

NIH Public Access

Author Manuscript

Mol Genet Metab. Author manuscript; available in PMC 2010 January 17.

Published in final edited form as:

Mol Genet Metab. 2006 August ; 88(4): 334–345. doi:10.1016/j.ymgme.2006.01.002.

Conserved family of glycerol kinase loci in Drosophila

melanogaster

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Abstract

Glycerol kinase (GK) is an enzyme that catalyzes the formation of glycerol 3-phosphate from ATP and glycerol, the rate-limiting step in glycerol utilization. We analyzed the genome of the model organism *Drosophila melanogaster* and identified five GK orthologs, including two loci with sequence homology to the mammalian Xp21 GK protein. Using a combination of sequence analysis and evolutionary comparisons of orthologs between species, we characterized functional domains in the protein required for GK activity. Our findings include additional conserved domains that suggest novel nuclear and mitochondrial functions for glycerol kinase in apoptosis and transcriptional regulation. Investigation of GK function in *Drosophila* will inform us about the role of this enzyme in development and will provide us with a tool to examine genetic modifiers of human metabolic disorders.

Keywords

Drosophila melanogaster; Glycerol kinase; Mitochondria; Nucleus; Apoptosis

Introduction

Glycerol kinase (GK) is an enzyme that catalyzes the formation of glycerol 3-phosphate from ATP and glycerol, the rate-limiting step in glycerol utilization [1]. Dihydroxy-acetone and L-glyceraldehyde can also act as acceptors [2]. UTP, and, in the case of the yeast enzyme, ITP and GTP, can act as donors [2]. GK provides a way for glycerol derived from fats or glycerides to enter the glycolytic pathway (Fig. 1). The enzyme can undergo a reversible subunit dissociation between tetramer and dimer [3] in bacteria. GK requires magnesium as a cofactor and is regulated by fructose 1,6-bisphosphate [4].

We have chosen to investigate GK because it is the rate-limiting enzyme and an obligatory step in glycerolipid production. As a consequence, the intermediates do not accumulate under standard conditions. It is known that decreased GK activity directly reduces levels of glycerolipids [5]. In mice, absence of glycerol 3-phosphate dehydrogenase leads to neonatal death [6]. GK knockout mice also die soon after birth [7]. However, the effects of reduction of glycerolipids and thus their role in biological processes are poorly understood.

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In mammals, GK activity is most abundant in liver [1], although it is present in adipose tissue under conditions of fasting, obesity, and diabetes [8,9]. Glycerol kinase activity increases 10fold in adipocytes that lack leptin receptor signaling [10]. Glycerol metabolism has been conserved and localized to the fat body in most insect species. In Drosophila, the fat body is the equivalent of the mammalian liver [11]. This structure expresses all of the enzymes required for carbohydrate and lipid metabolism [12]. Deletion of the fat body leads to death at 5 days of life and female sterility [13]. Its role in glycerol metabolism in the fruitfly is not well known. Incorporation of glycerol 3-phosphate into diacylglycerols and phosphoglycerides is higher in larval stages than adults [14]. GK expression parallels this developmental pattern. In the silkmoth, during the larval-pupal transformation, glycerol accumulation declines in the fat body at both larval and pupal stages in development while total GK activity increases [15]. In Drosophila, disruption of triacylglycerol production through mutations in diacylglycerol acyltransferase leads to death of oocyte nurse cells by apoptosis [16], suggesting that glycerol metabolism is essential for oogenesis. The locust has a complex that acylates phosphoglycerol into phosphatidate [17]. A variety of roles for glycerolipids in development is demonstrated by the differential entry of glycerol into triglycerides versus other glycerolipids between larval and adult stages [18].

Glycerol 3-phosphate is also a major metabolite for mitochondria in insect flight muscle [19]. GK activity has been demonstrated in the muscles of both vertebrates and invertebrates [20]. In the muscle of vertebrates, glycerol is incorporated into glycerolipids and used as energy fuel [21,22]. Flight in *Drosophila* requires active regulation of glycerolipid biosynthesis during activity [23]. Similarly, glycerol 3-phosphate dehydrogenase is also highly expressed in fat body and flight muscle [24]. All of these enzymes have been shown to colocalize in flight muscle [25]. Disruption of this colocalization leads to flightlessness [26].

Human GK deficiency (GKD, MIM 307030) is an X-linked disorder characterized by increased plasma concentration and urinary excretion of glycerol. It is recognized in two forms: complex GKD, the result of large deletions of multiple contiguous genes, and isolated GKD, resulting from point mutations and small intragenic deletions [1]. Patients who are true GK nulls resulting from gene deletion have facial dysmorphisms and mental retardation [27–32]. They also have seizures and developmental delay as well as hypotonia [30,32–35]. Additional anomalies include abnormal skeleton, spontaneous fractures, and premature loss of abnormal teeth [32,36]. Diabetes has been reported in these pedigrees [1,37,38]. In isolated forms of GKD, the phenotype may vary from a life-threatening childhood metabolic crisis with mental retardation and seizures, to asymptomatic 'pseudohypertriglyceridemia' from elevated glycerol levels [1,30]. This phenotypic heterogeneity is clearest within families, where identical mutations may have variable phenotypic manifestations in different family members.

As an initial approach to establishing *Drosophila* as a model organism for understanding the role of modifier genes on the phenotypic variability observed in individuals with hyperglycerolemia secondary to glycerol kinase deficiency, we have characterized glycerol kinase loci in *Drosophila melanogaster*.

Experimental procedures

Sequences for each organism were obtained from the following sources: the Unigene database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene), the *Drosophila* Genome Project's release 3.2.0 annotation [111] (http://flybase.org/), the *Saccharomyces* Genome Database 11/26/2003 annotations (http://www.yeastgenome.org), WormBase gene dump 3/1/2004 (http://www.wormbase.org), and (http://www.genome.org). The ExPASy proteomics server (http://us.expasy.org/) was used for protein sequence analysis. PSORT II (http://www.psort.org/) was used to analyze for subcellular localization sequences. Phylogeny

and alignment analyses were performed with the ClustalX [112] and MEGA 3.0 [113]. Pairwise multiple sequence alignment was performed using the Gonnet protein weight matrix. Evolutionary pairwise distances were estimated using a Poisson correction mode and a Grand Payhoff Matrix model with uniform substitution rates. Sequence analysis of the alignments was performed using the Sequencher program (version 4.0.5, Gene-Code, Madison, WI). In silico data mining was performed using the Gene expression omnibus (GEO) database at NCBI (http://www.ncbi.nlm.nih.gov/projects/geo/) as well as supplemental data files from published microarray studies as referenced. For the insect sequence alignments, the numbers after each species name correspond to the scaffold entry number in each organism's database. For Anopheles gambiae, the annotated protein sequences obtained from http://www.anobase.org/ and ENSEMBL are: ENSANGP00000018137, ENSANGP00000020314, ENSANGP0000 0010758, and ENSANGP00000028995 (also known as ENSANGP00000 013591). For Apis mellifera, a BLAST search was performed using the honeybee proteome database (http://azra.embl-heidel-berg.de/~zdobnov/Bee2/) and the predicted proteins compared by BLAST (http://www.ensembl.org/Multi/blastview) to the annotated sequences at ENSEMBL: Amel 13011 (ENSAPMP00000022551 and ENSAPMP000 00015828), Amel 17342 (ENSAPM P0000004685), Amel 9845 (ENS-APMP0 0000020060, ENSAPMP0000000 3731, and ENS-APMP00000003729), and Amel 3700 (ENSAPMP00000 011946 and ENSAPMP00000011947). For the silkmoth *Bombyx mori*, predicted protein sequences were obtained by BLAST (http://software.genomics.org.cn/softenv/right/run/index.jsp?class = SilkwormBlast&out = 1): Scaffold 006247, Scaffold001823, Scaffold013907, and Scaffold004254/Scaffold001818. Beetle protein sequences were obtained by BLAST searches of the Tribolium castaneum contig database

(http://www.hgsc.bcm.tmc.edu/blast/blast.cgi?organism=Tcastaneum): Contig1229, Contig1078, Contig2615, and Contig1405.

Results

Conserved family of GK genes in Drosophila

We have previously characterized the existence of a well-conserved family of GK genes [39]. Completion of the *Drosophila* genome sequences [40] has allowed us to identify GK loci in this organism suitable for genetic analysis. Our studies have shown that there are five GK-like genes in *D. melanogaster* (Fig. 2). One locus, termed Gyk (CG18374, located at 61B2), is the closest homolog to the X-linked GK gene in humans, sharing 53% identity over its entire 510-amino acid length. Another close homolog CG7995 (located at 62B1) shares 48% homology to human GK. In addition, three other loci CG1271 at 63A3, CG 1216 at 61B2, and CG8298 at 48D5 do not share the same degree of homology and contain transmembrane domains according to the Ward laboratory's *Drosophila* Membrane Protein Library database (http://www.cbs.umn.edu/fly/). They are closely related to the mammalian glycerol kinase-like pseudogene retroposons, which contain transmembrane domains and lack GK activity [41]. Sequence alignment of all *Drosophila* GK-like loci with glycerol kinase-like loci from other insect species further confirms the presence of five ancestrally well-conserved loci in this phylum (Fig. 3).

Using the latest version of the Unigene database, we confirmed the presence of five GK-like loci in vertebrates. The gene encoding human GK has been cloned and is comprised of 21 exons that map to chromosome Xp21.3 [42,43]. It was initially suggested that there were six genomic loci in humans with sequence homology to GK [43]. In addition to the X-linked locus at Xp21.3, a single exon non-coding pseudogene was also located at Xq22.1 (GK pseudogene 6, GKP6). Additional chromosome loci at 4q13 (GK2/GKP2) and 4q32.1 (GK pseudogene 3) were identified as intronless genes that encoded two different testis- and brain-specific

We have identified an additional locus at 4q26 (LOC201989) that is exclusively expressed in small intestine (Unigene). Initially described as the pseudogene at 1q41 (GK pseudogene 1) [43], it shares 95% homology to the Xp21.3 and the 4q GK loci. A search of the Unigene database did not identify loci for the pseudogenes previously described at chromosome loci 7p (GK pseudogene 4) or 10p (GK pseudogene 5). These have been removed or renamed as "regions" by the HUGO Gene Nomenclature Committee due to lack of sequence data [44]. Another locus at 3q23 (MGC40579) is more widely expressed (Unigene) and only shares 35% homology with GKs. Therefore in humans, based on the sequence data available at this time, there are five different loci identified with homology to GK, similar to the number identified in *D. melanogaster*.

Glycerol metabolic pathways in Drosophila

We next determined the suitability of *Drosophila* as a model for glycerol metabolism studies by identifying the enzymatic pathway components required for glycolysis and glycerolipid biogenesis, and comparing them to their human homologs. A search of the Flybase database identified multiple members of each of the enzymes involved in glycerol metabolism. Fig. 1 shows a schematic representation of the conserved metabolic networks present in *Drosophila*. Next, we performed a phylogenetic analysis of the *Anopheles* and *Drosophila* glycerol kinase-like genes and compared them to those of other species (Fig. 2). Bootstrap analysis identified a clade for the mosquito and *Drosophila* GK orthologs at a value of 55– 99%. Alignment of human, *Escherichia coli, Saccharomyces cerevisiae*, mouse, *Caenorhabditis elegans*, and *Drosophila* glycerol kinase protein sequences confirmed that all key functional residues for ADP and glycerol binding are conserved in CG7995 and Gyk, but not in the remaining three *Drosophila* GK loci (Fig. 4).

Conserved functional domains in Drosophila GK proteins

Protein sequence analysis identified potential protein interaction and phosphorylation modification sites that may regulate GK function. We performed a scan for protein motifs and identified multiple potential phosphorylation sites (Fig. 4). Those marked with a 'P' are potential phosphorylation sites recognized by protein kinase C (PKC) and casein kinase II (CKII). Some of these residues fall within regions that carry missense mutations in patients with GKD, identified in Fig. 4 by an asterisk. Two prominent and well-conserved examples are the missense mutations W503K and T278M. The tryptophan at position 503 is adjacent to the potential nuclear localization sequence (NLS) present in GK. This bipartite NLS is located in the C-terminus of *CG7995* (Fig. 4). The *C. elegans* GK homolog R11F4.1 also has the NLS HKRK at residues 198–201. The threonine at position 278 of human GK is part of a well-conserved PKC phosphorylation site at residues 296–298 in *CG7995*. This residue is conserved across species and mutated in GKD patients with the missense mutation T278M [30].

Many dileucine motifs important for protein–protein interactions and organelle targeting are present ('LL,' Fig. 4). Human GK also contains a domain between residues 416 and 490 that has 50% homology to zinc-finger domains. In *E. coli*, GK forms a stable multi-unit complex, GK exists at physiological concentration in an equilibrium between functional dimers and tetramers [45]. Human GK shares a subunit interface interaction region with *E. coli* GK. This region, when mutated, increases the thermal stability of the enzyme in *Flavobacterium meningosepticum* [46]. A PEST sequence in the amino terminus of Gyk shares 88% homology with the same conserved sequence in exon 1 of human GK. Also labeled is a conserved potential site for modification by SUMO-1 ('SSS,' Fig. 4). These domains suggest a role for proteolysis in GK activity regulation. Conserved residues required for ATP, fructose 1,6-bisphosphate,

and glycerol binding are marked A, F, and G, respectively (Fig. 4). A key glycine residue required for glucose-mediated regulation of GK activity in *E. coli* located at position 304 (G326 in human GK) [47] is conserved among all species. We identified the presence of an LXXLL motif at residues 154–158 ('LXXLL,' Fig. 4), involved in the interaction of many transcriptional coactivators with liganded nuclear hormone receptors, as well as a CtBP binding motif ('PXDLS,' Fig. 4) at the protein C-terminus. This latter domain is present and exclusively conserved in mammalian species but absent in other vertebrates and invertebrates (Fig. 4). Both the N- and the C-terminal domains of the FGGY family of carbohydrate kinases adopt a ribonuclease H-like fold that are structurally related to each other [48]. This domain is present in protein subunits of nuclear chromatin remodeling complexes [48]. The presence and evolutionary conservation of these protein domains suggests a potential role for GK in nuclear transcriptional regulation and chromatin remodeling.

Sequence analysis performed using the ExPASy protein sequence analysis website identified several domains associated with a role in mitochondrial apoptosis. A conserved sequence in the C-terminus contains a transmembrane hydrophobic domain homologous to that present in the pro-apoptotic protein Bax (Fig. 5) [49]. This domain mediates the translocation of Bax to the mitochondrial membrane in response to cell death signals and is required for its proapoptotic function [50]. Using the PSORTII software, we confirmed the likelihood of human GK localization to mitochondria. This is based on the amino acid composition of an alternatively spliced exon 18 that codes for the Bax-like hydrophobic transmembrane domain [51]. This domain is well conserved across species and is present in CG7995 (Fig. 5). Exon 18 is a tissue-specific, differentially spliced exon present in human, mouse, rat, zebrafish, and Drosophila. Sequences similar to human exon 18 are not present in glycerol kinase loci from C. elegans, E. coli, yeast, or plants. Alternative splicing of exon 18 produces an isoform initially described to be a brain-specific form [52], but which is expressed more widely [53]. This sequence targets GK to mitochondria [51]. CG7995 is the only GK ortholog that contains a sequence with similarity to human GK exon 18, while Gyk lacks this domain. It appears that while in Drosophila independent functions were segregated in these two genes, evolution may have merged them in vertebrates where they are differentially regulated at the splicing level. Other Drosophila proteins that share homology with this domain include the monoamine oxidase CG10561, the LDL receptor LpR1/CG31094, and CG5195. Exon 8A is also differentially spliced, and is present in *Xenopus* and isoforms expressed in testes and fetal liver [54]. Sequences with similarity to human GK exon 8A are not present in *Drosophila* GK genes.

In silico data mining for Drosophila GK-like loci expression

Using an in silico approach, we next pursued data mining analysis of previously unidentified gene expression data available for each of the *Drosophila* GK-like loci from large-scale microarray studies. These data include the developmental and tissue expression patterns of each of the *Drosophila* GK loci (Tables 1 and 2). These data have been deposited but not previously identified or analyzed for their relevance to GK function in *Drosophila*.

Gyk is expressed in testis, head, embryo, and larva, as well as the cell line Schneider S2. Based on microarray data across developmental stages, peak expression is in the early larval stage. CG7995 is present in head, embryo, larva, and Schneider S2 cells, but the very early embryo is the stage with peak expression. While CG1271 appears to be widely expressed, it is present in testis, demonstrating a conserved evolutionary function with its mammalian orthologs, which are primarily expressed in testis. CG8298 is expressed in the testis as well as the larval brain and imaginal discs. CG1216 shares little homology at the sequence level with GKs, except in a potassium channel tetramerization domain. However, an overlapping transcription unit exists where the 3' end of CG1216 shares antisense sequences with the Gyk 3' end sense transcript. This type of transcript overlap has been previously described for *Cs/CG10561* and

dopa decarboxylase/*ddc* loci in *Drosophila* [55]. CG10561 contains a conserved monoamine oxidase domain associated with dopamine breakdown. As part of the dopa decarboxylase complex, CG10561 is coregulated with other enzymes involved in dopamine synthesis and breakdown. Antisense transcripts have also been described for the human/mouse GK locus in mammals [56,57]. This overlapping transcript relationship potentially links CG1216 with Gyk activity, either as an antisense transcript or as a member of a shared pathway. Most key functional residues are missing in CG1216 suggesting it has no GK activity.

Discussion

Using an in silico approach, our analysis demonstrates the conservation of sequence and presence of glycerol kinase loci in *D. melanogaster*. The utility of this analysis is best supported by the finding of multiple conserved protein domains that suggest novel functions for this family of proteins, including roles in nuclear and mitochondrial activities. These functions appear to have been preserved across evolution.

GK catalyzes the ATP-dependent reaction from glycerol to glycerol 3-phosphate, a key link between carbohydrate and lipid metabolism [1]. GK is expressed at low levels in every human tissue but expressed at higher levels in liver, kidney, lymphocytes, testis, ovary, and lung [1] (Unigene). GKP2 on 4q13 is testis-specific [58]. GK is primarily expressed in hepatocytes, where it is induced by diabetes and fasting [9], not affected by glucagon, dexamethasone or cAMP [59], but increased by 20% linoleic acid and regulated by glycerol [60]. GK is also present in small intestine [61], and in kidney and pneumocytes its activity is increased by diabetes and normalized by insulin [62]. In adipose tissue there normally are low levels, but these increase in obesity [8] and are regulated by sympathetic innervation [63]. According to the Unigene database, GK transcripts have also been detected in isolated pancreatic beta cells, developing neuroectoderm and brain hippocampus, white blood cells, thymus, fetal heart, uterus, testis, stomach, ovary, skeletal muscle, and breast. In the developing mouse cerebellum, levels increase postnatally and peak at P14 [64]. GK is also selectively expressed in a number of cancers, including colon and squamous cell carcinoma [65].

Products of glycerolipid and sphingolipid metabolism are now known to fulfill second messenger functions in a variety of cellular signaling pathways [66]. The role of diacylglycerol in the regulation of protein kinase C (PKC) activity and its site of interaction with PKC are now well known. Recently, another glycerolipid second messenger, phosphatidic acid, was found to interact with the protooncogenic Raf-1 kinase. In cultured cells, a signal-induced generation of phosphatidic acid was critical for Raf-1 translocation to the cell membrane. Thus, different glycerolipid second messengers appear to regulate distinct targets with exquisite specificity [66]. Although the role of individual glycerolipids has been studied extensively, the relative contributions of their numerous potential downstream effectors remain uncertain.

Lysophosphatidic acid (LPA) and phosphatidic acid (PA) can act to promote cell survival, proliferation, and migration. Phosphatidic acid has been shown to affect migration of neutrophils [67], while lysophosphatidic acid stimulates migration of hematopoetic cells [68], induces neuronal shape changes, and leads to cell proliferation [69]. Mouse lipid phosphate phosphohydrolase (LPP3) affects development [70]. A *Drosophila* homolog of this gene, *wunen (wun)*, is important for guiding migrating germ cells [71] and is implicated in axon guidance [72]. Other enzymes such as rdgB are expressed in the developing nervous system, suggesting a role in its development [73]. This raises the possibility that phospholipids act as signals to guide cells in *Drosophila*. In *Neurospora crassa* glycerol kinase is induced by cold temperature [74]. Glycerol kinase is also upregulated 8-fold during the stress response to dehydration in the nematode *Steinerenema feltiae* [75].

The role of glycerol in cell survival has recently emerged with convincing evidence of its central role. Mice lacking the mitochondrial G3PDH have decreased survival, secondary to increased levels of cytoplasmic G3PDH compensatory activity [6]. In plants, GK is required for resistance against infection [76]. Flies with prolonged life span also have increased resistance to stress, and in particular, they survive extreme cold environments by increasing their levels of glycerol [77]. The role of GK in survival is further supported by its specific regulation during longevity in dauer larvae from *C. elegans* [78]. In *Drosophila*, elevation of diacylglycerol levels leads to increased cell death and shorter lifespan [79]. Mutations in *midway*, a DGAT involved in diacylglycerol removal into triglycerides, lead to cell death most likely from elevated DAG levels [16]. In photoreceptor cells, deficiency of phosphatidic acid leads to growth arrest and cell death. This is independent of glycerol 3-phosphate levels and depends on metabolites downstream of this metabolite [81].

Drosophila melanogaster is a useful model system for identifying second site modifier genes that genetically interact with the GK locus and their role in biological network regulation. Modifier screens in *D. melanogaster* have been invaluable for elucidating signal transduction pathways involved in human disease [82]. In *Drosophila*, 87% of genes known to be involved in human mental retardation are conserved [83]. *Drosophila* GK loci may be useful to develop model systems for investigations of human GK-related syndromes.

The realization of novel functions for GK from sequence analysis suggests experiments to investigate potential roles for members of this protein family in transcriptional regulation and apoptosis. ASTP or the ATP-stimulated glucocorticoid receptor translocation promoter is a cytosolic protein activity that enhances nuclear uptake of the activated glucocorticoidglucocorticoid receptor complex (G-GRC) in the presence of ATP and is involved in binding to chromatin [84], where it interacts with the argininerich histones H3 and H4, with preference for H4, via lysine residues [85]. Purification and peptide sequencing identified ASTP as identical to GK [86]. The ability of GK to facilitate glucocorticoid receptor nuclear translocation decreases with aging [87]. While mouse GK knockouts have autonomous glucocorticoid secretion and no resistance to its action [7], the role of GK in glucocorticoid receptor function or the roles of its chromatin interaction are still poorly understood. We have confirmed the nuclear translocation of GK in response to dexamethasone [88]. The glucocorticoid receptor, as a member of the nuclear receptor family of transcription regulators, contains a highly conserved, N-terminal zinc-finger domain that mediates specific binding to target DNA sequences. GK contains a domain that has homology to zinc-finger domains. Many of the missense mutations identified in patients with isolated GKD cluster within this domain. We have also identified an LXXLL domain in GK. This domain is present in coactivators of nuclear receptors. GK also contains a putative bipartite nuclear localization sequence at its Cterminus. The lack of LXXLL motifs in Drosophila and other invertebrates correlates with the absence of most nuclear receptors in these species. However, it is most intriguing that a phosphorylation residue adjacent to this motif (Serine 150) and which mediates receptor subtype specificity for nuclear receptor coactivators [89] has indeed been conserved. In addition, the T278 residue mutated in patients with GKD is part of a consensus site for PKC. PKC potentiates glucocorticoid receptor activity via phosphorylation of GK/ASTP [90]. ASTP is composed of two apparently identical subunits with molecular weights of 48,000 [91]. An additional minor band of about 50 kDa is also observed, consistent with GK being phosphorylated [92]. It remains to be determined if this ASTP function has been conserved in invertebrates as well.

Mitochondrial localization of GK has been previously described in response to apoptotic signals [93,94]. GK activity has been described in brain, particularly associated with mitochondria [1,95,96]. GK is associated with non-synaptic mitochondria [97], and appears to

be present in neurons [98], primarily in GABAergic neurons of the cortex and in cerebellar granule cells [99]. In the rat brain, the binding of GK to mitochondria is dependent on proapoptotic signals including divalent cations and glucose 6-phosphate, while glycerol 3phosphate and ATP reduce binding [100]. Blockers of mitochondrial functions, such as oligomycin, dinitrophenol, cyanide, and atractyloside, all inhibit GK activity [100]. Our observations suggest a dynamic subcellular localization for GK. Exon 18 codes for a conserved hydrophobic domain present in many other mitochondrial proteins, including Bax and Bcl-2 [101]. Mitochondrial localization of GK has previously been demonstrated in our laboratory, where it associates in response to calcium-induced cell death [93]. In addition, yeast GK localizes to mitochondria [102]. This evidence, as well as preliminary data in *Drosophila* showing modifiers of GK phenotypes to be involved in apoptosis [103] suggests GK to be a likely candidate in cell death processes.

The GK-like proteins CG1271, CG8298, and CG1216 do not have any glycerol kinase activity [41]. However, there have been suggestions of function. These proteins interact with the serotonin reuptake transporter, but do not affect its activity [104]. CG1216 contains a K+ channel tetramerization domain/BTB/POZ domain found in GMRP-1, *C. elegans* R05F9.2, and yeast SRP40, and conserved in *Anopheles gambiae* protein EAA04715.1. This domain is present in many other classes of proteins, mostly K channels. There are several potential transmembrane domains present (Fig. 6) (http://www.cbs.umn.edu/fly/). The POZ/BTB domain involved in protein interactions is present in zinc-finger proteins [105], and the K+ receptor interaction may be a cell-specific action of apoptosis [106]. Along with a role in neuronal function, a screen for olfactory mutants defective in an avoidance response to benzaldehyde identified a P-element insertion line smi61A in the region of the CG1216/Gyk locus [107]. This mutant interacts with a P-element insertion smi60E in the gene DSC1, a sodium channel [108,109]. In addition, CG1216 is expressed in the fly brain [110].

We have shown the presence of previously unidentified protein domains in glycerol kinase and their conservation across species. Their evolutionary preservation is suggestive of functional significance. Our observations open up new avenues for investigation of novel functions and roles for glycerolipid metabolism and GK activity in transcriptional regulation and apoptosis. We have also shown the presence and conservation of GK loci between *Drosophila* and humans. The presence of conserved protein domains not only provides insights into potential new functions for this family of proteins, but supports the utility of this model organism for in vivo genetic studies of GK function. Ongoing studies are defining the role of these novel potential functions in GK activity and their relationship to phenotypic variability in disease.

Acknowledgments

We thank the McCabe laboratory members past and present, George Jackson, Larry Zipursky, and Utpal Banerjee for all their support and helpful comments. This work was performed as part of the Intercampus Medical Genetics Training Program at UCLA and supported by NIH Training Program Grant T32GM08243 (J.A.M.) and R01HD022563 (E.R.B.M.).

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Fig. 1.

Glycerol metabolic pathways in *D. melanogaster*. Hex, hexokinase; Pgi, phosphoglucose isomerase; Pfk, phosphofructokinase; Ald, aldolase; Tpi, triosephosphate isomerase; Gpdh, NAD+ glycerol 3-phosphate dehydrogenase; Gpo, glycerophosphate oxidase/FAD glycerol 3-phosphate dehydrogenase; aay, astray; Gapdh, glyceraldehydes 3-phosphate dehydrogenase; Pgk, phosphoglycerate kinase; Pglym, phosphoglyceromutase; Eno, enolase; Pyk, pyruvate kinase; Gpt, Glutamate pyruvate transaminase; *rdgB, retinal degeneration B*; Dhapat, dihydroxyacetone phosphate acyltransferase; CdsA, CDP diglyceride synthetase; *eas, easily shocked*/ethanolamine kinase; *mdy, midway*/diacylglycerol *O*-acyltransferase; *wun, wunen*/ phosphatidate phosphatase. Loci responsible for these activities in *Drosophila* are shown above and/or below the arrows denoting the reactions.



Fig. 2.

Phylogenetic relationship of glycerol kinase (GK) proteins from *D. melanogaster* and *Anopheles* to other species. (A) Unrooted protein phylogeny radiation tree for GK proteins. Branch length denotes pairwise estimated evolutionary distances according to scale bar. (B) Unrooted consensus phylogeny of GK proteins showing tree topology not drawn to scale. Data were analyzed using the Neighbor-Joining method. Bootstrap percentage values are located at each node. Numbers below each branch correspond to pairwise estimated evolutionary distance values. (C) Unrooted consensus maximum parsimony tree. Bootstrap percentages are located at each node.



Fig. 3.

Alignment of insect glycerol kinase protein sequences across insect species.

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		M A SSS P	
Gyk[Mus]	1 :		90
Cult (Dath)			0.0
GYK[Rat]	1 :	MAAAKKAVLGPLVGAVDQGTSSTRELVENSKTASIJISHHQVEIKVEPPREGWDSDPKSLIQSVYECIEKTCENGQINIDISNIKAIGV :	90
GK[Hum]	1 :	MAASKKAVLGPLVGAVDQGTSSTRPLVENSKTADITSHHQVDIKQEPPREGVVDQDEKDIHSVYEGIEKTCEKEGQUNIDISNIKALGV	90
GK[Xen]	1 :	MAASNRVILGPLVGAUDOGTSSTREVENAKTABUTSHHOVETKOKEPKEGMBODEKBUTRSVYECVEKTCEKTTOINIDITNIKAIGV	90
CV [Fuend			60
GK[Fugu]	1 :	MILLQVFNTKTASIVCQRQVEINVSSERKEGNVBEDEQSILQSVTKCIERTCENTAQTNVDVSSERAVGV	69
2E383[Cele]	1 :	ROLFPHGEWOMDEMETSSSRIVEEADTGELVTSHQLEVROLFPHGEWOMDEMETYDTVVSCISKTIEKDENLGISADETKSVGV	81
<pre>glpK[Ecoli]</pre>	1 :	WIEKKYIVALDOGTISSRAVVMDHD-ANDISVSORDFEOLYPKPGWVDHD-MONAAOSSTLVEVVAKADISSDONAAGH	80
Guk[Droc]	1.	MOOD THE REPORT OF	97
GAKIDLOSI	1 :		91
CG7995[Dros	1 :	MTEGKNLPHSSADVPFRSKELRKQSLIQHTGLVGVIDEG*KAIGSSIYTTPDFKEHAAHRVDISVITPQDGYPQQELBMASINKCAEEAIKQPPEQGFSASDIVTVGI	110
30E57[Mosq]	1 :	WASLERSKLIGVIDACTNSVRFVI-KLPEFDELASHOIRITOIVERDENTENVEAURLOAVEACHOVEKUGFLVKDHAALGI	87
•		Exon 1 II Exon 2 II Exon 3	
		6	
		G*P G P PP PPG P LXXLL	
Gyk[Mus]	91 :	SNOREWYVVWDKV/GEPLYNAVVWDDURTOSTVENLSKRIPGNNNFVKSK/GDPLSTYFSAVKLRWIDDNVKKVOEAVEENRALFG/TDSWDWSD/GEIHGGVHCA:	197
Cut [Pat]	01 .		197
OYK[Kac]			197
GK[Hum]	91 :	SAQREWIVVADRINGEPEINAVVADDERIQSIVESISINGEPEININGEPEININGEPEININGEPEININGEPEININGEPEININGEPEININGEPIGEVAGGVAGGVAGGVAGGVAGGVAGGVAGGVAGGVAGGVA	197
GK[Xen]	91 :	SNQRETTVVNDKTTGEPLYNAIVNEDIRTQSTVERLIKRIPGKNKNFFKSRIGLPLSTYFSAVKIRNLIDNVEEIRHAVSEGRAMEGTVDSNILNSLACAKNGGVHCT	198
GK[Fugu]	70 :	TNOREWYVVNDKERGVPLYSATVNDDDRTOSTVENLINKAPGKDKNHLKHKTGDPTSTYFSAVKLKNTDDNVDEVROAVLSGRAMEGTVDSNTUNCDTGGSSGGVHCA	177
2E383[Cele]	82 .		184
-low(Real 4)	01 .		100
diby[Ecol1]	81 :	TARGENETIVE ENERGY PITTALVE QCRATAEICS HER R DGLEDYIRS AND VIDPYTS GTKVAULD HVEGSRERARRGED A GTVD WIDT RATO GTV	182
Gyk[Dros]	98 :	TNQRESTVVWDRNSGQPLVNAIIWDDNRTTSTVBELLETIPNNARNINYLRPLCGLPLSPYFSGVKLRWLRDNVPVVSQAMEKGTAMEGTIDTWLMYNLAGKDCGVHKT:	207
CG7995[Dros	111 :	TNOREWTIVNDAVNCKPLYNALLMKDIRTSTTVEOLVAKVODPNHFRSSTGUPISTYFSALKIRNIRDNVPEVROAIRERRCKAGTVDSNIVMNUNNGALHIA:	213
30E57[Mosa]	88 .		195
Sorry (mond)			100
		EXON4IIEXON 5IIEXON 6II	
		NNNN M P	
		* WD repeat PPP F ** G * AA LL * -Fork h	
Guk[Mue]	100 .		200
GAVINUSI	190 .		299
Gyk[Rat]	198 :	DVTNASRVMIPATHSTEWDKELCEFFGLPABILENVRSSSBTYGLAKAGADECVPISGCIGDQSAALVGQACFQDGQAKNTYGRGCFDCAUCHKCVFSEHG :	299
GK[Hum]	198 :	DVTNASRTMIFNIHSLENDKOICEFEGIPMETLENVRSSSELVGL.KISHSVKAGALECVPISGCLGDOSAALVGOMCFQIGOAKNTYG/GCFLICNTGHKCVFSDHG :	305
GK[Xen]	199 :		306
art Burnel	170		267
GK[Fugu]	1/8 :		201
2E383[Cele]	185 :	DVSNASRTALDDHKRKASTQLCEFDDDEIBILEEIRSSAEVYEHFDKGPLEE-VPLSGCLGDQQAAMVEHQCLNAGQTKNTYETGTEMLCNIETRPHISKNE:	286
<pre>glpK[Ecoli]</pre>	183 :		286
Gyk[Dros]	208 .		309
COTODE (Date	014		217
CG/995[DE05	214 :	DVMASKATANAKABAQAADPVALKTAGIREDANATAHSCSDITECHTSERSPIREATASGINGAQQASDIGQARGVARGQINAMARSGGINGCARPVESRAG	31/
30E57[Mosq]	196 :	DVTNASKTAAMNIETTHADPLITKTESVHPDMLPETRSSSETVEKUKDSSVIDEIPISAILENOOASIAVEORCLKEGOAKNTYRKEEDATYNTETRCVOSTHE	298
		Exon 7 Exon 8 8A Exon 9 Exon 9A Exon 10	
		and - 1 * D 3 33 3 * IPU2 domain! D 3*3 **	
		ead - * P A AA A * BH2 domain P A* A * *	
Gyk[Mus]	300 :	ead - * P A AA A * BH2 domain P A*A * * MMWWWAYKICRDK=VYYAPECSVAIACAVIRTIADHIGINKSSEETEKIKKE/GTSYGCY/VPASCI/APYTEPSARCIICC/MTOFNIKCHAPAADAVCPORETID :	409
Gyk[Mus] Gyk[Rat]	300 : 300 :	ead - I * P A AA A A * IBM2 domaini P A * A * IMWWAYKI GRDK VYYAMEGSVALI CAVIRINI DIIGI IKSSEETSKI. KEYGTSYGYI VPAESGVA PYTEPSARGI ICG/I/OFINI CHIAFAALSAVEFOLIETD : IMWWAYKI GRDK VYYAMEGSVALI GAVIRINI DIIGI IKSSEETSKI. KEYGTSYGYI VPAESAVAPYTEPSARGI ICG/I/OFINI CHIAFAALSAVEFOL	409 409
Gyk[Mus] Gyk[Rat] GK[Hum]	300 : 300 :	ead - * P A AA A * BH2 domain P A*A * IMTEVANKIGEDEVENTION OF A A A * IMTEVANKIGEDEVENTION OF A A A * IMTEVANKIGEDEVENTION OF A A A A * IMTEVANKIGEDEVENTION OF A A A A A A A A A A A A A A A A A A	409 409
Gyk[Mus] Gyk[Rat] GK[Hum]	300 : 300 : 306 :	ead - I * P A AA A * IBN2 domaini P A AA A * IBN2 domaini P A* A * * INVEVAYKI GROKEVYYANEGSYALI GAVIRALIDIIG IKSSEEISKI. KEYGTYGCY VPAESGVAPYKEPSARGIIG OD OF IN KEHAFALEAVEFORETID: INVEVAKI GROKEVYYALEGSYALI GAVIRALIDIIG IKSSEEISKI. KEYGTYGCY VPAESAVAPYKEPSARGIIG OD OF IN KEHAFALEAVEFORETID: INVEVAKI GROKEVYYALEGSYALI GAVIRALIDIIG IKSSEEISKI. KEYGTYGCY VPAESAVAPYKEPSARGIIG OD OF IN KEHAFALEAVEFORETID:	409 409 415
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen]	300 : 300 : 306 : 307 :	ead - * P A AA A * BH2 domain P A *A * M/WYVAYKLGRDK-VYYALEGSVALIGAVTRHADHIGTIKSSEETSKA-KEVGTSYGCY/VPAFSGLYAPYWEPSARGTICGL/OFAN-KCHTAFALEAVCFORETED : IA/WYVAYKLGRDK-VYYALEGSVALIGAVTRHADHIGTIKSSEETSKA-KEVGTSYGCY/VPAFSAL/APYWEPSARGTICGL/OFAN-KCHTAFALEAVCFORETED : L/WYVAYKLGRDK-VYYALEGSVALIGAVTRHADHIGTIKSSEETSKA-KEVGTSYGCY/VPAFSAL/APYWEPSARGTICGL/OFAN-KCHTAFALEAVCFORETED : L/WYVAYKLGRDK-VYALEGSVALIGAVTRHADHIGTIKSSEETSKA-KEVGTSYGCY/VPAFSAL/APYWEPSARGTICGL/OFAN-KCHTAFALEAVCFORETED :	409 409 415 416
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu]	300 : 300 : 306 : 307 : 268 :	ead - I * P A AA A * IBH2 domaini P A AA A * IBH2 domaini P A*A * * IM/WVAYKI CRKK-VYYALEGSVALI CAVIR/ILIDIIGI IKSSEISKI. KEYGTSYGCY VPAFSU/APYKEPSARGIICID/OFINIKCHIAFALEAVCFORETLD: IM/WVAYKI CRKK-VYYALEGSVALI CAVIR/ILIDIIGI IKSSEISKI. KEYGTSYGCY VPAFSU/APYKEPSARGIICID/OFINIKCHIAFALEAVCFORETLD: ILITYYAIKI CRKK-VYYALEGSVALI CAVIR/ILIDIIGI IKSSEISKI. KEYGTSYGCY VPAFSU/APYKEPSARGIICID/OFINIKCHIAFALEAVCFORETLD: ILITYYAIKI CRKK-VYYALEGSVALI CAVIR/ILIDIIGI IKSSEISKI. KEYGTSYGCY VPAFSU/APYKEPSARGIICID/OFINIKCHIAFALEAVCFORETLD: ILITYYAIKI CRKK-VYALEGSVALI CAVIR/ILIDIIGI IKSSEISKI. KEYGTSYGCY VPAFSU/APYKEPSARGIICID/OFINIKCHIAFALEAVCFORETLD:	409 409 415 416 377
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu] 2E383[Cele]	300 : 300 : 306 : 307 : 268 : 287 :	ead - I * P A AA A * IB42 domaini P A *A * IB42 domaini P A*A * * IL/IVVAYKI GRKFVYYALEGSVALI GAVI RHLADHIGI IKSSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH KCHI AFAALEAVCFORETLD : IL/IVVAYKI GRKFVYYALEGSVALI GAVI RHLADHIGI IKSSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH KCHI AFAALEAVCFORETLD : IL/IVVAYKI GRKFVYYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH KCHI AFAALEAVCFORETLD : IL/IVVAYKI GRKFVYYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH KCHI AFAALEAVCFORETLD : IL/IVVAYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH RHLAFAALEAVCFORETLD : IL/IVVAYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH RHLAFAALEAVCFORETLD : IL/IVVGY AYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH RHLAFAALEAVCFORETLD : IL/IVVGY AYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH RHLAFAALEAVCFORETLD : IL/IVVGY AYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH RHLAFAALEAVCFORETLD : IL/IVVGY AYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKTSEETSKI A KEYGTSYGCY VPAFSGLYAPYWEPSARGI ICG/IVOPH CHAFAALEAVCFORETLD : IL/IVVGY AYKI GRKFVYALEGSVALI GAVI RHLADHIGI IKSELSKI GYGY FYAFSGLYAPYWEPSARGI ICG/IVOPH CHAFAALEAVCFORETLD :	409 409 415 416 377 396
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu] 2E383[Cele] alpK[Ecoli]	300 : 300 : 306 : 307 : 268 : 287 : 287 :	ead - I * P A AA A * IB42 domaini P A AA A * IB42 domaini P A * A * A IMVIVATKI CROKE VYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI, KEYGTSYGCY VPAFALI APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKE VYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI, KEYGTSYGCY VPAFALI APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKE VYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI, KEYGTSYGCY VPAFALI APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI, KEYGTSYGCY VPAFALI APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI, KEYGTSYGCY VPAFALI APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYYALEGSVALI CAVERTIA DITIGITIKSSEETSKI KEYGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYYALEGSVALI CAVERTIA DITIGITIKSSETSKI KEYGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYYALEGSVALI CAVERTIA DITIGITIKSSETSKI KEYGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYALEGSVALI CAVERTIA DITIGITIKSSETSKI THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROKEVYALEGSVALI CAVERTIA DITIGITIKSSETSKI THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROVE CAVERTIA CAVERTIA DITIGITIKSSETSKI THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROVE CAVERTIA CAVERTIA DITIGITIKSSETSKI THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOFTH CETAFALE AVEFORETID : LIVIVATKI CROVE CAVERTIA CAVERTIA DITIGITIKSSETSKI THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOTHTHATICTCLATIFTY CONTINUE TO THATAGTSYGCY VPAFSUL APVEPSARG TCONTIFTS THATAGTSYGCY VPAFSUL APVEPSARG TCCDIFOTHTHATICTCLATIFTY C	409 409 415 416 377 396 394
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gvk[Dros]	300 : 300 : 306 : 268 : 287 : 287 : 287 :	ead - I * P A AA A * IBR2 domaini P A AA A * IBR2 domaini P A* A * * LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILLIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF II KCHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILLIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF II KCHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILLIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF II KCHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILLIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF II KCHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILLIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KCHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRILIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYYALEGSVALKGAVIRIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYALEGSVALKGAVIRIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYALEGSSVALKGAVIRIDI IG ILKSSEEISKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III KHIAFALEAVCFORSTLD: LIJVIVA KKI GRDK-VYALEGSSVALKGAVIRIDI IG ILKSSEEISKI ILLINKKKI KE GTSYGCY VPAFSGL/APYKEPSARGII CGL/OF III SHAFAKIKEPSILE VECONSTLD: LIJVIVA KKI GRDK-VYALEGSSVALKGAVIRIDI IF KFISDAKEMSGI CRSVEDISGAVI VESTGII TPYMDSTARGTI ILGI/OVI 0: EHI CLAALEAVCFORSTLD: LIJVIVI CONCOLUSION VALEGSSTIC APKKINDI ILGI INSATEDI ILLINKKINDI VESTGII TPYMDSTARGTI ILGI/OVI 0: EHI CLAALEAVCFORSTLD: LIJVIVI KKI GRDK-VYALEGSTICA APKKINDI ILI ILLINKSTUD ILI ILINKINDI VESTGII TPYMDSTARGTI ILGI/NEGANINHI I RATISSIN VESTGII DI ILINKINDI VESTGII TPYMDSTARGTI ILGI/NEGANINHI I RATISSIN VESTGII TPYMDSTARGTI ILGI/NEGANINHI I RATISSIN VESTGII TPYMDI ILINKINDI VESTGII TPYMDI ILINKINGVINDI ILINKINDI VESTGII TI ILINKINDI VESTGII TI ILINKIN VESTGII APKKINDI ILINKINI VESTGII TPYMDI ILINKIN VESTGII TPYMDI	409 409 415 416 377 396 394
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros]	300 : 300 : 306 : 268 : 287 : 287 : 310 :	ead - I * P A AA A A * IBH2 domaini P A AA A * IBH2 domaini P A * A * *	409 409 415 416 377 396 394 419
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros]	300 : 300 : 306 : 307 : 268 : 287 : 310 : 318 :	ead - I * P A AA A * IB42 domaini P A AA A * IB42 domaini P A* A * *	409 409 415 416 377 396 394 419 427
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 :	ead - I * P A AA A A * IBH2 domaini P A AA A A * IBH2 domaini P A * A * * IBH2 domaini P A * A * * IBH2 domaini P A * A * * IBH2 domaini A * P A AA * * IBH2 domaini A * * IBH2 domaini A * * IBH2 domaini A * * * IBH2 domaini A * * * * * * * * * * * * * * * * * *	409 409 415 416 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Yan] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq]	300 : 306 : 307 : 268 : 287 : 310 : 318 : 299 :	ead - * P A AA A A * IBN2 domaini P A AA A A * IBN2 domaini P A* A * * DWWYAYKI GROKAVYYALEGSVAI GAVIRALIDIIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN KCHAFALEAVCFORETID : LATYYAYKI GROKAVYALEGSVAI GAVIRALIDIIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN CHAFALEAVCFORETID : LATYYAYKI GROKAVYALEGSVAI GAVIRALIDIIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN CHAFALEAVCFORETID : LATYYAYKI GROTACYALEGSVAI GAVIRALIDIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN CHAFALEAVCFORETID : LATYYAYKI GROTACYALEGSVAI GAVIRALIDIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN CHAFALEAVCFORETID : LATYYAYKI GROTACYALEGSVAI GAVIRALIDIIGIIKSSEETSKI KE GTSYGGY VPAFSGLAPYKEPSARGIIGUTOPIN CHAFALEAVCFORETID : LATYYG GTGADSVVALEGSVAI GAVIRALIDIIGIIKSSETTISTY STYNTYN TATAGYYGYYGY TATAGYYGYOTATISTISTISTISTISTISTISTISTISTISTISTISTIST	409 409 415 416 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] GlpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 :	ead - I * P A AA A A * [BH2 domain] P A*A ** AMVWA,KI CROKEVYYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTGYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROKEVYYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTGYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROKEVYYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROKEVYYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROKEVYYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALGAVERTA DITIO ILGI IKSSEETSKI, KE GTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALGAVERTA DITIO ILGI IKSSETSTI ATAGTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALGAVERTA DITIO ILGI IKSSETSTI ATAGTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALGAVERTA DITIO ILGI IKSSETSTI ATAGTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVYA,KI CROV ACYALSOS VERTA DITIO ILGI IKSSETSTI ATAGTSYGCY V2 FSGLA DYKEPSARG I COLFOFIN COLAFA ILS VCPOTESTD : LIVYVYA,KI CROV ACYALSOS VERTA DITIO ILGI IKSSETSTI DISLOVY V2 FSGLA DYKEPSARG I COLFOFIN COLAFIA ILS VCPOTESTD : LIVYVYA,KI CROV ACYALSOS VERTA DITIO ILGI IKSSETSTI DISLOVY V2 FSGLA DYKEPSARG I COLFOFIN COLAFIA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALAKI, KI PINDIN V2 FSGLA DYKEPSARG I COLFOFIN COLAFIA ILS VCPOTESTD : LIVYVA,KI CROV ACYALSOS VALAKI, KI PINDIN V2 FSGLA DYKEPSARG I COLFOFIN COLFOFINALI DITIO ILGI INTO V2 FSGLA DYKEPSARG I COLFOFINALI ILGITIVI V2 FSGLA DYKEPSARG I COLFOFINALI ILGIT	409 409 415 416 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Yen] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 :	ead - * P A AA A A * IBN2 domain P A AA A A * IBN2 domain P A*A ** MUVVY AYKI GROKAVYYALEGSVAIL GAVIRALIDIIIGIIKSSEEISKI KE GTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYA KI GROKAVYALEGSVAIL GAVIRALIDIIGIIKSSEEISKI KE GTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYA KI GROKAVYALEGSVAIL GAVIRALIDIIGIIKSSEEISKI KE GTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYA KI GROVACYALEGSVAIL GAVIRALIDIIGIIKSSEEISKI KE GTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYA KI GROVACYALEGSVAIL GAVIRALIDIIGIIKSSEEISTIN ATAGTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYA KI GROVACYALEGSVAIL GAVIRALIDIIGIIKSSEEISTIN ATAGTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYAG GTGADSYVALEGSVAILGSVAILGAVIRALIDIIGIIKSSAEISTI ATAGTSYGCY VPASGU APYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYAG GTGADSYVALEGSVAILGSVAILGAVIRALIDIIGIIKSSESISTI ATAGTSUSYUVPASTI GLAPYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYAG GTGADSYVALEGSVAILGAVIRALIDIIGIIKSSESISTI STATAGTSUSYUVPASTI GLAPYYEPSARGIIGUTOPIN CHIAFALLSVCFORSTID: LATYYAG GTGADSYVALEGSVAILGAVIRALIDIIKIN DISSARGU CHIAFANISVIPATI GLAPYYEPSARGIIGUTOPIN CHIAFANISVEPSARGIICUS CHIAFANI	409 409 415 416 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 :	ead - I * P A AA A A ' [BH2 domain] P A*A * * AMYWA, KI CROK, VYYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & VCPOLETID : LATYVA, KI CROK, VYYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & VCPOLETID : LATYVA, KI CROK, VYYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & VCPOLETID : LATYVA, KI CROK, VYYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & VCPOLETID : LATYVA, KI CROK, VYYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & AVCPOLETID : LATYVA, KI CROK AVYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & AVCPOLETID : LATYVA, KI CROV ACYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & AVCPOLETID : LATYVA, KI CROV ACYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & AVCPOLETID : LATYVA, KI CROV ACYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I & AVCPOLETID : LATYVA, KI CROV ACYALSOS VALGAVIRTIA DITIO II KISEETSKI, KE GTSYGCY V2 IS GLA DYYEPSARG I COLOR FIN COLAFA I XI AVCPOLETID : LATYVA, KI CROV ACYALSOS VALGAVIRTIA DITIO II KISEETSKI I KA MAN SYNTY Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	409 409 415 416 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Kum] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 : 410 :	ead i P A A A * [B12 domain] P A* A * * IMVEVATKI CRKK VYYALSCOVIC CALCULATION TOTIKSTEEDEKIK KE (GTSYGCT VP) SCUVAPYAPSAROT COULDER THATALS VCFORETTD: : .	409 409 415 377 396 394 419 427 408
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 : 410 : 410 :	ead - I * P A AA A * IBH2 domaini P A*A * A * IBH2 domaini P A*A ** Advava, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO IN STREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYYALSGSVALGAVERTA DITO INSTREET SK. KE GTSYGGY V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYALSGSVALGAVERTA DITO INSTRUMENT SK. SEGTON V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVA, K. GRDK-VYALSGSVALGAVERTA DITO INSTRUMENT SK. SEGTON V2 IS GLA DYTEPSARG I COLOR F. H. CH AFALLS VCFOLES ID : LIVYVG-GLASSVALSGATEN GASTER, SEGTON VERTA DITO INSTRUMENT STATE ST	409 409 415 416 377 396 394 419 427 408 511 511
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat] GK[Hum]	300 : 306 : 307 : 268 : 287 : 310 : 318 : 299 : 410 : 410 :	ead i P A AA A * [B12 domain] P A* A * * IMVEVATKI GRDK VYYALSEGVALL GAVLENILDITGTIKSSEEISKI KE (GTSYGCT VD: FSGLY ADVEPSARG) IC GUTOPIN CELLAFALS VCFORETID : .	409 409 415 416 377 396 394 419 427 408 511 511
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat] GK[Hum]	300 : 306 : 307 : 268 : 287 : 310 : 318 : 299 : 410 : 410 : 416 :	ead - I * P A AA A * [BH2 domain] P A*A * * Advervar, K. GROK - VYYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROK - VYYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROK - VYYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROK - VYYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROK - VYYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROV - ACYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROV - ACYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, K. GROV - ACYALSGS VALGAV RTIA DITIG ILKSSEETSKI, KE GTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, GPG ABS VVYHAGS GSTG GAS V STL HATAGTS YGGY V2 IS GLA DYK PSARG I COLOR F H CCH AFALLS V FOUR STD : LAVYVAR, GPG ABS VVYHAGS GSTG GAS V STL HON 1985 SPT F KU GNH WY V2 STG GLAD STD STR MARKING H H H TO THE STAYON TO THE STANDAWST THE STAYON TO THE STANDAWST THE STANDAWST THE STANDAWST THE STANDAWST THE STANDAWST THE STANDAWST THE STAN	409 409 415 416 377 396 394 419 427 408 511 511 517
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Hum] GK[Kum]	300 : 306 : 306 : 268 : 287 : 287 : 310 : 318 : 299 : 410 : 410 : 416 : 417 :	ead i P A AA A * [B12 domain] P A* A * * IMVEVALKE CRDKE VYYALSES VALLOAVERIADITIG TIKSSEETSKE KE (GTSYGCT VD) FSGLVADYKEPSARGT CGUTOPEN CETAFALS VEFORETID : .	409 409 415 416 377 396 394 419 427 408 511 511 511 511
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecoli] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu]	300 : 300 : 307 : 268 : 287 : 310 : 318 : 299 : 410 : 410 : 410 : 417 : 378 :	ead - I * P A AA A * IBR2 domaini P A*A * * AMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CET AFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CET AFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CET AFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CET AFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CET AFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVA.K. GRDK-VYYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVA.K. GRDK-VYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVA.K. GRDK-VYALSGSVAL GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVG-GUT GALSVALSGATEN GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVG-GUT GALSVALSGATEN GAVERTA DIT GIT KSSEETSK. KE GTSYGGY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL STD : IMVVG-GUT GALSVALSGATEN GASTAR I INT MIN BIGGTT DI STD DISCHAFY V2 KSGLA DYKEPSARG I CGTAPEN CHAFAILS VCFOL DYTE : IMVVG-GUT GALSVALSGATEN GALSKAL DI KINDIN HINDING VY KSGLA DYKEPSARG I CGTAPEN CHART I STLAVETOR DYTE : IMVVG-GUT GALSVALSGATEN GALSKAL DI KINDING I INT MIN BIGTT DI STUDY VY KSGLA DYKEPSARG I I GALSKAL DYKEPSARG I CGTAPEN CHAFAILS I STUDYOUTS : IMVVG-GUT GALSVAL DYKARGATEN I I INT MIN BIGTT DI STUDY VY KSGLA DYKEPSARG I I GALSKAL DYKEPSARG I I GALSKAL DYKEPSARG I I GALSKAL DYKEPSARG I I GALSKAL DYKEPSARG I I I I I I I I I I I I I I I I I I I	409 409 415 377 396 394 419 427 408 511 511 511 517 518 479
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Rat] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele]	300 : 306 : 306 : 268 : 287 : 287 : 310 : 318 : 299 : 410 : 410 : 416 : 417 : 378 : 397 :	ead i P A A * [BH2 domain] P A* A * * IMPEVATE GREEVY MALESCALL CAVERIADING TESSETSKICKE (GTSYGCT VD: FSGL/ADVEPSARG) ICG/I/OPEN CETAFALS/VCPORETID: I <	409 409 415 377 396 394 419 427 408 511 511 511 517 518 479 498
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] 2E383[Cele]	300 : 306 : 307 : 287 : 287 : 287 : 310 : 318 : 299 : 410 : 410 : 410 : 416 : 417 : 378 : 397 :	ead - 1 * P A AA A * IBB2 domaini P A*A * * AMVVA.K. GRDK - VYYMEGOVIC GAURALIS DITIO II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS DITIO II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS ON II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS ON II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS ON II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS ON II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOVIC GAURALIS ON II GUINSSEETSKI, KE GTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, KI GRDV A CYMESONAI, GAV RILLID HIGUINSSEETSHI ATAGTSYGGY V2 IS GLA DYTEPSARG II GUINF HI GUIAFA HIS VEDOUCETD : MYVVAK, GRDK - VYMEGOSONAI, GAV RILLID HIGUINSSEETSHI ATAGTSYGGY V2 IS GLA DYTEPSARG II GUINF HIGUINAL VEDOUCETD : MYVVAK, GRDKAVPFYMEGONO, GAURALIS DY HIGUNSSEETSHI DA TAGTSYGOY V2 IS GLA DYTEPSARG II GUINF HIGUNAL TAFAN HIS VEDOUCETD : MYVVAK, GRDKAVPFYMEGONO, GAARGHIN, HIGUNSSEETSHI STONGANSY V2 IS GLA DYTEPSARG II GUINF HIGUNAL TAFAN HIGUNAL	409 409 415 377 396 394 419 427 408 511 511 511 511 517 518 479 498
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Rat] Gyk[Rat] GK[Hum] GK[Yen] GK[Fugu] 2E383[Cele] glpK[Ecoli] glpK[Ecoli]	300 : 300 : 268 : 287 : 287 : 310 : 310 : 299 : 410 : 410 : 410 : 417 : 378 : 397 : 395 :	ead - 1 * P A A * [BH2 domain] P A* A * * IMVEVALXEGRAR VYIALSGOVER, CAVERILED INGERESSEES AKE KENGTSYGGY VYALSGOVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES IMVEVALXEGRAR VYVIALSGOVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES IMVEVALXEGRAVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES IMVEVALXEGRAVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CHAPARA LEXVEGORETED INGERSEES IMVEVALXEGRAVER, CAVERILED INGERSSEES KENKENGTSYGGY VYALSGOVER, CAVERILES KENKENGTSYGGY VYALSGOVER, CAVERILES, VENKENGTSYGGY VYALSGOVER, VYALS	409 409 415 377 396 394 419 427 408 511 511 517 518 479 498 495 520
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros]	300 : 306 : 307 : 268 : 287 : 287 : 310 : 318 : 299 : 410 : 416 : 416 : 416 : 378 : 397 : 395 : 420 :	ead - I * P A AA A * IBH2 domaini P A*A * A * IBH2 domaini P A*A ** Adverted to the second se	409 409 415 377 396 394 419 427 408 511 511 517 518 479 498 495 520
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Rat] GK[Hum] GK[Xen] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros]	300 : 300 : 268 : 287 : 310 : 310 : 318 : 299 : 410 : 410 : 416 : 378 : 397 : 397 : 395 : 428 :	ead - 1 * P A A * [BH2 domain] P A* A * * IMPEVALXEGRAR VYIALSGAAL GAVERMEDING TEXPERSE KA KE GTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFALS VCFORE TD : IMPEVALXEGRAR VYIALSGAAL GAVERMEDING TEXPERSE KA KE GTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFA AFA * * IMPEVALXEGRAR VYIALSGAAL GAVERMEDING TEXPERSE KA KE GTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFALS VCFORE TD : IMPEVALXE GRONDAVALLSGAAL GAVERMEDING TEXPERSE KK KE GTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFA AFA * * IMPEVALXE GRONDAVALLSGAAL GAVERMEDING TEXPERSE KE GTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFA AFA * * IMPEVALXE GRONDAVALLSGAAL GAVERMEDING TEXPERSE ATAGTSYGCT VD. FSGLADY EPSARGIC GUTOPEN CETAFA AFA * * IMPEVALXE GRONDAVALLSGAAL MASSICTUD FK TESDAKENG TEXPERSE AFA * * TO T	409 409 415 416 377 396 394 419 427 408 511 517 518 517 518 479 498 495 520 531
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Hum] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] CG7995[Dros]	300 : 300 : 268 : 287 : 310 : 318 : 299 : 410 : 410 : 416 : 417 : 378 : 397 : 395 : 420 : 409 :	ead Image: P A AA A * [BB2 domain] P A* A * * Image: Arrow Arrow Construction of the sector of th	409 409 415 416 377 396 394 419 427 408 511 511 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Rat] GK[Hum] GK[Yam] GK[Fugu] 2E383[Cele] glpK[Ecoli] Gyk[Cros] CG7995[Dros] 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 287 : 318 : 299 : 410 : 410 : 410 : 416 : 417 : 378 : 397 : 397 : 428 : 428 : 400 : 428 : 400 : 410 : 400 : 40	ead - 1 * P A AA A * [BH2 domain] P AA * IMVEVALXEGRAR VYIALSGAAL GAVERMEDTEDTEDTESSEESKE KEIGTSGGT VD.FSGLADYTEPSARGTEGTUDER CTLAFALSAVEGTERETEDTE I I AA * [BH2 domain] P AA A * IMVEVALXEGRAR VYIALSGAAL GAVERMEDTEDTESSEESKE KEIGTSGYGCT VD.FSGLADYTEPSARGTEGTUDER CTLAFALSAVEGTERETEDTES I I I I I AA * I	409 409 415 416 377 396 394 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] dyk[Ecoli] Gyk[Ecoli] Gyk[Ecoli] Gyk[Dros] Gyk[Mus] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] dyk[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq]	300 : 300 : 306 : 268 : 287 : 310 : 318 : 299 : 410 : 410 : 416 : 417 : 397 : 395 : 420 : 409 :	ead - I * P A AA A * [BH2 domain] P AA * * AMYWA, K. GRDK, VYYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDK, VYYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDK, VYYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDK, VYYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDV, ACYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDV, ACYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDV, ACYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOP, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDV, ACYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTOFT, H. CELAPALE, VC PORTETTO I LMYWA, K. GRDV, ACYALSGOVAL, GAVERTADIT, GITKSEETSKI, KE, GTSYGCY, V2, IS GLA, DYK PESARGIT, GUTAF, H. KELAVEROPT, H. CELAPALE, VC PORTETTO I I I I I I I I I I I I I I I	409 409 415 416 377 396 394 427 408 511 511 511 511 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2B383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Rat] Gyk[Rat] GK[Hum] GK[Hum] GK[Fugu] 2B383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq]	300 : 306 : 307 : 268 : 287 : 287 : 287 : 310 : 318 : 299 : 410 : 410 : 410 : 410 : 378 : 378 : 395 : 420 : 428 : 409 :	ead - 1 * P A AA A * [BH2 domain] P A* A * * AMYEVANKI GERKEVYYAMSESVAH, GAVERMEDTIG IKSSEEISKI, KE/GTSYGCT V2, FSGLY, DYKEPSARGT CGMOPTA, CH AFALLS, VCEORETID : . <td>409 409 415 416 377 396 394 419 427 408 511 517 518 479 498 495 520 531 514</td>	409 409 415 416 377 396 394 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecoli] Gyk[Dros] 30E57[Mosq] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gk[Fugu] 2E383[Cele] Jpk[Ecoli] Gyk[Dros] 30E57[Mosq]	300 : 300 :	ead - I * P A AA A * [BH2 domain] P A* A * * AMAYUA, K. GRDK, VYYALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : . LMYYA, K. GRDK, VYYALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYYALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYYALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYALSGOVALSGOVAL, GAVIRTI, DILGI IKSSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI COTTOP, H. CELAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYALSGOVAL, GAVIRTI, DILGI H, SSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI LCHAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYALSGOVAL, GAVIRTI, DILGI H, SSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, EFARGI LCHAPALE, V2, ECRETTD : LMYYA, K. GRDK, VYALSGOVAL, GAVIRTI, DILGI H, SSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, ESARGI LCHAPALE, V2, ECRETTD : LMYYA, V. GRDKA, KI, KI, BATAGE, SSEEISKI, KE, GTSYGCY, V2, FSCH, 2017, ESARGI LCHAPALE, V2, ECRETTD : : LMYYA, GOVARA, KI, KI, KI, KI, KI, KI, KI, KI, KI, KI	409 409 415 416 377 396 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2B383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Hum] GK[Hum] GK[Fugu] 2B383[Cele] glpK[Ecoli] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus]	300 : 300 :	ead - 1 * P A AA A * [BH2 domain] P AA * * MAYEVALKI GERKE VYYAMSESVALL GAVERNIADILG ILKSSEEISKI KE (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFALS/CEORETID : [MYYYAKKI GERKEY VYMSESVALLGAVERNIADILG ILKSSEEISKI KE (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFALS/CEORETID : MYYYAKI GERKEY VYMSESVAL GAVERNIADILG ILKSSEEISKI KE (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFALS/CEORETID : [MYYYAKI GERKEY VYMSESVAL GAVERNIADILG ILKSSEEISKI KE (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFALS/CEORETID : MYYYAKI GERKEY VYMSESVAL GAVERNIADILG ILKSSEEISKI KE (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFARALS/CEORETID : [MYYYAKI GERKAY VYMSESVAL GAVERNIADILG IKKTEEVERKIA & (GTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFARALS/CEORETID : MYYYAKI GERKAY VYMSESVAL GAVERNIADILG ILKSSEEISTI AATAGTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFARALS/CEORETID : [MYYYAKI GERKAY VYMSESVAL GAVERNIADILG INKSENEISTI AATAGTSYGCT V2) FSGLY APKEPSARGI CGMOPTH CETAFARALS/CEORETID : MYYYAKI GERKAY VYMSESVALUS FKILDI FKILSSAREMET (FSGL) GYMYSTER (FSGL) APKEPSARGI CGMOPTHAR (FSGL) APKERANGT (FSGL) AFKERANGT (FSGL) AFKERANGT (FSGL) AFKERANGT (FSGL) AFKERANGT (FSGL) AFKERANGT (FSGL) AFKERANGT	409 409 415 416 377 396 427 408 511 511 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus]	300 : 300 :	ead - 1 * P A AA A * [BH2 domain] P A* A * * IMAYUA, K. GRDK, VYYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDK, VYYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDK, VYYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDK, VYYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDV, ACYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDV, ACYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDV, ACYALSGOVAL, GAVERIJ, DITG ILKSSEETSKI, KE GTSYGCY, V2 K5 GL, ADVEPSARGIL CUTOPEN CELLAPALEAVECURETTD I LIVYVA, K. GRDV, AVALSGOVAL, GAVERIJ, DITK, HUDDEN,	409 409 415 377 396 394 419 427 408 511 511 515 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Ecol1] Gyk[Toros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Mus] Gyk[Mus]	300 : 300 :	ead - 1 * P A AA A * [BH2 domain] P AA * * MAYEVALKE GREKE VYYALSGEVALL GAVERIALDING TIKSSEETSKE KE GTSYGCT V2 NEGLAPY TYPEPSARGT CGTOPT NCT AFA TA VCTORETTO :	409 409 415 377 396 394 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat]	300 : 300 :	ead - 1 * P A AA A * [BH2 domain] P A* A * * AMYWAKK (FRDK) VYMMEGSVAIL GAVERIL DITG INSSEEDSKINKE (GTSYGCY V215 GLA 2017) EPSARG IC GINOP IN CCH APA IE AVECURE TID I LIVYVAKK (FRDK) VYMMEGSVAIL GAVERIL DITG INSSEEDSKINKE (GTSYGCY V215 GLA 2017) EPSARG IC GINOP IN CCH APA IE AVECURE TID I LIVYVAKK (FRDK) VYMMEGSVAIL GAVERIL DITG INSSEEDSKINKE (GTSYGCY V215 GLA 2017) EPSARG IC GINOP IN CCH APA IE AVECURE TID I LIVYVAKK (FRDV ACYMESSOAIL GAVERIL DITG INSSEEDSKINKE (GTSYGCY V215 GLA 2017) EPSARG IC GINOP IN CCH APA IE AVECURE TID I LIVYVAKK (FRDV ACYMESSOAIL GAVERIL DITG INSSEEDSY EPK INS (MASSOCY V215 GLA 2017) EPSARG IC GINOP IN CCH APA IE AVECURE TID I LIVYVAKK (FRDV ACYMESSOAIL GAVERIL DITG INSSEEDSY EPK INS (MASSOCY V215 GLA 2017) EPSARG IC GINOP FIN COL APA IE AVECURE TID I LIVYVAK (FRDV ACYMESSOAICA (GAVERIL DITG INSSEEDSY EPK INS) (MASSOT FIN CONSERT) EPSARG IC GINOP FIN COL APA IE AVECURE TID I LIVYVAK (FRDV ACYMESSOAICA (GAVERIL DITM) FINISOST FIN CONSERT INSSEEDSY EPK INSSEEDSY EPK INSSEEDSY EPK INSSEEDSY EPK INSSEEDSY EPK INSSEEDSY EFK INSSEEDS	409 409 415 416 396 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Toros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Mus] Gyk[Mus] Gyk[Mus] Gyk[Mus]	300 : 300 :	ead - * P A AA A * [BH2 domain] P A* A * * IMTEVATE GROEN VYALEGEVALGAVERUED LGENSSEETSELENE KEUGSYGCY VALAGARGE GMOOTH CHAPA LEXVECORETED : IMTEVATE GROEN VYALEGEVALGAVERUED LGENSSEETSELENE KEUGSYGCY VALAATYE PEARGE CGMOOTH CHAPA LEXVECORETED : IMTEVATE GROEN VYALEGEVALGAVERUED LGENSSEETSELENE KEUGSYGCY VALABUARYE PEARGE CGMOOTH CHAPA LEXVECORETED : IMTEVATE GROEN VYALEGEVALGAVERUED LGENSSEETSELENE KEUGSYGCY VALABUARYE PEARGE CGMOOTH CHAPA LEXVECORETED : IMTEVATE GROEN VYALEGEVALGAVERUED LGENSSEETSELENE KEUGSYGCY VALABUARYE PEARGE CGMOOTH CHAPA LEXVECORETED : IMTEVATE GROEN VYALEGEVALGAVERUED (KETEEDSEN ALGUGSYGCY VALABUARYE PEARGE CGMOOTH KETEEDSELD : IMTEVATE GODO ACVALEG VENTERSEN DER KENGE (CES) GODESARY USE PEARGE CGMOOTH KETEEDSELD : IMTEVATE GODO ACVALEG STUCKAVERUED (KETEEDSEN SAAT VENTERSARCH VENTERSARCH LGEN VOOR STUCKAVERUEDSELD : IMTEVATE GOTO ACVALEG VENTERSEN OF THE KINNEN GUEST TATAATSYEED TATAATSYEED SARCH LGEN VOOR STUCKAVER STUCKAVER STUCKAVER STUCKAVER TEAVER VENTERSEN SAAT VENTERSE	409 409 415 416 377 396 394 419 408 511 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus] Gyk[Rus]	300 : 300 :	ead - 1 * P A AA A * [BH2 domain] P A* A * * IMTYVAX K GROKTVYALEGSVALGAVENTED LGTEKSEETSKE KE GTSYGCY VACA LATYN EPSARGT GATOPEN GTAPATES VEGOTETD : IMTYVAK GROKTVYALEGSVALGAVENTED LGTEKSEETSKE KE GTSYGCY VACA ALATYN EPSARGT GATOPEN GTAPATES VEGOTETD : IMTYVAK GROKTVYALEGSVALGAVENTED LGTEKSEETSKE KE GTSYGCY VACA ALATYN EPSARGT GATOPEN GTAPATES VEGOTETD : IMTYVAK GROKTVYALEGSVALGAVENTED LGTEKSEETSKE KE GTSYGCY VACA ALATYN EPSARGT GATOPEN GTAPATES VEGOTETD : IMTYVAK GROKTVYALEGSVALGAVENTED LGTEKSEETSKE KE GTSYGCY VACA SELATYN EPSARGT GATOPEN	409 409 415 416 377 396 394 419 427 408 511 517 518 479 498 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] GK[Hum] GK[Kum] GK[Kum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Mus] Gyk[Rat] Gyk[Mus] Gyk[Mus] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele]	300 : 300 :	ead - * P A AA A * [BH2 domain] P A* A ** IMPROVIKE (DRNK VY TALESCE ALL GAVER TALE) IT GENESSEET KE LEVER (SUCE (V2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (409 409 415 416 377 396 394 419 408 511 511 517 518 517 518 479 498 495 5200 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gk[Hum] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gk[Fugu] 2E383[Cele] Jpk[Ecol1]	300 : 300 :	ead - * P A AA A * [BH2 domain] P A*A ** MARWY XK (GRDK VY TALEGC ALL GAVIERTED) IG THASSEET KIN KE (GTS YECT V2) (2 ALL GAVIERTED) (ALL AT ALL V COTTONET D) IARY VX (K) GRDK VY TALEGC ALL GAVIERTED) IG THASSEET KIN KE (GTS YECT V2) (2 ALL GAVIERTED) (ALL AT ALL V COTTONET D) MATY VX (K) GRDK VY TALEGC ALL GAVIERTED) (ALL RESSEET KIN KE) (GTS YECT V2) (2 ALL GAVIERTED) (ALL AT ALL V COTTONET D) IARY VX (K) GRDK VY TALEGC ALL GAVIERTED) (ALL AT ALL V COTTONET D) MATY VX (K) GRDK ACTALEGC ALL GAVIERTED) (ALL AT ALL V) (COTTONET D) IARY (X) (COTTONET D) IARY (X) (COTTONET D) MATY VX (K) GRDK ACTALEGC ALL GAVIERTED) (ALL ALL GES ED SCALE VIEW (V2) (COTTONET D) IARY (V) (COTTONET D) IARY (V) (COTTONET D) MATY VX (K) GRDY (ACTALEGC ALL GAVIERTED) (K) (MDAYDS YF (K) (MAHOV (V2) (COTTONET D)) IARY (V) (COTTONET D) IARY (V) (COTTONET D) MATY VX (K) GRDY (ALL GAVIERTED) (K) (MDAYDS YF (K) (MAHOV (V2) (COTTONET D)) IARY (V) (COTTONET D)) IARY (V) (COTTONET D) IARY (V) (COTTONET D)) MATY VX (K) GRDY (V2) (V2) (V2) (V2) (V2) (V2) (V2) (V2)	409 409 415 377 396 419 427 408 511 517 518 479 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gk[Fugu] 2E383[Cele] glpk[Ecol1] glpk[Ecol1] Gk[Fugu]	300 : 300 :	ead - * P A AA A * [BH2 domain] P A* A ** ATWW YKK (GRNK VY TALEGC VAL GAVIR TALE) IG IKSSEETSKIK KE (GTS YECT V2 AT SALVEPSARGT CGM OF AL CHAPA TE VCG OF BETD : MAYY YKK (GRNK VY TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SALVEPSARGT CGM OF AL CHAPA TE VCG OF BETD : ATWY YKK (GRNK VY TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SALVEPSARGT CGM OF AL CHAPA TE VCG OF BETD : MAYY YKK (GRNK AC TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SGM DY TEPSARGT CGM OF AL CHAPA TE VCG OF BETD : ATWY YKK (GRN AC TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SGM DY TEPSARGT CGM OF AL CHAPA TE VCG OF BETD : MAYY YKK (GRN AC TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SGM DY TEPSARGT CGM OF AL CHAPA TE VCG OF BETD : MYYY YKK (GRN AC TALEGC VAL GAVIR TED) IG IKSSEETSKIK KE (GTS YECT V2 AT SGM DY TEPSARGT CGM OF AL CHAPA TE VCG OF BETD : MAYY YKK (GRN AC TALEGC VAL GAVIR TED) IG IKSSEETSKIK YKK (YVE YE GAVIR TED) STARGT IG IKSKEETD : MYYY YKK (GRN AC TALEGC VAL GAVIR TED) IK (MD AND YD STFT TK (VAL HEW YWE YE FARGT CGM AC TED STARGT IG IKSKEETD : MAYY YKK (YKE YA MAESSET) CAA FEM OF OF TH YKE YYKE YKE YKE YKE YKE YKE YKE YKE YK	409 409 415 377 396 394 419 427 408 511 511 517 518 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gk[Fugu] 2E383[Cele] Jpk[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat]	300 : 300 :	ead - 1 * P A A A * [B82 domain] P A* A ** MARY VARIAGEN VIALEGENALI CAVER THEOTIGET MIKESEETENE NEUGTSYGCY VOIG CLAUCE THEORIGET CHAPPER AGGING CHAPPE	409 409 415 3196 394 419 427 408 511 517 518 479 498 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Hum] GK[Yuu] 2E383[Cele] glpk[Ecol1] Gyk[Nros] CG7995[Dros] Gyk[Mus] Gyk[Rat]	300 : 300 :	ead - * P A AA A * [B12 domain] P A* A ** MARY VAR (GEDK VY MALES VALL (GAV R H-D) LIG INSSEETS KILKE (GT YGC) VALGUAR (GT YGC) CHAC FINGUAR (GT PARLE) VALGUAR (GT	409 415 416 377 396 419 427 408 511 517 518 479 498 495 520 5314
Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] Gyk[Rat] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] Gyk[Rat] GK[Fugu] 2E383[Cele] JPK[Ecol1] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Col1] Gyk[Dros] CG7955[Dros] CG7955[Dros]	300 : 300 :	ead - * P A AA A * [B12 domain] P A* A ** MARVEY, VIK, GEDK, VYYMASGYAR, GAV, RYM, DITG, IKSSEEDSKI, KEV GYSGY, VYR, GAV, GYK, ESP, NG, IC, GMA, EM, GURAPA, UZ, VGCTORESTD, I MARVEY, VIK, GEDK, VYYMASGYAR, ICAVIR, ULDITG, IKSSEEDSKI, KEV GYSGY, VYR, GAV, GYK, ESP, NG, IC, GMA, EM, GURAPA, UZ, VGCTORESTD, I MARVEY, VIK, GEDK, VYYMASGYAR, ICAVIR, ULDITG, IKSSEEDSKI, KEV GYSGY, VYR, GAV, GYK, ESP, NG, IC, GMA, EM, ATA, UZ, VGCTORESTD, I MARVEY, VIK, GEDK, AV, MARVE, ULDITG, IKSSEEDSKI, KEV, GYSGY, VYR, GAV, GYK, ESP, NG, IC, GMA, EM, ATA, UZ, VGCTORESTD, I MARVEY, VIK, GEDV, ALL, GAV, RIZ, DITG, IKSSEEDST, ANARGY, GYSGY, VYR, BAR, ULDY, EPS, NG, IC, GMA, EM, ATA, UZ, VGCTORESTD, III, MARVEY, VIK, GEDV, GAV, GYK, GAR, GAVGA, GA	409 409 415 416 377 396 419 427 408 511 517 518 498 495 520 531 514
Gyk[Mus] Gyk[Rat] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] 30E57[Mosq] Gyk[Mus] GK[Hum] GK[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Dros] CG7995[Dros] Gyk[Mus] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Rat] Gyk[Fugu] 2E383[Cele] glpk[Ecol1] Gyk[Fuso] CG7995[Dros] 30E57[Mosq]	300 : 300 :	ead - * P A AA A * [BH2 domain] P A* A ** MATELY INCORDANTY ALLEGY AT GAVER THOLD INFORM SSEEDS ALLEY GY YOR DIG IN A DYNEPS ARGIN CHAPT, HILL ALLEY OF CONTROL MATELY INCORDANTY ALLEGY AT GAVER THOLD INFORM SSEEDS ALLEY GY YOR DIG IN A DYNEPS ARGIN CHAPT, HILL ALLEY OF CONTROL MATELY INCORDANTY ALLEGY AT GAVER THOLD INFORM SSEEDS ALLEY GY YOR DIG IN A DYNEPS ARGIN CHAPT, HILL ALLEY ALLEY OF CONTROL MATELY INCORDANTY ALLEGY AT GAVER THOLD INFORM SSEEDS ALLEY GY YOR DIG INTO THE DEGY ALLEY ALL	409 409 415 416 377 396 419 427 408 511 511 511 517 518 498 495 520 514

Fig. 4.

Alignment of glycerol kinase protein sequences across species. Limits of each human exon are labeled below the aligned sequences. F, fructose 1,-bisphosphate binding site; P, phosphorylation site; G, glycerol binding residue; *, residue mutated in patients with GKD; M, magnesium binding residue; A, ATP binding residue; SSS, SUMOylation sequence; LXXLL, coactivator interaction domain; LL, dileucine repeats; N, nuclear localization sequence; PXDLS, CtBP binding site. The protein residues encoded by each exon are labeled below the sequences.

Martinez Agosto and McCabe

GK[Hum]	524	:	SPESGDPSIFCSLPLGFFIVSSMVMLIGARYISGIP-	:	559
GK1 [Hum]	518	:	SPEGGDPSVFCSLPLGFFIVSSMAMLIGARYISGIP-	:	553
GK2 [Hum]	518	:	SPEGGDPSIFSSLPLGFFIVSSMVMLIGARYISGVP-	:	553
GK[Xen]	527	:	SNGNGDCSIFCSLPLGLYIVSSMVLLIGAKYISGLNK	:	563
gk2-prov[X	527	:	SNGNGDCSIFCSLPLGLYIVSSMVLLIGAKYISGLNK	:	563
CG7995[Dro	540	:	TREYTEENYR <mark>M</mark> LS <mark>SLP</mark> ASI FLISS FAMLVHSLAASGQ	:	576
30E57[Mosq	522	:	SEAMTDERYSLLA <mark>SIP</mark> AGLFLATTFLMLVHSQRR	:	555

Fig. 5.

Alignment of human glycerol kinase exon 18 to homologous sequences in other species. Gk2-prov (*Xenopus* GK2 homolog), 30E57 (*Anopheles* GK homolog).





Alignment of divergent glycerol kinase protein sequences. TM, transmembrane domain.

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Table 1

Expression patterns of Drosophila glycerol kinase-like genes

	Eye disc	Leg disc	Epidermis	Salivary gland	midgut	Head	Wing disc	Testis	Ovary	Brain 8.5 h embryo	Kc167	SL2 cells
CG7995		ŧ	+++++++++++++++++++++++++++++++++++++++	+	+	+	+	+		+	+	+
CG18374 (Gyk)	+	‡			+ (EcR dependent)	+		+			+	+
CG1216	+	+++++++++++++++++++++++++++++++++++++++		+				+	+	+	+	+
CG1271			+	+	+++ (up in metamo)	+		+	+		+	+
CG8298	++	‡				+		+				
Ref.	I	Ι	2	2, 3	2	7	2	4	4	5	4	4
¹ L. Michaut, S. Fliste 4029.	π, M. Neeb, K.	.P. White, U. (Certa, W.J. Gehr	ing, Analysis of the ϵ	sye developmental pathw	ay in <i>Dro</i> o	<i>ophila</i> using DN	A microan	ays. Proc. N	latl. Acad. Sci. US	A 100 (7) (2	003) 4024–
² T.R. Li, K.P. White,	Tissue-specifi	c gene expres	sion and ecdyso	ne-regulated genomic	c networks in <i>Drosophila</i>	. Dev Cell	5 (1) (2003) 59-	72.				
³ Unigene database.												

⁵T. Brody, C. Stivers, J. Nagle, W.F. Odenwald, Identification of novel *Drosophila* neural precursor genes using a differential embryonic head cDNA screen. Mech Dev. 113 (1) (2002) 41–59. ⁴GEO, gene expression omnibus.

Table 2

Developmental pattern of expression

	Embryo	Larval–early pupal	Schneider L2 cells	Adult ovary	Adult testes	Adult head
CG7995	+	+	+	I	+	+
CG18374 (Gyk)	+	+	+	I	+	+
CG1216	+	+	1	+	+	+
CG1271	Ι	I	I	I	+	+
CG8298	+	I	I	I	+	+
Ref.	1,2	2	2	I	1,2	1,2

² BDGP database: M. Stapleton, G. Liao, P. Brokstein, L. Hong, P. Carninci, T. Shiraki, Y. Hayashizaki, M. Champe, J. Pacleb, K. Wan, C. Yu, J. Carlson, R. George, S. Celniker, G.M. Rubin, The *Drosophila* gene collection: identification of putative full-length cDNAs for 70% of *D. melanogaster* genes, Genome Res. 12 (8) (2002) 1294–1300.