MINIREVIEW

5′-AMP-Activated Protein Kinase Signaling in *Caenorhabditis elegans*

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5′-AMP-activated protein kinase (AMPK) has been called "the metabolic master switch" because of its central role in regulating fuel homeostasis. AMPK, a heterotrimeric serine/threonine protein kinase composed of α, β, and γ subunits, is activated by upstream kinases and by 5′-AMP in response to various nutritional and stress signals. Downstream effects include regulation of metabolism, protein synthesis, cell growth, and mediation of the actions of a number of hormones, including leptin. However, AMPK research represents a young and growing field; hence, there are many unanswered questions regarding the control and action of AMPK. This review presents evidence for the existence of AMPK signaling pathways in *Caenorhabditis elegans*, a genetically tractable model organism that has yet to be fully exploited to elucidate AMPK signaling mechanisms. Exp Biol Med 233:12–20, 2008

Key words: AMPK; *Caenorhabditis elegans*; signaling; model systems; genetics

Introduction

The central role of 5′-AMP-activated protein kinase (AMPK) as a "metabolic master switch" has come to light in part as a result of discoveries that mutations cause a glycogen storage disorder and cardiomyopathy, and activation ameliorates metabolic syndrome conditions such as atherosclerosis, diabetes, and obesity (1–11). AMPK signaling is thus considered a prime target for new therapies. As proof of this principle, the widely used antidiabetic drugs metformin and rosiglitazone act, at least in part, by activating AMPK via separate mechanisms to improve insulin sensitivity (12, 13). Such discoveries have led to an exponential growth of research in this emerging field. Indeed, as of July 2007, there are 1565 publications indexed in PubMed, but ~25% and ~58% of those have appeared in the past 1 year and 3 years, respectively. Nevertheless, much is left to be discovered about AMPK signaling pathways. The objective of this review is to point out the tremendous unexploited value of *Caenorhabditis elegans* as a genetically tractable metazoan to uncover AMPK signaling mechanisms and thus discover new targets for therapies and diagnostics.

C. elegans as a Model Organism

Much has been written about *C. elegans*; thus, only a brief overview will be provided here (e.g., see 14–16). Biochemists in particular are referred to the review by Corsi (16). Three notable features have made *C. elegans* an important model organism: 1) they are cheap and easy to culture; 2) many biological processes are conserved across species such that discoveries in *C. elegans* are often applicable to humans; and 3) genetic approaches to biological questions can be applied readily. This will become clearer with the following brief explanations of each of these three points.

First, *C. elegans* is a free-living nematode that feeds on bacteria. They are very easy and inexpensive to grow in the laboratory. Moreover, their generation times are about 3–4 days and fecundity is high—a single hermaphrodite (the predominant sex) can produce approximately 200 self-progeny. To place that in perspective, a single adult hermaphrodite (~1 mm in length and ~0.05 mm in diameter) can generate well over 10,000 worms with a total

This work was supported by Grants from Texas Tech University Health Sciences Center School of Medicine and the Center for Cardiovascular Disease and Stroke.

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DOI: 10.3181/0705-MR-117
1535-3702/08/2331-0012$15.00

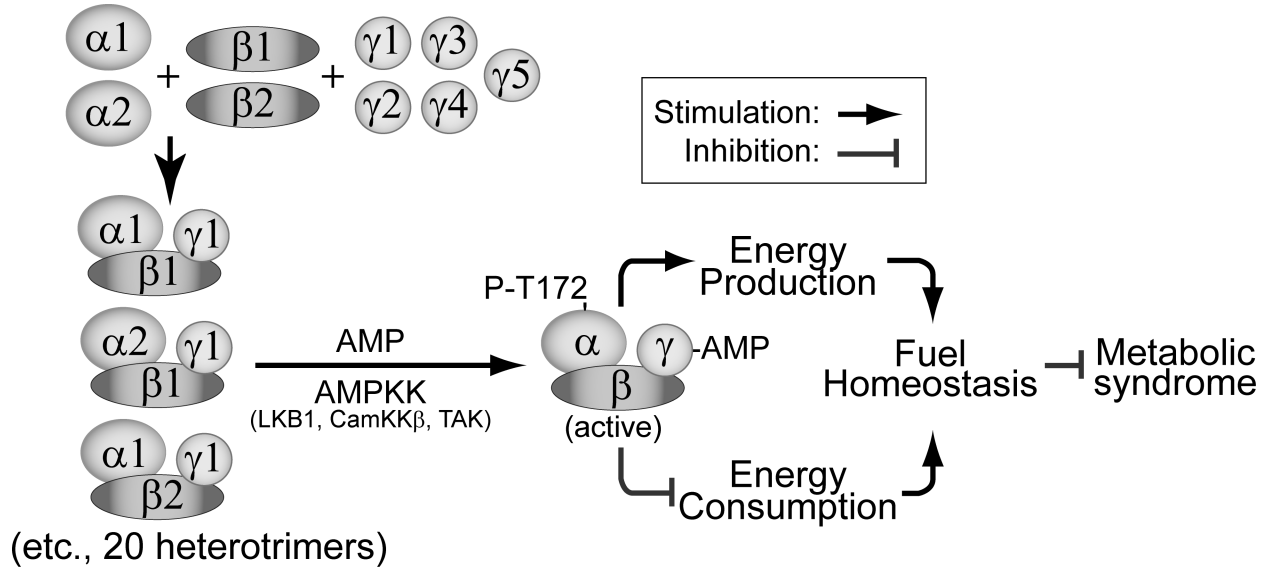

Figure 1. Fuel homeostasis through AMPK signaling. AMPK is a serine/threonine protein kinase composed of a catalytic subunit, α, and two regulatory subunits, β and γ (39). As illustrated here, each of the many possible heterotrimers is allosterically activated by AMP (40, 41) when phosphorylated at threonine 172 of the α subunit by an upstream kinase, AMPKK (42). Multiple genes encode each subunit—two α, two β, and three γ subunits (mammals) or five γ subunits (*C. elegans*) (see Figs. 3–6). Thus, "AMPK" is constituted by as many as twelve mammalian heterotrimers. *C. elegans* has the potential for twenty heterotrimers. Questions for future studies are whether these heterotrimers have different functions and whether AMPK signaling pathways can serve as targets for new antimetabolic syndrome treatments.

mass of 50 mg or more in about 10 days while feeding on *E. coli* in a single Petri dish.

Second, there are many examples of cross-species conservation of biological processes in *C. elegans*. Notable examples include apoptosis (17), RNA silencing (18), neural pathways (19), insulin-like signaling and aging (20), and fat metabolism (21). Conservation is a critical issue for any biologist considering the use of *C. elegans* (or any model organism for that matter) in their research. Indeed, the major focus of this paper centers on the conservation of AMPK from *C. elegans* to humans.

Third, *C. elegans* is highly amenable to genetic analysis for many reasons. As already mentioned, they have short reproductive cycles and produce large numbers of offspring. The *C. elegans* genome is small—8×10^6 bp of DNA in six nuclear chromosomes. The genome is completely cloned and sequenced (it was the first metazoan genome sequenced: 22, 23). It contains in excess of 19,000 genes, which is similar to mammalian genomes. A plethora of valuable resources further enhance *C. elegans* as a model organism. Specific examples include bioinformatics via www.wormbase.org; thousands of inexpensive mutant strains from the NIH-funded Caenorhabditis Genetics Center (www.cbs.umn.edu/CGC) at the University of Minnesota; a Gene Knockout Consortium (http://celeganskoconsortium.omrf.org) that seeks to generate a complete library of knockout strains at no charge to the research community; robust RNAi suppression of gene transcription by an RNAi feeding library (21, 24, 25); and online worm anatomy resources at www.wormatlas.org. Further, *C. elegans* is the only metazoan whose entire developmental fate map is known (26), making them particularly valuable for developmental studies.

Overview of AMPK

Since several recent reviews (1, 8, 27–29) provide an excellent overview of the current state of knowledge about AMPK signaling pathways, only a brief description will be provided here.

The enzyme is an α/β/γ heterotrimer (Fig. 1). In mammals, these subunits are encoded by two α, two β, and three γ genes. As will be discussed below, *C. elegans* differs in that they have five γ subunit genes. Why so many different genes are needed for each subunit is only now being investigated. Preliminary possibilities are that the twelve possible mammalian and twenty possible *C. elegans* heterotrimers generate functional diversity.

As indicated in Figure 1, AMPK functions to keep fuel levels in check. This may be important to the effects of exercise and drugs such as metformin on insulin sensitivity in mammalian species. Indeed, future therapies that target AMPK might be useful in treating a number of disorders associated with metabolic syndrome as suggested in Figure 1. In general, AMPK promotes ATP production and inhibits ATP consumption. It does this by rapid mechanisms involving phosphorylation of metabolic enzymes and by slow mechanisms involving gene regulation. Known AMPK substrates are shown in Figure 2. While the concept of AMPK as a metabolic master switch was initially thought to be restricted to intracellular fuel homeostasis, it is now recognized that whole body, or intercellular, mechanisms

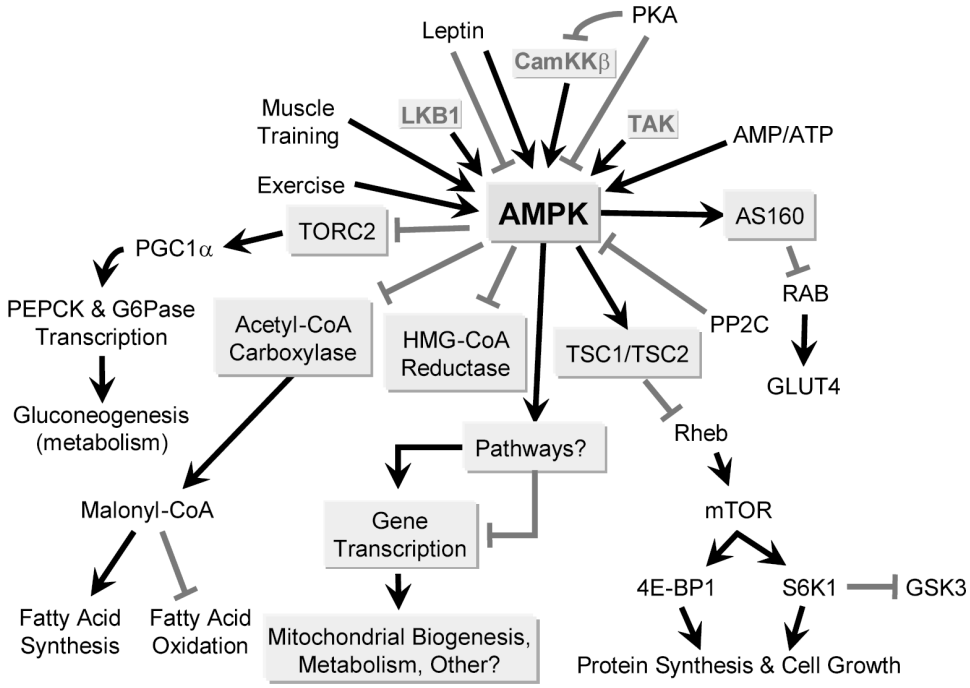

Figure 2. AMPK signaling is pleiotropic. This is a selected overview of the numerous and complex signaling pathways discussed at the 4th FASEB Summer Research Conference on AMPK held in August 2006. Numerous factors affect AMPK, most by unknown mechanisms. Many inputs require one of three known AMPK kinases—LKB1, a tumor suppressor (43); CaMKKβ (44–46); and TGFβ-activating kinase/TAK1 (47). The best-understood role of AMPK is its effect on lipid metabolism via acetyl-CoA carboxylase inhibition (1–3, 8). The details of the other pathways in this figure are less certain. Some AMPK pathways impinge on transcription such as: 1) PEPCK inhibition via TORC2/PGC1α; and 2) actions of AMPK on undefined pathways to control transcription of genes, many of which appear to be involved in various metabolic pathways. Other notable actions of AMPK include regulation of the TSC/TOR pathway, glucose transport, and insulin signaling (not shown since the mechanisms are unclear). There is currently little information available about which of these pathways are conserved in *C. elegans*. A bioinformatic analysis suggests that worms do not have genes encoding orthologs of acetyl-CoA carboxylase, HMG-CoA reductase, and TSC2. However, there is strong evidence supporting the existence of other AMPK signaling pathways in *C. elegans*. For example, the *C. elegans* orthologs of the AMPK-activating kinases LKB1, CamKKβ, and TAK1 are PAR-4, CKK-1, and MOM-4, respectively (35, 48–52). LKB-1/PAR-4 has been experimentally linked to AMPK in *C. elegans* (35).

are involved as well because AMPK is involved in mediating the actions of hormones such as leptin (1, 8).

AMPK's protein kinase activity resides within the α subunit, which must by phosphorylated on threonine residue 172 (or its equivalent) to be active. Activity also depends upon formation of the heterotrimeric complex and binding of either AMP or ATP to the γ subunit. A high AMP:ATP ratio stimulates, whereas a low ratio inhibits activity. Thus AMPK receives upstream input by phosphorylation/dephosphorylation, and it directly senses intracellular fuel status by binding either AMP or ATP, which are in equilibrium via adenylate kinase, which interconverts two ADPs with ATP + AMP. AMP and ATP bind to two regions known as Bateman Domains, each of which is constituted by two cystathionine β-synthase (CBS) motifs in the γ subunit (Fig. 6). Recent crystal structures of SNF4, the γ subunit orthologs from yeast, have shed new light on the underlying mechanisms (30, 31).

AMPK was initially discovered in 1973 as an inhibitor of acetyl-CoA carboxylase and HMG-CoA reductase, key enzymes of fatty acid and cholesterol synthesis, respectively (32, 33). These and numerous other actions are shown in Figure 2. The points illustrated by Figure 2 are that AMPK signaling is varied, complex, and important in a variety of pathways. Moreover, there remain many key unanswered questions about AMPK signaling. Indeed, the details of many of the signaling pathways shown in Figure 2 are poorly understood. Hopefully, research in *C. elegans* can contribute to a better understanding.

AMPK Is Conserved Between *C. elegans* and Humans

As indicated above, the first question to ask when considering *C. elegans* as a model system is whether the proteins of interest are conserved. Several observations support this idea, which was first suggested by Gao *et al.* (34). Figures 3–6 and Table 1 illustrate the author's *in silico* analysis, indicating that worms have two α, two β, and five γ gene orthologs (generating the twenty possible heterotrimer combinations indicated in Fig. 1).

The extent of this conservation is much better appreciated in the multiple sequence alignments shown in Figures 4–6, which compare the α, β, and γ subunits, respectively, from humans, mice, and *C. elegans*. Strikingly, 30% of the residues in the α subunit sequence alignment are identical (Fig. 4). If one includes conservative substitutions,

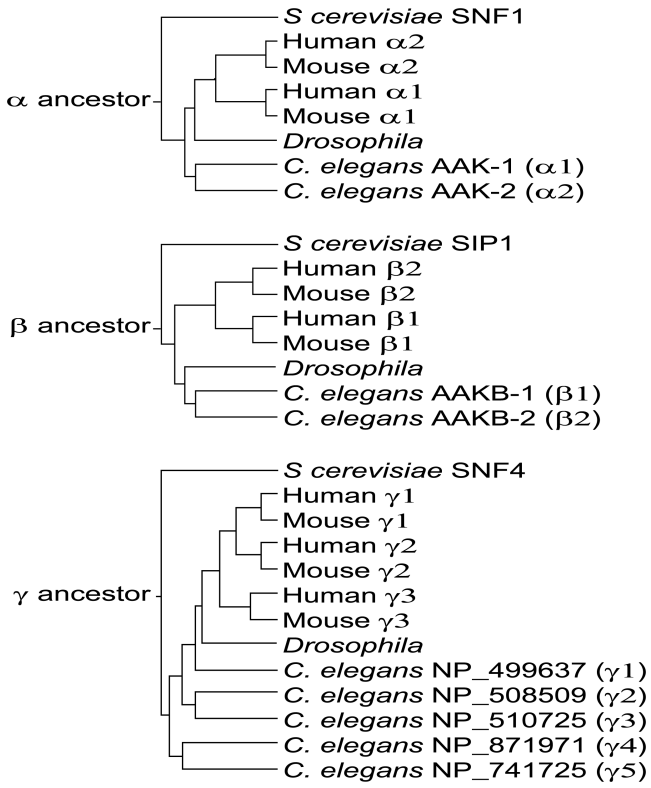

Figure 3. All three AMPK subunits are conserved in *C. elegans.* These phylogenetic trees are redrawn from data found at www.treefam.org. The α and β subunits have all been named in Wormbase. However, the γ subunits have not been named and are listed by their RefSeq IDs.

then the aligned regions are 78% similar from *C. elegans* to humans. The β subunit alignment is also striking in that 30% of the residues are identical and 84% are similar (Fig. 5).

While the γ subunits are also conserved, there is more divergence among these proteins than among the α and β subunits as illustrated in Figure 6 and Table 1. Among the human γ subunits, the similarities range from 56% to 80%, whereas the similarities range from 25% to 47% among the *C. elegans* γ subunits. It is particularly noteworthy that the region of maximum homology (76% similarity across all 11 sequences in the alignment) spans the two AMP/ATP–binding Bateman domains. For reasons yet to be discovered, the *C. elegans* γ subunits are most similar to the human γ1 subunit—ranging from 37% to 69% similar.

Since the bioinformatic analyses suggest that *C. elegans* have genes that encode functional AMPK, the next question to ask is whether there is any direct experimental evidence that *C. elegans* express AMPK activity. Such evidence has been published by two groups who have examined AMPK in *C. elegans* (35–37).

First, AMPK affects life span in *C. elegans*, a popular model of aging (35, 36). As in mammals, restricted caloric intake appears to be correlated with increased longevity and vice versa. These investigators found that knockout of the AMPKα2 catalytic subunit gene causes a 12% reduction in life span, whereas overexpression lengthens life span by 13% compared with wild-type worms. Additional genetic experiments indicated that AMPKα2 acts downstream of the insulin-like receptor, DAF-2, but in parallel with the canonical insulin target, FOXO (DAF-16), to regulate life span. This implicates AMPK in both nutritional control and in longevity regulation. The underlying mechanisms are yet to be deciphered. Likewise, it is yet to be determined if AMPK affects life span in humans. However, when considered in the light that insulin-like signaling affects life span in worms, these observations suggest the possibility that *C. elegans* could contribute to a better understanding of how AMPK activation improves insulin sensitivity.

Second, Narbonne and Roy investigated the mechanisms associated with suppression of germ line cell division during a special developmental state known as dauer (37). Briefly, when *C. elegans* are starved and crowded they execute an alternative developmental program to generate enduring "dauer" larvae that can live many times longer than well-fed worms (16). Dauer formation is regulated by insulin-like (DAF-2), TGFβ-like (DAF-7), and cGMP signaling pathways. The genes encoding dauer-controlling proteins are referred to as "DAF" genes (for DAuer Formation). Dauer larvae do not feed, are resistant to a harsh environment, and do not produce mature gametes.

Table 1. Similarities Among Human and *C. elegans* AMPK γ Subunits[a]

	C. elegans γ2 (%)	*C. elegans* γ3 (%)	*C. elegans* γ4 (%)	*C. elegans* γ5 (%)	Human γ1 (%)	Human γ2 (%)	Human γ3 (%)
C. elegans γ1	42/27	45/28	30/17	30/17	69/54	49/36	47/33
C. elegans γ2	—	47/28	26/13	25/9.7	48/31	24/11	26/14
C. elegans γ3	—	—	28/11	27/13	47/29	34/19	38/22
C. elegans γ4	—	—	—	32/16	37/17	31/14	32/16
C. elegans γ5	—	—	—	—	37/20	23/11	32/18
Human γ1	—	—	—	—	—	80/70	71/58
Human γ2	—	—	—	—	—	—	56/43

[a] To determine the extent of similarity seen in the multiple sequence alignment shown in Figure 6, each γ subunit was aligned pairwise using AlignX/VectorNTI software (Invitrogen) with the PAM120 scoring matrix within species and PAM200 across species. For each comparison, the percent similar and percent identical residues for the aligned regions are given. Note that the Bateman Domain regions of the gamma subunits have much higher levels of similarity than the overall similarities indicated here (see Fig. 6 and the associated text).

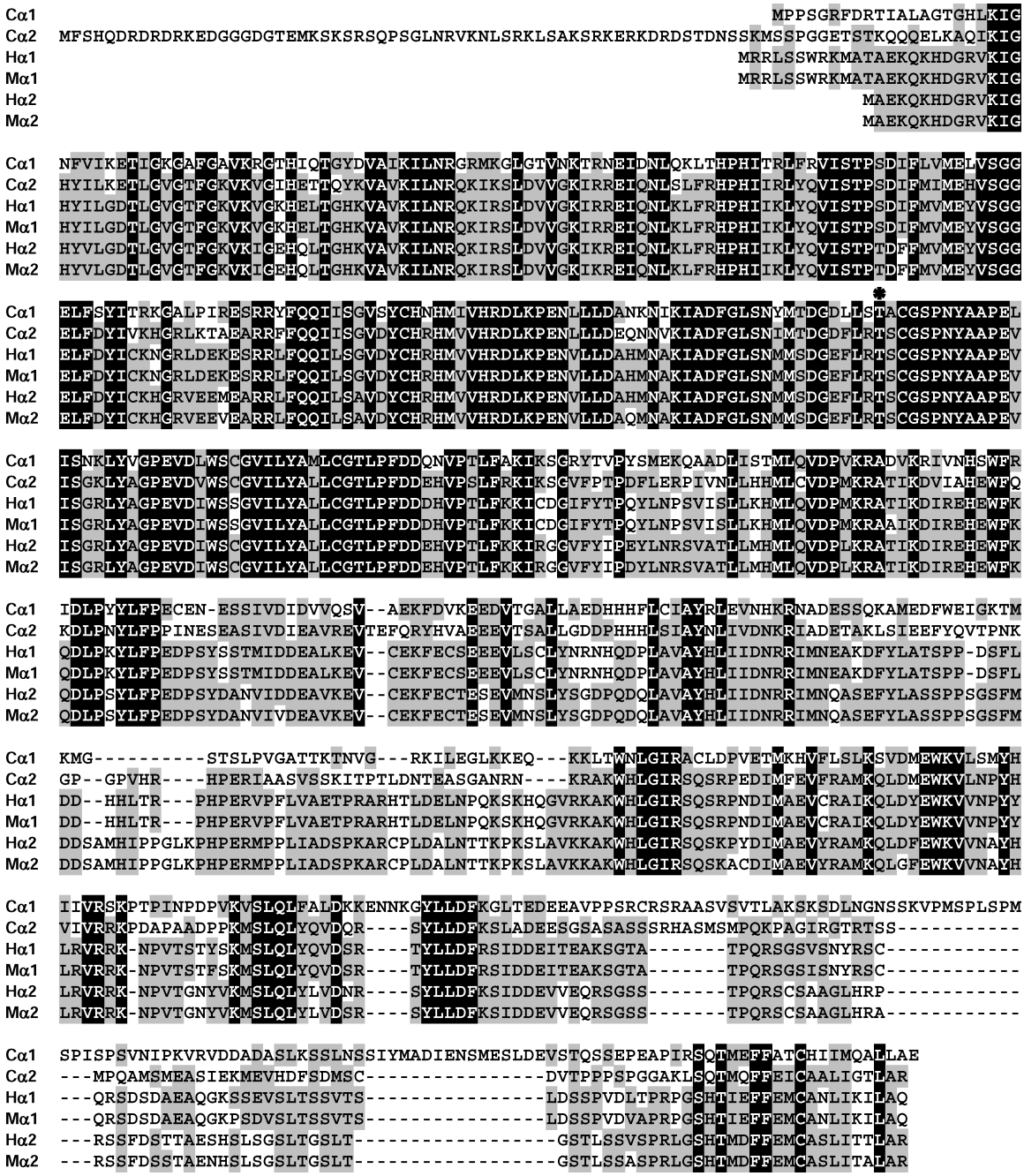

Figure 4. AMPKα subunits are conserved. AMPKα subunits from *C. elegans* (AAK-1 and AAK-2 designated as Cα1 and Cα2, respectively), human (AMPKα1 and AMPKα2 designated as Hα1 and Hα2), and mouse (AMPKα1 and AMPKα2 designated as Mα1 and Mα2) were aligned using a PAM250 scoring matrix with Vector NTI/AlignX (Invitrogen). Identical residues are shown in white type on a black background, while conserved/similar residues are shown in black type on a gray background. The conserved threonine residue that is phosphorylated by an AMPK-activating kinase is marked by an asterisk (equivalent to T172 in human and mouse AMPKα2).

Narbonne and Roy mutagenized worms and screened for those that produced excess numbers of germ cell precursors in dauer larvae. They found a single mutant, identified the mutation as the AMPKα2 subunit gene, and showed that DAF-2 and DAF-7 converge to regulate germ cell proliferation via LKB1 (an AMPK kinase, Figs. 1 & 2), AMPKα2, and PTEN (a.k.a., DAF-18). This implicates AMPK in the control of cell proliferation in worms. Moreover, the data suggest cross-talk with insulin-like and TGFβ-like signaling pathways. Similar control has been documented in mammalian systems via the TSC/mTOR pathway (38) as shown in Figure 1. However, since worms do not have TSC orthologs (based upon a bioinformatic search for TSC2 orthologs) alternative mechanisms must be involved in AMPK control of proliferation. We postulate that such mechanisms will involve transcriptional control.

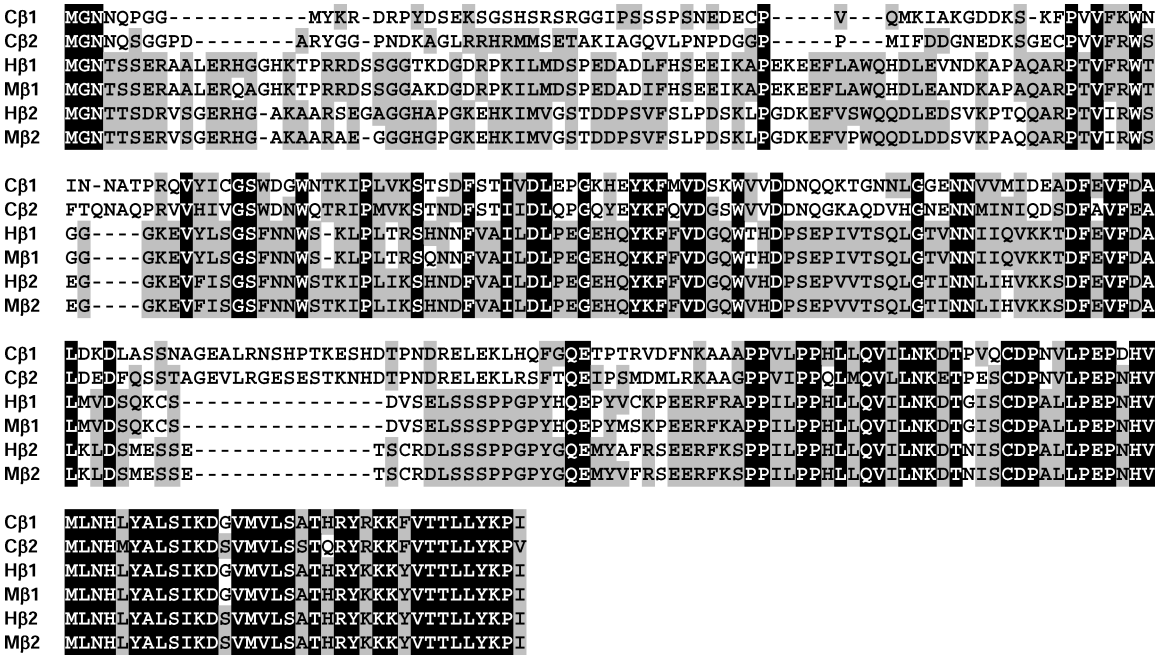

Figure 5. AMPKβ subunits are conserved. AMPKβ subunits from *C. elegans* (AMPKβ1 and AMPKβ2 designated as Cβ1 and Cβ2, respectively), human (AMPKβ1 and AMPKβ2 designated as Hβ1 and Hβ2), and mouse (AMPKβ1 and AMPKβ2 designated as Mβ1 and Mβ2) were aligned and displayed as described in Figure 4.

Summary and Speculations

As alluded to above and in Figure 2, AMPK signaling pathways are numerous, and many of the details are poorly understood. Genetic approaches provide a powerful adjunct to biochemistry, cell biology, and molecular biology in solving biological problems. As genetically tractable organisms, yeast, and, to a much lesser extent, fruit flies have been the only model systems used to investigate AMPK. Collectively, the studies described above (35–37) along with the bioinformatic analyses presented here validate worms as a model organism to study many aspects of AMPK signaling via a genetic approach. Table 2 presents a listing of the AMPK subunits in *C. elegans* as well as in other important model organisms including flies, yeast, mice, and humans.

Currently, two labs are using *C. elegans* to investigate the role of AMPK in biological processes. One lab is studying how AMPK pathways slow aging (35, 36). That pathway appears to cross-talk with insulin-like signaling, though the specific mechanisms are as yet unknown. The other lab is investigating how AMPK signaling inhibits germ cell proliferation (37). This pathway also appears to be related to insulin-like signaling by unknown mechanisms. Both groups have discovered the insulin-like pathway connection as a direct result of the power of genetic analysis and the availability of a large library of mutant *C. elegans* strains. Further progress will most certainly draw on other genetic tricks along with "mining" of resources available through www.wormbase.org.

Of special note is that there is clear interaction of AMPK with insulin-like signaling in *C. elegans* (35–37). It is thus tempting to speculate that one important unanswered

Table 2. AMPK Subunit Orthologs in Five Species[a]

Subunit	*C. elegans*	*D. melanogaster*	*S. cerevisiae*	Human	Mouse
α	AAK-1 (PAR2.3)	SNF1A	SNF1	PRKAA1 (5562)	Prkaa1 (105787)
	AAK-2 (T01C8.1)			PRKAA2 (5563)	Prkaa2 (108079)
β	AAKB-1 (F55F3.1)	CG8057-PA	SIP1, SIP2, GAL83	PRKAB1 (5564)	Prkab1 (19079)
	AAKB-2 (Y47D3A.15)			PRKAB2 (5565)	Prkab2 (108097)
γ	Y111B2A.8; T20F7.6; Y41G9A.3; T01B6.3; R53.7	SNF4	SNF4	PRKAG1 (5571)	Prkag1 (19082)
				PRKAG2 (51422)	Prkag2 (108099)
				PRKAG3 (53632)	Prkag3 (241113)

[a] The official names are listed except for the *C. elegans* AMPK γ subunits and the *Drosophila* β subunit, which have not as yet been officially named. The designations in parentheses for *C. elegans* indicate the GenePair names in Wormbase, while those for the human and mouse genes indicate Entrez Gene IDs (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene). The designation for the *Drosophila* AMPK β subunit is the Flybase peptide ID (http://flybase.bio.indiana.edu). The five *C. elegans* γ subunits are designated γ1 – γ5 in Figures 3 and 6.

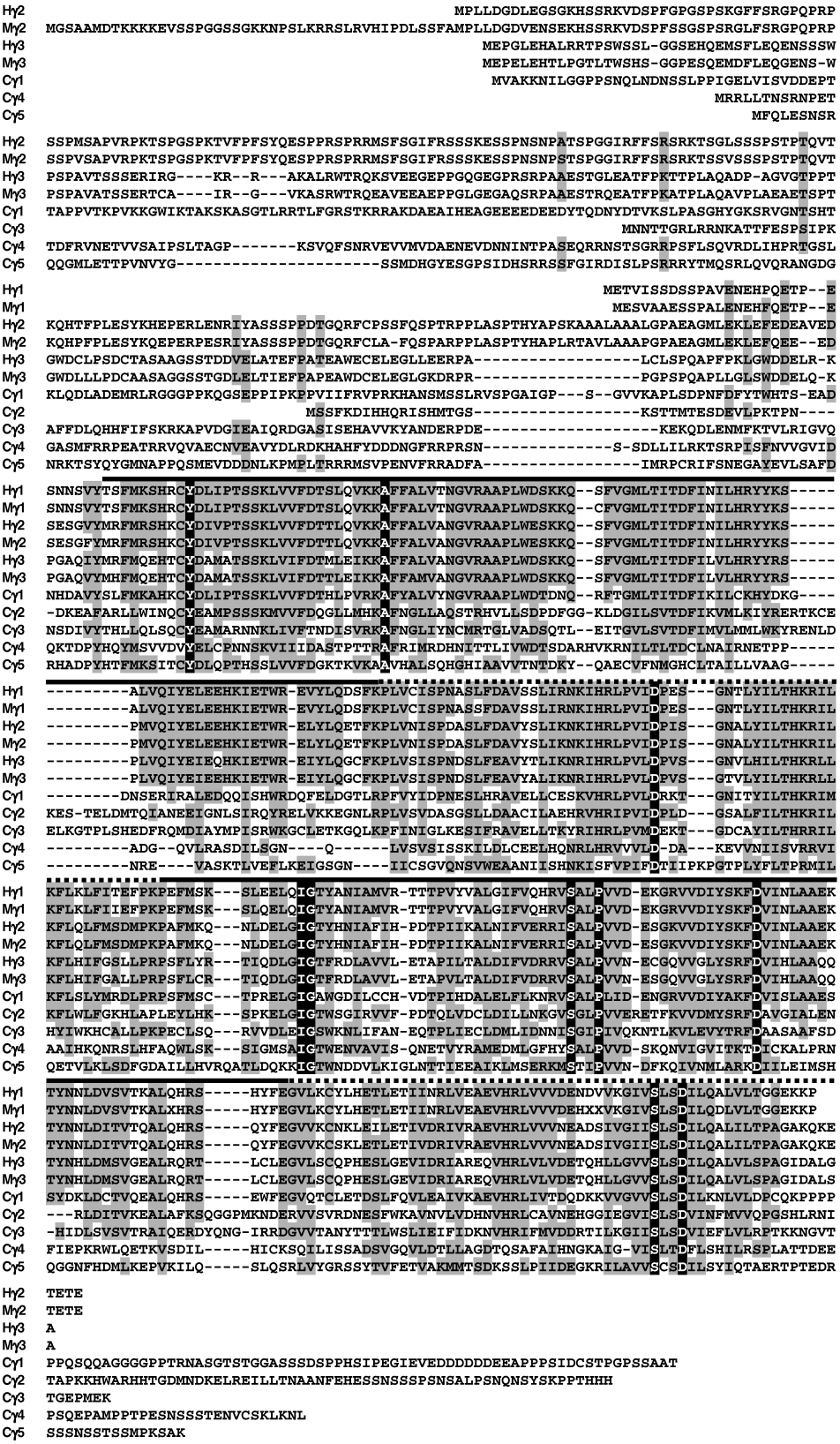

Figure 6. AMPKγ subunits are conserved. AMPKγ subunits from *C. elegans* (designated as Cγ1 through Cγ5), human (AMPKγ1, AMPKγ2, and AMPKγ3 designated as Hγ1, Hγ2, and Hγ3), and mouse (AMPKγ1, AMPKγ2, and AMPKγ3 designated as Mγ1, Mγ2, and Mγ3) were aligned using a PAM200 scoring matrix with Vector NTI/AlignX (Invitrogen). Identical and similar/conserved residues are illustrated as described in Figure 4. The four cystathionine β-synthase (CBS) motifs that make up the two AMP/ATP-binding Bateman domains are overlined. CBS1 and CBS3 are marked with a solid line, whereas CBS2 and CBS4 are designated with a dashed line.

question that could be approached in worms is the mechanism by which AMPK activation (e.g., by the antidiabetic drugs metformin and rosiglitazone) improves insulin sensitivity. If so, perhaps new and better antidiabetic therapies could be developed for use in humans.

Another important question centers on the mechanisms that underlie regulation of gene expression. Transcriptional regulation by AMPK is important in many mammalian tissues such as mitochondrial biogenesis in response to exercise in skeletal muscle and gluconeogenic enzyme repression in liver in response to fuel deprivation (1). Because worm strains bearing AMPK catalytic subunit knockout genes are freely available (see www.wormbase.org), it should be possible to determine whether these knockouts affect gene expression in *C. elegans*. Perhaps a simple approach would be with microarray analysis in wild type and knockout worms subjected to stresses such as oxidative stress or food deprivation. Such an approach would test the hypothesis that worm AMPK regulates mitochondrial biogenesis through the production of mitochondrial proteins.

In summary, conservation of AMPK subunits coupled with the ease of genetic analysis in *C. elegans* provides a relatively unexploited model system in which many of the central questions of AMPK action can be addressed. One of the immediate issues that must be addressed is the generation of immunological reagents for *C. elegans* AMPK subunits. There are currently numerous commercial suppliers of antibodies for the AMPK subunits of other species, but none are likely to cross-react with their *C. elegans* orthologs. It is hoped that this review will point out the need for new antibodies and that it will foster new research in this model organism. Only imagination limits what new discoveries can be made using *C. elegans*.

The author thanks Drs. Daniel Castracane and Clinton MacDonald for their helpful comments.

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