Honey Bee PTEN – Description, Developmental Knockdown, and Tissue-Specific Expression of Splice-Variants Correlated with Alternative Social Phenotypes

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Abstract

**Background:** Phosphatase and TEnsin (PTEN) homolog is a negative regulator that takes part in IIS (insulin/insulin-like signaling) and Egfr (epidermal growth factor receptor) activation in Drosophila melanogaster. IIS and Egfr signaling events are also involved in the developmental process of queen and worker differentiation in honey bees (Apis mellifera). Here, we characterized the bee PTEN gene homologue for the first time and begin to explore its potential function during bee development and adult life.

**Results:** Honey bee PTEN is alternatively spliced, resulting in three splice variants. Next, we show that the expression of PTEN can be down-regulated by RNA interference (RNAi) in the larval stage, when female caste fate is determined. Relative to controls, we observed that RNAi efficacy is dependent on the amount of PTEN dsRNA that is delivered to larvae. For larvae fed queen or worker diets containing a high amount of PTEN dsRNA, PTEN knockdown was significant at a whole-body level but lethal. A lower dosage did not result in a significant gene down-regulation. Finally, we compared same-aged adult workers with different behavior: nursing vs. foraging. We show that between nurses and foragers, PTEN isoforms were differentially expressed within brain, ovary and fat body tissues. All isoforms were expressed at higher levels in the brain and ovaries of the foragers. In fat body, isoform B was expressed at higher level in the nurse bees.

**Conclusion:** Our results suggest that PTEN plays a central role during growth and development in queen- and worker-destined honey bees. In adult workers, moreover, tissue-specific patterns of PTEN isoform expression are correlated with differences in complex division of labor between same-aged individuals. Therefore, we propose that knowledge on the roles of IIS and Egfr activity in developmental and behavioral control may increase through studies of how PTEN functions can impact bee social phenotypes.

Introduction

Insulin/insulin-like signaling (IIS) and epidermal growth factor receptor (Egfr) are important and highly conserved signal transduction pathways, spanning from yeast to fruit flies to humans [1], [2], [3], [4]. Research on invertebrate model organisms shows that many physiological processes are influenced by these pathways, including nutrient metabolism, growth, development, reproduction, and aging. Thus, IIS and Egfr cascades play major roles in invertebrate life-history regulation [1]. Also, in vertebrate systems, IIS and Egfr are part of the energy sensing systems of individual cells, and defects in these pathway components can lead to serious illness, including growth abnormalities, diabetes, and cancer [4], [5], [6].

The tumor suppressor gene PTEN is a dual specificity phosphatase that is conserved from nematode worms to humans [1], [7], [8]. PTEN can down-regulate both IIS and Egfr by dephosphorylating PI(3,4,5)-tris-phosphate to PI(4,5)P2, making it a direct antagonist of phosphoinositide-3-kinase (PI3K). Thereby, PTEN acts antagonistically to the growth-promoting signals from the activated insulin receptor [9], [10] and Egfr [3], [4]. The D. melanogaster PTEN homolog, dPTEN plays a critical role in regulation of cell proliferation, cell size, and organ/tissue size during development [11], and dPTEN homozygosity and trans-homozygosity causes lethality during embryonic and early larval stages [10]. In the nematode worm Caenorhabditis elegans, the PTEN homolog daf-18 regulates dauer formation, i.e. developmental and life-extendening arrest during the ‘L1’ larval stage [12]. Loss of daf-18 bypasses this arrest and results in inappropriate growth [12]. PTEN has been studied primarily for its effects on longevity and cell size, in both worms and flies [10], [13], [14]. Yet, the recent insight that PTEN influences complex behavior, even in humans [15], suggests that research should be...
expanded to models of social behavior. The honey bee *A. mellifera* provides an attractive model for studies of molecular mechanisms that contribute to variation in social phenotype.

In honey bees, female larvae can develop into two reproductive castes: fecund queens or essentially sterile workers. During larval ontogeny, a bee goes through five larval instars, and caste fate is determined in the 3rd instar by nutrition. Adult workers that exhibit nursing behavior control the food provisions of the larvae. A queen-destined larva receives nutrient-rich diet (i.e. food rich in royal jelly) throughout development, whereas worker-destined larvae receive a less nutrient-rich diet from the 3rd instar and onward. Larvae respond to this difference in nutrition by changing the expression of genes involved in IIS, Egfr and Target of Rapamycin (TOR) nutrient sensing cascades [2], [16], [17]. Consistent with nutrition being causal to female caste fate, Rapamycin (TOR) nutrient sensing cascades [2], [16], [17]. ontogeny can be experimentally decoupled from age. Although usually chronological in sequence, this behavioral ontogeny is non-viable. Lower dosage of dsRNA did not lead to measurable higher dosage of RNAi-inducing double-stranded RNA (dsRNA) is dosage dependent and the phenotype associated with using the dsRNA synthesis and larval feeding

The dsRNA targeted all three isoforms and following forward and reverse primers were used for dsRNA synthesis 3′ TAA-TACGACTCATATAGGGCAGTGTGCTCAAAATTG-TGGAA 3′ and 5′ TAA-TACGACTCATATAGGGGAGTGTGCTCAAAATTG-TGGAA 5′. The dsRNA was fed for four consecutive days at either 150 μg/ml or 450 μg/ml concentration at 12 h intervals for two consecutive days. The details of the feeding regime for rearing queens were previously described by Patel and colleagues [19]. Due to methodological challenges, in vitro rearing typically does not yield a high proportion of queens. It is desirable that the frequency of successfully raised individuals with full queen morphology is higher than 50% before a diet is characterized as ‘queen-inducing’ diet. For the second set up, the feeding regime was modified and larvae were nutritionally restricted and fed every 24 h on the VS diet throughout larval ontogeny. This feeding regime yields primarily worker caste (Kafianaghi O, Amdal GM, Page RE, unpublished data). The dsRNA was fed for four consecutive days at either 150 μg/ml or 450 μg/ml concentration at 24 h intervals. The larvae were collected 24 h after the final dsRNA feeding for knocking down PTEN isoforms in adults, and thus age-associated, manner, resulting in a temporal division of endocrine physiology and complex behavior [26], [27], [28], [29], [30], [31], [32], and can be combined with dietary manipulations, pharmacology, and RNAi mediated gene knockdown to unravel causal relationships [33], [34], [35], [36], [37].

PTEN is alternatively spliced and has three and six isoforms in *D. melanogaster* [32] and the mosquito *Aedes aegypti*, respectively [32]. In mosquitoes, isoforms show developmental- and tissue-specific mRNA levels [39]. A putative PTEN ortholog was identified by the Honey Bee Genome Sequencing project and its transcript was detected in developing larvae [18]. Yet, the corresponding gene structure, as well as intragenomic expression patterns, is unknown. Here, we have cloned and characterized the PTEN gene. Alternate splicing was identified, resulting in three splice variants. We have used RNA interference (RNAi) to down-regulate PTEN expression in larvae, and show that the efficacy of the knockdown is dosage dependent and the phenotype associated with using the higher dosage of RNAi-inducing double-stranded RNA (dsRNA) is non-viable. Lower dosage of dsRNA did not lead to measurable PTEN down-regulation and produced a viable phenotype from larvae raised on queen diet. Finally, we have explored the tissue-specific gene regulation patterns for the PTEN isoforms in adults, and show how these patterns correlated with social behavior using a controlled single cohort set up.

**Methods**

**Bees**

Wild type (unselected commercial stock) honey bees were used for all experiments. Bees were reared at the Honey Bee Research Facility on the ASU Polytechnic campus in Mesa, AZ.

**Cloning of PTEN isoforms**

Total RNA isolated from worker larval instars (4th and 5th) and from worker adult brain and fat body was used for cloning PTEN. Total RNA was treated with DNaseI (Ambion) and 5′ and 3′ RACE experiments were carried using the GeneRacer Kit (Ambion) according to the manufacturer’s instructions. For the 5′ RACE following three reverse primers (5′ TCAACAA-TAGGTCGACCTCCC 3′, 5′ TCCCCACGTAGAGTAAC 3′, 5′ GTATCAAGCTGGCTGAAGTA 3′) were used in combination with the 5′ RACE supplied with the kit. For the 3′ RACE following three forward primers (5′ TGAATGTGTTGTCAAAATTTGTGGAA 3′, 5′ TTTCCATGGAGGTCAAGGAT 3′ and 5′ CGCAAAAGAATGCATACGA 3′) were used in combination with the 3′RACE primers supplied with the kit. Four independent RACE experiments were carried out. RNA from different sources (larvae, adult brain and fat body) was used for RACE experiments. The PCR products were cloned into pCR®-TOPO® vector using TOPO TA cloning kit (Invitrogen), several clones (6–10 per experiment) were randomly picked and verified by sequencing. Subsequent to sequence analysis, full-length mRNA corresponding to the three PTEN isoforms was amplified and re-verified by sequencing.

**dsRNA synthesis and larval feeding**

The dsRNA targeted all three isoforms and following forward and reverse primers were used for dsRNA synthesis 5′ TACGACTCATATAGGGGAGTGTGCTCAGTAAATTGTTGGAA 3′ and 5′ TAA-TACGACTCATATAGGGGAGTGTGCTCAAAATTG-TGGAA 5′. The dsRNA was fed for four consecutive days at either 150 μg/ml or 450 μg/ml concentration at 12 h intervals for two consecutive days. The details of the feeding regime for rearing queens were previously described by Patel and colleagues [19]. Due to methodological challenges, in vitro rearing typically does not yield a high proportion of queens. It is desirable that the frequency of successfully raised individuals with full queen morphology is higher than 50% before a diet is characterized as ‘queen-inducing’ diet. For the second set up, the feeding regime was modified and larvae were nutritionally restricted and fed every 24 h on the VS diet throughout larval ontogeny. This feeding regime yields primarily worker caste (Kafianaghi O, Amdal GM, Page RE, unpublished data). The dsRNA was fed for four consecutive days at either 150 μg/ml or 450 μg/ml concentration at 24 h intervals. The larvae were collected 24 h after the final dsRNA feeding for knocking down PTEN isoforms and both set ups were independently replicated.

**Scoring morphological characters that distinguish queens, intercastes and workers**

Queens were identified as having >100 ovarioles/ovary, notched mandibles, smooth stinger and absence of corbicula (pollen basket). Workers were identified as having 2–30 ovarioles, barbed stinger and presence of corbicula. Intercastes have characters reminiscent of queens but had smaller ovary size (ranging between 40–70 ovarioles/ovary). Ovariode scoring was carried out as described previously [[19]]. Detailed data on morphological characters are not shown, as their occurrence was in agreement with our earlier results reported in [19],[21].
Preparing single cohort colonies

Two single cohort colonies were prepared in 4-frame standard Langstroth-size nucleus hives (19 inches in length and 19 1/8 inches in depth. (483 mm×232 mm). Queens (n = 2) were caged for 24 h to obtain newly laid eggs. The combs with the newly laid eggs were numbered and left in the colonies. These combs were removed 24 h prior to the emergence of adult workers and placed into an incubator at 35°C and 65% RH, in order to collect newly emerged bees. Single cohort colonies were established by placing about 7,000 newly emerged bees into the 4-frame nucleus hives. Each hive had 1 frame of honey, 1 frame of pollen, 2 fully drawn combs for queens to lay eggs.

Sample collections

Foragers were marked in both single cohort colonies after 15 days. Foragers identified at the hive entrance when returning from foraging flights were marked on the abdomen with a dot of paint and then allowed to enter the hive and continue foraging. Five days later, nurses and marked foragers were sampled directly into liquid nitrogen. Nurses were identified on the brood with their heads inside the brood cells. For RNA isolation and mRNA quantification, materials from three bees were pooled to make up one biological sample per tissue (brain, ovaries, and fat body). Three such biological replicates were derived from each single cohort colony, to make up a total sample size of 6 for each tissue and behavioral group (nurse bee and forager).

RNA Isolation and quantitative real-time RT-PCR (RT-qPCR)

For RNA was isolated from brain, ovaries and fat body using standard Trizol procedure except that the RNA was precipitated overnight in the presence of glycogen. For the RT-qPCR, total RNA was treated with DNaseI (Ambion) following standard instructions. RNA was diluted to 25 ng/µl and 2.0 µl was used a template. The RT-qPCR was run in triplicate (i.e. three technical replicates of the same sample on the same plate) using ABI Prism 7500 Applied Biosystems, and the data were analyzed using the comparative CT method [42] with actin (XM_623378) used as an reference gene. RT-qPCR conditions as described earlier by Wang and colleagues [43] were used. Following PTEN isoform-specific qPCR primers were used: PTEN_A Fp 5’ TCTGCGATCTCCTGGTTGGA 3’ and PTEN_A Rp 5’ TTGTGTTTTCGCGGATGACTA 3’; for the B isoform, PTEN_B Fp 5’ ACCATGCTCAATAGAATGGGTG 3’ and PTEN_B Rp 5’ ACAATTAGGTGACCTCCCTGTG 3’; and for the C isoform, PTEN_C Fp 5’ AAGGCGACAGCAGT-GAAGT 3’ and PTEN_C Rp 5’ AAAATGTGGCTCCCGCTGGT- TTT 3’. The amplification products were verified by sequencing prior to quantification. For actin, forward and reverse primers were Fp 5’ TGCCACACACTGGCCTCTG 3’ and Rp 5’ AGAATTGACCACCACTGCA 3’ respectively. Negative control (without reverse transcriptase) for every sample was used to verify that the RT-qPCR assay was not confounded by DNA contamination or primer dimers. To determine the primer efficiencies, we checked melting curves for each set of primers and run the PCR products on agarose gels. Each primer pair had a single peak in melting curve analysis and a single sharp band of expected size on the agarose gel. Additionally, amplification curves of each PTEN isoform paralleled with those of actin, which indicated that primers for each gene had equal and comparable efficiencies.

Statistics

For gene knockdown verification, PTEN expression levels were log transformed [43],[44] and Main Effect ANOVA was used for statistical analysis after validating that the data conformed the assumptions of Levene’s test. Treatment group and qPCR plate (technical factor) were categorical predictors. Adult expression data were also log transformed and main effects ANOVA was conducted separately for each tissue. Behavioral caste, qPCR plate and colony were categorical predictors. Comparisons were not made between tissues or between isoforms because i) actin transcript levels (our reference gene within tissue) can be assumed to vary between tissues, and because ii) amplification efficacies may differ between the three isoform-specific primer sets. The data conformed to assumptions of ANOVA, as determined by Levene’s tests. Fishers’ LSD tests were used to identify the pattern of significance for each isoform. Statistica 6.0 (StatSoft) was used for all analyses. For ovary and adult wet weight, the data was not normally distributed, therefore we used the non-parametric Kruskal-Wallis test, and thereafter Mann-Whitney U tests for post hoc comparisons between the treatment groups.

Results

Identification of alternate splice variants

A single honey bee PTEN gene was identified based on in silico genomic analysis by the Honey Bee Genome Consortium [45]. We used RACE (Rapid Amplification of cDNA Ends) to clone alternate splice variants of this gene, and to demonstrate that the gene, overall, contains eleven exons and ten introns. The intron/exon organization of PTENs is not shared between honey bee, D. melanogaster, mosquitoes (A. aegypti and Anopheles gambiae), C. elegans and human sequences ([39] and our Figs. 1 A and B). Despite these differences at the nucleotide level, the encoded proteins show relatively high amino acid sequence homology (Fig. 2). The degree of shared identity is 47% to D. melanogaster, 51% to A. aegypti, 37% to C. elegans, and 45% to the human proteins. The highest degree of identity is toward another hymenopteran insect, Nasonia vitripennis (73%).

As determined before for D. melanogaster [38], A. aegypti [39] and human [46], we found that the honey bee PTEN gene is alternatively spliced. Three splice variants were cloned (Fig. 1B). All isoforms encode the putative phosphatase domain (residues 52–198), essential for its activity as a tumor suppressor in humans [47] and the putative C2 lipid binding domain (residues 244–338), which has affinity to phospholipid membranes [47],[48]. The PTEN signature motif representing active site residues ‘HCXXGXXR’ (HCKAGKGR in honey bee) [48] was found at position 132–137 in all honey bee PTEN splice variants, and thus it is identical between all the available insect sequences so far (Fig. 2). The signature motif is also present in tyrosine phosphatases and dual specificity protein phosphatases but no sequence homology occurs outside this motif between these phosphatases and PTEN [49],[50]. The PTEN PDZ binding motif (XTXL/V), which has a potential role in protein-protein interaction [49],[50], was only present in honey bee PTEN isoform B. This finding is similar to the data on fly and mosquito PTEN, where only one isoform with PDZ binding motif was detected [38],[39]. Furthermore, of the three honey bee PTEN isoforms, only isoform A encodes multiple serine and threonine toward the C-termini that provide sites for post-transcriptional modification, particularly for phosphorylation (Fig. 2). Honey bee PTEN isoform C, thereby, was characterized as lacking both the PDZ binding motif (isoform B-specific) and the multiple serine and threonine residues (isoform A-specific).

Effects of reducing PTEN gene expression during larval development

Knockout of PTEN in flies and mice is embryonic lethal [51],[52] and most of the studies to gain insights into PTEN
function during development have been conducted on mutants of eye [10] or wing [51] tissue generated by somatic recombination. Here, we combined in vitro (laboratory) rearing of 1st–5th instar honey bee larvae with suppression of PTEN activity by introduction of dsRNA against the PTEN gene in the larval food. The PTEN dsRNA targeted all the three isoforms of the gene (180–741 bp). Previously, we have used the in vitro dsRNA feeding technique to reduce larval expression of TOR and the insulin receptor substrate, IRS [19],[21]. Feeding of dsRNA is also successful in down-regulating gene expression in C. elegans, termites, tsetse fly and ticks [53],[54],[55],[56]. In our study, we tested two amounts of PTEN dsRNA in both of two diets to study efficacy and effects of PTEN gene knockdown. Each dsRNA/diet combination was replicated twice.

Larvae (n = 100, per treatment group) were either given a diet made primarily of royal jelly that supports queen development (queen diet) [19] or reared on a modified diet that supports worker development (worker diet) (see Methods for details). Additionally, we also tested the dosage dependent RNAi response using two different dsRNA dosages (150 μg/ml and 450 μg/ml). First, the diets contained dsRNA at a concentration of 450 μg/ml and were fed over two or four consecutive days depending upon the diet (details in the methods). Controlling corresponding groups were raised on both diets mixed with dsRNA against green fluorescent protein (GFP) sequence, which does not share homology with genes in the honey bee genome. This approach led to a significant reduction in PTEN expression in the animals reared on worker diet (main effects ANOVA: F (1,21) = 5.55, p = 0.03, Fig. 3A) and on queen diet (main effects ANOVA: F (1,43) = 5.40, p = 0.03, Fig. 3B). As before, the controls developed normally into either queens (>50%) or workers (>70%), depending upon the diet. Regardless of diet, however, none of the PTEN knockdowns completed metamorphosis. When they reached the pupal stage, PTEN RNAi phenotypes had body region deformities, such as distorted heads, thoraces and abdomens. Mortality was 100% within 5 days after the onset of pupation. Second, we tested a lower dose of dsRNA (150 μg/ml). This approach did not affect PTEN expression at the whole-body level irrespective the rearing conditions (Fig. S1A, B). As before, the controls developed normally into either queens or workers depending upon the feeding regime. Whereas, the larvae reared on queen diet and this low dosage emerged with intercastes characteristics (queen/worker intermediates) (See Fig. S2), while those reared on worker diet failed to complete development.

Expression profiling of brain, ovary and fat body in different behavioral groups

Using two replicate single cohort colonies, we studied PTEN mRNA expression in honey bee nurses and foragers (Fig. 4, Table 1). We found that all honey bee PTEN isoforms were expressed at a significantly higher level in the brains and ovaries of foragers compared to age-matched nurses (Fig. 4, A–F, see the legend for details on the statistics). In contrast, only PTEN isoform B was significantly elevated in the fat body of nurses when compared to age-matched foraging bees (Fig. 4H).

Within tissue, we next plotted the correlative relationships between isoforms (Fig. 5). This approach allowed us to study putative associations between the transcript amounts, while taking into account that the absolute expression levels of the isoforms could not be directly compared. This is because we used a semi-quantitative measure for gene expression that is scaled separately for each gene product (see Methods). We found that PTEN isoforms A and B were significantly positively correlated in brain (Pearson’s correlation, n = 6, p<0.0005) and in ovary (p<0.05), but not associated in fat body (p>0.4) (Fig. 5A). Similarly, isoforms A and C were positively correlated in brain (p<0.01) and ovary (p<0.01), but not in fat body (p>0.85) (Fig. 5B), and the pattern was repeated for isoforms B and C: significant correlation in brain (p<0.01) and ovary (p<0.007), but not in fat body (p>0.2) (Fig. 5C).

Discussion

PTEN is a conserved lipid and protein phosphatase

As a key negative affector of IIS and Egfr signaling, PTEN homologs have been identified in all eukaryote genomes sequenced so far. Our study has identified three PTEN isoforms in honey bees. Similar to fly and human PTEN, these isoforms all share the highly conserved PTEN signature motif ‘HC(X)5R’ [46],[48], the phosphatase domain [48] and the C2 domain [48]. Crystal structure provided insights into functional motifs responsible for mediating different PTEN functions [48]. Subsequent studies based on structure-functional analysis of these motifs, and, by mutating key residues within these motifs, strongly suggested that PTEN activity is required for normal growth and loss in its activity contributes to tumorigenesis [57]. These genetic approaches have also shown the link between the phosphatase domain and PI(3,4,5) P3 phosphatase activity [49],[58], and between the C2 lipid

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Figure 1. Schematic diagram of the organization of the honey bee PTEN gene. (A) cDNA sequences of three honey bee PTEN isoforms (A, B and C) were compared to the genomic sequences gleaned from the Honey Bee Genome Resources database (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=7460) to define the exons (including alternative exons) and introns. (B) Three alternate splice forms were cloned and their structure is shown. doi:10.1371/journal.pone.0022195.g001
Binding domain’s phospho-lipid membrane affinity [47],[48] and Ca$^{2+}$ dependent recruitment of kinases [59].

We found that the PDZ binding motif at the carboxy terminal is only present in honey bee PTEN isoform B. In mice, mutations in the PDZ domain greatly reduced PTEN ability to inhibit Akt/PKB signaling and lead to rapid degradation of the PTEN product, suggesting that PDZ domain stabilize PTEN through different protein-protein interactions [60],[61]. We also found that honey bee PTEN isoform A encodes putative phosphorylation sites toward the C-termini. Phosphorylation of these residues can result in conformation change that affects recruitment of PTEN-associated complexes to plasma membrane [62], and can further stabilize PTEN, control its subcellular location, and/or its association with other signaling molecules [49],[63],[64]. Thus, our data indicate how PTEN isoforms may have different stabilities that may be linked to different functions in honey bees.

**Knockdown of PTEN during larval development**

We made a first effort to test honey bee PTEN function by down-regulating PTEN during larval development using RNAi. The dsRNA targeted all three isoforms during larval ontogeny. We used two different feeding protocols and two different dsRNA amounts. We fed the larvae with a high amount of dsRNA (450 μg/ml of each diet) (see Methods for details). This dosage produced a significant reduction of PTEN expression in larvae irrespective of the feeding regime (Fig. 3A, B). These knockdown larvae failed to complete development and died during pupation. Similar lethal phenotypes are also reported for the PTEN knockout mice [65] and flies [51]. We also tested a lower dsRNA concentration (150 μg/ml) (details in Methods). This approach did not reduce PTEN expression significantly (Fig. S1A, B). These results lead us to conclude that knockdown efficacy is dosage dependent. Dosage-dependent gene suppression is reported in mice, where efficacy of knockdown in different tissues (liver, kidney and lung) was directly proportional to dosage of siRNA administered [66]. Similar results are also reported for rat tissues [67] and in human cell lines [68].

Although PTEN expression was not reduced with lower dsRNA dosage, the phenotype was different from control: while larvae reared on queen diet completed metamorphosis and achieved intercaste or worker morphology. These adults had enlarged abdomens (see Fig. S2 B). Phenotypic effects in the absence of significant PTEN down-regulation might be explained by the target gene being affected in some regions and tissues but not in others. This heterogeneous response could make whole-body RNAi-detection difficult. In adult honey bees, where it is easier to conduct tissue specific studies of gene expression, regional RNAi efficiencies are already confirmed [43]. Overall, our study did not provide evidence that PTEN is specifically involved in queen-worker differentiation because PTEN gene suppression was lethal in both phenotypes during development. Our inference from the results, therefore, is limited to PTEN playing a central role in nutrient sensing, presumably conferred by conserved effects on IIS and Egfr activity; and thus perturbation of PTEN can negatively impact larval development.

The significant down-regulation was achieved with a high dosage that resulted in non-viable phenotype. It can always be a concern that a non-viable phenotype is unspecific. However, many studies in honey bees and other organisms have highlighted the specificity of RNAi [54],[69],[70],[71],[72]. Recent results from C. elegans, moreover, suggest that regions must share 40 bp with 95% identity for off-target effects to occur [73]. A modified BLASTn search at the NCBI non-redundant database (with somewhat similar sequences option enabled) of our PTEN dsRNA region produced only one significant hit of 19 bp against another honey bee gene: a putative cytochrome P450. This makes off-target effects unlikely. In addition, controls reared on the same amount of GFP dsRNA developed normally, ruling out negative effects of dsRNA per se. Our study does not explicitly address the lethality of PTEN down-regulation, but the phenotype is well known from experiments in mice and flies [51],[52], where the effect is explained by various developmental defects reported in different tissues and organs [51],[52]. Future studies may explore if similar explanation can be found in bees. In this context, isofrom-specific approaches may yield viable phenotypes and be more informative.

**PTEN expression and correlation to social behavior**

We demonstrate that the honey bee PTEN isoforms are transcribed at different levels within the brain, ovaries and fat tissues and organs [51],[52]. Future studies may explore if similar explanation can be found in bees. In this context, ISO-form-specific approaches may yield viable phenotypes and be more informative.
body of same-aged nurses and foragers (Fig. 4). In brain, all isoforms are expressed in higher levels in foragers. Since PTEN generally suppresses growth, our finding is not consistent with the volume increase of the neuropil of the mushroom body, a brain region that is generally expanded during honey bee foraging [74],[75]. This apparent discrepancy may be explained by the increase in neuropil volume being due to dendritic arborization rather than neurogenesis [76]. Dendritic arborization determines the nature and extent of innervation of a neuron in response to intrinsic and extrinsic signals and is a result of cytoskeleton changes in neurons [77]. PTEN is essential for proper localization of an F-actin-myosin II-based cytoskeleton in *Dictyostelium discoideum*, permitting the formation of filopodia necessary for both locomotion and chemotaxis [78]. In honey bees, increased locomotion and olfactory learning are both associated with foraging activities [79],[80]. Therefore, increased PTEN expression may be involved in cytoskeleton changes in mushroom bodies that occur during behavioral transition from in-hive work to foraging. However, we measured gene expression in the entire brain, and it is unclear how this overall transcript level reflects on dynamics in smaller sub-compartments like mushroom bodies.

Likewise, all isoforms show higher transcript levels in forager ovaries when compared to nurses of the same chronological age. In vertebrates, ovarian activity increases when PTEN expression is suppressed [81], and similarly, the propensity for ovary activation and egg-laying is increased in nurse bees compared to 21-day-old worker bees that are of forager age [82]. Thus, we hypothesize that elevated PTEN expression in the ovaries of forager bees contributes to their reduced propensity of reproductive activation [83].

In fat body (the abdominal adipose tissue), only the PTEN isoform B was transcribed at significantly different levels between the bees of different behavioral phenotype, and with increased expression in nurses. Nurse bee fat body also expresses vitellogenin (Vg) at high levels [25],[84],[85]. Vg is a yolk protein precursor and behavioral effector protein that is hypothesized to suppress IIS and perhaps Egfr signaling in honey bees [19],[86]. In general, IIS is anticipated to be reduced in nurse bee fat body [32]. Our result from PTEN isoform B is consistent with this expectation.

Finally, we found that, while the relative expression levels of all isoforms were correlated in brain and ovaries, none were correlated in fat body (Fig. 5). This pattern is largely driven by the expression dynamics described above: in brain and ovary, isoforms show highly variable expression levels that diverge consistently between nurse bees and foragers. In fat body, however, the variance is less pronounced and – with the exception of isoform B – the transcript levels do not diverge between the two behavioral groups.

### Honey bees as a model system to study PTEN function

Honey bees provide a model for understanding the molecular mechanisms that regulate complex behavior. In insects as well as mammalian systems, behavior can be affected by physiological feedback between brain, gonad and adipose tissue [26],[87],[88],[89],[90],[91]. This feedback is at least partly linked to nutrient- and energy sensing signals. In honey bees, IIS, partly...
through insulin-like peptides (ilp-1 & 2), can form a complex regulatory network influencing social behavior, which includes Vg and juvenile hormone (JH) (Fig. 6) [32],[86]. Reduced Vg can accelerate foraging behavior through feedback with JH, a systemic hormone with pleiotropic effects on metabolic biology and development [34],[86],[92],[93]. TOR can facilitate vg gene expression [19], while IRS, a central gene in IIS, can affect food-related behavior — presumably in interplay with Vg and JH [43]. Our gene expression results suggest that PTEN may be involved in adult social behavior (Fig. 6). Since PTEN influences Egfr signal transduction, Egfr could potentially also influence this regulatory loop to modulate the behavioral repertoire of worker bees. However, the role of Egfr in adult behavior is yet to be determined for honey bees.

PTEN knockdown mice are characterized by brain overgrowth and deficits in female social behavior [15]. Mutant alleles are also associated with human behavioral disease [15]. Thus, researchers have already established links between PTEN activity and complex behavior. To understand these relationships more fully, future studies on honey bees may provide insights that are less accessible in vertebrate systems or difficult to probe in flies (D. melanogaster), which lacks complex social behavior. In this context,

| Tissue      | Test    | Main effect | F value | d.f. | p     |
|-------------|---------|-------------|---------|------|-------|
| Brain       | ANOVA   | Behavior    | 13.8    | 3    | <0.01 |
|             |         | qPCR plate  | 0.12    | 3    | 0.94  |
|             |         | Colony      | 0.32    | 6    | 0.91  |
|             | Post hoc comparison of isoform b/w nurses and foragers | Isoform A | 0.005 |
|             |         | Isoform B   | <0.01   |
|             |         | Isoform C   | 0.01    |
| Ovary       | ANOVA   | Behavior    | 6.75    | 3    | 0.03  |
|             |         | qPCR plate  | 0.26    | 3    | 0.85  |
|             |         | Colony      | 0.72    | 6    | 0.64  |
|             | Post hoc comparison of isoform b/w nurses and foragers | Isoform A | 0.04 |
|             |         | Isoform B   | <0.005  |
|             |         | Isoform C   | <0.005  |
| Fat body    | ANOVA   | Behavior    | 16.7    | 3    | <0.01 |
|             |         | qPCR plate  | 0.6     | 3    | 0.63  |
|             |         | Colony      | 0.8     | 6    | 0.58  |
|             | Post hoc comparison of isoform b/w nurses and foragers | Isoform A | 0.11 |
|             |         | Isoform B   | 0.01    |
|             |         | Isoform C   | 0.08    |

Behavioral caste (nurse bee and forager), qPCR plate and colony were categorical predictors. Post hoc comparison using Fisher’s LSD were used to determine the effect of behavioral caste on each isoform (see Materials and Methods for details). Significant differences are highlighted in green.

doi:10.1371/journal.pone.0022195.t001

Figure 5. PTEN isoform-specific correlation plots by tissue. (A) Isoforms A and B, (B) Isoforms A and C (C) Isoforms B and C. Relative levels in brain (red circles), ovary (blue squares) and fat body (green triangles) are shown. Open circles, squares and triangles represent the forager data points while the closed symbols represent the nurse bee data points. As a general pattern, expression of PTEN isoforms is positively correlated in brain and ovary (Pearson’s correlation, p<0.05), but not in the fat body of the workers (p>0.05).

doi:10.1371/journal.pone.0022195.g005
our isoform- and tissue-specific data provide a platform for future experimental planning.

Supporting Information

Figure S1  
PTEN RNAi during larval development.  
Test of gene knockdown in honey bee larvae fed worker (A) vs. queen (B) diet in each of two separate experiments (n = 24). The larvae were fed with a lower dosage (150 μg/ml). Compared to the controls, the low dosage of dsRNA did not lead to measurable PTEN down-regulation at the whole-body level, neither for the queen diet, (main effects ANOVA: F (1,43) = 0.50, p = 0.48, A) nor for the worker diet (main effects ANOVA: F (1,21) = 0.38, p = 0.54, A). For the queen diet treatment, the controls primarily emerged as queens (56%) relative to 28% intercastes (individuals with mixed caste traits) and 14% workers, while those that received PTEN dsRNA emerged with intercaste phenotypes (44%) or with worker traits (52%). The phenotypic distributions of the bees, thereby, were different between the control and the PTEN dsRNA-containing queen diets (Chi-square test: χ² = 83.1, df = 2, p<0.0001). Bars represent mean ± s.e, different letters (a, b or c) denotes significantly different groups (A and B, Kruskal-Wallis test followed by post hoc Mann-Whitney U test, p<0.001).

(TIF)

Figure 6. Possible tissue-specific PTEN functions in a regulatory network of honey bee behavior. In brain and ovary, PTEN (red circles) isoforms A, B and C, which could potentially down-regulate insulin/insulin-like signaling (IIS), are more abundant in foragers than in nurses. However, foraging behavior is positively associated with IIS via the release of insulin-like peptide 1 (ilp-1, orange ellipse) in the brain (pink) [32]. Ilp-1 may cause juvenile hormone (JH, green ellipse) levels to increase [91]; JH is also positively correlated with foraging behavior [94] and may enhance IIS by feedback suppression of vitellogenin (Vg, violet ellipse), a proposed negative regulator of IIS [34],[86],[92],[93]. These relationships contradict the repression of IIS by elevated PTEN in forager brain tissue. In contrast, suppressed IIS by PTEN in ovary tissue is consistent with the reduced reproductive propensity of foragers [31]. In fat body (yellow), PTEN isoform B, ilp-1 and insulin-like peptide 2 (ilp-2) are elevated in nurses compared to foragers (K. Ihle, unpublished data; and results in this paper), but effects on metabolic biology are currently unclear. The ilp gene products from fat body or brain may also take part in remote signaling to other organs [32]. In this illustration, larger-size circles/ellipses, and thicker arrows (positive)/blocked arrows (negative) denote higher levels of expression, enhancement and suppression, respectively. Dotted arrows indicate the yet unresolved effects on worker phenotypes. doi:10.1371/journal.pone.0022195.g006

Acknowledgments

We thank J. Bozler for assistance with RT-qPCR and members of Amdam lab for discussions and suggestions. We are also grateful to J. Evans, O. Halskau, K. Haight, D. Moore, A. Dolezal, F. Wolschin, K. Dolezal and K. Traynor for helpful comments on the manuscript.

Author Contributions

Conceived and designed the experiments: NSM GVA. Performed the experiments: NSM YW OK GVA. Analyzed the data: NSM YW GVA. Contributed reagents/materials/analysis tools: GVA. Wrote the paper: NSM YW GVA.
References

1. Barbieri M, Bonafè M, Franceschi C, Paolino G (2003) Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to man. Proc Natl Acad Sci USA 100: 11350–11355.

2. Oldham S, Hafen E (2003) Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. Trends Cell Biol 13: 79–85.

3. Guo A, Villen J, Kornhauser J, Lee KA, Stokes MP, et al. (2008) Signaling networks assembled by oncogenic EGFR and c-Met. Proc Natl Acad Sci U S A 105: 692–697.

4. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, et al. (2006) Epidermal growth factor receptor (EGFR) signaling in cancer. Gene 366: 2–16.

5. Oldham S, Kim JJ, Lee J (2003) Obesity, insulin resistance and cancer risk. Oncogene 22: 449–455.

6. Schinner S, Scherbaum WA, Bornstein SR, Barthel A (2005) Molecular mechanisms of insulin resistance. Diabet Med 22: 674–682.

7. Fukuyama M, Rougvie AE, Rothman JH (2006) C. elegans DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germ-line. Curr Biol 16: 773–779.

8. Page RE, Jr., Scheiner R, Amdam GV (2006) Social exploitation of honey bee workers (Apis mellifera) affects colony-level selection effects on individual foraging behavior. Proc Natl Acad Sci USA 103: 16068–16075.

9. Wolschin F, Amdam GV (2007) Comparative proteomics reveal characteristics of life-history transitions in a social insect. Science 315: 145–149.

10. Amdam GV, Norberg K, Fondrk MK, Page RE, Jr. (2004) Reproductive ground signaling networks assembled by oncogenic EGFR and c-Met. Proc Natl Acad Sci U S A 101: 11350–11355.

11. Amdam GV, Page RE, Jr. (2006) Regulation of honey bee (Apis mellifera) life histories by vitellogenin. In: Pfäff D, Arnold A, Egen A, Fahrbach S, Rubin E, eds. Hormones, Brain and Behavior. 2 ed. San Diego: Elsevier Academic Press.

12. Amdam GV, Norberg K, Hagen A, Omholt SW (2003) Social exploitation of vitellogenin. Proc Natl Acad Sci U S A 100: 1799–1802.

13. Crailshiem K, Stößberg E (1989) Influence of diet, age and colony condition upon intestinal morphology and size of the hypertrophic glands in the honeybee (Apis mellifera L.). J Insect Physiol 35: 595–602.

14. Marco Antonio DS, Guichard-Lazzarini KR, Nascimento AM, Simões ZLP, Hartfelder K (2008) RNA-mediated silencing of vitellogenin gene functions honey bee (Apis mellifera) workers into extremely precocious foragers. Naturwissenschaften 95: 953–961.

15. Schulz DJ, Huang ZY, Robinson GE (1998) Effects of colony food shortage on behavioral development in honey bees. Behav Ecol Sociobiol 42: 293–303.

16. Smith N, Amdam G, Paule S, Cabello C, Leevens SJ, et al. (1999) Alternative splicing of the Drosophila PTEN gene. Biochim Biophys Acta 1447: 313–317.

17. Riehle MA, Brown JM (2007) Characterization of phosphatase and tensin homolog expression in the mosquito Aedes aegypti: six splice variants with developmental and tissue specificity. Insect Mol Biol 16: 277–286.

18. Amdam GV, Norberg K, Page RE, Jr., Erber J, Scheiner R (2006) Downregulation of vitellogenin gene activity increases the gustatory responsiveness of honey bee workers (Apis mellifera). Behav Brain Res 169: 201–205.

19. Mutti NS, Lespin J, Pappan K, Begum K, et al. (2008) A protein from the salivary glands of the pea aphid, Acyrthosiphum pisum, is essential in feeding on a host plant. Proc Natl Acad Sci U S A 105: 9965–9969.

20. Luvak KJ, Schmitgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402–408.

21. Wang Y, Mutti NS, Jile KE, Siegel A, Dolezal AG, et al. (2010) Down-regulation of honey bee IRS gene biases behavior toward food rich in protein. PLoS Genet 6: e1000896.

22. Grozinger CM, Sharabash NM, Whitfield CW, Robinson GE (2003) Pheromone-mediated gene expression in the honeybee brain. Proc Natl Acad Sci U S A 100: Suppl 2: 14313–14325.

23. Souza FC, Delatte H, Teixeira MA (2006) Effect of storage temperature on the morphology of the honeybee (Apis mellifera) colonies. Insect Mol Biol 15: 777–787.

24. Das S, Dixon JE, Cho W (2003) Membrane-binding and activation mechanism of PTEN. Proc Natl Acad Sci U S A 100: 7491–7496.

25. Lee JO, Yang H, Georgescu MM, Di Cristofano A, Machetta T, et al. (1999) Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell 99: 323–334.

26. Gerike A, Runcos M, Ross AH (2006) Regulation of the PTEN phosphatase. Cancer Res 66: 3741–3746.

27. Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H (1999) The tumor-suppressor activity of PTEN is regulated by its carboxy-terminal region. Proc Natl Acad Sci U S A 96: 10182–10187.

28. Gehrbach DC, Paricio N, Mlodzik M, Wilson C (1999) Splicing of the Drosophila PTEN gene. Biochim Biophys Acta 1447: 313–317.

29. Rojas LM, Pradhan V, Chauhan V, Raftery M, Lhuillier J, et al. (2006) Drosophila tumor suppressor PTEN controls cell size and number by antagonizing the Chico/Pi3ks-nasine signaling pathway. Genes Dev 13: 3244–3258.

30. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, et al. (1999) High expression of phosphatase and tensin homolog (PTEN) in a panel of colorectal cancer cell lines. Cancer Res 59: 6542–6547.

31. Yano M, Palavecino A, Stoppacciaro A, Petraglia C, D’Incalci M, et al. (2001) Down-regulation of PTEN in colorectal cancer cell lines is associated with increased cell migration and invasion. Oncogene 20: 5019–5027.

32. Amend SA, Corona M, Pollock HS, Robinson GE (2000) Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. Proc Natl Acad Sci USA 107: 20073–20078.

33. Amdam GV, Bale KE, Page RE, Jr. (2009) Regulation of honey bee (Apis mellifera) life histories by vitellogenin. In: Pfäff D, Arnold A, Egen A, Fahrbach S, Rubin E, eds. Hormones, Brain and Behavior. 2 ed. San Diego: Elsevier Academic Press.

34. Amdam GV, Norberg K, Hagen A, Omholt SW (2003) Social exploitation of vitellogenin. Proc Natl Acad Sci U S A 100: 1799–1802.

35. Crailshiem K, Stößberg E (1989) Influence of diet, age and colony condition upon intestinal morphology and size of the hypertrophic glands in the honeybee (Apis mellifera L.). J Insect Physiol 35: 595–602.

36. Marco Antonio DS, Guichard-Lazzarini KR, Nascimento AM, Simões ZLP, Hartfelder K (2008) RNA-mediated silencing of vitellogenin gene functions honey bee (Apis mellifera) workers into extremely precocious foragers. Naturwissenschaften 95: 953–961.

37. Schulz DJ, Huang ZY, Robinson GE (1998) Effects of colony food shortage on behavioral development in honey bees. Behav Ecol Sociobiol 42: 293–303.

38. Smith N, Amdam G, Albacete S, Cabello C, Leevens SJ, et al. (1999) Alternative splicing of the Drosophila PTEN gene. Biochim Biophys Acta 1447: 313–317.

39. Riehle MA, Brown JM (2007) Characterization of phosphatase and tensin homolog expression in the mosquito Aedes aegypti: six splice variants with developmental and tissue specificity. Insect Mol Biol 16: 277–286.

40. Amdam GV, Norberg K, Page RE, Jr., Erber J, Scheiner R (2006) Downregulation of vitellogenin gene activity increases the gustatory responsiveness of honey bee workers (Apis mellifera). Behav Brain Res 169: 201–205.

41. Mutti NS, Lespin J, Pappan K, Begum K, et al. (2008) A protein from the salivary glands of the pea aphid, Acyrthosiphum pisum, is essential in feeding on a host plant. Proc Natl Acad Sci U S A 105: 9965–9969.

42. Luvak KJ, Schmitgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402–408.

43. Wang Y, Mutti NS, Jile KE, Siegel A, Dolezal AG, et al. (2010) Down-regulation of honey bee IRS gene biases behavior toward food rich in protein. PLoS Genet 6: e1000896.
multi-PDZ domain containing scaffold protein MAGI-2. Proc Natl Acad Sci U S A 97: 4233–4238.

61. Valiente M, Andres-Pons A, Gomez B, Torres J, Gil A, et al. (2005) Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. J Biol Chem 280: 28936–28943.

62. Vaucheret H, Vazquez F, Crete P, Bartel DP (2004) The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev 18: 1187–1197.

63. Griffin RJ, Moloney A, Kelhimer M, Johnston JA, Ravid R, et al. (2005) Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer’s disease pathology. J Neurochem 93: 105–117.

64. Marahama T (2006) [Regulation of the lipid phosphatase PTEN]. Seikagaku 78: 135–137.

65. Di Cristofano A, Pesce B, Cordes Cardoza C, Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression. Nature Genetics 19: 348–355.

66. Kobayashi N, Matsui Y, Kawase A, Hirata K, Miyagishi M, et al. (2004) Vector-mediated knockdown of AURKB and EGFR shows enhanced therapeutic efficacy in prostate tumor regression. PLoS Biol 2: E98.

67. Dann CT, Alvarado AL, Hammer RE, Garbers DL (2006) Heritable and stable gene knockdown in rats. Proc Natl Acad Sci U S A 103: 11246–11251.

68. Addepalli MK, Ray KB, Kumar B, Ramanath RL, Chile S, et al. (2009) RNAi-mediated knockdown of AURKB and EGFR shows enhanced therapeutic efficacy in prostate tumor regression. Gene Ther 17: 352–359.

69. Tomoyasu Y, Miller SC, Tomita S, Schoppmeier M, Grossmann D, et al. (2008) Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in Tribolium. Genome Biol 9: R10.

70. Reye M, Hartel S, Hagen A, Hasselmann M, Omholt SW (2002) Specific developmental gene silencing in the honey bee using a homeobox motif. Insect Mol Biol 11: 527–532.

71. Ambros V, Chen X (2007) The regulation of genes and genomes by small RNAs. Mol Biol Med 18: 348–355.

72. Vaucheret H, Vazquez F, Crete P, Bartel DP (2004) The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev 18: 1187–1197.

73. Farris SM, Robinson GE, Fahrbach SE (2001) Experience- and age-related inhibition of small RNA function. PLoS Biol 2: E96.

74. Rual JF, Klitgord N, Achaz G (2007) Novel insights into RNAi off-target effects. PLoS Biol 5: e62.

75. Nelson CM, Biale KE, Fondrik MK, Page RE, Amdam GV (2007) The gene vitellogenin Has Multiple Coordinating Effects on Social Organization. PLoS Biol 5: e62.

76. Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, et al. (2007) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. FEBS Lett 579: 4961–4965.

77. Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic arborization. Nat Rev Neurosci 11: 316–328.

78. Wessel D, Lasche DF, Kuhi S, Heid P, Steid DR (2007) PTEN plays a role in the suppression of lateral pseudopod formation during Dictyostelium motility and chemotaxis. J Cell Sci 120: 2517–2531.

79. Fahrbach SE, Robinson GE (1995) Behavioral development in the honey bee: toward the study of learning under natural conditions. Learn Mem 2: 199–224.

80. Scheimer R, Page RE, Jr., Erber J (2001) Responsiveness to sucrose affects tactile and olfactory learning in foraging honey bees of two genetic strains. Behav Brain Res 120: 67–73.

81. Reddy P, Liu L, Adhikari D, Jagalamudi K, Rajareddy S, et al. (2008) Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science 319: 611–613.

82. Amdam GV Social context, stress, and plasticity of aging (Aging Cell).

83. Hansen IA, Attardo GM, Park JH, Peng Q, Raikhel AS (2004) Target of Rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc Natl Acad Sci U S A 101: 10626–10631.

84. Pinto LZ, Bitondi MMG, Simões ZLP (2000) Inhibition of vitellogenin synthesis for embryonic development and tumour suppression. Nature Genetics 19: 133–137.

85. Griffin RJ, Moloney A, Kelliher M, Johnston JA, Ravid R, et al. (2005) Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer’s disease pathology. J Neurochem 93: 105–117.

86. Lin H, Winston ML, Haunerland NH, Slesor KN (1999) Influence of age and population size on ovarian development, and of trophallaxis on ovarian development and vitellogenin titres of queenless worker honey bee (Hymenoptera: Apidae). Can Entomol 131: 693–706.

87. Scheimer R, Page RE, Jr., Erber J (2001) Responsiveness to sucrose affects tactile and olfactory learning in foraging honey bees of two genetic strains. Behav Brain Res 120: 67–73.

88. Broue F, Liere P, Kenyon C, Baulieu EE (2007) A steroid hormone that extends life-span by insulinlike signaling in the nervous system. Science 290: 147–150.

89. Reddy P, Liu L, Adhikari D, Jagalamudi K, Rajareddy S, et al. (2008) Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science 319: 611–613.

90. Nelson CM, Biale KE, Fondrik MK, Page RE, Amdam GV (2007) The Gene vitellogenin Has Multiple Coordinating Effects on Social Organization. PLoS Biol 5: e62.

91. Amdam GV (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. J Theor Biol 223: 451–464.

92. Reddy P, Liu L, Adhikari D, Jagalamudi K, Rajareddy S, et al. (2008) Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science 319: 611–613.

93. Broue F, Liere P, Kenyon C, Baulieu EE (2007) A steroid hormone that extends the lifespan of Caenorhabditis elegans. Aging Cell 6: 87–94.

94. Pinto LZ, Bitondi MMG, Simões ZLP (2000) Inhibition of vitellogenin synthesis for embryonic development and tumour suppression. Nature Genetics 19: 133–137.

95. Elekonich MM, Schulz DJ, Bloch G, Robinson GE (2001) Juvenile hormone and reproductive signals in lifespan determination. Aging Cell 6: 715–721.

96. Vaucheret H, Vazquez F, Crete P, Bartel DP (2004) The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev 18: 1187–1197.

97. Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic arborization. Nat Rev Neurosci 11: 316–328.

98. Wessel D, Lasche DF, Kuhi S, Heid P, Steid DR (2007) PTEN plays a role in the suppression of lateral pseudopod formation during Dictyostelium motility and chemotaxis. J Cell Sci 120: 2517–2531.

99. Fahrbach SE, Robinson GE (1995) Behavioral development in the honey bee: toward the study of learning under natural conditions. Learn Mem 2: 199–224.

100. Scheimer R, Page RE, Jr., Erber J (2001) Responsiveness to sucrose affects tactile and olfactory learning in foraging honey bees of two genetic strains. Behav Brain Res 120: 67–73.

101. Reddy P, Liu L, Adhikari D, Jagalamudi K, Rajareddy S, et al. (2008) Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science 319: 611–613.

102. Amdam GV Social context, stress, and plasticity of aging (Aging Cell).

103. Hansen IA, Attardo GM, Park JH, Peng Q, Raikhel AS (2004) Target of Rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc Natl Acad Sci U S A 101: 10626–10631.

104. Pinto LZ, Bitondi MMG, Simões ZLP (2000) Inhibition of vitellogenin synthesis for embryonic development and tumour suppression. Nature Genetics 19: 133–137.