REVIEW / SYNTHÈSE

cGMP-dependent protein kinase: linking foraging to energy homeostasis

Karla R. Kaun and Marla B. Sokolowski

Abstract: Successful foraging is necessary for procurement of nutritional resources essential for an animal's survival. Maintenance of foraging and food acquisition is dependent on the ability to balance food intake and energy expenditure. This review examines the role of cGMP-dependent protein kinase (PKG) as a regulator of foraging behaviour, food acquisition, and energy balance. The role of PKG in food-related behaviours is highly conserved among worms, flies, bees, ants, and mammals. A growing body of literature suggests that PKG plays an integral role in the component behaviours and physiologies underlying foraging behaviour. These include energy acquisition, nutrient absorption, nutrient allocation, nutrient storage, and energy use. New evidence suggests that PKG mediates both neural and physiological mechanisms underlying these processes. This review illustrates how investigating the role of PKG in energy homeostasis in a diversity of organisms can offer a broad perspective on the mechanisms mediating energy balance.

Key words: cGMP-dependent protein kinase, foraging behaviour, food intake, energy homeostasis, obesity, diabetes, *Drosophila melanogster*, *Caenorhabditis elegans*, social insects, mammals, thrifty genotype hypothesis.

Résumé : La recherche de nourriture est nécessaire à l'obtention des ressources nutritionnelles essentielles à la survie d'un animal. Le maintien de la recherche et de l'acquisition de nourriture repose sur une capacité à équilibrer la prise alimentaire et la dépense énergétique. Cet article de synthèse examine le rôle de la protéine kinase dépendante du GMPc (PKG) en tant que régulateur du comportement de recherche alimentaire, d'acquisition de nourriture et d'équilibre énergétique. Le rôle de la PKG dans les comportements alimentaires est très conservé chez les vers, les mouches, les abeilles, les fourmis et les mammifères. Un corpus croissant au sein de la littérature suggère que la PKG joue un rôle intégral dans les composantes d'ordre comportemental et physiologique qui sous-tendent les comportements de recherche de nourriture. Celles-ci comprennent l'acquisition d'énergie, l'absorption de nutriments, l'allocation des nutriments, l'entreposage des nutriments et la consommation énergétique. De nouvelles évidences suggèrent que la PKG contrôle à la fois des mécanismes neuraux et physiologiques sous-tendant ces processus. Cette synthèse illustre comment l'étude du rôle de la PKG dans l'homéostasie énergétique chez divers organismes peut offrir une perspective large sur les mécanismes déterminant l'équilibre énergétique.

Mots-clés : protéine kinase dépendante du GMPc, comportement de recherche alimentaire, prise alimentaire, homéostasie énergétique, obésité, diabète, *Drosophila melanogaster*, *Caenorhabditis elegans*, insectes sociaux, mammifères, hypothèse du génotype économe.

Introduction

cGMP-dependent protein kinases (PKGs) are serine/threonine kinases activated by cGMP (Feil et al. 2005). They are found in a diverse range of eukaryotic organisms from *Paramecia* to humans (Hofmann 2005). PKGs have been implicated in a number of functions including smooth muscle tone, regulation of smooth muscle proliferation, platelet aggregation, intestinal fluid secretion, bone growth, circadian rhythmicity, long-term potentiation, long-term depression, learning and memory, and food search behaviour (Hofmann 2005).

The role of PKG in food-related behaviours is highly conserved across species from nematodes to humans (Fitzpatrick and Sokolowski 2004). This suggests a role for PKG in the processes underlying the hunger sensation that drives

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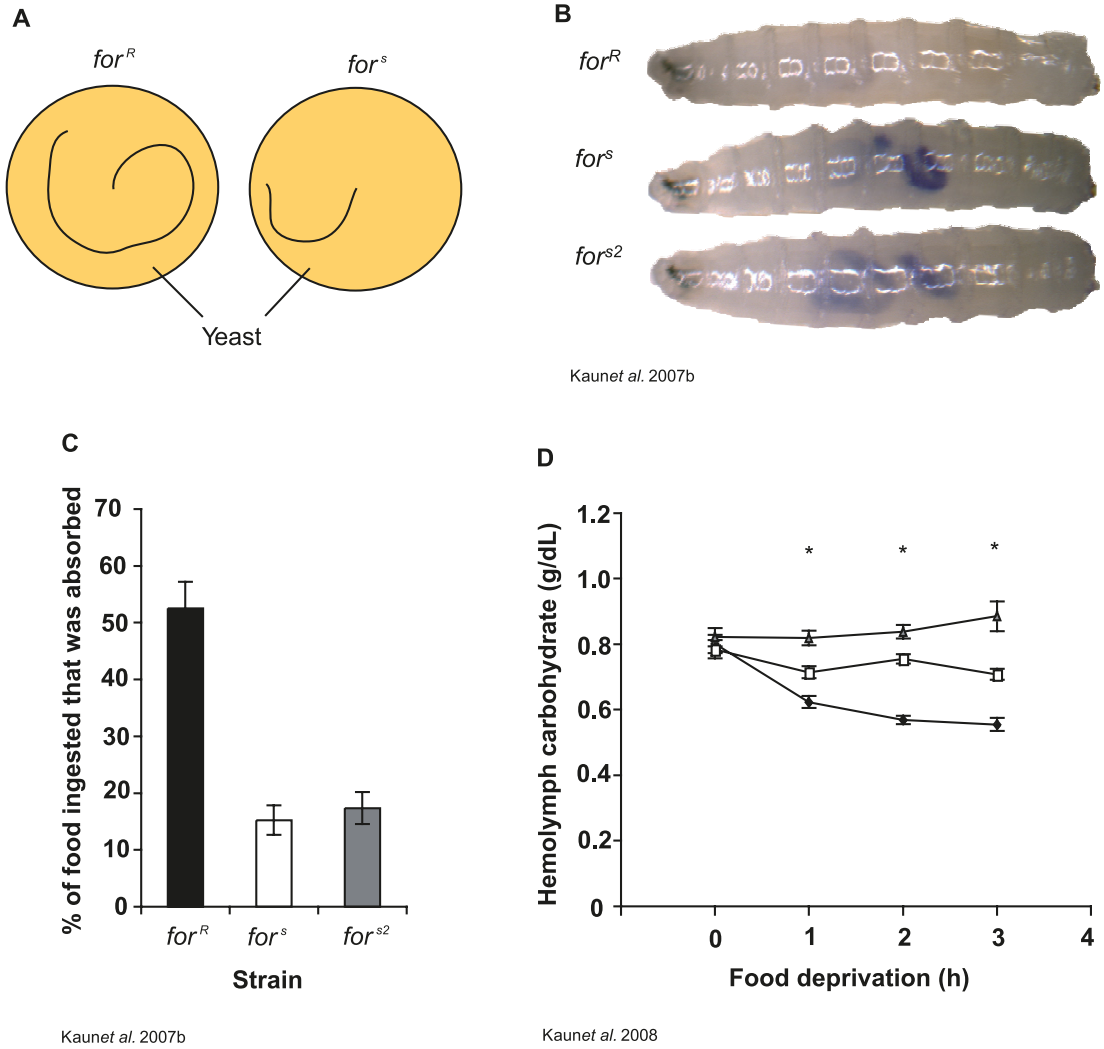
Fig. 1. *for*, PKG, and *Drosophila melanogaster*. (A) Natural variants of *for* differ in their locomotion on food. Rovers move more within a food substrate compared with sitters (Sokolowski, 2001). (B) *for* affects food acquisition. Rover (for^R) larvae ingest less food than sitter (for^s) and sitter mutant (for^{s2}) larvae (Kaun et al. 2007*b*). (C) *for* affects glucose absorption. for^R larvae absorb a larger percentage of ingested ^{14}C-labelled glucose mixed in with yeast paste compared with for^s and for^{s2} larvae (Kaun et al. 2007*b*). (D) *for* affects glucose homeostasis. Following 2 h of food deprivation, hemolymph glucose levels decrease in for^R but not in for^s or for^{s2} larvae (Kaun et al. 2008).



food search behaviour. In fact, growing evidence suggests that PKG plays an integral part in mediating the balance between energy acquisition and use. Here, we review the emerging role of PKG in food acquisition and energy homeostasis.

PKG, foraging, and energy homeostasis in *D. melanogaster*

foraging (*for*) gene and food-related behaviours

The most in-depth analysis of the role of PKG in food-related behaviours and energy homeostasis has been carried out in the fruit fly *Drosophila melanogaster*. Naturally occurring variation in a gene encoding PKG, the *for* gene, affects foraging behaviour in *D. melanogaster* larvae and adults (Sokolowski 1980; de Belle and Sokolowski 1987; de Belle et al. 1989; Pereira and Sokolowski 1993). Rover (for^R) variants move more within and between patches of food compared with sitter (for^s) variants, whereas in the absence of food, both genotypes move at similar rapid speeds (Fig. 1A) (Sokolowski 1980, 2001). Growing evidence suggests that *for* affects a suite of food-related behaviours that may aid in foraging in nature. For example, *for* affects the ability of adult flies to detect and migrate towards the source of a food odour (Shaver et al. 1998). *for* also modifies sucrose responsiveness and sucrose habituation in adult flies, behaviours that have been associated with foraging proficiency in honeybees (*Apis mellifera*) (Scheiner et al. 2004). Additionally, *for* affects the ability to associate an odour with the presence of food, another important skill in food seeking (Kaun et al. 2007*a*). The role of *for* in such an array of food-related traits suggests that the gene is important for triggering the hunger response that drives foraging behaviour and thus influences energy homeostasis.

Natural variation in food acquisition due to *for*

Natural variation in *for* affects larval food acquisition in an environmentally dependent fashion (Kaun et al. 2007*b*).

When fed ad libitum, for^R larvae have lower food intake (Fig. 1B), higher glucose absorption (Fig. 1C), and preferential glucose allocation to lipids compared with sugars than for^s larvae. Importantly, larvae harbouring a hypomorphic mutation in the *for* gene but otherwise genetically rover (for^{s2}) display sitter phenotypes, indicating that variation in the *for* gene underlies observed behavioural and metabolic differences. When reared in environments with low food levels, for^R, for^s, and for^{s2} larvae increase food intake to a common maximal level, but for^R larvae retain their increased absorption efficiency. In such environments, for^R larvae also have higher survivorship and faster development than for^s and for^{s2} larvae.

Depriving larvae completely of food for short periods of time also elicits differences in responses between the natural variants of *for*. Following such acute food deprivation, for^R larvae show a greater decrease in blood (hemolymph) sugar levels and a slower rate of increase in food intake compared with for^s and for^{s2} larvae (Fig. 1D). This is linked to differences in adipokinetic hormone mRNA levels. Adipokinetic hormone acts to maintain stable hemolymph sugar levels by converting stored glycogen and lipid into usable glucose, similar to the role of mammalian glucagon (Vroemen et al. 1998; Van der Horst 2003; Kim and Rulifson 2004; Lee and Park 2004; Isabel et al. 2005). Accordingly, for^s larvae recover more quickly from acute food deprivation compared with for^R larvae. Collectively, these results show that natural variation in *for* affects a suite of phenotypes involved in the regulation of food acquisition and that changes in *for* expression can induce corrective behavioural modifications in response to food deprivation.

Mechanisms underlying *for*-dependent energy homeostasis

for encodes one of two PKGs in *D. melanogaster* (Osborne et al. 1997). Although the precise nucleotide polymorphism distinguishing for^R from for^s has not yet been identified, PKG enzyme activities and mRNA levels are higher in for^R than in for^s and for^{s2} animals (Osborne et al. 1997). Transgenic expression of *for*-cDNA in for^s larvae changes larval behaviour and PKG activity from for^s to for^R levels, demonstrating the role for functional PKG activity in foraging behaviour (Osborne et al. 1997). Until recently, the molecular and cellular mechanisms underlying the naturally occurring behavioural polymorphism were unknown. New evidence suggests that soluble guanylyl cyclase, which affects synthesis of cGMP, may play a role in *for*-mediated foraging behaviour (Riedl et al. 2005). Interactions between *for* and molecules known to affect energy homeostasis in mammals are currently unknown or are under investigation (K.R. Kaun, unpublished data).

for and the thrifty genotype hypothesis

The thrifty genotype hypothesis states that adaptations allowing an organism to rapidly store energy in times of food surplus will confer a survival advantage during reciprocal periods of food shortages and famine (Neel 1962; Prentice et al. 2005). The rover/sitter system provides an enticing model for further investigation of the effects of "thrifty" genes that regulate efficiency of nutrient homeostasis. for^R larvae appear to acquire nutrients more efficiently compared with for^s larvae, which aids in survivorship under conditions of chronic food depletion. This suggests that the for^R allele may tend to be selected over the for^s allele when larvae are reared in a poor nutritive environment. Intriguingly, larvae with the for^R allele exhibit a selective advantage in crowded environments, which may be associated with poor food conditions, compared with for^s homozygotes (Sokolowski et al. 1997).

However, experiments investigating frequency-dependent selection at the *for* locus suggest that for^R larvae do not always have an advantage over sitters; during times of larval competition, sitters show a higher survivorship when rare relative to rovers (Fitzpatrick et al. 2007). Intriguingly, for^s larvae may have an advantage over for^R larvae when faced with short bouts of food deprivation: for^s larvae increase food intake after a short period of food deprivation and are more resilient to expenditure of hemolymph sugar stores compared with for^R larvae (Kaun et al. 2008). Whether *for* affects rapid fat gain, which may further confer a survival advantage, is currently under investigation (A. Belay, unpublished results).

PKG, foraging, and energy homeostasis in *Caenorhabditis elegans*

The *C. elegans* homolog of *for*, *egl-4*, is also important for the regulation of food-related behaviour. A loss-of-function mutation in *egl-4* changes characteristic dwelling behaviour in the presence of food to roaming behaviour (Fig. 2A) (Fujiwara et al. 2002). Loss of *egl-4* function has also been associated with defects in long-term regulation of olfactory adaptation, extended life span, and increased body size (Fig. 2B) (L'Etoile et al. 2002; Hirose et al. 2003). Increased body size has been attributed to a difference in the transforming growth factor-beta (TGF-β) pathway regulating fluid content in cells (Nagamatsu and Ohshima 2004) and in the SMA–MAB pathway mediating food-dependent endoreduplication (Tain et al. 2008). Alternations in longevity are thought to require the insulin signalling pathway (Hirose et al. 2003). *egl-4* loss-of-function mutants also show increased feeding rate and locomotion on food following a short period of fasting (You et al. 2008).

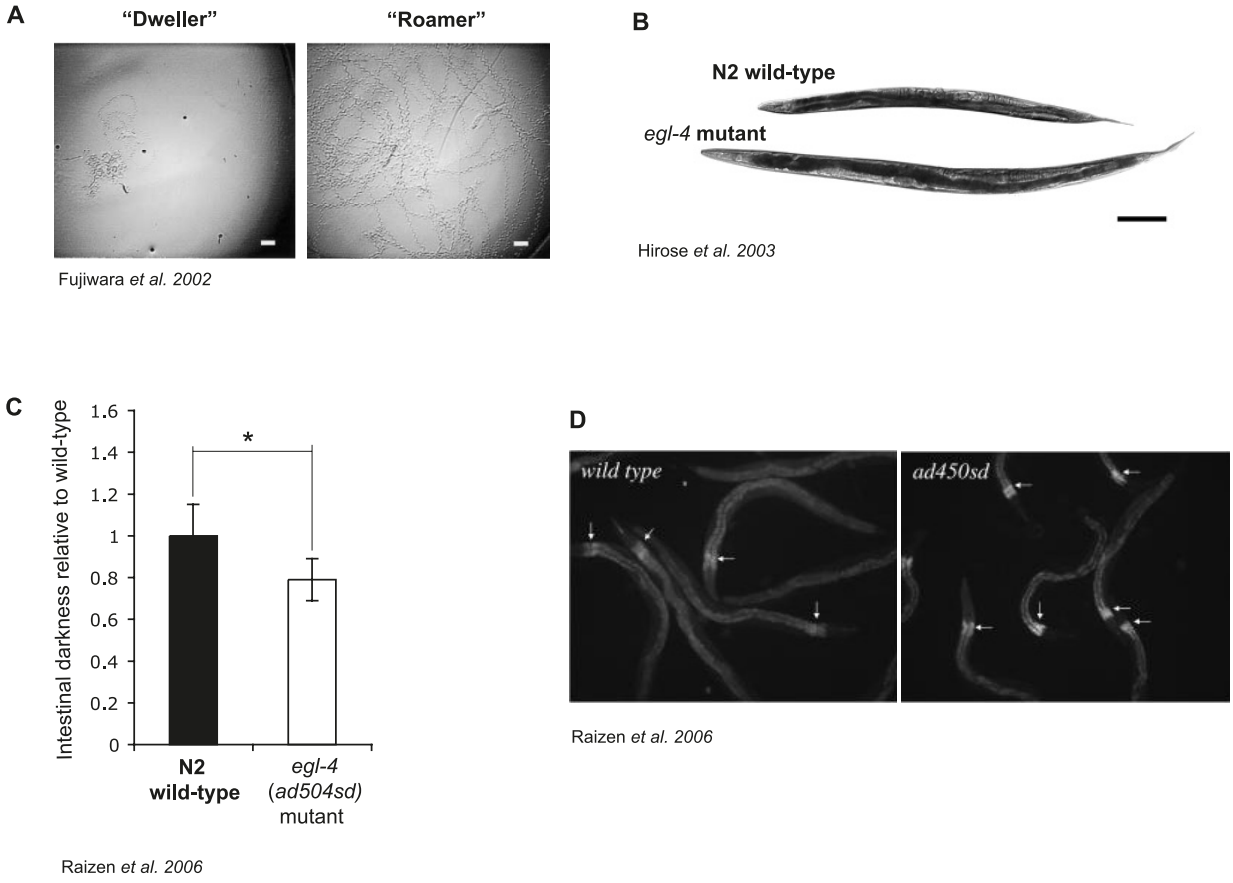
Recently, a dominant *egl-4* mutation associated with increased *egl-4* activity despite reduced EGL-4 protein levels was shown to confer a pale intestine and increased intestinal nile red staining that is associated with fat storage (Figs. 2C and 2D) (Raizen et al. 2006). In addition, the increased quiescence duration of *egl-4* gain-of-function mutants is unaffected by fasting (You et al. 2008). You et al. (2008) suggested that this increased quiescence mimics satiety in mammals and may be controlled by a signalling pathway in which PKG is activated by insulin, cGMP, and TGF-β pathways.

Thus, a number of phenotypes implicate *egl-4* in mediating energy acquisition and use. The high-throughput nature of genetic research in *C. elegans* makes this organism a strong candidate to investigate genes and neural networks that interact with PKG to affect energy homeostasis.

PKG and foraging in social insects

In the honeybee, there is an age-related increase in

Fig. 2. *egl-4*, PKG, and *Caenorhabditis elegans*. (A) *egl-4* affects food search behaviour. A loss-of-function mutation in *egl-4* increases locomotion on food from dweller to roamer behaviour (Fujiwara et al. 2002). (B) *egl-4* affects body size. *egl-4* mutants are significantly greater in size compared with wild-type *C. elegans* (Hirose et al. 2003). (C) *egl-4* affects the gut. An *egl-4* mutation that decreases protein levels (allele *ad450sd*) also decreases intestinal opacity (Raizen et al. 2006). (D) *egl-4* may affect fat stores. An *egl-4* mutation that decreases protein levels (allele *n479*) also decreases the opacity of nile red staining associated with the presence of fat in the fat bodies (Raizen et al. 2006).



expression of *Amfor*, the honeybee *for* homolog, associated with a transition from hive work to out-of-hive foraging (Fig. 3A) (Ben-Shahar et al. 2002). Furthermore, *Amfor* may mediate this transition from nurse to forager by modulating phototaxis (Ben-Shahar et al. 2003). Whether *Amfor* affects components of energy homeostasis other than food seeking is currently unknown. However, foraging bees show lower lipid levels than nurse bees, independent of the age of the bee, suggesting a potential link between *Amfor* and lipid metabolism (Toth and Robinson 2005). In red harvester ants (*Pogonomyrmex barbatus*), expression of *Pbfor* differs between young worker ants and foraging ants (Ingram et al. 2005).

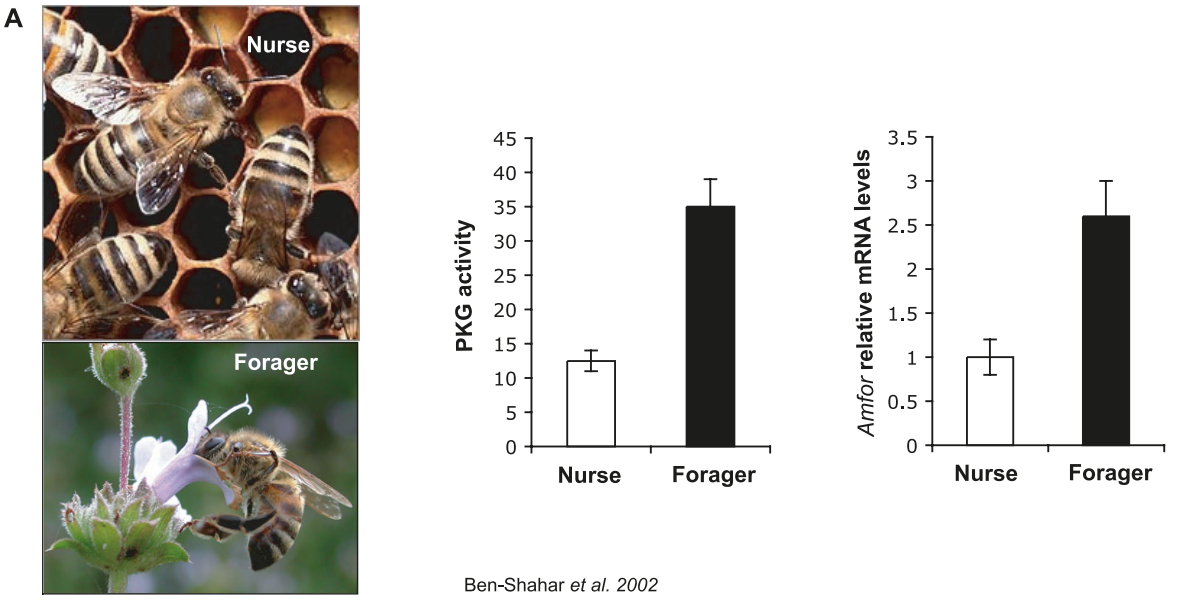
PKG and energy homeostasis in mammals

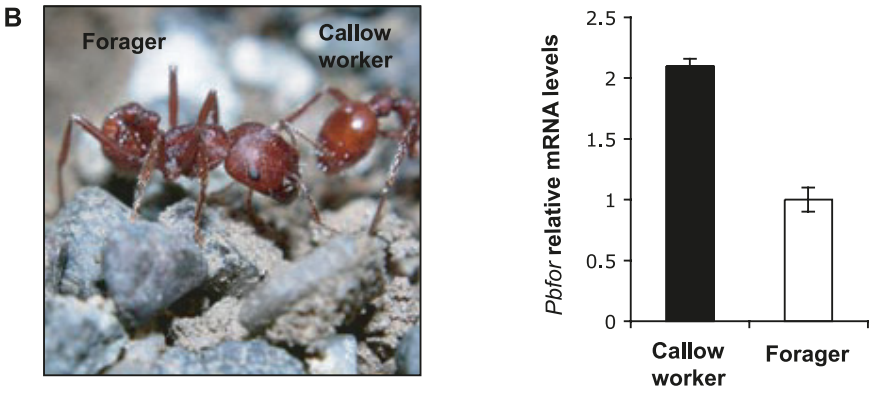
The role of PKG in mammalian food-related behaviours is currently unknown, although the molecule has been implicated in intestinal muscle function, obesity, and high blood glucose concentration characteristic of diabetes (Su et al. 2003; Chang et al. 2004; Engeli et al. 2004; Wang et al. 2004; Hofmann 2005; Zanetti et al. 2005).

Mammals have two PKG genes, *prkg1* and *prkg2*, that encode cGK1 and cGK2. *prkg1* is homologous to *D. melanogaster for*. Although a polymorphism in *prkg1* has not yet been found between obese and healthy-weight human populations (Zakharkin et al. 2005), some evidence suggests a link between cGK1 and obesity. *prkg1* is expressed at higher levels in adipocytes of obese women (Engeli et al. 2004). In addition, natriuretic peptides stimulate lipid mobilization through a cGMP-dependent pathway (Lafontan et al. 2005; Langin 2006). Atrial natriuretic peptides increase intracellular cGMP, which, in turn, activates cGK1 leading to perilipin and hormone-sensitive lipase phosphorylation and lipolysis (Lafontan et al. 2008).

PKG is associated with diabetes in mammals through a number of different mechanisms. For example, diabetes induces a decrease in cGK1 activity, *prkg1* mRNA, and cGK1 protein expression in rat corpus cavernosum and aortic vascular smooth muscle (Chang et al. 2004; Jacob et al. 2004). Pancreatic beta cells and human islet cells also express *prkg1* where it regulates the viability of the cells by preventing cell death in insulin-dependent diabetes mellitus (Loweth et al. 1997; Zaitsev et al. 2001; Kaminski et al. 2004). Changes in PKG are also associated with disorders resulting from hyperglycemia. For example, cGK1 inhibition is associated with diabetic nephropathy. Excessive TGF-β activity in hyperglycemia contributes to the development of

Fig. 3. *Amfor,* PKG, and *Apis mellifera* and *Pbfor*, PKG, and *Pogonomyrmex barbatus*. (A) *Amfor* affects foraging behaviour in *A. mellifera*. An increase in *Amfor* mRNA and PKG activity levels is associated with the transition from nurse to forager. (B) *Pbfor* affects foraging behaviour in *P. barbatus*. High levels of foraging behaviour as seen in older foraging ants is associated lower *Pbfor* mRNA levels (Ingram et al. 2005).





diabetic nephropathy (Wang et al. 2004). cGK1 inhibition facilitates TGF-β bioactivity, which leads to the development of diabetic disease of the kidney (Wang et al. 2004).

Thus, growing evidence places a role for PKG in both diabetes and obesity-related phenotypes in mammals. This suggests that PKG plays an integral role in the processes underlying energy homeostasis in humans.

Conclusions

Investigation of the mechanisms underlying PKG-mediated food acquisition and energy homeostasis is facilitated by conservation of this role among a diversity of organisms. For example, natural variation of *for* in *D. melanogaster* makes flies a good model to study the evolutionary significance of PKG in energy homeostasis. The genetic tools available in both *D. melanogaster* and *C. elegans* allow ease of investigation of genetic and molecular mechanisms underlying PKG-mediated food acquisition and energy homeostasis. Physiological aspects of the role of PKG in energy balance can be best studied in larger insects such as bees and ants and in mammals such as mice and rats.

Importantly, the highly conserved role of PKG in food-related traits and energy homeostasis phenotypes suggests that the information acquired using these model organisms is transferable to humans. Therefore, further investigation into the role of PKG in energy homeostasis may reveal treatments for disorders associated with an imbalance in energy homeostasis such as obesity and diabetes.

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