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Differential Gene Expression in the Hypopharyngeal Glands of Worker Honeybees (*Apis mellifera* L.) Associated with an Age-Dependent Role Change

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Associated with the age-dependent role change of worker honeybees (*Apis mellifera* L.) from nurse bees to foragers, some structural and functional changes occur in the hypopharyngeal glands (HPGs): nurse bee HPGs are well developed and synthesize major royal jelly proteins (MRJPs), while forager HPGs shrink and synthesize α -glucosidase, which converts nectar into honey. To identify candidate genes involved in the structural and functional HPG changes associated with the age-dependent role change of worker honeybees, we searched for genes whose expression in the HPGs depends on the role of workers, by using differential display and quantitative reverse transcription-polymerase chain reaction. Here, we newly identified a *buffy* homolog encoding a Bcl-2-like protein as a gene whose expression, like *MRJP*, is higher in nurse bees than in foragers, and a *matrix metalloproteinase 1 (MMP1)* homolog as a gene whose expression, like α -glucosidase, is higher in foragers than in nurse bees, suggesting that both suppression of inhibition of the caspase cascade by *buffy* and degradation of the extracellular matrix by *MMP1* are involved in the functional and structural changes of the HPGs. Furthermore, although both *buffy* and *MMP1* were highly expressed in various tissues other than the HPGs, *buffy* expression in the other tissues did not differ significantly between nurse bees and foragers, whereas *MMP1* expression in midgut was also significantly higher in foragers than in nurse bees, as in the HPGs. These results suggest that in *buffy* and *MMP1*, expression is regulated in a tissue-preferential manner according to the age-dependent role change of workers.

Key words: honeybee, hypopharyngeal gland, age-dependent role change, matrix metalloproteinase, *buffy*, tissue-preferential gene expression

INTRODUCTION

The European honeybee, *Apis mellifera* L., is a eusocial insect, and females differentiate into either queens (the reproductive caste) or workers (the labor caste) (Winston, 1987). Queens devote their life to reproduction, whereas workers engage in various tasks related to colony growth and maintenance, such as brood rearing, comb building, and foraging. In addition, the role of workers changes depending on their age after eclosion (Winston, 1987). The lifespan of a worker is usually 30 to 40 days from spring to autumn (Dayer, 1991); young workers (generally, less than 13 days after eclosion) take care of the brood in the hive by secreting royal jelly (nurse bees), whereas old workers (more than 18 days) collect nectar and pollen outside the hive (foragers) (Lindauer, 1952; Sakagami, 1953; Winston, 1987).

Some physiological changes in certain organs accompany this age-dependent role change in workers. For example, the hypopharyngeal glands (HPGs), a pair of exocrine

glands in the worker's head, undergo structural and functional changes associated with this age-dependent role change in workers. In nurse bees, the HPGs are well developed and synthesize major royal jelly proteins (MRJPs) (Kubo et al., 1996; Ohashi et al., 1997), whereas in foragers, they shrink and synthesize carbohydrate-metabolizing enzymes that process nectar into honey, such as α -glucosidase, α -amylase, and glucose oxidase (Kubo et al., 1996; Ohashi et al., 1996; 1997; 1999). We previously reported that the mode of gene expression for MRJP isoforms and the carbohydrate-metabolizing enzymes changes at the level of single secretory cells in the HPG acini associated with the age-dependent role change in workers (Ohashi et al., 1996; Ohashi et al., 1997).

Both the roles and the physiology of workers have plasticity to be modulated concurrently depending on the colony demand. For example, in colonies where brood decreases in number, older workers tend to retain well-developed HPGs, like nurse bees (Hrassnigg and Crailsheim, 1998). Furthermore, in orphan colonies, where no newly emerging workers are supplied and thus older workers need to take care of their brood, the older workers continue to synthesize MRJP in the HPGs and work as nurse bees (Ohashi et al., 2000). These findings strongly suggest a close link between

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the physiology of the HPGs and the role of workers. Therefore, honeybee HPGs could be a model organ whose structure and function change at the cellular level associated with the animal's behavior. The molecular mechanisms underlying the structural and functional changes of the HPGs associated with the role change of workers, however, remain unknown.

In the present study, to identify candidate genes involved in the structural and functional changes of the HPGs, we searched for genes whose expression in the HPGs differs between nurse bees and foragers, using differential display and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). We newly identified a *buffy* homolog, *Ambuffy*, as a nurse bee-selective gene, and a *matrix metalloproteinase 1* homolog, *AmMMP1*, as a forager-selective gene. The findings respectively suggest possible involvement of the caspase cascade and extracellular matrix degradation in the structural and functional changes of the worker HPGs.

MATERIALS AND METHODS

Animals and tissues

European honeybee (*Apis mellifera* L.) colonies were purchased from the Kumagaya bee farm (Saitama, Japan) and maintained at the University of Tokyo (Tokyo, Japan). Nurse bees were collected when they were feeding brood, and foragers were collected when they returned to the colony after foraging pollen and honey (Kubo et al., 1996). After the workers were anesthetized on ice, the heads were removed and the HPGs were dissected from them with fine tweezers and a surgical knife under a binocular microscope. Nurse bees with well-developed HPGs and foragers with shrunken HPGs were used in experiments, as described previously (Ohashi et al. 1999). Tissues to be used for extraction of RNA were stored frozen at -80°C until use.

Differential display

Total RNA was extracted from HPGs using TRIzol Reagent (Invitrogen, Carlsbad, CA), treated with DNase I, and reverse-transcribed by using SuperScript III (Invitrogen) with rhodamine-labeled downstream primer 1 (TaKaRa, Tokyo, Japan). Differential display was performed using a Fluorescence Differential Display Kit, Rhodamine Version (TaKaRa) and *LA Taq* (TaKaRa) with a combination of 24 primers, as described previously (Ito et al., 1994; Kamikouchi et al., 1998). PCR conditions were ($94^{\circ}\text{C} \times 2 \text{ min} + 40^{\circ}\text{C} \times 5 \text{ min} + 72^{\circ}\text{C} \times 5 \text{ min}$) $\times 1 \text{ cycle} + (94^{\circ}\text{C} \times 30 \text{ sec} + 40^{\circ}\text{C} \times 2 \text{ min} + 72^{\circ}\text{C} \times 1 \text{ min}) \times 34 \text{ cycles} + 72^{\circ}\text{C} \times 5 \text{ min}$. To ensure the reproducibility of the differential display profiles, duplicate reactions with two lots of RNA obtained from two honeybee colonies were performed.

Bands whose signal intensities differed between nurse bees and foragers in the two lots of RNA samples were selected as candidate bands. Bands whose intensities differed between nurse bees and foragers were excised from gels, and the DNA in the bands was reamplified by PCR. The resulting PCR products were cloned into pGEM-T easy vector (Promega, Madison, WI) and transfected into *Escherichia coli* DH5 α competent cells (TaKaRa). The cDNA sequences were determined using an ABI PRISM 3100 Genetic Analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit. Similarity searches of cDNA sequences were performed by using NCBI Honey Bee Genome Resources.

Cloning of full-length open reading frame sequences

RT-PCR was performed using *Ex Taq* (TaKaRa), with cDNA derived from the HPG as the template. Primers (*Ambuffy*, 5'-GAAATGAATCGTTCGTTGTAG-3' and 5'-CAAGATGAAATTTAATGAATCATTATGAGAGG-3'; *AmMMP1*, 5'-GACGATCTACGG-

GAACAC-3' and 5'-GATGACACAATATTGCATGCGAC-3') were designed on the basis of sequences in the 5' and 3' untranslated regions, obtained from the NCBI Honey Bee Genome Resources. Cloning and sequencing were performed as described above. Domain and motifs in deduced amino acid sequences were predicted by using the Pfam database (GenomeNet Database Resource: <http://motif.genome.jp/>), SignalP (The Center for Biological Sequence Analysis at the Technical University of Denmark: <http://www.cbs.dtu.dk/services/SignalP/>), and information previously reported by Llano et al. (2000) and Igaki and Miura (2004). The C-terminal hydrophobic membrane anchor (MA) was predicted by using TMHMM (The Center for Biological Sequence Analysis at the Technical University of Denmark: <http://www.cbs.dtu.dk/services/TMHMM/>). The amino acid sequences of *Ambuffy* and *AmMMP1* were aligned by using ClustalX (Thompson et al., 1997).

Quantitative RT-PCR

To investigate tissue-preferential gene expression, we simply dissected worker bodies into HPGs, the head except for the HPGs, thorax, midgut, and abdomen except for the midgut. We dissected the midgut from the abdomen, because the midgut contained much food pollen, which might have disturbed the RNA extraction procedure. We washed the pollen from the midgut and used the remaining midgut also for RNA extraction. Total RNA was extracted from these tissues with TRIzol Reagent (Invitrogen), treated with DNase I, and reverse-transcribed by using SuperScriptIII (Invitrogen) with the oligo dT primer. Real-time PCR was performed with SYBR *premix Ex Taq* II (TaKaRa) and LightCycler (Roche, Basel, Switzerland) according to manufacturers' protocols, using gene-specific primers. The amount of transcript was normalized with that of *elongation factor 1 α -F2* (*EF1 α -F2*; Danforth and Ji, 1998) or *ribosomal protein 49* (*rp49*) (Ben-Shahar et al., 2003). We also examined the expression of *mrjp2* and *α -glucosidase* as reference genes that are expressed preferentially in nurse bee and forager HPGs, respectively, which we previously showed by Northern blotting analysis (Ohashi et al., 1997).

Gene-specific primers (*Ambuffy*, 5'-CATGGCACTTCTCATC-CTTTTC-3' and 5'-GAGAACGGTTTCAGCATCAATC-3'; *AmMMP1*, 5'-GCTTCCCATAATCTTGATG-3' and 5'-CATCCGAACCACCAGTAAG-3'; *MRJP2*, 5'-AAATGGTCGCTCAAATGACAGA-3', and 5'-ATTCATCCTTTACAGGTTTGTTC-3'; *α -glucosidase*, 5'-TACCTG-GCTTCGTGTCAAC-3' and 5'-ATCTTCGGTTTCCCTAGAGAATG-3'; *EF1 α -F2*, 5'-CATCAAAAACATGATTACTGGTACCTC-3' and 5'-CAGAATACGGTGGTTCAGTGG-3'; and *rp49*, 5'-AGAAACTGGCG-TAAACCTAAAG-3' and 5'-GTTCTTGACATTATGTACCAAAAC-3') were derived from cDNA sequences for each gene and information from Honey Bee Genome Resources. PCR conditions were: ($95^{\circ}\text{C} \times 30 \text{ s}$) + ($95^{\circ}\text{C} \times 5 \text{ sec} + 60^{\circ}\text{C} \times 15 \text{ s} + 72^{\circ}\text{C} \times 20 \text{ sec}$) $\times 45 \text{ cycles}$.

RESULTS

Screening of nurse bee and forager HPG-preferential genes

To search for genes whose expression in the HPGs differs between nurse bees and foragers, we used the differential display method. By screening approximately 900 bands, 48 candidate bands were obtained (23 nurse bee-selective and 25 forager-selective bands). Among these, 14 bands were selected based on the extent of differences in intensity between nurse bees and foragers in the differential display images. Sequence analysis assigned these bands to seven genes: *MRJP1*, 3 (Ohashi et al, 1997), and 7 (Stefan and Jaroslav, 2004), *α -glucosidase* (Ohashi et al, 1996), and three other clones (1 to 3). In the present study, we focused on clones 1 and 2, as we could not detect significant expression of clone 3 (data not shown).

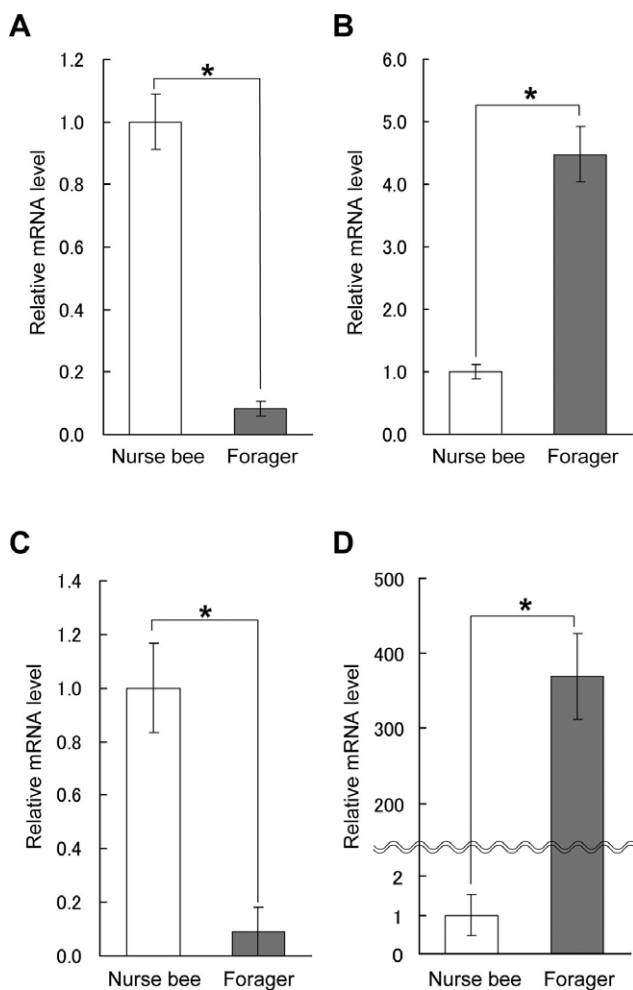


Fig. 3. Quantitative analysis of *Ambuffy* and *AmMMP1* transcripts in the HPGs. Nurse bees or foragers (n=9–14/group) were collected as one batch, and four batches prepared from four different colonies were subjected to real-time RT-PCR. Bars indicate mean relative mRNA levels with the standard deviation for (A) *Ambuffy*, (B) *AmMMP1*, (C) *MRJP2*, and (D) α -glucosidase, with the amount of mRNA in nurse bee HPGs defined as 1. Asterisks indicate significant differences between nurse bees and foragers (* p <0.01; Welch's t-test).

(Fig. 3), the amount of *Ambuffy* transcript was normalized to that of *elongation factor 1 α -F2* (*EF1 α -F2*) (Danforth and Ji, 1998), which was expressed almost equally in both nurse bee and forager HPGs (data not shown). However, we used *rp49* (Ben-Shahar et al., 2003) as a reference to normalize *Ambuffy* and *AmMMP1* expression in various tissues, because we could not detect any significant *EF1 α -F2* expression in the other tissues, and quantitative RT-PCR revealed that there were almost equivalent levels of *rp49* expression in all these tissues (data not shown).

Ambuffy expression was high not only in the HPGs but also in the other body parts and tissues examined. However, interestingly, although the differential *Ambuffy* expression in the HPGs between nurse bees and foragers was again confirmed in this experiment, the expression level of *Ambuffy* in nurse bee HPGs was largely comparable to that in the other body parts and tissues of nurse bees, and the *Ambuffy*

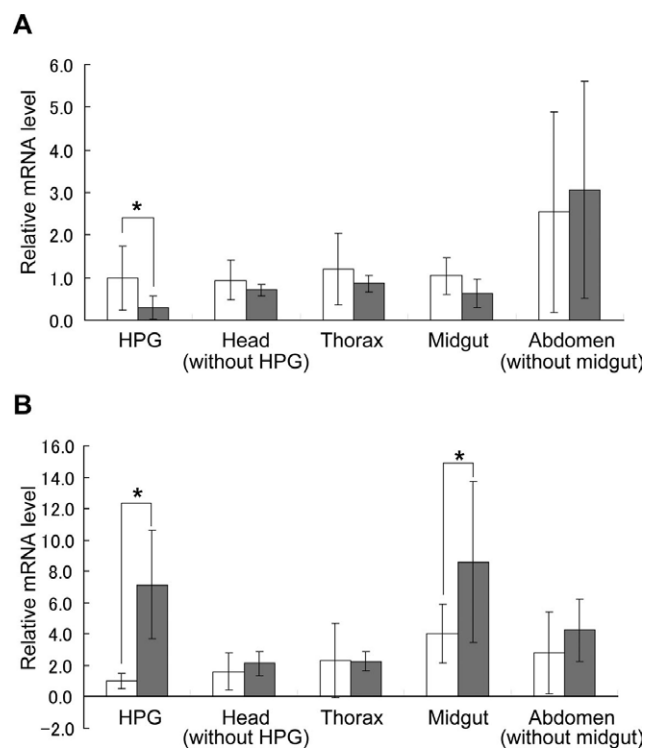


Fig. 4. Quantitative analysis of *Ambuffy* and *AmMMP1* transcripts in various body parts and tissues. Samples were collected from two or three nurse bees or foragers for each batch, and eight batches of samples were prepared from three colonies and subjected to real-time RT-PCR. HPGs of foragers were collected from only seven batches due to a failure in sample preparation. Bars indicate the mean relative mRNA levels with the standard deviation for (A) *Ambuffy* and (B) *AmMMP1* in various body parts and tissues, with the amount of mRNA in nurse bee HPGs defined as 1. Asterisks indicate significant differences between nurse bees and foragers (* p <0.05; Welch's t-test).

expression in other body parts or tissues other than the HPGs did not differ significantly between nurse bees and foragers (Fig. 4A).

Similarly, all body parts or tissues examined showed high *AmMMP1* expression, and the differential *AmMMP1* expression in the HPGs between nurse bees and foragers was again confirmed in this experiment (Fig. 4B). *AmMMP1* expression varied to a larger extent among body parts and tissues than *Ambuffy* expression. *AmMMP1* expression was distinct from *Ambuffy* expression in that it was significantly higher in foragers than in nurse bees, not only in the HPGs but also in the midgut, indicating that *Ambuffy* and *AmMMP1* expression differed between nurse bees and foragers in a tissue-preferential manner.

DISCUSSION

In the present study, we newly identified honeybee homologs of *buffy* (*Ambuffy*), whose expression was higher in nurse bee HPGs than in forager HPGs, and *matrix metalloproteinase 1* (*AmMMP1*), whose expression was higher in forager HPGs than in nurse bee HPGs. This is the first identification of genes that are possibly involved in the regulation of HPG structure and/or function (intracellular sig-

naling and extracellular matrix degradation, respectively), rather than the products of the HPGs as an exoscretory gland, such as MRJPs, which are the components of royal jelly, and carbohydrate-metabolizing enzymes that process nectar into honey (Kubo et al, 1996; Ohashi et al., 1996, 1997, 1999).

In *Drosophila*, buffy (*Dmbuffy*) is a Bcl-2-like protein with anti-apoptotic activity (Brachmann et al., 2000; Quinn et al., 2003). Genetic analysis suggests that *Dmbuffy* functions upstream of the apical caspase Dronc (Quinn et al., 2003). Based on these proposed functions for *Dmbuffy* and the fact that *Ambuffy* expression in the HPGs was higher in nurse bees than in foragers (Figs. 3A, 4A), *Ambuffy* may repress the caspase cascade in nurse bee HPGs, and this repression is released in forager HPGs, in which *Ambuffy* expression is decreased. We previously demonstrated that the number of secretory cells was almost the same in individual acini of nurse bee and forager HPGs, suggesting that cell death does not occur markedly during the age-dependent role change of workers, and that apoptotic degeneration might not be involved in the HPG morphological change (Ohashi et al., 1997). Hence, our present findings suggest that part, but not all, of the caspase cascade plays a role in the structural and/or functional changes of the HPGs without inducing cell death. In mammals, caspases are also involved in skeletal muscle cell differentiation and osteogenic differentiation of bone marrow stromal stem cells (Fernando et al., 2002; Miura et al., 2004). Caspases can also act as signal-transducing molecules that are not directly related to cell death (Kroemer and Martin, 2005). Therefore, caspases may function as signal-transducing molecules not leading to apoptosis in forager HPGs. In addition, that *Ambuffy* expression decreased only in the HPGs, but not in other body parts or tissues in foragers (Fig. 3A), suggests that *Ambuffy* transcription is regulated in an HPG-specific manner. The caspase cascade may also be activated specifically in the forager HPGs.

On the other hand, based on similarity in sequence and motif structures (Fig. 1B), *AmMMP1* is expected to function similarly to *Dm1-MMP*. *Dm1-MMP* degrades fibronectin and type-IV collagen, which are extracellular matrix and basement membrane proteins (Llano et al., 2000). Therefore, it is plausible that *AmMMP1* functions to degrade the extracellular matrix in the forager HPGs, leading to shrinkage of the gland tissue. That *AmMMP1* expression was higher in foragers than in nurse bees not only in the HPGs but also in the midgut (Fig. 4) suggests that *AmMMP1* expression might be regulated in a tissue-preferential manner (HPG and midgut), and that *AmMMP1* functions to degrade extracellular matrix not only in the HPGs but also in the midgut in foragers. The food of adult workers changes depending on their role; nurse bees feed mainly on protein-rich food such as pollen for synthesizing and secreting royal jelly (Winston, 1987; Crailsheim et al., 1992; Szolderits and Crailsheim, 1993), whereas foragers feed mainly on carbohydrate-rich food such as nectar and honey for flying (Winston, 1987). Therefore, it might be that the structure of the midgut, as a digestive organ, also changes in association with the role change of workers and that *AmMMP1* is involved in this process. In the present study, we did not discriminate whether the differential expression was due to the

change in the age or in the role of the workers. Nonetheless, our findings further support our previous notion that the physiological change (*Ambuffy* and *AmMMP1* expression, in this study) occurs in association with the behavioral change of the workers (Kubo et al, 1996; Ohashi et al., 1996; 1997; 1999).

We showed that the extent of role-dependent change in *Ambuffy* expression in HPGs was almost comparable to that in *MRJP2* expression (Fig. 3A). Although the extent of differential expression of *AmMMP1* associated with the role change of workers was smaller than that of α -glucosidase, the synergistic effects of multiple regulators, each of which shows a small level of differential expression, often have an amplified effect on the expression of final products (Cobb, 1999). Therefore, we propose *Ambuffy* and *AmMMP1* as good candidates for genes involved in structural and functional changes in HPGs. In general, behavior (role) and physiology are correlated in social insects, and both can be plastically modulated in accordance with social interactions. For example, bumblebee (*Bombus terrestris*) workers change enormously in body size, and body size and division of labor are closely correlated (Wilson, 1975). Our future study of functional changes in HPGs might also shed light on the related molecular mechanisms underlying the correlated changes in behavior and physiology observed in social insects.

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REFERENCES

- Bateman A, Birney E, Cerruti L, Durbin R, Etwiller L, Eddy SR, Griffiths-Jones S, Howe KL, Marshall M, Sonnhammer EL (2002) The Pfam protein families database. *Nucleic Acids Res* 30: 276–280
- Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 340: 783–795
- Ben-Shahar Y, Leung H-T, Pak WL, Sokolowskiv MB, Robinson GE (2003) cGMP-dependent changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. *J Exp Biol* 206: 2507–2515
- Brachmann CB, Jassim OW, Wachsmuth BD, Cagan RL (2000) The *Drosophila* bcl-2 family member dBorg-1 functions in the apoptotic response to UV-irradiation. *Curr Biol* 10: 547–550
- Cobb MH (1999) MAP kinase pathways. *Prog Biophys Mol Biol* 71: 479–500
- Colussi PA, Quinn LM, Huang DC, Coombe M, Read SH, Richardson H, Kumar S (2000) Debcl, a proapoptotic Bcl-2 homologue, is a component of the *Drosophila melanogaster* cell death machinery. *J Cell Biol* 148: 703–714
- Crailsheim K, Schneider LHW, Hrassnigg N, Bühlmann G, Brosch U, Gmeinbauer R, Schöffmann B (1992) Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function. *J Insect Physiol* 38: 409–419
- Danforth BN, Ji S (1998) Elongation factor-1 alpha occurs as two copies in bees: implications for phylogenetic analysis of EF-1

- alpha sequences in insects. *Mol Biol Evol* 15: 225–235
- Dayr FC (1991) Coadaptation of colony design and worker performance in honeybees. In "Diversity in the Genus *Apis*" Ed by DR Smith, Westview Press, Boulder, pp 2133–2245
- Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA (2002) Caspase 3 activity is required for skeletal muscle differentiation. *Proc Natl Acad Sci USA* 99: 11025–11030
- Gomis-Ruth FX, Gohlke U, Betz M, Knauper V, Murphy G, Lopez-Otin C, Bode W (1996) The helping hand of collagenase-3 (MMP-13): 2.7 Å crystal structure of its C-terminal hemopexin-like domain. *J Mol Biol* 264: 556–566
- Hrassnigg N, Crailsheim K (1998) Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies. *J Insect Physiol* 44: 929–939
- Igaki T, Miura M (2004) Role of Bcl-2 family members in invertebrates. *Biochim Biophys Acta* 1644: 73–81
- Igaki T, Kanuka H, Inohara N, Sawamoto K, Nunez G, Okano H, Miura M (2000) Drob-1, a *Drosophila* member of the Bcl-2/CED-9 family that promotes cell death. *Proc Natl Acad Sci USA* 97: 662–667
- Ito T, Kito K, Adati N, Mitsui Y, Hagiwara H, Sakaki Y (1994) Fluorescent differential display: arbitrarily primed RT-PCR fingerprinting on an automated DNA sequencer. *FEBS Lett* 351: 231–236
- Kamikouchi A, Takeuchi H, Sawata M, Ohashi K, Natori S, Kubo T (1998) Preferential expression of the gene for a putative inositol 1,4,5-trisphosphate receptor homologue in the mushroom bodies of the brain of the worker honeybee *Apis mellifera* L. *Biochem Biophys Res Commun* 242: 181–186
- Kroemer G, Martin SJ (2005) Caspase-independent cell death. *Nat Med* 11: 725–730
- Kubo T, Sasaki M, Nakamura J, Sasagawa H, Ohashi K, Takeuchi H, Natori S (1996) Change in the expression of hypopharyngeal gland proteins of the worker honeybees (*Apis mellifera* L.) with age and/or role. *J Biochem* 119: 291–295
- Lindauer M (1952) Ein Beitrag zur Frage der Arbeitsteilung im bienenstaat. *Zeitschr Vergleich Physiol* 34: 299–345
- Llano E, Pendas AM, Aza-Blanc P, Kornberg TB, Lopez-Otin C (2000) Dm1-MMP, a matrix metalloproteinase from *Drosophila* with a potential role in extracellular matrix remodeling during neural development. *J Biol Chem* 275: 35978–35985
- Miura M, Chen XD, Allen MR, Bi Y, Gronthos S, et al. (2004) A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J Clin Invest* 114: 1704–1713
- Ohashi K, Sawata M, Takeuchi H, Natori S, Kubo T (1996) Molecular cloning of cDNA and analysis of expression of the gene for alpha-glucosidase from the hypopharyngeal gland of the honeybee *Apis mellifera* L. *Biochem Biophys Res Commun* 221: 380–385
- Ohashi K, Natori S, Kubo T (1997) Change in the mode of gene expression of the hypopharyngeal gland cells with an age-dependent role change of the worker honeybee *Apis mellifera* L. *Eur J Biochem* 249: 797–802
- Ohashi K, Natori S, Kubo T (1999) Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.). *Eur J Biochem* 265: 127–133
- Ohashi K, Sasaki M, Sasagawa H, Nakamura J, Natori S, Kubo T (2000) Functional flexibility of the honey bee hypopharyngeal gland in a dequeen colony. *Zool Sci* 17: 1089–1094
- Page-McCaw A, Serano J, Sante JM, Rubin GM (2003) *Drosophila* matrix metalloproteinases are required for tissue remodeling, but not embryonic development. *Dev Cell* 4: 95–106
- Quinn L, Coombe M, Mills K, Daish T, Colussi P, Kumar S, Richardson H (2003) Buffy, a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *EMBO J* 22: 3568–3579
- Sakagami S (1953) Untersuchungen über die Arbeitsteilung in einem Zwergvolk der Honigbienen. *Beiträge zur Biologie des Bienenvolkes, Apis mellifera* L. I. *Jap J Zool* 11: 117–185
- Sonnhammer EL, von Heijne G, Krogh A (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc Int Conf Intell Syst Mol Biol* 6: 175–182
- Stefan A, Jaroslav K (2004) The MRJP/YELLOW protein family of *Apis mellifera*: identification of new members in the EST library. *J Insect Physiol* 50: 51–59
- Szolderits MJ, Crailsheim K (1993) A comparison of pollen consumption and digestion in honeybee (*Apis mellifera carnica*) drones and workers. *J Insect Physiol* 39: 877–881
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25: 4876–4882
- Wilson EO (1975) *Sociobiology: The New Synthesis*. Harvard University Press, Cambridge
- Winston ML (1987) *The Biology of the Honey Bee*. Harvard University Press, Cambridge
- Zhang H, Huang Q, Ke N, Matsuyama S, Hammock B, Godzik A, Reed JC (2000) *Drosophila* pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanisms. *J Biol Chem* 275: 27303–27306

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