Published in final edited form as:

EXS. 2016 ; 107: 389–401. doi:10.1007/978-3-319-43589-3_16.

The Role of AMPK in *Drosophila melanogaster*

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Abstract

In the fruit fly, *Drosophila melanogaster*, mono-allelic expression of AMPK- α , - β , and - γ yields a single heterotrimeric energy sensor that regulates cellular and whole-body energetic homeostasis. The genetic simplicity of *Drosophila*, with only a single gene for each subunit, makes the fruit fly an appealing organism for elucidating the effects of AMPK mutations on signaling pathways and phenotypes. In addition, *Drosophila* presents researchers with an opportunity to use straightforward genetic approaches to elucidate metabolic signaling pathways that contain a level of complexity similar to that observed in mammalian pathways. Just as in mammals, however, the regulatory realm of AMPK function extends beyond metabolic rates and lipid metabolism. Indeed, experiments using *Drosophila* have shown that AMPK may exert protective effects with regard to life span and neurodegeneration. This chapter addresses a few of the research areas in which *Drosophila* has been used to elucidate the physiological functions of AMPK. In doing so, this chapter provides a primer for basic *Drosophila* nomenclature, thereby eliminating a communication barrier that persists for AMPK researchers trained in mammalian genetics.

Keywords

Drosophila melanogaster; AMPK; LKB1; Gal4; neurodegeneration

16.1 Introduction

16.1.1 Overview of AMPK Signaling in *Drosophila*

In the fruit fly, mono-allelic expression of AMPK- α , - β , and - γ yields a single heterotrimeric energy sensor that regulates cellular and whole-body energetic homeostasis (Johnson et al. 2010; Pan and Hardie 2002). In contrast to the streamlined fly genome, the more complex mammalian genome encodes multiple AMPK subunit isoforms (α_{1-2} , β_{1-2} , and γ_{1-3}) that can theoretically form 12 unique heterotrimers (Iseli et al. 2005; Pan and Hardie 2002). Thus, the genetic simplicity of *Drosophila*, with only a single gene for each subunit, makes the fruit fly an appealing organism for elucidating the effects of AMPK mutations on signaling pathways and phenotypes.

Understandably, the phylogenetic separation between *D. melanogaster* and *H. sapiens* may make some AMPK researchers reluctant to explore the elegant genetic tools (e.g., temporally and/or spatially driven gene expression systems) that are easily available to fly geneticists. Two lines of evidence, however, point to the utility of *Drosophila* as a useful model organism for studying AMPK. First, functionally critical amino acids in AMPK subunits are either conserved or replaced by biochemically similar residues across species (Figs. 16.1 and 16.2) (Chen et al. 2012; Kazgan et al. 2010; Pan and Hardie 2002; Spasic et al. 2008; Xiao et al. 2007). These conserved amino acid residues include those that regulate AMPK localization, subunit interactions, and activity. Several examples are highlighted here: (1) Cytoplasmic localization of AMPK- α_2 is controlled by a C-terminal export signal that is conserved in mammals and flies (Kazgan et al. 2010). Deletion of this export signal has been shown to restrict localization of *Drosophila* AMPK (dAMPK)- α to nuclei (Kazgan et al. 2010). (2) The scaffolding β_1 subunit relies on its C-terminal residues (amino acids 186–270 in rat) to bind AMPK- α_1 and - γ_1 in vitro (Iseli et al. 2005). Although N-terminal AMPK- β residues have diverged among species, the C-terminal residues in dAMPK- β are almost perfectly identical to those of human and rat AMPK- β_1 (Fig. 16.1). (3) Finally, amino acid residues that mediate binding of AMP at two regulatory sites in AMPK- γ_1 (i.e., the allosteric activation site and dephosphorylation inhibition site) are conserved in *Drosophila* (Fig. 16.2) (Chen et al. 2012; Xiao et al. 2007). In addition, key AMPK- γ amino acid residues that have been shown to alter cardiac AMPK activity and cause human cardiomyopathies (when mutated) are conserved in both human and fly genomes (Fig. 16.2) (Burwinkel et al. 2005; Liu et al. 2013; Moffat and Harper 2010).

A second line of evidence pointing to the utility of *Drosophila* is the conserved expression of the AMPK kinase LKB1 and the AMPK substrate acetyl CoA carboxylase (ACC-1) (Amin et al. 2009; Andersen et al. 2012; Martin and St Johnston 2003; Pan and Hardie 2002). The conserved expression of LKB1 and ACC underscores the evolutionary importance of this signaling pathway and raises another key point regarding the merit of *Drosophila* as a model organism: whereas the streamlined *Drosophila* genome simplifies genetic loss- or gain-of-function studies in vivo, the complexity of LKB1 signaling—with both AMPK-*dependent* and AMPK-*independent* effects—is also conserved in both mammals and flies (Amin et al. 2009; Andersen et al. 2012; Choi et al. 2015). This conserved signaling complexity means that researchers can use *Drosophila* to help identify (or eliminate) the role of AMPK with respect to various LKB1-dependent developmental and physiological processes. In mice, flies, and even nematodes, LKB1 genetic loss-of-function phenotypes can be more severe than those of AMPK genetic loss-of-function phenotypes (yeast do not have an obvious homologous sequence for LKB1) (Amin et al. 2009; Apfeld et al. 2004; Hardie and Alessi 2013; Nakada et al. 2010; Williams et al. 2011). For example, although LKB1-null and AMPK-null *Drosophila* embryos both exhibit defects in mitosis, loss of AMPK (in eye tissue) does not phenocopy the pitted surface or rhabdomyere fusion observed in adult LKB1-null fly eye tissue (Amin et al. 2009; Lee et al. 2007). In addition, constitutively active AMPK cannot rescue all phenotypic abnormalities induced by LKB1 loss in *Drosophila*. In the absence of LKB1, for example, constitutively active AMPK (AMPK- α^{T184D}) rescues epithelial polarity defects in embryos but fails to rescue decreased lipid storage levels in the fat body (adipose- and liver-like tissue) of adult *Drosophila* (Choi et al. 2015; Lee et al.

2007). Instead, overexpression of an AMPK-related kinase, salt-inducible kinase 3, rescues lipid storage levels in LKB1-null fat bodies (Choi et al. 2015).

In summary, *Drosophila* is a model organism that allows researchers to use straightforward genetic approaches to elucidate conserved signaling pathways that can be characterized by a level of complexity similar to that of mammalian pathways. For an overview of *Drosophila* AMPK's (dAMPK's) role as a metabolic regulator—particularly in the context of dietary restriction—we invite readers to review the biochemical and behavioral experiments published by Johnson et al. (2010). AMPK-dependent feeding behaviors published by Johnson and colleagues include hyperphagia triggered by genetically reduced dAMPK function as well as a normally pro-survival “foraging” behavior induced in starved flies with genetically reduced AMPK function (Johnson et al. 2010). Just as in mammals, however, the regulatory realm of AMPK extends beyond metabolic rates and lipid metabolism. Indeed, as Sect. 2 will show, experiments using *Drosophila* have shown that AMPK may exert protective effects with regard to life span and neurodegeneration (Ng et al. 2012; Pimenta de Castro et al. 2012; Ulgherait et al. 2014). This chapter addresses a few of the research areas—many of which translate to higher organisms—in which *Drosophila* has been used to elucidate the physiological functions of AMPK. In doing so, this chapter will also function as a primer for basic *Drosophila* nomenclature, thereby eliminating a communication barrier that persists between AMPK researchers trained in either mammalian genetics or *Drosophila* genetics.

16.1.2 Overview of Tools Available to *Drosophila* Geneticists

Many readers of this chapter may have limited or no experience working with *Drosophila* and the genetic tools that are uniquely available to fly geneticists. These tools include P elements, the Gal4-upstream activating sequence (UAS) gene expression system, and genome-wide availability of *Drosophila* transgenic RNAi approaches. Understandably, researchers with expertise in mammalian AMPK may have difficulty mining *Drosophila* literature for insights into cellular signaling pathways. Thus, we have illustrated the technical approaches to help readers understand the ways in which *Drosophila* has contributed to AMPK research (Fig. 16.3). The Gal4-UAS expression system is an especially noteworthy and commonly used tool, as it allows geneticists to manipulate the AMPK signaling pathway spatially and/or temporally (Brand and Perrimon 1993; McGuire et al. 2004). Essentially, this approach relies on a yeast transcription activator (Gal4) (that has no known function in *Drosophila*) to precisely control the expression of any gene placed after the yeast UAS promoter within transgenic *Drosophila*. A detailed description of this system has been published by McGuire et al. (2004). In short, Gal4 binds to UAS targets upstream of a transgene that has been inserted into the fly genome. To achieve either ubiquitous or spatially restricted transgene expression, researchers can select from a number of freely available fly lines (from stock centers) that express Gal4 drivers under various well-characterized enhancers (Table 16.1). To temporally regulate transgene expression, researchers can use two derivatives of the classical Gal4-UAS system: a pharmacologically inducible Gene-Switch system and a temperature-controllable TARGET system (McGuire et al. 2004; Osterwalder et al. 2001). Mechanistic explanations of these systems are beyond the scope of this paper but are provided alongside excellent illustrations in the above-mentioned

review (McGuire et al. 2004). For this chapter, it is sufficient for readers to simply know that fly geneticists have established tools for silencing (via Gal4-driven RNAi expression) or overexpressing AMPK subunits both spatially and temporally.

16.2 Selected Disease Models Studied in *Drosophila*

16.2.1 Overview of Disease Models

Before beginning a discussion of translational research in *Drosophila*, we would like to first acknowledge an excellent review that explains how *Drosophila* has helped to expand our understanding of human neurodegenerative diseases (Lessing and Bonini 2009).

“Maintaining the Brain: Insight into Human Neurodegeneration From *Drosophila* Mutants,” by Lessing and Bonini, touches upon research areas that are also described herein (e.g., Parkinson’s disease (PD) and Alzheimer’s Disease (AD)). We recommend this published review to readers and will expand upon Lessing’s and Bonini’s work by discussing dAMPK-related research published during or after 2010. As stated earlier, we will focus on recent studies that demonstrate the protective role of dAMPK with regard to life span and neurodegeneration (Lin et al. 2014; Ng et al. 2012; Pimenta de Castro et al. 2012; Ulgherait et al. 2014).

16.2.2 Role of AMPK in Longevity

With its short life span, *Drosophila melanogaster* is a convenient model organism for longevity studies in the lab (Linford et al. 2013). Multiple publications have explored the role of AMPK in regulating life span. To begin, the Gal4-UAS system has been used to show that—depending on the strength of the Gal4 driver—a decrease in dAMPK activity may be either lethal during the larval stage or may shorten the life span of adult transgenic animals (Gal4 was used to drive the near ubiquitous overexpression of either AMPK RNAi or a kinase-dead AMPK mutant) (Johnson et al. 2010). In agreement with this observation, a second lab has shown that *increased* pan-neuronal dAMPK activity *extends* adult female life span (Ulgherait et al. 2014). Furthermore, genetic knockdown of dAMPK- α (in the fat body) has been shown to prevent the increased life span induced by β -sitosterol, a natural product that promotes AMPK phosphorylation in *Drosophila* S2 cell culture (Lin et al. 2014). In light of these 3 lines of evidence, it may be tempting to simply conclude that life span positively correlates with dAMPK activity in flies. During starvation, however, this correlation may not always be observed. For example, overexpression of AMPK in neurons has been shown to *shorten* the life span of starved transgenics, while overexpression of dominant-negative AMPK in neuroendocrine cells has been shown to *extend* the life span of starved transgenic animals (Braco et al. 2012; Ulgherait et al. 2014). Interestingly, decreased triglyceride levels—an indicator of altered metabolism—have been documented for both fed mutants with reduced (ubiquitous) AMPK function and starved mutants with increased neuronal AMPK function (Johnson et al. 2010; Ulgherait et al. 2014). Together, these findings highlight the complex relationships among AMPK activity, diet, and life span. In addition, these findings indicate that ubiquitous and tissue-specific modulation of AMPK activity may have different consequences on life span (Braco et al. 2012; Johnson et al. 2010).

Finally, before continuing to the next section, we would be remiss in omitting a revelatory finding described in Ulgherait et al.'s lifespan studies. Just as increased pan-neuronal dAMPK activity extends adult life span, the reciprocal experiment (increased intestinal dAMPK activity) also extends *Drosophila* life span *while upregulating autophagy in the brain* (Ulgherait et al. 2014). Importantly, Poirier and colleagues have confirmed that the intestinal Gal4 driver TIGS-2 permits UAS-transgene expression solely within the digestive system of male and female *Drosophila*—without detectable levels of UAS-transgene expression in the brain (Poirier et al. 2008). The non-cell autonomous effects demonstrated by Ulgherait and colleagues are particularly timely in light of an increasingly widespread interest regarding the interdependence of brain and intestinal health (Duca et al. 2015; Reardon 2014; Ulgherait et al. 2014).

16.2.3 Role of AMPK in Neurodegenerative Disorders

Many experts in mammalian AMPK signaling may be familiar with the regulation or alteration of autophagy by AMPK, as well as the physiological relevance of autophagy in proteinopathic diseases (Cai et al. 2012; Vingtdoux et al. 2011). In cell cultures, for example, activation of AMPK by resveratrol has been shown to promote autophagy and—as a result—decrease levels of amyloid- β ($A\beta$), a secreted peptide that contributes to the development of Alzheimer's disease (AD) (Vingtdoux et al. 2010). In agreement with in vitro findings, dietary resveratrol has been shown to significantly increase AMPK phosphorylation and decrease $A\beta$ deposition in the brains of mice modeling AD (Vingtdoux et al. 2010).

More recently, *Drosophila* researchers have used genetic tools to address a different and perhaps lesser-known facet of proteinopathic diseases: *mitochondrial* pathology. Researchers have generated genetically distinct *Drosophila* models of PD-related mitochondrial pathology. This chapter addresses two *Drosophila* models: one in which transgenic animals express misfolded ornithine transcarbamylase (dOTC) and another in which transgenic *Drosophila* express mutated leucine-rich repeat kinase 2 (LRRK2) (Ng et al. 2012; Pimenta de Castro et al. 2012). The former model was motivated by the observation that mutated PTEN-induced putative kinase 1 (PINK1) is accompanied by increased levels of misfolded mitochondrial protein (in both PD patients and fly mutants) (Pimenta de Castro et al. 2012). By mutating the mitochondrial matrix protein OTC instead of PINK1, researchers were able to examine the effects of *organelle-restricted* protein misfolding (Pimenta de Castro et al. 2012). Misfolded mitochondrial protein was accompanied by an accumulation of light chain 3-II (LC3-II; an autophagy marker) in flies expressing dOTC but not in flies expressing dOTC with Gal4-driven AMPK- α -RNAi (Pimenta de Castro et al. 2012). Pimenta de Castro and colleagues have speculated that AMPK-dependent autophagy may exert a protective role in this fly model of protein misfolding (2012). Notably, shortly after the *Drosophila* model of dOTC was published, researchers published a mouse model of PD where dOTC expression is driven by the tyrosine hydroxylase promoter (Moiso et al. 2014). This progression from early experiments in flies to ensuing studies in mice underscores the translational merit of *Drosophila* research.

As alluded to earlier, a second *Drosophila* model of PD-related mitochondrial pathology was achieved by using the Gal4-UAS system to express mutated LRRK2 (a disease gene mutated

in PD) in muscle tissue (Ng et al. 2012). In addition to exhibiting mitochondrial pathology, these flies exhibited locomotor dysfunction which was rescued by ectopic expression of parkin (mutations in parkin can cause PD) (Ng et al. 2012). Researchers demonstrated that ectopic expression of constitutively active AMPK rescued both mitochondrial and locomotor abnormalities in LRRK2 G2019S mutants as well as in *parkin* mutants (Ng et al. 2012).

Finally, no discussion of the role of dAMPK in the brain would be complete without mentioning *loechrig* (*loe*) (also reviewed by Lessing and Bonini 2009). *Loe* mutants have reduced (“hypomorphic”) dAMPK- γ expression in the nervous system (Tschaape et al. 2002). This reduced AMPK function allows adult flies to develop past the earlier lethality found in AMPK-null mutations, but results in a severe vacuolar pathology and widespread neuronal death in the adult fly brain (Kazgan et al. 2010; Tschaape et al. 2002). More recently, Kretschmar and colleagues have demonstrated that *loe* mutants have increased prenylation of Rho1 (Rho1 is orthologous to mammalian RhoA) (Cook et al. 2012). Although *loechrig* does not model the etiology of AD, Cook et al. stress the translational value of their research by discussing the link between activated RhoA/Rho-associated protein kinase (ROCK) signaling and AD (Cook et al. 2012). Pharmacological inhibition of ROCK, for example, has been shown to decrease A β 42 levels in vivo (Cole and Vassar 2006; Zhou et al. 2003).

16.3 Summary

Drosophila is a model organism that allows researchers to use straightforward genetic approaches to elucidate conserved, AMPK-dependent signaling pathways. Fly geneticists have used traditional loss-of-function or hypomorphic AMPK subunit alleles and the versatile Gal4-UAS gene-expression system to elucidate the physiological functions of AMPK in starvation and longevity studies as well as in in vivo models of neurodegenerative disorders (Cook et al. 2012; Johnson et al. 2010; Ng et al. 2012; Pimenta de Castro et al. 2012; Tschaape et al. 2002; Ulgherait et al. 2014). Importantly, translational research in *Drosophila* may help provide new insights into the etiology of mammalian disease models, as demonstrated by recently published models of PD in transgenic flies and mice (Moiso et al. 2014; Pimenta de Castro et al. 2012).

Acknowledgments

We would like to thank Dr. Rob Onyenwoke and Dr. Nevzat Kazgan for critiquing this manuscript prior to submission. This work was funded by NS080108 to J.B.

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hAMPK-β ₁	152	...I IQVKKTD FEVFDALMVDSQKCS DVSELSSSPPGPYHQEPYVCKPEERFRAPPILPPH	209
rAMPK-β ₁	152	...I IQVKKTD FEVFDALMVDSQKCS DVSELSSSPPGPYHQEPYISKPEERFKAPPILPPH	209
dAMPK-β	102	...EGQRNNLVS VRESDFEVFOALAKDSENVNTNYAEKEYSQEV PQVKPWEKVS GPPVLPPH	159
hAMPK-β ₁	210	LLQVILNKDTGISCDPALLPEPNHVMLNHL YALSIKDGVMVLSATHRYKKKYVTTLLYKPI	270
rAMPK-β ₁	210	LLQVILNKDTGISCDPALLPEPNHVMLNHL YALSIKDGVMVLSATHRYKKKYVTTLLYKPI	270
dAMPK-β	160	LLQVILNKDTPLSCEPTLLPEPNHVMLNHL YALSIKDGVMVLSATHRYRKKYVTTLLYKPI	220

Fig. 16.1. Conservation of carboxy-terminus residues in AMPK-β
Most amino acids in the C-terminus of human AMPK-β₁ (NM_006253.4), rat AMPK-β₁ (GenBank: X95577.1), and *Drosophila* AMPK-β (NP_995783.1) are either invariant (*gray*) or have residues with conserved hydrophilicity (*green*) or hydrophobicity (*yellow*). Amino acids 246–270 (*underlined*) in rAMPK-β₁ are required for binding to AMPK-γ; amino acids 186–270 in rAMPK-β₁ are required for heterotrimer formation (Iseli et al. 2005)

dAMPK- γ	941	AKLVVFD	TQLLVKKAFYALVYNGVRAAPLWDSEKQQFV			GMLTITDFIKILQMYKSPNAS	1000
hAMPK- γ_1	46	SKLVVFD	TSLQVKKAFFALVTNGVRAAPLWDSKKQSFV			VLRALSCPLGMLTITDFINILHRYYSKALVQ	114
hAMPK- γ_2	234	SKLVVFD	TTLQVKKAFFALVANGVRAAPLWESKKQSFV			GMLTITDFINILHRYYSKSPMVQ	293
hAMPK- γ_3	201	SKLVIFD	TMLEIKKAFFALVANGVRAAPLWDSKKQSFV			GMLTITDFILVLRHYYSPLVQ	260
				*			

dAMPK- γ	1001	MEQLEE	HKLDTWRSV_LHNQVMPLVSI	GPDASLYDAIKILHSRIHRLPVIDPATGNVLYILTHKRILRF			1069
hAMPK- γ_1	115	IYELEE	HKIETWREVLQDSFKPLVCI	SPNASLFDVSSLIIRNKIHRPVIDPESGNTLYILTHKRILKF			184
hAMPK- γ_2	294	IYELEE	HKIETWRELYLQETFKPLVNI	SPDASLFDVSYSLIKNKIHRPVIDPISGNALYILTHKRILKF			363
hAMPK- γ_3	261	IYEIEQ	HKIETWREIYLOGCFKPLVSI	SPNDSLFEAVYTLIKNRIHRLPVLDPVSGNVLIHILTHKRLLKF			330
						*	
						*	
dAMPK- γ	1097	NIETADE	TSIITALKKFVERVSALPLVDS	GRGLVDIYAKFDVINLAAEKT		YNNLDVSLRKANEHRNEW	1166
hAMPK- γ_1	212	NIAMVRT	TPVYVALGIFVQHRVSALPVV	DEKGRVVDIYSKFDVINLAAEKT		YNNLDVSVTKALQHRSHY	281
hAMPK- γ_2	391	NIAFIHP	DTPIIKALNIFVERRISALPVV	DESGKVVDIYSKFDVINLAAEKT		YNNLDITVTQALQHRISQY	460
hAMPK- γ_3	358	DLAVVLE	TAPILTALDIFVDRRV	SALPVVNECGQVGLYSRFDVIHLAACT		YNNHLDMSVGEALQRTLC	427
						*	
						*	
dAMPK- γ	1167	FEGVQK	CNLDSELYTIMERIVRAEVH	RLVVVDENRKGIIISLSDILLY			1215
hAMPK- γ_1	282	FEGVLK	CYLHETLETIINRLVEAEVH	RLVVVDENDVVGIVSLSDILQA			330
hAMPK- γ_2	461	FEGVVK	CNKLEILETIVDRIVRAEVH	RLVVVNEADSIKGIISLSDILQA			509
hAMPK- γ_3	428	LEGVLS	CPHESLGEVIDRIAREQVH	RLVLVDETQHLLGVVSLSDILQA			476
						*	

Fig. 16.2. Alignment of human and *Drosophila* AMPK- γ

CBS domains of AMPK are highly conserved between *H. sapiens* and *D. melanogaster*.

Most amino acids in the CBS domains of *Drosophila* AMPK- γ (NP_732598.1) and human AMPK- γ_{1-3} (γ_1 , NP_001193638.1; γ_2 , NP_001035723.1; γ_3 , NP_059127.2) are either invariant (gray) or have residues with conserved hydrophilicity (green) or hydrophobicity (yellow). In SNF4 (dAMPK- γ), the first pair of CBS domains spans from amino acids 941–1069, while the second pair spans from amino acids 1097–1215 (Marchler-Bauer et al. 2015). Human AMPK- γ_2 amino acids highlighted in red represent conserved residues that, when mutated, can lead to cardiomyopathy in humans (Burwinkel et al. 2005; Liu et al. 2013; Moffat and Harper 2010). Stars indicate some of the conserved amino acid residues that have been shown to mediate nucleotide binding at regulatory sites in crystal structures containing a specific transcript variant of rat AMPK- γ_1 (Chen et al. 2012; Xiao et al. 2007). *Note:* Amino acid numbering will vary among transcript variants; the numbering listed in the figure corresponds to the provided accession numbers

Fig. 16.3. Tools used in *Drosophila* research

Techniques used by fly geneticists are illustrated. (Clockwise from *top left*) *P-elements*: A transgene plasmid (e.g., encoding cherry-tagged AMPK- α) and helper plasmid (encoding transposase) can be mixed and injected into *Drosophila* embryos (Bachmann and Knust 2008; Beall and Rio 1997). The inverted terminal repeats flanking the transgene are recognized by transposase, which randomly inserts the transgene into genomic DNA (Bachmann and Knust 2008; Beall and Rio 1997). Embryos will then develop into adults in which some germ cells carry a transgene insertion. Adult males can be crossed to females to generate offspring that retain the insertion in every cell (Bachmann and Knust 2008; Majumdar and Rio 2015). *Drosophila* offspring carrying the P-element insertion can be identified by a marker (typically a gene for red eyes) that is also encoded separately in the transgene plasmid (Bachmann and Knust 2008; Majumdar and Rio 2015). *Gal4-UAS gene expression systems*: Gal4 and UAS-transgene parental lines can be crossed to generate offspring in which transgene expression is driven by Gal4 (Brand and Perrimon 1993). Kazgan et al., for example, used Ubiq-Gal4 to drive ubiquitous (Ubiq) expression of both full-length GFP-tagged dAMPK- α and mCherry (ch)-tagged truncated dAMPK- α in offspring, thereby allowing them to identify a nuclear export signal in dAMPK- α (Kazgan et al. 2010). The figure depicting Kazgan et al.'s technical approach is adapted from the earliest publication describing the Gal4-UAS system (Brand and Perrimon 1993). *RNA interference*: Gal4 can also be used to drive the expression of RNA (Perkins et al. 2015). In the example shown, knockdown of AMPK- α can be used to help identify changes in

AMPK-dependent signaling pathways in larvae (Onyenwoke et al. 2012). *High-throughput screening*: *Drosophila* cell lines allow researchers to take advantage of *Drosophila*'s streamlined genome without having to breed stable fly lines. In the example shown, cells can be rapidly plated onto wells that have been pretreated with an RNAi library. Moser and colleagues have used this approach to screen for host factors (such as AMPK) that regulate viral infection (Moser et al. 2010a, b)

Table 16.1

Use of Gal4-UAS system in AMPK research

Expression	Gal4 driven	Transgene	Study
Ubiquitous	Tubulin ¹	AMPK- α -DN AMPK-RNAi (α , γ) AMPK- α	Effect of AMPK on lethality
	Actin ^{1,6}	AMPK- α -DN AMPK-RNAi (α , γ) AMPK- α	Effect of AMPK on lethality
		AMPK- α -RNAi	Effect of AMPK on NDPK activity
	Ubiquitin ^{1,5}	AMPK- α -DN AMPK- γ -RNAi	Effect of AMPK on life span and feeding behavior
		AMPK- α ($\pm$ mCh) truncated AMPK- α ($\pm$ mCh)	Identification of nuclear export signal in AMPK- α
	Hsp70 ^{1,a}	AMPK- α -DN AMPK-RNAi (α , γ)	Effect of AMPK on life span during starvation
Panneuronal	ELAV ^{4,b}	AMPK- γ truncated AMPK- γ	Effect of WT or mutated AMPK- γ on vacuolar pathology in the brain
	ELAV GS ^{3,c}	AMPK- α ($\pm$ mCh)	Effect of neuronal AMPK overexpression on life span ($\pm$ starvation), intestinal aging, and the expression of autophagy related genes (ATG)
Intestinal	TIGS-2 GS ^{3, c}	mCh-AMPK- α	Effect of intestinal AMPK overexpression on intestinal aging, life span
Fat body, digestive system	S106 GS ^{2,7, c}	AMPK- α -RNAi	Role of AMPK in mediating life span extension induced by β -sitosterol
Sensory neurons	109(2)80 ^{1,5}	Actin-GFP, AMPK- α -DN AMPK-RNAi (α , γ) truncated AMPK- α	Effect of AMPK on neuronal morphology

^aHeat shock protein (Hsp)70-Gal4 is temperature controllable (McGuire et al. 2004)

^bELAV, embryonic lethal abnormal vision promoter (McGuire et al. 2004) is pan-neuronal

^cGS Gene-Switch Gal4s that are expressed after feeding flies with RU486 (Osterwalder et al. 2001). *mCh* mCherry fluorescent tag.

(1) Johnson et al. (2010),

(2) Lin et al. (2014),

(3) Ulgherait et al. (2014),

(4) Tschape et al. (2002),

(5) Kazgan et al. (2010),

(6) Onyenwoke et al. (2012),

(7) Poirier et al. (2008)