

MINIREVIEW

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

ELMUS G. BEALE¹

Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

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.

¹ To whom correspondence should be addressed at Cell Biology & Biochemistry, Mail Stop 6540, 3601 4th Street, TTUHSC, Lubbock, Texas 79430. E-mail: elmus.beale@ttuhsc.edu

DOI: 10.3181/0705-MR-117
1535-3702/08/2331-0012\$15.00
Copyright © 2008 by the Society for Experimental Biology and Medicine

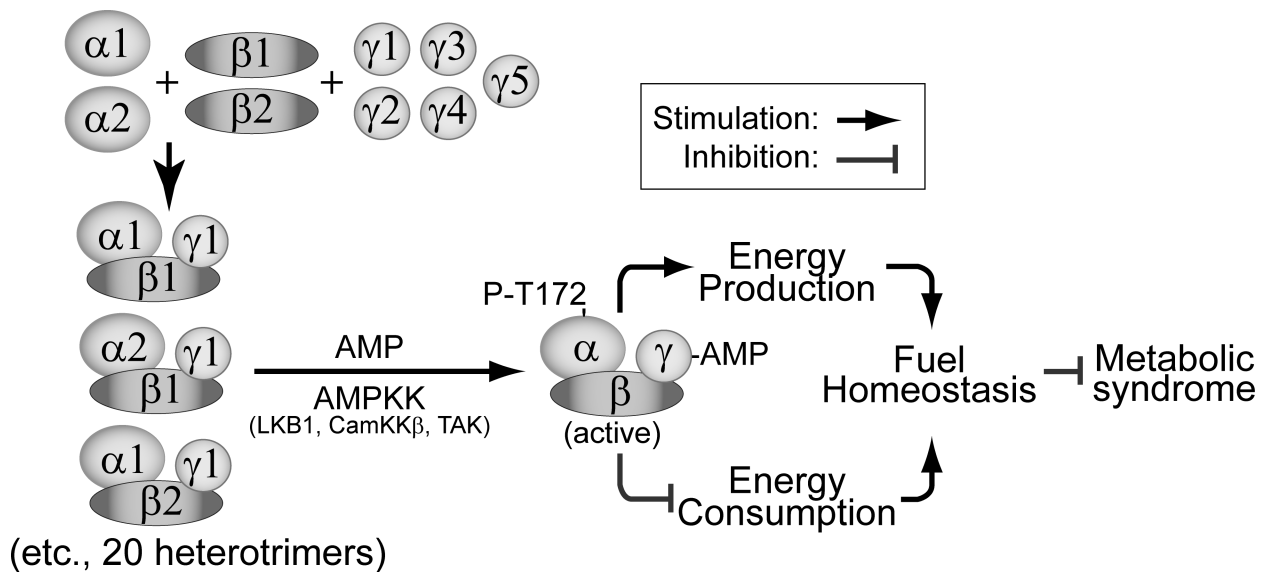


Figure 1. Fuel homeostasis through AMPK signaling. AMPK is a serine/threonine protein kinase composed of a catalytic subunit, α , and two regulatory subunits, β and γ (39). As illustrated here, each of the many possible heterotrimers is allosterically activated by AMP (40, 41) when phosphorylated at 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 $\alpha/\beta/\gamma$ 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

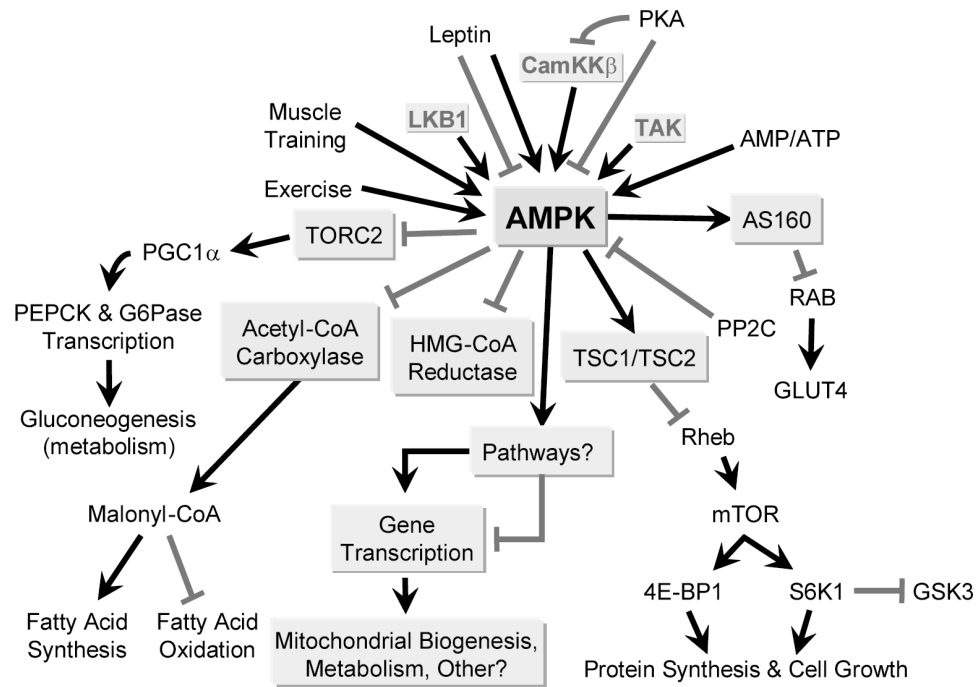


Figure 2. AMPK signaling is pleiotropic. This is a selected overview of the numerous and complex signaling pathways discussed at the 4th FASEB Summer Research Conference on AMPK held in August 2006. Numerous factors affect AMPK, most by unknown mechanisms. Many inputs require one of three known AMPK kinases—LKB1, a tumor suppressor (43); CamKK β (44–46); and TGF β -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/PGC1 α ; 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, CamKK β , 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 be 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,

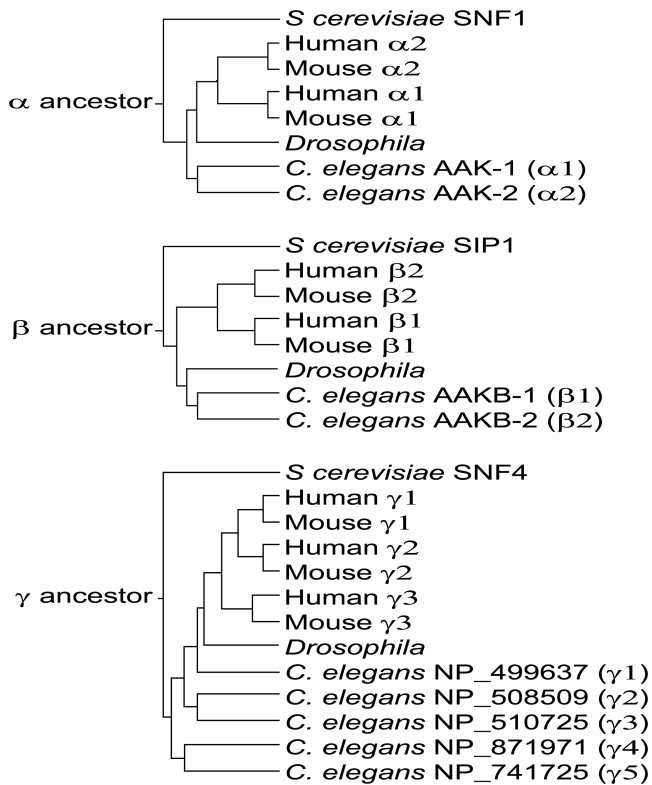


Figure 3. All three AMPK subunits are conserved in *C. elegans*. These phylogenetic trees are redrawn from data found at www.treefam.org. The α and β subunits have all been named in Wormbase. However, the γ subunits have not been named and are listed by their RefSeq IDs.

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 $\gamma 1$ 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 AMPK $\alpha 2$ 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 AMPK $\alpha 2$ 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), TGF β -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

	<i>C. elegans</i> $\gamma 2$ (%)	<i>C. elegans</i> $\gamma 3$ (%)	<i>C. elegans</i> $\gamma 4$ (%)	<i>C. elegans</i> $\gamma 5$ (%)	Human $\gamma 1$ (%)	Human $\gamma 2$ (%)	Human $\gamma 3$ (%)
<i>C. elegans</i> $\gamma 1$	42/27	45/28	30/17	30/17	69/54	49/36	47/33
<i>C. elegans</i> $\gamma 2$	—	47/28	26/13	25/9.7	48/31	24/11	26/14
<i>C. elegans</i> $\gamma 3$	—	—	28/11	27/13	47/29	34/19	38/22
<i>C. elegans</i> $\gamma 4$	—	—	—	32/16	37/17	31/14	32/16
<i>C. elegans</i> $\gamma 5$	—	—	—	—	37/20	23/11	32/18
Human $\gamma 1$	—	—	—	—	—	80/70	71/58
Human $\gamma 2$	—	—	—	—	—	—	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).

Co1 MPPSGRFDRTIALAGTGHKIG
 Co2 MFSHQDRDRDRKEDGGDGTEMSKSRSPQPSGLNRVKNLSRKLAKSRKERKDRDSTDNSSKMSSPGGETSTKQQQELKAQIKIG
 Ha1 MRLSSWRKMATAEKQKHDGRVKIG
 Ma1 MRLSSWRKMATAEKQKHDGRVKIG
 Ha2 MAEKQKHDGRVKIG
 Ma2 MAEKQKHDGRVKIG

Co1 NFVIKETICKGAFCAVAKRGTHIQGTGYDAIKILNRGRMKGLGTVMKTRNEIDNLQKLTHPHITRLFRVISTPSDIFLVMELVSGG
 Co2 HYILKETLGVGTFGKVKVGIIHETIQYKAVKILNRQIKISLDVVGKIRRETONLSLFRHPHIIRLQVISTPSDIFMIMEHVSGG
 Ha1 HYILGDTLGVGTFGKVKVGKHELTGHKVAVKILNRQIKISLDVVGKIRRETONLKLFRHPHIKLYQVISTPSDIFMMEYVSGG
 Ma1 HYILGDTLGVGTFGKVKVGKHELTGHKVAVKILNRQIKISLDVVGKIRRETONLKLFRHPHIKLYQVISTPSDIFMMEYVSGG
 Ha2 HYVLGDTLGVGTFGKVKIGEHQLTGHKVAVKILNRQIKISLDVVGKIKRETONLKLFRHPHIKLYQVISTPTDFMMEYVSGG
 Ma2 HYVLGDTLGVGTFGKVKIGEHQLTGHKVAVKILNRQIKISLDVVGKIKRETONLKLFRHPHIKLYQVISTPTDFMMEYVSGG

Co1 ELFSYITRKGALPIRESRRYFQOILISGVSYCHNHMIVHRDLKPENLLLDANKNIKIADFGLSNYMTDGDLLSTACGSPNYAAPEL
 Co2 ELFDYIVKHGRLKTAEARRFQOILISGVSYCHRHMVVHRDLKPENLLLEQNNVKIADFGLSNIMTDGDFLRTSCGSPNYAAPEV
 Ha1 ELFDYICKNGLRDEKESRRLFQOILSGVDYCHRHMVVHRDLKPENVLDDAHMNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEV
 Ma1 ELFDYICKNGLRDEKESRRLFQOILSGVDYCHRHMVVHRDLKPENVLDDAHMNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEV
 Ha2 ELFDYICKHGRVEEMEARRLFQOILSAVDYCHRHMVVHRDLKPENVLDDAHMNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEV
 Ma2 ELFDYICKHGRVEEVEARRLFQOILSAVDYCHRHMVVHRDLKPENVLDDAQMNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEV

Co1 ISNKLTVGPEVDLWSCGVILYALLCGTLPFDDQNVPTLFAKIKSCRYTVPYSMEKQAADLISTMLOVDPVKRADVKRIVNHSWFR
 Co2 ISGKLYAGPEVDVWSCGVILYALLCGTLPFDDHEVPSLFRKIKSGVFPPTDFLERPIVNLHMHMLQVDPMKRATIKDVIAHEWFQ
 Ha1 ISGRLYAGPEVDIWSCGVILYALLCGTLPFDDHVPTLFFKIKCDGIFYTPQYLNPSVISLLKHMLOVDPMKRATIKDIREHEWFK
 Ma1 ISGRLYAGPEVDIWSCGVILYALLCGTLPFDDHVPTLFFKIKCDGIFYTPQYLNPSVISLLKHMLOVDPMKRAAIKIDIREHEWFK
 Ha2 ISGRLYAGPEVDIWSCGVILYALLCGTLPFDDHEVPTLFFKIRGCVFYIPYLNRSVATLLMHMLQVDPKRAATIKDIREHEWFK
 Ma2 ISGRLYAGPEVDIWSCGVILYALLCGTLPFDDHEVPTLFFKIRGCVFYIPDYLNRSVATLLMHMLQVDPKRAATIKDIREHEWFK

Co1 IDLPYVLFPEECEN-ESSIVDIDVQSV--AEKFDVKEEDVTGALLAEDHHHFLCTAYRLEVNHKRNADESSQKAMEDFWEIGKTM
 Co2 KDLPNLYLFPPISEASIVDIEAVREVTEFQRVHVAEEVTSALLGDDPHHLSIAYNLIVDNKRITADETAKLSIEEFYQVTPNKK
 Ha1 QDLPKYLFPEDPSSYSTMIDDEALKEV--CEKFCSEEEVLSCLYNNRHQDPLAVAYHLIDNRRIMNEAKDFYLATSPP-DSFL
 Ma1 QDLPKYLFPEDPSSYSTMIDDEALKEV--CEKFCSEEEVLSCLYNNRHQDPLAVAYHLIDNRRIMNEAKDFYLATSPP-DSFL
 Ha2 QDLPSYLFPEDPSSYDANVIDEAVKEV--CEKFECTESEVMNSLYSGDPQDQLAVAYHLIDNRRIMNQASEFYLASSPPSGSFM
 Ma2 QDLPSYLFPEDPSSYDANVIDEAVKEV--CEKFECTESEVMNSLYSGDPQDQLAVAYHLIDNRRIMNQASEFYLASSPPSGSFM

Co1 KMG-----STSLPVGATTKINVG---RKILEGLKKEQ---KKLTWNLGIRACLDPVETMKHVFSLKSVDMEWKVLSMYH
 Co2 GP--GPVHR---HPERIAVASVKSTIPTLDNTEASGANRN---KRAKWHLGIRSQSRPDIIMEFVFRAMKQLDMEWKVLNPNYH
 Ha1 DD--HHLTR---PHPERVPFLVAETPRARHTLDELNPQSKHQGVRAKAKWHLGIRSQSRPNDIMAEVCRAIKOLDYEWKVVNPYY
 Ma1 DD--HHLTR---PHPERVPFLVAETPRARHTLDELNPQSKHQGVRAKAKWHLGIRSQSRPNDIMAEVCRAIKOLDYEWKVVNPYY
 Ha2 DDSAMHIPGGLKPHPERMPPLIADSPKARCPLDALNTTPKPSLAVKKAHWHLGIRSQSKPYDIMAEEVYRAMKOLDFEWKVVNAYH
 Ma2 DDSAMHIPGGLKPHPERMPPLIADSPKARCPLDALNTTPKPSLAVKKAHWHLGIRSQSKACDIMAEEVYRAMKOLDFEWKVVNAYH

Co1 IIVRSKPTPINPDVPKVSQQLFALDKKENNKGYYLDFKGLTEDEEAVPPSRCRSRAASVSVTLAKSKSDLNGNSSKVPMSPLSPM
 Co2 VIVRRKPDAPADPPKMSLQLYQVDQR---SYLLDFKSLADEESGSASASSSRHASMSMPQKPAIGRGTSTSS-----
 Ha1 LRVRRK-NPVTSTYSKMSLQLYQVDSR---TYLLDFRSIDDEITEAKSGTA-----TPQRSISVSNYRSC-----
 Ma1 LRVRRK-NPVTSTFSKMSLQLYQVDSR---TYLLDFRSIDDEITEAKSGTA-----TPQRSISVSNYRSC-----
 Ha2 LRVRRK-NPVTGNYVKMSLQLYLVDSR---SYLLDFKSIDDEVVEQSRGSS-----TPQRSCSAAGLHRP-----
 Ma2 LRVRRK-NPVTGNYVKMSLQLYLVDSR---SYLLDFKSIDDEVVEQSRGSS-----TPQRSCSAAGLHRA-----

Co1 SPISPSVNPVKVRVDDADASLKSSLNSSIYMADIENSMSLDESVTQSSEPEAPIRSOTMEFFATCHIIMQALLAE
 Co2 ---MPQAMSMEASIEKMEVHDFSDMSC-----DVTPPPSPGGAKLSQTMQOFFEICAALIGTLAR
 Ha1 ---QRSDSDAEAQGKSSEVSLTSSVTS-----LDSSPVDLTPRPGSHTIEFFEMCANLIKILAQ
 Ma1 ---QRSDSDAEAQGKPSDVSLSSTSSVTS-----LDSSPVDVAPRPGSHTIEFFEMCANLIKILAQ
 Ha2 ---RSSFDSTTAESHLSGSLTGSLT-----GSTLSSVSPRLGSHTMDFFEMCASLITLAR
 Ma2 ---RSSFDSTTAESHLSGSLTGSLT-----GSTLSSASPRLSGHTMDFFEMCASLITLAR

Figure 4. AMPK α subunits are conserved. AMPK α subunits from *C. elegans* (AAK-1 and AAK-2 designated as C α 1 and C α 2, respectively), human (AMPK α 1 and AMPK α 2 designated as H α 1 and H α 2), and mouse (AMPK α 1 and AMPK α 2 designated as M α 1 and M α 2) 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 AMPK α 2).

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 AMPK α 2 subunit gene, and showed that DAF-2 and DAF-7 converge to regulate germ cell proliferation via LKB1 (an AMPK kinase, Figs. 1 & 2), AMPK α 2, 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 TGF β -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.

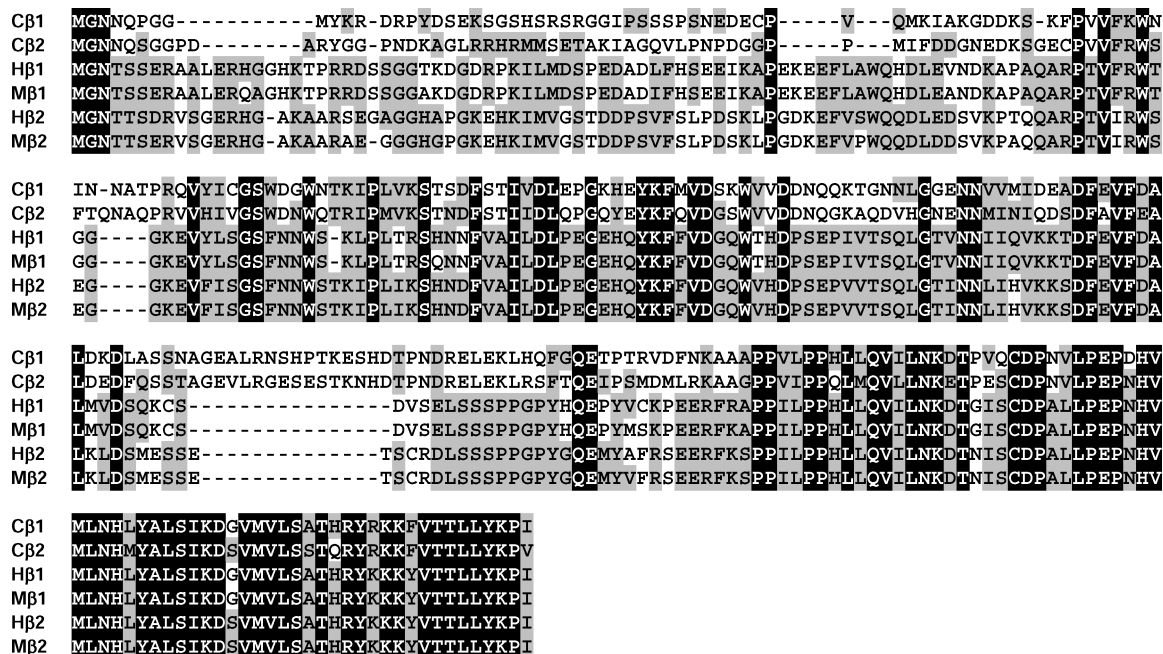


Figure 5. AMPK β subunits are conserved. AMPK β subunits from *C. elegans* (AMPK β 1 and AMPK β 2 designated as C β 1 and C β 2, respectively), human (AMPK β 1 and AMPK β 2 designated as H β 1 and H β 2), and mouse (AMPK β 1 and AMPK β 2 designated as M β 1 and M β 2) were aligned and displayed as described 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	<i>C. elegans</i>	<i>D. melanogaster</i>	<i>S. cerevisiae</i>	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;	SNF4	SNF4	PRKAG1 (5571)	Prkag1 (19082)
	T01B6.3; R53.7			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 γ 1 – γ 5 in Figures 3 and 6.

```

Hy2      MFLDGDLESGKHSSRKVDSPFGPGSPKGFSSRGFPQPRP
Hy2      MGSAAAMDTKKKKEVSSPGGSSGKKNPSLKRRLRVHIDPSSFAMPLLDGVDENSEKHSSRKVDSPFGSPSRGLFSRGFPQPRP
Hy3      MEPGLEHALRRTPSWSSL-GGSEHQEMSFLQENSSSW
Hy3      MEPELEHTLPGTLTWSHS-GGPESEQEMDFLEQGENS-W
Cy1      MVAKKNILGGPPSNQLNDNSSLPIGELVIVSDDEFT
Cy4      MRRLTNSRNPET
Cy5      MFQLESNSR

Hy2      SSPMSAPVRPKTSPGSPKTVFFSYQESPSPRRMSFSGIFRSSSKESPNSNPATSPGGIRFFSRSRKTSGLSSSPSTPTQVT
Hy2      SSPVSAVVRPKTSPGSPKTVFFSYQESPSPRRMSFSGIFRSSSKESPNSNPSTSPGGIRFFSRSRKTSGLSSSPSTPTQVT
Hy3      PPAVATSSSERIRG---KR---AKALRWTRQKSVEEGEPGQEGEPSPRPAEATSTGLEATFPKKTPLAQADP-AGVGTPTPT
Hy3      PPAVATSSSERIRG---IR---VKASRWTRQEAEEAEPPLGEGAQSRPAEATSTQEAATFPKATPLAQAVPLAEATSTPT
Cy1      TAPPVTKPVKKGWIKTAKSKASGTLRLRTLFGRSTKRRADAEATHEAGEEDEEDYTDQNDYTVKSLPASGHYKSRVGNSTSH
Cy3      MNNTTGLRLRNKATTFESFSPK
Cy4      TDFRVNETVVSAPSLTAGP-----KSVQFSNRVEVMVDAENEVDNNINTPASEQRNNTSGRRPSFSLQVRDLIHPRTGSL
Cy5      QQGMLETTPVNVYG-----SSMDHGYESGPSIDHSRRSSFGIRDISLPSRRRYTMSQRLQVRANGDG

Hy1      METVISDDSSPAVENEHPQETP--E
Hy1      MESVAAESSPALENEHPQETP--E
Hy2      KQHTFPLESYKHEPERLENRIYASSPPDTGQRFCSFQSPTRPPPLASPHYAPSKAAALAAALGPAEAGMLEKLEFEDEAVED
Hy2      KQHPFPLESYKQEPERPESTRYASSPPDTGQRFCLA-FQSPARPPLASPHYAPLRTAVLAAAPGPAEAGMLEKLEFEDE--ED
Hy3      GWDCPLSDCTASAAGSSTDDVELATEFPATEAWECELEGLLEERPA-----LCLSPQAPFPKLGWDDDEL-K
Hy3      GWDLLLPDCAASAGGSSTGDELTIETFPAPAWDCELEGLKDRPR-----PGPSFPQAPLLGLSWDDDELQ-K
Cy1      KLQDLADEMRLRGGGPPKQSGSEPIPKPVIIFRVPRKHANSMSLRSVPGAIGP--S-GVVKAPLSDPNFDFYTWHTS-EAD
Cy2      MSFDFDIHQRIISHMTGS-----KSTMTTESDEVLPKTFN----
Cy3      AFFDLQHFFISFKRKAPVDGIEAIQRDQASISHAHVVKYANDERPDE-----KEKQDLENMFTVLRIGVQ
Cy4      GASMFRRPEATRVRVQAEACNEAVYDLRDKHAHFYDDDDNGFRPRSN-----S-SDLLILRKTSPISFNVGVDIG
Cy5      NRKTSYQYQMEVDDNLKMPLETRRRMSVPEVFRADFA-----IMRPCRITSNEGAEVLSAFE

Hy1      SNNSVYTSFMKSHRCYDLIPTSSKLVVFTSLQVKKAFVALVTNGVRAAPLWDSKKQ--SFVGMLTITDFINILHRYYS----
Hy1      SNNSVYTSFMKSHRCYDLIPTSSKLVVFTSLQVKKAFVALVTNGVRAAPLWDSKKQ--CFVGMLTITDFINILHRYYS----
Hy2      SEGCVYMRFMRSKCYDIVPTSSKLVVFTTLQVKKAFVALVANGVRAAPLWESKKQ--SFVGMLTITDFINILHRYYS----
Hy2      SEGCVYMRFMRSKCYDIVPTSSKLVVFTTLQVKKAFVALVANGVRAAPLWESKKQ--SFVGMLTITDFINILHRYYS----
Hy3      PGAQIYMRFMQEHCTYDAMATSSKLVIFDTMLEIKKAFVALVANGVRAAPLWDSKKQ--SFVGMLTITDFILVLHRYYS--
Hy3      PGAQVYMHFMQEHCTYDAMATSSKLVIFDTMLEIKKAFVALVANGVRAAPLWDSKKQ--SFVGMLTITDFILVLHRYYS--
Cy1      NHDAVSLFMKAHKCYDLIPTSSKLVVFTDLHVRKAFVALVANGVRAAPLWDTDNQ--RFTGMLTITDFIKILCKHYDKG---
Cy2      -DKEAFARLLWINQCYEAMPSSSKMVVFDQGLLMHKAFLNGLLAQSTRHVLSDPFDGG-KLDGILSVTDFIKVLMKIYRETKCE
Cy3      NSDIVYTHLLQLSQCYEAMARNKLVFTNDISVRKAFNGLIYNCMTGLVADSQTL--EITGVLSVTDIFIMVLMMLWKYRENLD
Cy4      QKTFDYHQYMSVVDVYELCPNNSKVIIIDASTPTTFRFRIMRDHNTTLIVWDTSDARHVKNRILTLTDCNLAARNETPP--
Cy5      RHADPYHTFMKSITCYDLQPHSSLVVDGKTKVKAHVALSOHGHIAAVVTNTDKY--QAECVFNMGHCITALLVAAG----

Hy1      -----ALVQIYELEEHKIETWR-EVYLQDSFKPLVCISPNASLFDVSSLIKNKIHRLPVDPIS--GNTLYILTHKRIL
Hy1      -----ALVQIYELEEHKIETWR-EVYLQDSFKPLVCISPNASSFDVSSLIKNKIHRLPVDPIS--GNTLYILTHKRIL
Hy2      -----PMVQIYELEEHKIETWR-ELYLQETFKPLVNISPDASLFDVAVSLIKNKIHRLPVDPIS--GNALYILTHKRIL
Hy2      -----PMVQIYELEEHKIETWR-ELYLQETFKPLVNISPDASLFDVAVSLIKNKIHRLPVDPIS--GNALYILTHKRIL
Hy3      -----PLVQIYELEEHKIETWR-EIYLQGCFFKPLVSI SPNDSLFEAVYTLIKNRIHRLPVDPIS--GNVLIHLTHKRIL
Hy3      -----PLVQIYELEEHKIETWR-EIYLQGCFFKPLVSI SPNDSLFEAVYALIKNRIHRLPVDPIS--GTVLIHLTHKRIL
Cy1      -----DNSLRALQEDQISHWDQFELDGTLRPFVYIDPNESLHRAVELLCEKSVHRLPVDRKT--GNITYILTHKRIM
Cy2      KES-TELDMTQIANEEIGNLSIRQYRELVKKEGNLRPLVSDASGLDAACTLAELHVRHRIHPVDPIS--GSALYILTHKRIL
Cy3      ELKGTPLSHEDFRQMDIAYMPSIRWKGCELTGKQLKPFINILKESIFRAVELLTKYRIHRLEVMDEKT--GDCAYILTHKRIL
Cy4      -----ADG--QVLRASDILSGN---Q-----LVSVSISSKILDLCEELHQNRLHRVVVD-DA--KEVVNIISVRRVI
Cy5      -----NRE---VASKTLVEFLKEIGSGN---IICSGVQNSVWEAANIISHNKISFVPIFTIIPKPGTPLYFLTPRMIL

Hy1      KFLKLEITEFPKPEFMSK---SLEELQIGTYANIAMVR-TTPVYVALGIFVQHRVSALPVVD-EKGRVVDIYSKFDVINLAAEK
Hy1      KFLKLEITEFPKPEFMSK---SLEELQIGTYANIAMVR-TTPVYVALGIFVQHRVSALPVVD-EKGRVVDIYSKFDVINLAAEK
Hy2      KFLQLFMSDMPKPAFMKQ---NLDELIGIGTYHNIAFIH-PDTPIIKALNIFVERRISALPVVD-ESGKVVDIYSKFDVINLAAEK
Hy2      KFLQLFMSDMPKPAFMKQ---NLDELIGIGTYHNIAFIH-PDTPIIKALNIFVERRISALPVVD-ESGKVVDIYSKFDVINLAAEK
Hy3      KFLHIFGSLPRPSFLYR---TIQDLGIGTFRDLAVVL-ETAPILTALDIFVDRRVSALEPVN-ECGQVVGYSRFDVIHLAAQQ
Hy3      KFLHIFGALLPRPSFLCR---TIQDLGIGTFRDLAVVL-ETAPVLTALDIFVDRRVSALEPVN-ESGQVVGYSRFDVIHLAAQQ
Cy1      KFLSLYMRDLPRPSFMSC---TPRELIGAGWDILCCH-VDTPIHDALELFLKNRVSALPLID-ENGRVVDIYAKFDVSLAAES
Cy2      KFLWLWEGKHLAPLEYLHK---SPKELGIGTWSGIRVVF-PDTQLVDCDLILLNKGVSGLEPVVERETFKVVDMSRFPVAGIALEN
Cy3      HIYWKHCALLPKPECLSQ---RVVDLEIGSWKNLIFAN-EQPLIECLDMLIDNNISGHPVQKNTLVLEVYTRFADAASAFSD
Cy4      AAITHKQNSRLSHFAQWLSK---SIGMSAIGTWENVAVIS-QNETVYRAMEMDLGFHYSALPVVD-SKQNVIGVITPTKICALPRN
Cy5      QETVVLKSLDFGDALLHVRQATLDQKKIGTWNDVVKIQLNTTIEEAIKLMSEKMSSTHEPVN-DFKQIVNMLARKITILEIMSH

Hy1      TYNNLDVSVTKALQHRSS---HYFEGVLKCYLHETLETIINRLVEAEVHRLVVVDENVVKGIVSLSDILQALVLTGGEKKP
Hy1      TYNNLDVSVTKALXHRSS---HYFEGVLKCYLHETLETIINRLVEAEVHRLVVVDENVVKGIVSLSDILQDLVLTGGEKKP
Hy2      TYNNLDITVTQALQHRSS---QYFEGVVKCNKLEILETIVDRIVRAEVHRLVVVNEADSVIGIISLSDILQALILTTPAGAKQKE
Hy2      TYNNLDITVTQALQHRSS---QYFEGVVKCNKLEILETIVDRIVRAEVHRLVVVNEADSVIGIISLSDILQALILTTPAGAKQKE
Hy3      TYNNHLDMSVGEALRQRT---LCLEGVLSQCPHESLGEVIDRIAREQVHRLVLVDETQHLLGVVSLSDILQALVLSFAGIDALG
Hy3      TYNNHLDMSVGEALRQRT---LCLEGVLSQCPHESLGEVIDRIAREQVHRLVLVDETQHLLGVVSLSDILQALVLSFAGIDALS
Cy1      SYDKLCTVTEALQHRSS---ENFEGVQTCLETDSLQVLEAIVKAEVHRLIVTDQDKKVVGVVSLSDILKLVLSFCQKPPFP
Cy2      ---RLDITVKEALAFKSGQGPKNDRVSVRDNESFWKAVNVLDHNVHRLCAVNEHGGIEGVISLSDVINFMVQVPGSHLRNI
Cy3      -HIDLSVSVTRAIQERDYQNG-IRRQGVVTANYTTTWSLIEIFIDKNVHRIFMVDDRTILKGITSLSDVIEFLVLRFTKKNQVT
Cy4      FIEPKRWLQETRVSDIL---HICKSQLLISSADSVGQVLDTLTLAGDTQSAFAIHNGKAIG-VISLDFLSHILRSEPLATDEE
Cy5      QGGNFHMDLKEPVKILQ---SLQSRVLVGRSSYTVFETVAKMNTSDKSSLPIDEGKRILAVVSCSDILSYIQTAERTPTEDR

Hy2      TETE
Hy2      TETE
Hy3      A
Hy3      A
Cy1      PPQSQQAGGGGPPTRNASGTSTGGASSSDSPHSIFEGIEVEDDDDDDEEAPPSIDCTPFGSSAAT
Cy2      TAPKKHWAHHTGDMDNKELREILLTNAANFEHSSNSSSPNSALPSNQNSYKPPTHHH
Cy3      TGEPMEK
Cy4      PSQEPAMPPTPESNSSSTENVCCLKNL
Cy5      SSSNSTSSMPKSAK

```

Figure 6. AMPK γ subunits are conserved. AMPK γ subunits from *C. elegans* (designated as Cy1 through Cy5), human (AMPK γ 1, AMPK γ 2, and AMPK γ 3 designated as Hy1, Hy2, and Hy3), and mouse (AMPK γ 1, AMPK γ 2, and AMPK γ 3 designated as My1, My2, and My3) 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 overlaid. 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.

- Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest* 116:1776–1783, 2006.
- Hardie DG, Hawley SA, Scott JW. AMP-activated protein kinase—development of the energy sensor concept. *J Physiol* 574:7–15, 2006.
- Jorgensen SB, Richter EA, Wojtaszewski JF. Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. *J Physiol* 574:17–31, 2006.
- Reznick RM, Shulman GI. The role of AMP-activated protein kinase in mitochondrial biogenesis. *J Physiol* 574:33–39, 2006.
- Viollet B, Foretz M, Guigas B, Horman S, Dentin R, Bertrand L, Hue L, Andreelli F. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. *J Physiol* 574:41–53, 2006.
- Daval M, Foulle F, Ferre P. Functions of AMP-activated protein kinase in adipose tissue. *J Physiol* 574:55–62, 2006.
- Motoshima H, Goldstein BJ, Igata M, Araki E. AMPK and cell proliferation—AMPK as a therapeutic target for atherosclerosis and cancer. *J Physiol* 574:63–71, 2006.
- Xue B, Kahn BB. AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. *J Physiol* 574:73–83, 2006.
- Ramamurthy S, Ronnett GV. Developing a head for energy sensing: AMP-activated protein kinase as a multifunctional metabolic sensor in the brain. *J Physiol* 574:85–93, 2006.
- Dyck JR, Lopaschuk GD. AMPK alterations in cardiac physiology and pathology: enemy or ally? *J Physiol* 574:95–112, 2006.
- Evans AM. AMP-activated protein kinase and the regulation of Ca^{2+} signalling in O_2 -sensing cells. *J Physiol* 574:113–123, 2006.
- Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 277:25226–25232, 2002.
- Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–1174, 2001.
- Wood WB, Ed. The Nematode *Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp667, 1988.
- Riddle DL, Blumenthal T, Meyer BJ, Priess JR, Eds. *C. elegans* II. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp1222, 1997.
- Corsi AK. A biochemist's guide to *Caenorhabditis elegans*. *Anal Biochem* 359:1–17, 2006.
- Hengartner MO. Cell death. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR, Eds. *C. elegans* II. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp383–416, 1997.
- Tomari Y, Zamore PD. Perspective: machines for RNAi. *Genes Dev* 19:517–529, 2005.
- Chalfie M, White J. The nervous system. In: Wood WB, Ed. The Nematode *Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp337–392, 1988.
- Porte D Jr, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* 54:1264–1276, 2005.
- Ashrafi K, Chang FY, Watts JL, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421:268–272, 2003.
- Liu LX, Spoerke JM, Mulligan EL, Chen J, Reardon B, Westlund B, Sun L, Abel K, Armstrong B, Hardiman G, King J, McCague L, Basson M, Clover R, Johnson CD. High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res* 9:859–867, 1999.
- C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012–2018, 1998.
- Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421:231–237, 2003.
- Wang D, Kennedy S, Conte D, Jr., Kim JK, Gabel HW, Kamath RS, Mello CC, Ruvkun G. Somatic misexpression of germline P granules and enhanced RNA interference in retinoblastoma pathway mutants. *Nature* 436:593–597, 2005.
- Sulston J. Cell lineage. In: Wood WB, Ed. The Nematode *Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp123–156, 1988.
- Luo Z, Saha AK, Xiang X, Ruderman NB. AMPK, the metabolic syndrome and cancer. *Trends Pharmacol Sci* 26:69–76, 2005.
- Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res* 100:328–341, 2007.
- Witters LA, Kemp BE, Means AR. Chutes and ladders: the search for protein kinases that act on AMPK. *Trends Biochem Sci* 31:13–16, 2006.

30. Rudolph MJ, Amodeo GA, Iram SH, Hong SP, Pirino G, Carlson M, Tong L. Structure of the Bateman2 domain of yeast Snf4: dimeric association and relevance for AMP binding. *Structure* 15:65–74, 2007.
31. Townley R, Shapiro L. Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase. *Science* 315:1726–1729, 2007.
32. Beg ZH, Allmann DW, Gibson DM. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity with cAMP and with protein fractions of rat liver cytosol. *Biochem Biophys Res Commun* 54:1362–1369, 1973.
33. Carlson CA, Kim KH. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *J Biol Chem* 248:378–380, 1973.
34. Gao G, Widmer J, Stapleton D, Teh T, Cox T, Kemp BE, Witters LA. Catalytic subunits of the porcine and rat 5'-AMP-activated protein kinase are members of the SNF1 protein kinase family. *Biochim Biophys Acta* 1266:73–82, 1995.
35. Apfeld J, O'Connor G, McDonagh T, DiStefano PS, Curtis R. The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev* 18:3004–3009, 2004.
36. Curtis R, O'Connor G, DiStefano PS. Aging networks in *Caenorhabditis elegans*: AMP-activated protein kinase (*aak-2*) links multiple aging and metabolism pathways. *Aging Cell* 5:119–126, 2006.
37. Narbonne P, Roy R. Inhibition of germline proliferation during *C. elegans* dauer development requires PTEN, LKB1 and AMPK signalling. *Development* 133:611–619, 2006.
38. Avruch J, Hara K, Lin Y, Liu M, Long X, Ortiz-Vega S, Yonezawa K. Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase. *Oncogene* 25:6361–6372, 2006.
39. Davies SP, Hawley SA, Woods A, Carling D, Haystead TA, Hardie DG. Purification of the AMP-activated protein kinase on ATP-gamma-sepharose and analysis of its subunit structure. *Eur J Biochem* 223:351–357, 1994.
40. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett* 223:217–222, 1987.
41. Sim AT, Hardie DG. The low activity of acetyl-CoA carboxylase in basal and glucagon-stimulated hepatocytes is due to phosphorylation by the AMP-activated protein kinase and not cyclic AMP-dependent protein kinase. *FEBS Lett* 233:294–298, 1988.
42. Stein SC, Woods A, Jones NA, Davison MD, Carling D. The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J* 345(Pt 3):437–443, 2000.
43. Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, Cantley LC. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A* 101:3329–3335, 2004.
44. Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab* 2:9–19, 2005.
45. Hurley RL, Anderson KA, Franzone JM, Kemp BE, Means AR, Witters LA. The Ca²⁺/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem* 280:29060–29066, 2005.
46. Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M, Carling D. Ca²⁺/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab* 2:21–33, 2005.
47. Momcilovic M, Hong SP, Carlson M. Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem* 281:25336–25343, 2006.
48. Watts JL, Morton DG, Bestman J, Kemphues KJ. The *C. elegans* par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. *Development* 127:1467–1475, 2000.
49. Kimura Y, Corcoran EE, Eto K, Gengyo-Ando K, Muramatsu MA, Kobayashi R, Freedman JH, Mitani S, Hagiwara M, Means AR, Tokumitsu H. A CaMK cascade activates CRE-mediated transcription in neurons of *Caenorhabditis elegans*. *EMBO Rep* 3:962–966, 2002.
50. Shin TH, Yasuda J, Rocheleau CE, Lin R, Soto M, Bei Y, Davis RJ, Mello CC. MOM-4, a MAP kinase kinase kinase-related protein, activates WRM-1/LIT-1 kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Mol Cell* 4:275–280, 1999.
51. Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, Barker N, Waterman M, Bowerman B, Clevers H, Shibuya H, Matsumoto K. The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. *Nature* 399:798–802, 1999.
52. Meneghini MD, Ishitani T, Carter JC, Hisamoto N, Ninomiya-Tsuji J, Thorpe CJ, Hamill DR, Matsumoto K, Bowerman B. MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* 399:793–797, 1999.