

Arginine kinase is highly expressed in the compound eye of the honey bee, *Apis mellifera*

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Abstract

We have cloned and sequenced a 1.68-kb cDNA encoding arginine kinase in the honey bee, *Apis mellifera*. The predicted protein shows a high level of identity to known arginine kinases in invertebrates and to other proteins belonging to the conserved family of ATP: guanidino phospho-transferases. The pattern of expression of arginine kinase has been investigated for the first time in various tissues including the brain, antennae and compound eye. Our results show that three isoforms of arginine kinase, transcribed from a single gene, are expressed in a characteristic pattern in major tissues of the honey bee. Arginine kinase mRNA is relatively abundant in the central nervous system and in the antennae. However, the highest level of expression, that is at least two to three times higher than in the brain, is found in the compound eye of the bee. By contrast, the levels of mRNAs encoding another metabolically important enzyme, α -glycerolphosphate dehydrogenase (α -GPDH), are low in the eye. These findings suggest that arginine kinase is an important component of the energy releasing mechanism in the visual system that has high and fluctuating energy demands. Furthermore, our results support the role of phosphagen kinases in energy transport in polarised cells and are consistent with the role of arginine kinase as an energy shuttle that delivers ATP generated by mitochondria to high energy-requiring processes, such as massive membrane turnover and pigment regeneration in the retina. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In order to detect brief flashes of light and adjust to the enormous range of ambient intensities, photoreceptors face very different problems from cells responding to slower and less variable stimuli. In particular, the visual system must cope with the formidable task of providing energy for such processes as the massive membrane turnover and rapid pigment regeneration in the retina (Schwemer, 1989; Stowe et al., 1990).

In living organisms, ATP is utilised as a convenient store of energy to drive many different chemical reactions that cells need. However, depending on the cell type and its activity, the metabolic half-life of an ATP molecule varies from seconds to minutes. Tissues with a high ATP turnover, such as brain and muscles, have

only a few seconds of supply of ATP and rely on a free energy reservoir that functions to regenerate ATP rapidly. In invertebrates, phosphoarginine functions in this capacity (Dumas and Camonis, 1993; Strong and Ellington, 1995). It is synthesised by the reversible phosphorylation of arginine by ATP as catalysed by arginine kinase (ArgK). Under standard conditions, the reaction: $\text{ATP} + \text{arginine} \rightleftharpoons \text{phosphoarginine} + \text{ADP}$, operates close to equilibrium. However, at times of high metabolic activity, when ATP is low, the equilibrium shifts so as to yield net synthesis of ATP. Phosphoarginine thereby acts as an ATP buffer in cells that contain ArgK. The physiological importance of this reaction is seen by some authors as providing the metabolic capacitance whereby ArgK (or creatine kinase in vertebrates) allows reduction of peak rates of ATP synthesis in cells that alternate between periods of high- and low-energy consumption. Furthermore, facilitated diffusion of ATP and ADP leads to spatial buffering in addition to temporal buffering (Sweeney, 1994).

ArgK is a member of a highly conserved family of

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Abbreviations: ArgK, arginine kinase; ORF, open reading frame; PCR, polymerase chain reaction; SDS, sodium dodecyl sulphate.

phosphagen kinases and is found in invertebrate species where it serves a function analogous to that of creatine kinase (CrK) in vertebrates (Dumas and Camonis, 1993; Strong and Ellington, 1995). Although ArgK is the major guanidino kinase found in invertebrates, other members of this protein family have also been reported in annelids, molluscs and arthropods (Muhlebach et al., 1994; Suzuki and Furukohri, 1994). By contrast, vertebrate tissues express exclusively various isoenzymes of CrK that are transcribed from multiple loci (Muhlebach et al., 1994; Suzuki and Furukohri, 1994). For example, four different nuclearly encoded CrK isoforms are found in birds and mammals, including two mitochondrial isoenzymes (Muhlebach et al., 1994). Distinct mitochondrial isoenzymes of ArgK have also been reported in a number of invertebrate species including the fly, *Drosophila melanogaster* (Chen and Lehninger, 1973; Munneke and Collier, 1988; Doumen and Ellington, 1990; Muhlebach et al., 1994). However, the report on the mitochondrial isoform of ArgK in the fly remains controversial after strong evidence against its occurrence has been published recently (Wyss et al., 1995). In evolutionary terms, ArgK is considered as the primordial phosphagen kinase and has been studied in some detail in a few animals, in particular in the lobster and in a number of molluscs (Dumas and Camonis, 1993; Suzuki and Furukohri, 1994; Strong and Ellington, 1995). The major focus of those studies has been on the biochemical and evolutionary properties, using the arthropod muscle ArgK as a model, with less attention directed towards molecular and regulatory aspects. Consequently, the spatio-temporal patterns of ArgK expression are not well understood. Recently, a number of cDNAs encoding ArgKs in major groups of invertebrates have been made available. This allowed the consensus peptide regions to be elucidated and a hypothesis on the role of particular residues in catalytic activity to be made (Dumas and Camonis, 1993; Suzuki and Furukohri, 1994; Strong and Ellington, 1995).

As part of our effort to understand the functional significance of genes expressed in the central nervous system, we have cloned and sequenced a cDNA encoding arginine kinase in an important behavioural model organism, the honey bee (*A. mellifera*) and examined its pattern of expression in various tissues. We show that three isoforms of ArgK, transcribed from a single gene, are expressed in major tissues in the bee. Interestingly, the highest level of ArgK is found in the compound eye of this insect, suggesting that ArgK plays a key role in the energy-releasing mechanism in the visual system. Our findings are consistent with the proposed role of phosphagen kinases in providing an energy reservoir and facilitating energy transport in cells that alternate between high- and low-energy consumption and, moreover, have a form of metabolic polarisation.

2. Materials and methods

2.1. Organism and tissue preparation

Adult foraging bees were captured near a free-standing feeder and immediately snap-frozen in liquid nitrogen (LN). In order to obtain late-stage pupae and young, newly emerged bees, a single brood frame was removed from the hive and incubated at 32°C. Collected pupae and newly emerged bees were also quickly frozen in LN. Organ separation and head dissection were carried out on a plastic plate placed on dry ice. Brains were dissected under permeant LN cooling.

2.2. cDNA cloning

The original partial cDNA corresponding to the coding sequence of ArgK in the honey bee has been identified via differential display RT-PCR by comparing patterns of gene expression in heads of newly emerged bees with those in adult foragers (not shown, but see Fig. 3A for differences in the level of ArgK mRNA in heads of young, NH, versus heads of adult bees, FH). To obtain the entire coding sequence of the honey bee ArgK, the original cDNA clone was extended via 3'- and 5'RACE amplification using total bee brain RNA and brain cDNA library, respectively. The unidirectional brain cDNA library has been constructed in pcDNA3.1 (Invitrogen). The primers were, 3'RACE: F-5' CCT TGC TTG ACC GAG GCT C and R-5'GAC TCG AGT CGA CAT CGA T₁₇. 5'RACE: F-T7 promoter, R-5' CCT GAC AGA GTG CTG GAG ACT. The longest clone amplified from the library is 1683 bp long. PCR fragments were extracted from agarose gels using the Qiagen kit and cloned into an *EcoRV*-digested BlueScript II KS vector (Stratagene) that was modified by incubation with Taq DNA polymerase (Promega) at 75°C.

2.3. RNA isolation

Total brain RNA was extracted with the Trizol reagent (Gibco-BRL) following the manufacturer's protocol. The final RNA pellets were dissolved in H₂O and used immediately. For isolation of poly(A)⁺ RNA from various tissues, the following procedure has been employed: 200 mg of tissue were homogenised in 0.5 ml of the lysis/binding buffer (0.1 M Tris-HCl, 5 M LiCl, 10 mM EDTA, 1% LiDS and 5 mM DTT, pH 8.0) and passed through a QIA-shredder column (Qiagen). The homogenate was combined with 2.5 mg of Dynabeads Oligo (dt)₂₅ (Dynal) and allowed to anneal for 5 min at room temperature. Subsequent steps were conducted essentially as described in the Dynal protocol. mRNA was eluted from magnetic beads with 50 ml of H₂O at 65°C and stored at -80°C.

2.4. RNA electrophoresis, blotting and hybridisation

mRNA samples were denatured by mixing with an equal volume of formamide containing 0.05% Bromophenol Blue and 0.05% xylencyanol, incubated at 90°C for 5 min and immediately chilled on ice. Electrophoresis was performed in small horizontal tanks (Hoeffer HE33) using 1.2% agarose gels submerged in TBE buffer (50 mM Tris-borate, 1 mM EDTA, pH 8.2) at 20 V/cm. In accordance with previous reports (Liu and Chou, 1990) we find that omission of formaldehyde in the running buffer does not affect the separation of RNA. Following electrophoretic resolution, the gels were soaked in 1 M ammonium acetate, 0.02M NaOH and blotted on to Hybond N+ nylon membranes (Amersham) by capillary transfer. RNA was cross-linked to the membrane by UV irradiation, and after a brief wash in 2 × SSC, the blots were pre-hybridised in 5 ml of the ExpressHyb solution (Clontech) for 30–60 min. Hybridisation was carried out at 68°C for 1–16 h using P³²-labelled probes (RediPrime kit, Amersham). Blots were washed three or four times in 2 × SSC, 0.1% SDS at 50°C and exposed to a phosphorstorage screen without drying. Computer-generated images (MD Phosphor-Imager 400S) of individual gels were analysed using ImageQuant software. In all cases, the ArgK probe was a cDNA fragment spanning the middle part of clone AmARGK (residues 483–1327). The *a*-GPDH probe was a 333-bp PCR fragment of the bee gene, GenBank Accession No. AF023666 (Wilanowski and Gibson, 1998), amplified with primers: F-5' GGC CAA TAG CTG TAG GCT TTA T and R-5' CGT ATT TGT ATC GTT GGA TCA GG.

2.5. Quantitative analysis and other methods

To standardise the amount of RNA loaded per lane, all samples were stained with the RNA dye, SyberGreen II (FMC Bioproducts) and quantified with the Vistra FluorImager. We estimate the loading accuracy to be 10–15%. Other molecular and bioinformatics methods were applied as described previously (Maleszka et al., 1996; Maleszka and Stange, 1997).

3. Results

3.1. Sequence analysis of a cDNA clone encoding arginine kinase in the honey bee

The nucleotide sequence of the 1683-bp cDNA fragment designated AmARGK is shown in Fig. 1. Judging from the size estimates of ArgK isoforms in the bee (Fig. 3, see below), the length of this clone coincides with the smallest ArgK isoform, designated *c*, that is approximately 1.7 kb. However, we cannot rule out the

possibility that AmARGK corresponds to a 5'-truncated longer isoform. AmARGK contains a long open reading frame (ORF) starting at position 27 and terminating at position 1142. Since the 5' region of this sequence contains a stop codon (TAA) at position 24 upstream of the first in-frame methionine, we conclude that ATG codon at position 75 is the translation start. This notion is supported by the remarkable conservation of the ArgK sequences shown in Fig. 1. The TAA stop codon at position 1140 interrupts the ORF leaving 541 bp of untranslated trailer at the 3' end. The clone is polyadenylated (30 residues) and has three consensus motifs AATAAA (underlined) located at positions 1366, 1396 and 1510 that may represent polyadenylation signals.

The alignment of the 355 amino acid predicted polypeptide encoded by AmARGK with the corresponding proteins in the grasshopper and lobster is shown in Fig. 2. Over the whole length of the protein, AmARGK shares 87% identical amino acids with the grasshopper ArgK and 85% identical amino acids with the lobster protein. This remarkable homology includes a peptide motif CPTNLGT around a reactive cysteinyl residue (position 270) that has been implicated in guanidine binding (Dumas and Camonis, 1993). Moreover, the bee ArgK also shares 30–50% identical residues with various creatine kinases in vertebrates, including the human enzyme (not shown).

3.2. Pattern of ArgK expression in the honey bee

Although we have not recovered ArgK cDNAs longer than 1.7 kb from our cDNA brain library, Northern blot hybridisations show that the honey bee has three isoforms of ArgK, designated *a*, *b* and *c*, that are 3.5, 2.5 and 1.7 kb long, respectively (Fig. 3A and B). Since the Southern blot analysis is consistent with the presence of a single gene encoding ArgK in the honey bee (not shown), we conclude that all three isoforms are transcribed from one gene.

Fig. 3A illustrates a typical Northern blot analysis of ArgK expression in different body compartments in both young and adult bees and in pupal heads. The tissue-specific pattern of expression shows considerable differences in the amount of each of these isoforms between various tissues as well as a variability in their relative proportions in individual tissues. Two isoforms, ArgK-*b* and ArgK-*c*, are clearly detectable in all of the analysed tissues, whereas ArgK-*a* is either missing or its expression is marginal in the abdomen (see also lane O in panel B). The levels of ArgK-*a* are low in pupal heads, but they increase in heads of young individuals and reach high levels in forager head (Fig. 3A). The strongest hybridisation intensities are yielded by ArgK-*b*, whereas ArgK-*c* yields the weakest signals. The latter also appears to be the least variable. A notable feature of the hybridisation pattern shown in Fig. 3A is the high

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1   cgcgtagcac  gtgcctcgag  gagtaactca  gaacacttca  attttctttt
51  gtctgccaaa  gcaaaaaaag  caatATGGTT  GACCAAGCTG  TTTTGGATAA
101 ACTAGAGACC  GGCTTCAGCA  AATTGTCTAG  CTCAGATAGC  AAATCATTGC
151 TGAAGAAGTA  TTTGTCCAAG  GATGTCTTTG  ATCAACTAAA  AACGAAGAAA
201 ACCTCCTTCG  ATTCTACTCT  TCTAGATTGT  ATCCAATCTG  GTATAGAGAA
251 TTTGGATTCT  GGCCTTGGCA  TTTACGCCCC  TGATGCTGAG  GCTTACACAC
301 TTTTGTGCTG  CCTCTTCGAC  CCCATTATTG  AAGATTATCA  TGGTGGTTTC
351 AAGAAGACTG  ACAAGCATCC  ACCAAAAGAT  TTTGGAGACG  TTGATTCTCT
401 TGGCAACCTT  GATCCAGCTA  ATGAATTCAT  CGTTTCCACT  CGTGTAAGAT
451 GCGGTCGCTC  CTTAGAAGGT  TATCCGTTCA  ATCCTTGCTT  GACCGAGGCT
501 CAGTACAAAG  AGATGGAAGA  AAAAGTCTCC  AGCACTCTGT  CAGGTTTAGA
551 GGGTGAACCTG  AAGGGGACTT  TCTATCCTTT  AACCGGCATG  AGCAAAGAAA
601 CTCAACAAAA  ATTGATCGAC  GATCACTTCC  TGTTTAAAGA  AGGTGATCGC
651 TTTTGTCAAG  CTGCCAATGC  TTGCCGCTTC  TGGCCCACTG  GCCGTGGTAT
701 TTACCACAAC  GATGATAAGA  CCTTCTTGGT  CTGGTGCAAC  GAAGAAGATC
751 ATCTTCGTAT  TATTTCCATG  CAGATGGGCG  GTGACCTTGG  ACAGGTTTAT
801 CGTCGTTTGG  TACATGCTGT  AAACGAAATC  GAGAAACGGC  TTCTTTTCTC
851 ACACAATGAT  CGTCTCGGTT  TCCTGACTTT  CTGCCCGACT  AACTTGGGTA
901 CTACAGTCCG  TGCTTCTGTG  CACATCAAGC  TGCTTAAAT  AGCCGCCAAC
951 AGGGCAAAAC  TTGAGGAAAT  CGCAGGGAAA  TTCAATCTCC  AGGTCCGTGG
1001 TACTCGCGGG  GAGCACACCG  AGGCTGAAGG  GGGCATCTAT  GATATTTCTA
1051 ATAAACGGCG  TCTTGGTCTG  ACCGAGTATC  AGGCTGTGAA  GGAGATGCAC
1101 GATGAATCG  CCGAGTTGAT  TAAACTCGAA  AAGGAACCTt  aaataacgcg
1151 ccagagacaa  agcaatcacg  cgatcatttc  cctttattcg  tccttttagac
1201 tctcttgata  catcgaggat  cctttctttg  aaaaaaaaga  caagagataa
1251 agatcttcaa  ttatcacatc  atttttgtaa  aaactttgaa  acaagcgatt
1301 gagaagtatt  cgaaaaaaat  aaaaaaatgt  aaagtattat  aattcgtttc
1351 taaagagtga  gaaaaaataa  aagaattgtg  aaattcgttc  tgaaaaataa
1401 acaaaaaaat  aaaaacattt  ctaaagggaa  caatggggca  ttttttgaat
1451 tagccatttt  gttcacgtga  aataaaaaat  gatagacaat  tttttgtctt
1501 ctaattttta  ataaatccta  atcctcgggt  tgctaagatc  tcttcaacgc
1551 agctacaagc  ggggtacttct  tagagttcta  aaaaggagtc  gatgagagct
1601 tggttcacga  tgagataatt  cctacaaagg  tttagttcgt  ctagtcgaaa
1651 ctgaaaaaaa  aaaaaaaaaa  aaaaaaaaaa  aaa

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Fig. 1. Nucleotide sequence of the honey bee cDNA clone designated AmARGK. The coding region is in capital letters. The ATG start-codon and the TAA terminator are in bold. The in-frame stop codon TAA at position 24 and putative polyadenylation signals (AATAAA) are underlined.

level of ArgK mRNA, in particular isoform *b*, in the head as compared to the brain, suggesting that other cephalic tissues or appendages contribute significantly to the intensity of the head-specific pattern. Following this observation, we examined the ArgK expression in the eyes and antennae and compared it with that in other tissues. The results shown in Fig. 3B clearly indicate that the highest intensity of hybridisation for both isoforms ArgK-*a* and ArgK-*b* is found in the compound eye. We estimate the ArgK expression in the eye to be at least two to three times higher than that in the brain. The antennal expression of ArgK is also relatively high. The lowest expression is found in ovaries, where isoform *a* is undetectable. Although ArgK-*b* is the most abundant isoform in all examined samples, the ratio of ArgK-*b* to ArgK-*a* varies significantly depending on the tissue. For example, the *b/a* ratio is 6.5 in the eye, 2.5 in the brain and 6.8 in the thorax.

In a control Northern experiment, we examined the expression pattern of the soluble α -glycerolphosphate dehydrogenase (α -GPDH), an important enzyme that is found in metabolically active cells. The envisaged role(s) of α -GPDH is distinct from that of ArgK. This protein is involved in maintaining a proper NAD⁺ level, providing reducing equivalents to the electron transport chain and serving a gluconeogenic function (Bewley and Miller, 1983). In contrast to ArgK, the highest level of α -GPDH is found in the ovaries. The levels of both

isoforms of α -GPDH are also high in the brain and in the antennae, but not in the eye. This result clearly demonstrates that metabolic requirements of different tissues can vary considerably, and they must be provided by functionally distinct enzymes.

By using primers that amplify the coding region of clone AmARGK, we have obtained preliminary evidence that ArgK isoforms in the honey bee differ at their 3' ends rather than being products of differential splicing (not shown). Thus, we believe that all three isoforms in this organism encode the same polypeptide. The 3' untranslated regions of eukaryotic transcripts often contain genetic information that has been implicated in various regulatory mechanisms controlling the expression pattern of the encoded proteins. These include tissue specificity, intracellular localisation and responses to physiological stimuli (Hentze et al., 1988; Macdonald and Kerr, 1997; Mahon et al., 1997; Parthasarathy et al., 1997). Hence, it is conceivable that the unique 3' regions of AmARGK isoforms harbour specific signals that determine their spatio-temporal pattern of expression.

4. Discussion

We have characterised at the molecular level a cDNA encoding ArgK in the honey bee, *A. mellifera*. The predicted protein shows a remarkable level of identity

A. m	1	MVDQAVLDKLETGFSKLSST-DSKSLLKKYLSKDVFQDLK
S. a	1	MVDAAVLEKLEAGFKKLEAS-DSKSLLKKYLTRVFDKLLK
H. g	1	MADAATIAKLEEGFKKLEAATDCKSLLKKYLSKDVFDSLK
A. m	40	TKKT-SFDSTLLDCIQSGIENLD SGVGIYAPDAEAYTLFA
S. a	40	TKKTPSEGSTLLDCIQSGIENHDSGVGIYAPDAEAYTVFA
H. g	41	AKKT-SLGAATLLDV IQSGVENLD SGVGIYAPDAEAYS LFA
A. m	79	DLFDPIIEDYHGGFKKTDKHPKDFGDVDSLGNLDPANEF
S. a	80	DLFDPIIEDYHGGFKKTDKHPKDFGDVDTLANLDPNGEY
H. g	80	PLFDPIIEDYHKGFKQTDKHPAKDFGDVSKFINVDPEGTF
A. m	119	IVSTRVRCGRSLEGYPFNPCLTEAQYKEME EKVSSTLSGL
S. a	120	VISTRVRCGRSMQGYPFNPCLTEAQYKEME QKVS TLSSSL
H. g	120	VISTRVRCGRSMEGYPFNPCLTEAQYKEME EKVSSTLSGL
A. m	159	EGELKGTFYPLTGMSKETQQKLIDDHFLFKEGDRFLQAAN
S. a	160	EGELKGQFYPLTGMSKEVQQKLIDDHFLFKEGDRFLQAAN
H. g	160	EGELKGSYFPLTGMTEKEVQQKLIDDHFLFKEGDRFLQAAN
A. m	199	ACRFWPTGRGIYHND DKTFVLWCNEEDHLRIISMOMGGDL
S. a	200	ACRFWPSGRGIYHNDNKTFVLWCNEEDHLRIISMOMPGGDL
H. g	200	ACRYWPA GRGIYHNDNKTFVLWCNEEDHLRIISMOMGGDL
A. m	239	GQVYRRLVHAVNEIEKRLLFSHNDRLGFLTFCPTNLGTTV
S. a	240	GQVYRRLVHAVNEIEKRIPFSHNDRLGFLTFCPTNLGTTT
H. g	240	GQVYRRLVSAVNDIEKRVFFSHNDRLGFLTFCPTNLGTTV
A. m	279	RASVHIKLPKLAANRAKLEELAGKFNLQVRGTRGEHTEAE
S. a	280	RASVHIKLPKLAADRTEKLEEVAGKFNLQVRGSTGEHTEAE
H. g	280	RASVHIKLPKLAANREKLEEVAAKFSLQVRGTRGEHTEAE
A. m	319	GGIYDISNKRRGLTEYQAVKEMHDGIAELIKIEKEL
S. a	320	GGIYDISNKRRMGLTEYDAVKEMNDGILELIKIEGSL
H. g	320	GGIYDISNKRRMGLTEYQAVKEMNDGILELIKIEKEM

Fig. 2. Alignment of amino acid sequences of ArgKs in the bee (A. m), grasshopper, *Schistocerca americana* (S. a) and lobster, *Homarus gammarus* (H. g). Identical and similar residues are shown in black and grey boxes, respectively. Accession Nos. A. m: AF023619, S. a: U77580, H. g: P14208.

to known ArgKs in invertebrates and to other proteins belonging to the conserved family of ATP: guanidino phospho-transferases (phosphagen kinases). This finding supports a number of previous studies that provided evidence for an excellent conservation of both primary and secondary structures amongst phosphagen kinases (Muhlebach et al., 1994; Suzuki and Furukohri, 1994).

Our analysis of the spatio-temporal pattern of ArgK expression demonstrates that ArgK mRNA is relatively abundant in the brain and in the thorax of the honey bee. In addition, it is also highly expressed in other tissues, in particular in the antennae. However, our data show that the highest level of ArgK expression (two to three times higher than in the brain) is found in the compound eye of the bee. The elevated level of ArgK expression in the visual system can be related to a need for an efficient energy system that transfers ATP generated by mitochondria to high energy-consuming processes that occur in distal regions of the retina. The visual pigment molecules are inactivated after triggering the excitation of the photo-receptor cell, and they must be regenerated in order to maintain the sensitivity of the sensory cells (Schwemer, 1989; Stowe et al., 1990).

Moreover, a continuous renewal of the light-sensitive components, including the breakdown of membranes and the addition of newly synthesised visual pigment and membranes to the existing light-sensitive structures is also associated with photoreceptor regeneration. These molecular processes are seen as a mechanism to prevent inefficient functioning of the receptor due to aged light-damaged constituents as well as an adaptation to ambient light conditions (Stowe et al., 1990). As these processes are expensive in terms of both energy and substrate consumption, they represent a demanding task for the energy-releasing mechanisms in the eye. Our results suggest that a high abundance of ArgK mRNA in the eye provides a molecular solution to such a massive demand of energy. By acting as an energy shuttle, and/or as an energy reservoir, ArgK can provide both spatial and temporal buffering in delivering energy in sensory cells. It remains to be established, however, as to whether the abundance of AmARGK in the bee eye correlates with high levels of the corresponding protein, or reflects the requirement for a rapid regulatory response that would be facilitated by an unstable mRNA.

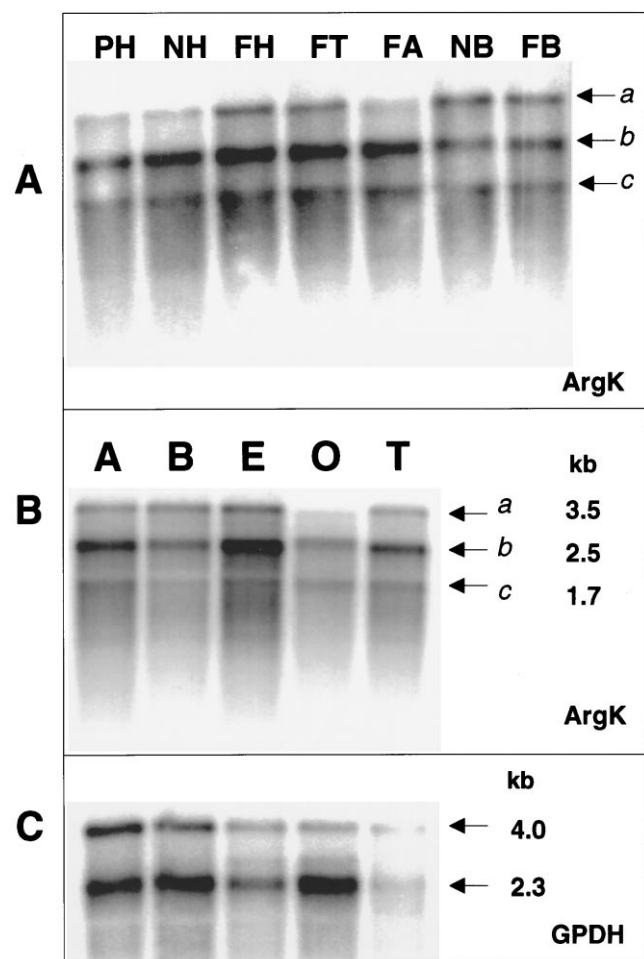


Fig. 3. Northern blot analysis of ArgK expression in the bee. The tissue used as the source of poly (A)⁺ RNA is indicated above each lane. To standardise the amount of RNA loaded per lane, all samples were stained with the RNA dye, SyberGreen II (FMC Bioproducts) and quantified with the Vistra FluorImager. We estimate the loading accuracy to be 10–15%. RNA ladder from NEN BioLabs was used as a size marker. Panels A and B show hybridisation with the ArgK probe, panel C with the α -GPDH probe (see Materials and methods for coordinates). Arrows indicate the positions of three isoforms *a*, *b* and *c* of ArgK. The computer-generated image was obtained with the PhosphorImager (MD). (A) Pupae head (PH), 48-h-old nurse-bee head (NH), adult forager head (FH), forager thorax (FT), forager abdomen (FA), 48-h-old nurse brain (NB) and adult forager brain (FB). (B, C) Antennae (A), brain (B) eyes (E), ovaries (O) and thorax (T).

Although the tissue-specific pattern of ArgK expression has not been investigated in detail in other invertebrates, a study by Langer et al. (1990) reports a high specific activity of ArgK in the eyes of the ghost crab, *Ocypode ryderi*. As a result of feeding habits that are controlled by water tides independently from the day–night cycle, the visual system of this arthropod must adapt to extremely different light intensities. Consequently, the amount of visual pigment in the crab's eyes changes by a factor of 6 during a 1-day cycle. By comparison, the specific activity of ArgK in the com-

pound eyes of the blowfly (*Calliphora erythrocephala*) that do not face a similar challenge, was found to be almost three times lower (Langer et al., 1990). It has also been shown that oxygen consumption and carbohydrate utilisation are greatly increased in insect eyes following illumination (Autrum and Hoffman, 1960). In this context, the high level of ArgK expression in the honey bee's eye may be advantageous for the foraging behaviour of this insect. Typically, a worker bee makes many foraging trips per day, returning each time to a dark hive where she remains for a period of time ranging from minutes to hours. An efficient system controlling energy release ensures that the regeneration process in the bee's eye can cope with the frequently changing light intensities.

A relevant study by Tombes and Shapiro (1989) provided evidence that phosphagen kinases are key elements in the energy-releasing metabolism in polarised cells, such as certain types of sperm, whereby energy production and consumption are spatially separated. These authors also found that the specific activity of creatine kinase in other polarised cells, namely, frog photoreceptors and rabbit oviduct epithelial cells, was considerably high. Our findings in the compound eye add more weight to the notion that phosphagen kinases facilitate energy transport in metabolically polarised cells in both vertebrates and invertebrates.

The notion that ArgK can facilitate energy transport in metabolically polarised cells leads to the suggestion that different isoforms of ArgK in the bee may be distributed in distinct cellular compartments as elements of the energy shuttle. Although this hypothesis needs to be tested experimentally, the presence of three isoforms in various tissues is consistent with such a suggestion.

Finally, database searches reveal that ArgK, and its vertebrate counterpart CrK, have not been found in simple eukaryotes, or in plants, and are most likely a metazoan invention. They clearly belong to an interesting class of proteins that evolved to provide molecular solutions to demanding problems facing specialised tissues and organs in animal species, in particular, in the central nervous system and in sensory organs.

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