohei	Kyoto Institute of Technology	2012/6/17-6/19	Pusan National University

For Final Fiscal Year(FY2012)	For Final Fiscal Year(FY2012)
Total: 3 persons	Total: 9days man-days

Numbers of Exchanges during the Past Fiscal Years
FY2010: Total 6 persons FY2011: Total 4 persons

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution

For Final Fiscal Year(FY2012)	For Final Fiscal Year(FY2012)
Total: 0 persons	Total: 0 man-days

Numbers of Exchanges during the Past Fiscal Years
FY2010: Total 1 persons FY2011: Total 0 persons

6. Objective of Research

In mammals, gut homeostasis is maintained by the balance between intestinal stem cell (ISC) proliferation, differentiation and removal of dead cells in adult (Casali A and Batlle E., Cell Stem Cell 4:124-127, 2009). However detailed mechanisms how ISC homeostasis is regulated in mammalian gut are still unknown. Recently, *Drosophila* melanogaster has been shown to be an excellent model system for the study of ISC biology. It is demonstrated that proliferating progenitor cells reside within the gut epithelium of adult *Drosophila*, similar to vertebrate gut (Ohlstein B and Spradling A. , Science 315:988-992, 2006). Moreover, in *Drosophila* model, it is revealed that many important developmental signaling pathways such as Notch, Wg and JAK/STAT signaling pathways contribute to ISC homeostasis (Casali A and Batlle E., Cell Stem Cell 4:124-127, 2009). Although protein-protein interaction and protein regulation mechanisms have been studied well, RNA level study has not been performed in *Drosophila* adult gut, despite the importance.

Yoo group has developed the adult *Drosophila* midgut system to investigate proliferation and differentiation of ISC. Yamaguchi group has already prepared antibodies and mutants for various epigenetic regulators such as histone methyltransferases and demethylases. Yamaguchi group has also established RNA fluorescent *in situ* hybridization technique with *Drosophila* embryos. Therefore both groups can complement to each other to establish a unique approach in studying epigenetic regulation during proliferation and differentiation of ISC. Moreover no other research group in the world is working on studies on modification of the epigenetic regulation by aging and oxidative stress. Th objective of our collaborative research is therefore the followings.

1) Identification of epigenetic regulators that are involved in epigenetic switch from proliferation to differentiation of intestinal stem cells (ISC).
2) Modification of the epigenetic regulation by aging and oxidative stress along with study of mechanism eliminating the aged cells.
3) Establishment of RNA fluorescent *in situ* hybridization on *Drosophila* gut model system.
4) Application of the findings in *Drosophila* system to mammals.

7. Methodology

Immunostaining of ISC, enteroblasts (EB), enteroendocrine cells (EE) and enterocytes (EC) with antibodies to histone methylation-related enzymes such as G9a was performed. Analyses of methylation state of histone H3 lisine 9 (H3K9) were carried out by immunostaining with monoclonal antibodies to mono-, di-, and tri-methylated H3K9. Gal4/UAS targeted expression system will be used to overexpress or knockdown histone methylation-related enzymes such as G9a. Transgenic flies carrying Pvf2-lacZ, p38b-lacZ or BSK-lacZ were used to monitor expression of genes that play important roles in ISC. EE was marked wit immunostaining with anti-prospero antibody. In addition, esg-GFP flies were used to detect the esg-positive small cells which marks ISC and EB. The paraquat treatment was given to flies to monitor any change by oxidative stress. Activation of stress-responding signals and target gene expression was examined by monitoring lacZ and GFP reporters. Highly sensitive RNA in situ hybridization using tyramide was applied to monitor expression of endogenous genes during proliferation and differentiation of ISC.