

การกระตุ้นรังไข่ในผึ้งงานของผึ้งพันธุ์ *Apis mellifera* L. และการระบุยีนที่เกี่ยวข้อง



นางสาวปรียดา โทยวิวัฒน์ตระกูล

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**OVARY ACTIVATION OF WORKER HONEY BEES *Apis mellifera* L. AND
IDENTIFICATION OF GENES INVOLVED**

Miss Preeyada Koywiwattrakul

**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biochemistry**

Department of Biochemistry

Faculty of Science

Chulalongkorn University

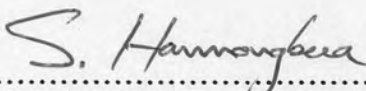
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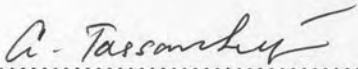
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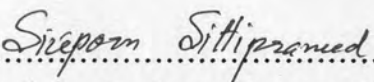
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Thesis Advisor Associate Professor Siriporn Sittipraneed, Ph.D.
Thesis Co-advisor Professor Ryszard Maleszka, Ph.D.

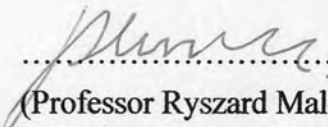
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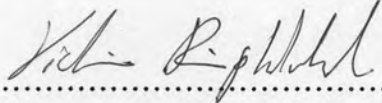

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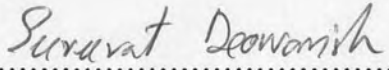
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ปริยดา โภยวิวัฒน์ตระกูล: การกระตุ้นรังไข่ในผึ้งงานของผึ้งพันธุ์ *Apis mellifera* L. และการกระตุ้นที่เกี่ยวข้อง. (OVARY ACTIVATION OF WORKER HONEY BEES *Apis mellifera* L. AND IDENTIFICATION OF GENES INVOLVED) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. ศิริพร สิริพิริยะนิต, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.ดร. Ryszard Maleszka, 285 หน้า.

เพื่อที่จะทราบว่ามียีนใดบ้าง ที่เกี่ยวข้องกับการกระตุ้นรังไข่ในผึ้งงาน เทคนิค Northern blot hybridization และ Quantitative real-time PCR ถูกนำมาใช้ในการตรวจสอบการแสดงออกที่แตกต่างกันของยีน ระหว่างผึ้งงานที่มีและไม่มีการพัฒนาของรังไข่ การเปรียบเทียบการแสดงออกของยีนระหว่างผึ้งงานปกติและผึ้งงาน anarchist ที่มีการพัฒนารังไข่ด้วย Northern blot hybridization พบว่าทั้งยีน Vitellogenin และ Transferrin มีการแสดงออกที่ต่างกันอย่างชัดเจน โดยในส่วนท้อง ยีนทั้งสองจะมีการแสดงออกในผึ้งงาน anarchist สูงกว่าผึ้งงานปกติ ขณะที่ในส่วนหัวของผึ้งงานตัวเดียวกัน ยีนทั้งสองจะมีการแสดงออกในผึ้งงาน anarchist ต่ำกว่าผึ้งงานปกติ เมื่อใช้ Quantitative real-time PCR ยืนยันผล พบว่า Vitellogenin มีการแสดงออกในส่วนท้องของผึ้งงาน anarchist ที่มีการพัฒนารังไข่เพิ่มขึ้น 4 เท่าเปรียบเทียบกับผึ้งงานปกติที่ไม่มีพัฒนารังไข่นอกจากนั้นได้สร้างผึ้งงานให้มีการพัฒนารังไข่ เพื่อใช้เป็นตัวอย่างในการทดลองหาอินทรีย์ที่มีหน้าที่ควบคุม หรือเกี่ยวข้องในกระบวนการกระตุ้นรังไข่ ได้ใช้คาร์บอน ไดออกไซด์และฟีโรโมนเป็นกุญแจสำคัญในการสร้างตัวอย่างผึ้งที่มีการพัฒนารังไข่ขึ้นมา คาร์บอนไดออกไซด์มีผลต่อการยับยั้งการพัฒนารังไข่ในผึ้งงาน ส่วนฟีโรโมนก็มีผลเช่นเดียวกัน ทั้งยีน Vitellogenin และยีน Transferrin มีการแสดงออกที่แตกต่างกันอย่างเห็นได้ชัดทั้งในส่วนท้องและส่วนหัว เมื่อได้รับก๊าซคาร์บอนไดออกไซด์ โดยทั้งสองยีนนี้มีการแสดงออกที่เพิ่มขึ้นประมาณ 4 เท่า ในส่วนท้องของผึ้งที่มีการพัฒนารังไข่ ในทางตรงกันข้าม ทั้งสองยีนนี้กลับมีการแสดงออกที่ลดลงประมาณ 3 เท่า ในส่วนหัวของผึ้งตัวเดียวกัน ผลการทดลองในส่วนนี้ยืนยันผลจากเทคนิค Northern blot hybridization ก่อนหน้านี้ในแง่ของการพัฒนารังไข่ ยีน Vitellogenin และ ยีน Transferrin มีการแสดงออกที่เพิ่มขึ้นประมาณ 14 เท่า และ 9 เท่า ตามลำดับ ในส่วนท้องของผึ้งงานซึ่งอยู่ในรังที่ขาดนางพญาและมีการพัฒนารังไข่ เมื่อเปรียบเทียบกับผึ้งงานซึ่งอยู่ในรังที่มีนางพญาและไม่มีพัฒนารังไข่นอกจากนี้ยีนสำหรับ Phosphoinositolyglycan-peptide และ Tyramine receptor ก็มีแนวโน้มที่จะมีการแสดงออกที่เพิ่มขึ้น ในส่วนท้องของผึ้งงานที่มีการพัฒนารังไข่เช่นเดียวกัน

สาขาวิชา ชีวเคมี
สาขาวิชา ชีวเคมี
ปีการศึกษา 2551

ลายมือชื่อนิสิต ปริยดา โภยวิวัฒน์ตระกูล
ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก *Ryszard Maleszka*
ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม *Priscilla*

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PREEYADA KOYWIWATTRAKUL: OVARY ACTIVATION OF WORKER
HONEY BEES *Apis mellifera* L. AND IDENTIFICATION OF GENES
INVOLVED. THESIS ADVISOR: ASSOC.PROF. SIRIPORN SITTIPRANEED,
Ph.D., THESIS CO-ADVISOR: PROF. RYSZARD MALESZKA, Ph.D., 285 pp.

In an effort to uncover genes associated with ovary activation in honey bee workers, northern blot hybridization and quantitative real-time PCR were used to examine differential expression of the candidate genes in the workers with and without ovary development. From northern blot hybridization, the expression of vitellogenin and transferrin genes showed significantly different between wild-type workers and ovary activated anarchist workers. These two genes had more expressed in the abdomen of anarchist workers while they were less expression in the head part of those workers compared with wild-type workers. The quantitative real-time PCR was used to confirm the results and we found that vitellogenin was up-regulated about 4-fold in the abdomen of ovary activated anarchist workers when compared with ovary non-activated wild-type workers. Moreover, the honey bee workers that had ovary activation were constructed to use as the samples for examining the genes that regulate or involve in the network of ovary activation. Carbon dioxide and pheromone were the important keys for constructing these samples. Carbon dioxide affected the ovary retardation in the honey bee workers and also pheromone did the same effect. Vitellogenin and transferrin genes did show significantly differences in the abdominal tissue and head tissue part following the CO₂ narcosis. These two genes were up-regulated about 4-fold in the abdomen of ovary activated workers. In contrast, they were down-regulated about 3-fold in the head of those workers. These confirmed the previous results from northern blot analysis in the case of ovary activation. These vitellogenin and transferrin were up-regulated, about 14-fold and 9-fold respectively, in the abdomen of ovary activated queenless workers relative to ovary non-activated queenright workers. Moreover, gene encoding phosphoinositolyglycan-peptide and tyramine receptor seem to be up-regulated in the abdomen of ovary activated queenless workers too.

Department: Biochemistry.....

Student's signature: Preeyada koywiwattrakul

Field of study: Biochemistry.....

Advisor's signature: Siriporn Sittipraneed

Academic year: 2008.....

Co-advisor's signature: Ryszard Maleszka

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LIST OF ABBREVIATIONS

°C	degree celcius
A	absorbance
bp	base pair
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
DEPC	diethylpyrocarbonate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dTTP	deoxythymidine triphosphate
EDTA	ethylene diamine tetraacetic acid
EtBr	ethidium bromide
hr	hour
kb	kilobase
kDa	kilodalton
M	molar
mg	milligram
MgCl ₂	magnesium chloride
min	minute
ml	milliliter
mM	millimolar
mRNA	messenger ribonucleic acid
ng	nanogram
nm	nanometer
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
RNA	ribonucleic acid

RNase	ribonuclease
RT	reverse transcription
SDS	sodium dodecyl sulfate
sec	second
TE	tris EDTA
Tris	tris (hydroxyl methyl) aminomethane
μg	microgram
μl	microliter
μM	micromolar