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REVIEW

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The translation factors of *Drosophila melanogaster*

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ABSTRACT

Synthesis of polypeptides from mRNA (translation) is a fundamental cellular process that is coordinated and catalyzed by a set of canonical 'translation factors'. Surprisingly, the translation factors of *Drosophila melanogaster* have not yet been systematically identified, leading to inconsistencies in their nomenclature and shortcomings in functional (Gene Ontology, GO) annotations. Here, we describe the complete set of translation factors in *D. melanogaster*, applying nomenclature already in widespread use in other species, and revising their functional annotation. The collection comprises 43 initiation factors, 12 elongation factors, 3 release factors and 6 recycling factors, totaling 64 of which 55 are cytoplasmic and 9 are mitochondrial. We also provide an overview of notable findings and particular insights derived from *Drosophila* about these factors. This catalog, together with the incorporation of the improved nomenclature and GO annotation into FlyBase, will greatly facilitate access to information about the functional roles of these important proteins.

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Introduction

The process of protein synthesis can be divided into 4 phases: initiation, elongation, termination (or release) and recycling. Each phase involves the ribosome, transfer RNA (tRNAs) and a series of sequentially acting 'translation factors' operating upon messenger RNA (mRNA) substrates. Together, these components comprise the core translational machinery that converts the genetic code into polypeptide chains in all cells.

The translational machinery operating in the cytoplasm and mitochondria of eukaryotic cells is distinct. Cytoplasmic (or 'eukaryotic') translation factors are encoded by the nuclear genome and direct the bulk of cellular protein synthesis. The initiation phase of cytoplasmic translation is the most highly regulated, which is reflected by the large number of cytoplasmic initiation factors – in mammals, at least 12 distinct initiation factors (involving ~35 separate proteins) have been characterized, compared to just 2–3 elongation factors, 2 release factors and 1 major recycling factor.[1,2] Mitochondrial translation utilizes a similar, albeit much smaller, set of mitochondria-specific factors, which are more closely related to prokaryotic translation factors. These are also encoded by nuclear genes but are targeted to the mitochondria where they catalyze production of the 13 proteins encoded by the mitochondrial genome. Only 8 mitochondrial translation factors exist in mammals and, in contrast to the situation in the cytoplasm, there is no expansion in the set of initiation factors.[3]

Interest in cytoplasmic and mitochondrial translation factors has grown beyond basic research in recent years because their deregulation is linked to an increasing number of human cancers and other diseases.[4-6] The fruit fly, *Drosophila melanogaster*, has proved to be an effective model in which to study both the canonical functions and pathological roles of the protein synthesis machinery,[7] and many individual fly translation factors have been characterized. However, the full set of translation factors in this model organism has not yet been systematically identified or

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annotated, thereby hindering access to existing data as well as the development of further research.

We are aware of 2 previous studies that have attempted to catalog the translation factors of *D. melanogaster* (Supplementary Table 1). The first was performed immediately after the publication of the initial genome sequence - it identified 47 cytoplasmic translation factors based on similarity to their mammalian counterparts.[8] However, this early list omitted several proteins that have only since been recognized as translation factors, while mitochondrial translation factors were not examined. A later study identified 68 potential translation factors based on Gene Ontology (GO) annotations and sequence similarity searches (see additional data file 6 in ref.[9]). Although this longer list included more *bona fide* cytoplasmic factors, it is now apparent that it also contained many false positive hits; again, mitochondrial factors were not identified. The absence of an accurate reference set of fly translation factors has resulted in a haphazard nomenclature for these genes/proteins in FlyBase, the online database of *Drosophila* research.[10]

In order to directly address these issues, we have conducted a new analysis of the complement of genes encoding the canonical translation factors in *D. melanogaster*, taking into account recent molecular, genetic and biochemical studies conducted in flies and in other eukaryotes. We find that there are 55 genes encoding cytoplasmic translation factors and 9 genes coding for mitochondrial translation factors, making 64 factors in total. Moreover, we have updated all relevant GO annotations in FlyBase and propose a systematic nomenclature based on common usage in flies and the wider community. We also provide an overview of the current state of characterization of the different translation factors in flies, highlighting particularly significant or noteworthy aspects.

Identification of translation factors

We employed a 3-pronged approach to identify the full set of genes encoding the canonical cytoplasmic and mitochondrial translation factors in *D. melanogaster*. (We define 'canonical translation factors' as those classified as such in several recent reviews in the field.[1-3,6,11,12]). First, the sets of genes identified by previous studies were compiled and integrated.[8,9] Second, a *de novo* search was conducted by searching FlyBase [10] (release FB2016_03) for *D. melanogaster* genes annotated with relevant GO terms. Third, the established sets of mammalian cytoplasmic and mitochondrial translation factors [1-3,6,11,12] were compiled and their *D. melanogaster* orthologs were determined using the DIOPT integrative ortholog prediction tool.[13] These analyses identified a total of 64 canonical translation factors - 13 of which are identified/named for the first time here (including two-thirds of the mitochondrial complement), while a further 16 are renamed to use conventional nomenclature. Furthermore, the functional annotations for all identified genes have been updated to reflect current knowledge, resulting in the addition of >30 GO annotations and the removal of >60 GO annotations. These data are given in full in Supplementary Table 1 and are summarized in Tables 1 and 2.

Cytoplasmic translation factors

Initiation factors

Translation initiation involves the assembly of elongation-competent 80S ribosomes in which the anticodon of the initiator methionyl-tRNA is base-paired with the AUG start codon of the mRNA. (See refs. [1,6,14] for recent reviews and details of individual protein functions.) In brief, initiation begins with the formation of a ternary complex of initiator methionyl-tRNA and GTP-bound eukaryotic initiation factor (eIF)2, which then assembles into a preinitiation complex (PIC) with the small (40S) ribosomal subunit and eIF1, eIF1A, eIF3 and eIF5. Next, the PIC attaches to the capped region of the mRNA in a step that involves the poly(A)-binding protein (PABP), the helicase eIF4A (whose activity is enhanced by eIF4B and eIF4H), the cap-binding protein eIF4E, and the scaffold protein eIF4G. The PIC then scans along the 5′ untranslated region (UTR) to the initiation codon, at which point eIF5 promotes the conversion of eIF2 to its GDP-bound state, resulting in the displacement of most eIFs. The GTPase eIF5B then mediates the joining of the large (60S) ribosomal subunit to form the 80S ribosome, after which eIF5B is released together with eIF1A. The guanine exchange factor eIF2B is required to reload eIF2 with GTP, and may also dissociate an eIF5–eIF2 complex, in order that these factors may participate in subsequent rounds of initiation.

We identified a total of 41 genes encoding cytoplasmic eIF proteins in flies (Table 1). As in mammals, most eIF genes are present as single copies, including those encoding eIF1, eIF1A,[15] the 3 subunits of

Table 1. *D. melanogaster* cytoplasmic translation factors.

Symbol	AKA*	CG number**	Human ortholog (identity)***	Primary reference(s)****		
				Genetic identification	Molecular function analyses	Phenotypic analyses
Initiation factors						
eIF1		CG17737	EIF1 (70%)	—	—	—
eIF1A		CG8053	EIF1AX (78%)	15	—	—
eIF2α		CG9946	EIF2S1 (62%)	17	19	74
eIF2β		CG4153	EIF2S2 (56%)	18	—	—
eIF2γ		CG43665	EIF2S3 (28%)	16	—	—
eIF2Bα		CG7883	EIF2B1 (50%)	19	—	—
eIF2Bβ		CG2677	EIF2B2 (47%)	19	—	—
eIF2Bγ		CG8190	EIF2B3 (35%)	19	—	—
eIF2Bδ		CG10315	EIF2B4 (40%)	19	—	—
eIF2Bε		CG3806	EIF2B5 (32%)	19	19	—
eIF3a	eIF3-S10	CG9805	EIF3A (44%)	—	65	—
eIF3b	eIF3-S9	CG4878	EIF3B (50%)	—	65	—
eIF3c	eIF3-S8	CG4954	EIF3C (51%)	—	65	—
eIF3d1	eIF-3p66, eIF3-S7	CG10161	EIF3D (58%)	—	—	—
eIF3d2	eIF3-S7	CG4810	EIF3D (54%)	—	—	—
eIF3e	eIF-3p48, Int6, eIF3-S6	CG9677	EIF3E (60%)	75		—
eIF3f1	eIF3-S5	CG9769	EIF3F (48%)	—	—	—
eIF3f2	eIF3-S5	CG8335	EIF3F (37%)	—	—	—
eIF3g1	eIF3-S4, eIF3ga	CG8636	EIF3G (42%)	—	—	—
eIF3g2	eIF3-S4, eIF3gb	CG10881	EIF3G (41%)	—	—	—
eIF3h	eIF-3p40, eIF3-S3	CG9124	EIF3H (45%)	—	—	—
eIF3i	Trip1, eIF3-S2	CG8882	EIF3I (61%)	—	65	—
eIF3j	Adam, eIF3-S1	CG12131	EIF3J (40%)	—	65	—
eIF3k		CG10306	EIF3K (49%)	—	—	—
eIF3l		CG5642	EIF3L (53%)	—	—	—
eIF3m	Tango7	CG8309	EIF3M (47%)	—	—	—
eIF4A		CG9075	EIF4A2 (74%)	22,23	22	23
eIF4B		CG10837	EIF4B (29%)	20	20	20
eIF4E1	eIF4E, eIF4E1,2	CG4035	EIF4E1B (42%)	26,27,76	24	31
eIF4EHP	4EHP, eIF4E8	CG33100	EIF4E2 (47%)	24,29	24, 28-30, 77	29,30,78
eIF4E3		CG8023	EIF4E1B (45%)	24	24,32	31,32
eIF4E4		CG10124	EIF4E1B (44%)	24	24	—
eIF4E5		CG8277	EIF4E1B (45%)	24	24	—
eIF4E6		CG1442	EIF4E1B (52%)	24	24	—
eIF4E7		CG32859	EIF4E1B (43%)	24	24	—
eIF4G1	eIF4G	CG10811	EIF4G1 (27%)	37	—	31
eIF4G2	ofs	CG10192	EIF4G3 (21%)	36	36	31,35,36
eIF4H1	Rbp2	CG4429	EIF4H (34%)	—	—	—
eIF4H2		CG1340	EIF4H (25%)	—	—	—
eIF5		CG9177	EIF5 (49%)	—	—	—
eIF5B	IF2	CG10840	EIF5B (50%)	21		21
Elongation factors						
eEF1α1	Ef1α48D	CG8280	EEF1A1 (86%)	56	—	—
eEF1α2	Ef1α100E	CG1873	EEF1A1 (85%)	56	—	—
eEF1β		CG6341	EEF1B2 (58%)	—	—	—
eEF1γ		CG11901	EEF1G (58%)	57	—	57,72
eEF1δ		CG4912	EEF1D (50%)	—	—	—
eEF2		CG2238	EEF2 (78%)	58	—	—
eEF5	eIF5A	CG3186	EIF5A (68%)	59	—	59
eEFSec		CG9841	EEFSEC (42%)	60	—	60,79
Release/Termination factors						
eRF1		CG5605	ETF1 (87%)	62	—	62
eRF3	Elf	CG6382	GSPT1 (59%)	62	—	62
Recycling and reinitiation factors						
ABCE1	pix	CG5651	ABCE1 (77%)	64	65	64
MCTS1		CG5941	MCTS1 (58%)	66	66	66
DENR		CG9099	DENR (48%)	66	66	66
eIF2D	ligatin	CG31426	EIF2D (35%)	66	—	—

Note. Factors in bold have enriched expression in the testis (see text for details).
*Common alternative symbols, where these are significantly different from the symbol used in the first column.
**Unique FlyBase annotation symbol
***High scoring orthologous human gene, with percentage identity between the encoded proteins given in parentheses, as given at DIOPT[13]. Where two human genes give similar DIOPT orthology scores, the one with the highest percentage identity is shown.
****Major reference(s) identifying/characterizing the translation role of the *D. melanogaster* protein.

Table 2. *D. melanogaster* mitochondrial translation factors.

Symbol	AKA	CG number	Human ortholog (identity)	Primary Reference(s)		
				Genetic identification	Molecular function analysis	Phenotypic analysis
Initiation factors						
mIF2		CG12413	MTIF2 (46%)	—	—	—
mIF3		CG13163	MTIF3 (30%)	—	—	—
Elongation factors						
mEFTu1	EfTuM	CG6050	TUFM (62%)	—	—	—
mEFTu2		CG12736	TUFM (51%)	—	—	—
mEFTs		CG6412	TSFM (38%)	—	—	—
mEFG1	ico	CG4567	GFM1 (67%)	68	—	68
Release/Termination factors						
mRF1		CG5705	MTRF1L (43%)	—	—	—
Recycling factors						
mRRF1		CG4447	MRRF (35%)	—	—	—
mRRF2	EF-G2	CG31159	GFM2 (42%)	68	—	—

Note. See footnotes to Table 1.

eIF2,[16-18] the 5 subunits of eIF2B,[19] most eIF3 subunits, eIF4B,[20] eIF5 and eIF5B.[21] Flies also harbor a single gene encoding functional eIF4A,[22,23] whereas mammals possess 2 paralogs.[1,6] Of these single copy genes, *eIF2α* and *eIF2γ* are notable for being characterized as haplo-insufficient – *eIF2α* is a likely *Minute* locus, while *eIF2γ* appears to be haplo-lethal.[9]

Other eIFs are encoded by multiple genes in flies (Table 1). Most strikingly, there are 7 genes encoding eIF4E isoforms, as opposed to just 3 in mammals.[24,25] Fly eIF4E1 is the canonical form and is expressed ubiquitously.[24,26,27] eIF4EHP and eIF4E6 are exceptional in that they harbor amino acid substitutions that prevent binding to eIF4G [24]; indeed, eIF4EHP acts as a translational inhibitor of *caudal, hunchback* and *belle* mRNAs, and thus plays a key role in patterning the oocyte and the early embryo.[28-30] eIF4E3 is a testis-specific protein that is required for spermatogenesis and male fertility.[31,32] Although little functional information is available for eIF4E4, eIF4E5, eIF4E6 and eIF4E7, it is notable that their expression is also enriched in the male germline [33,34] (Supplementary Table 2), suggesting specialized roles in this tissue. Flies also have duplicate genes encoding eIF4G, eIF4H, and the eIF3 subunits eIF3d, eIF3f and eIF3g. Again, it is significant that the expression of one member of each of these pairs (eIF4G2, eIF4H2 eIF3d2, eIF3f2, and eIF3g2) is specifically enriched in the testis [33-36] (Supplementary Table 2). Of these 5 pairs, only eIF4G has so far been characterized functionally: eIF4G1 is the major, canonical form,[37] whereas eIF4G2 has divergent N- and C-termini and is essential for the proper meiotic divisions during spermatogenesis.[31,35,36]

An additional 6 proteins are classified as 'non-canonical' initiation factors (Supplementary Table 1).[1] Fly eIF2A (CG7414) is the ortholog of human EIF2A, which is an alternative initiator tRNA-binding protein, and fly NAT1 (CG3845) [38-40] is the ortholog of human eIF4G2 (also known as NAT1/p97/DAP5), which has sequence similarity to the C-terminus of EIF4G1. Both human proteins are implicated in mediating cap-independent (i.e. internal ribosome entry site, IRES) translation under conditions of cellular stress,[41,42] while fly NAT1 regulates translation of specific mRNAs important for germband extension, metamorphosis and circadian rhythm.[39,40] CG1582 appears to be the fly ortholog of the human DHX29 helicase, which promotes ribosomal scanning through highly structured 5′ UTRs.[43,44] Mxt (CG2950) appears to be an eIF4G analog that binds eIF3 and eIF4E and may serve as an alternative to the canonical eIF4G to promote translation in certain tissues.[45] It has no clear mammalian ortholog. PolyA-binding protein, encoded by the *pAbp* (*CG5119*) gene in flies,[46] is a multifunctional RNA-binding protein with an essential role in enhancing translation initiation.[47] Finally, fly eIF6 (CG17611) [48] is the ortholog of human EIF6, which has an indirect role in translation by preventing ribosomal subunit joining until large subunit biogenesis is complete.[49] In flies, eIF6 down-regulates Wnt signaling through selective inhibition of *β*-catenin translation.[48]

It is worth noting that a few other fly genes and proteins have acquired symbols or synonyms that suggest a role in translational initiation that they do not in fact possess. Two proteins, originally named eIF5A and eIF2D, have since been implicated in translation

elongation or recycling, respectively, rather than in the initiation phase - these cases are discussed in the relevant sections below. Fly eIF4AIII (CG7483) is the ortholog of mammalian EIF4A3 (88% identity) – this protein has sequence similarity to eIF4A but has no known initiation factor activity.[25] Rather, it has been demonstrated to be a core component of the exon junction complex that functions in nonsense-mediated mRNA decay.[50] CG4849 has been referred to as EFTUD2 or eEF2, implying a role in translation elongation, but despite sequence similarity to eEF2, it actually functions in pre-mRNA splicing.[51] Finally, eIF2C1 and eIF2C2 are obsolete designations for the Argonaute family proteins [52] (AGO1 and AGO2 in flies), while eIF5C is an obsolete term for the translational inhibitor now known as Krasavietz.[53]

Elongation factors

Translation elongation is the step-wise increase in the length of the growing peptide chain by the addition of single amino acids through the action of the ribosome, aminoacyl-tRNAs and 2 major eukaryotic elongation factors, eEF1 and eEF2. (See refs. [2,11] for recent reviews and details of individual protein functions.) Briefly, the eEF1 complex, comprising 4 subunits, catalyzes the delivery of aminoacyl-tRNA to the ribosome in a GTP-dependent manner – eEF1α is the main player in this process, while eEF1β and eEF1δ are guanine exchange factors for eEF1α, while eEF1γ is thought to have a structural role. eEF2 acts as the translocase to move the ribosome down the mRNA one codon at a time. Recently, the role of the protein originally designated eIF5A has been clarified, and it has been shown to function in the elongation phase. We therefore refer to it as eEF5 herein, as proposed in ref. [54] eEFSec is a specialized elongation factor that is required for decoding of the UGA stop codon as selenocysteine in mRNAs containing a specific SECIS element, resulting in the production of selenoproteins.[55]

Flies contain the same complement of genes encoding eEFs as mammals: 2 copies of *eEF1α*,[56] and one copy each of *eEF1β*, *eEF1γ*[57], *eEF1δ*, *eEF2*,[58] *eEF5* [59] and *eEFSec* [60] (Table 1). Of the 2 eEF1α isoforms, eEF1α1 is expressed at high levels in all tissues throughout development and appears to be the major form, while eEF1α2 expression is more restricted with peaks in the nervous system during pupal stages.[33,61] As in other eukaryotes, eEF5 is the only protein in flies known to harbor a hypusine residue, which is a post-translational modification of a lysine.[54,59] Importantly, the function of hypusine-modified eEF5 in translation elongation has been confirmed in flies.[59]

Release/termination factors

Translation termination occurs at the end of the coding sequence of the mRNA, when the ribosome encounters a stop codon - UAA, UAG or UGA (in the absence of a SECIS element). (See refs.[2,12] for further details.) Just 2 release factors are involved, eRF1 and eRF3. (Note that there is not a eukaryotic release factor named 'eRF2'.) eRF1 recognizes the stop codon and catalyzes the hydrolysis of the peptidyl-tRNA to release the completed polypeptide chain, while eRF3 enhances this process in a GTP-dependent manner.

As in mammals, the 2 fly eRFs are encoded by single-copy genes (Table 1). Interestingly, mutations in each of the fly genes were isolated in a genetic screen as 'nonsense suppressors' because disruption of eRF function results in increased read-through of a subset of nonsense mutations in other genes, leading to phenotypic suppression.[62]

Recycling and reinitiation factors

The process of recycling involves the disassembly and release of the 80S ribosome, deacylated tRNA and eRF1 from the mRNA. (See refs. [2,12,63] for further details.) The first step is the dissociation of the 60S ribosomal subunit, which is mediated by the ABCE1 ATPase. This is followed by the ejection of the deacylated tRNA and dissociation of the 40S subunit – this step appears to be stimulated either by a subset of canonical initiation factors, including eIF1, eIF1A and eIF3j, or through a second mechanism involving eIF2D (also known as ligatin) or the MCTS1–DENR complex. The separated ribosomal subunits then associate with initiation factors again to allow further rounds of initiation. The related process of translation reinitiation, where the termination reaction is followed by initiation on the same mRNA molecule, is mechanistically similar to recycling and at least some of the same factors are implicated.

Flies contain single copy genes encoding all these factors (Table 1). Notably, the first indications that ABCE1 (encoded by the *pixie* gene) had a role in translational regulation came through genetic studies in flies,[64] and follow-up studies were among the first to demonstrate the association of ABCE1 with eIFs and the 40S ribosome.[65] Furthermore, research in flies

has demonstrated a specific function of the MCTS1–DENR complex in translation reinitiation - mRNAs containing upstream open reading frames selectively require MCTS1–DENR for their proper translation in proliferating cells.[66]

Mitochondrial translation factors

The mitochondrial translation system of eukaryotic cells is much simpler than its cytoplasmic counterpart, comprising a total of just 9 canonical translation factors that are homologous to bacterial factors. (See refs. [3,5,67] for recent reviews and detailed information on the function of individual proteins.) All key components of the translation system in this organelle (i.e., ribosomal proteins, tRNAs, aminoacyl tRNA synthetases, in addition to translation factors) exist as mitochondrial-specific forms, distinct from their cytoplasmic equivalents.

The mitochondrial translation process may be summarized as follows. Initiation begins with the association of mitochondrial initiation factor 3 (mIF3) to the 28S small ribosomal subunit, followed by GTP-bound mIF2 and the initiator tRNA. (Note that there is not a mitochondrial initiation factor named 'mIF1'.) The substrate mRNA then binds, followed by association of the large 39S ribosomal subunit, GTP hydrolysis and exiting of the mIFs. The elongation phase proceeds through cycles of mEFTu-GTP bringing the aminoacyl-tRNA to the ribosome coupled with GTP hydrolysis – mEFTs acts to recharge mEFTu with GTP, and mEFG1 catalyzes the translocation of the ribosome along the mRNA to the next codon. The UAA or UAG mitochondrial stop codons are recognized by the MTRF1L release factor that acts to hydrolyse the peptidyl-tRNA bond and thereby liberate the completed polypeptide. Non-canonical release factors encoded by the mammalian *MTRF1, C12orf65* and *ICT1* genes are also thought to play a role in the termination step. Finally, the mitochondrial recycling factors mRRF1 and mRRF2 promote ribosomal dissociation and release of the deacylated tRNA and mRNA substrate.

As in mammals, flies have single copy genes encoding the initiation factors, mIF2 and mIF3, and the elongation factors mEFTs and EFG1 (Table 2). However, unlike mammals, flies have 2 distinct mEFTu proteins, one of which (mEFTu2) is enriched in the testis [33,34] (Supplementary Table 2). Of the fly mIFs and mEFs, only mEFG1 (encoded by the *iconoclast* gene) has been characterized to date.[68] Interestingly, it contains a nuclear localization sequence in addition to a mitochondrial targeting sequence, and may relay regulatory signals from the mitochondrion to the nucleus.[68]

A single release factor-encoding gene, *mRF1*, exists in flies that is orthologous to the mammalian genes, *MTRF1L* and *MTRF1* (Table 2). The fly orthologs of mammalian C12orf65 and ICT1 are CG30100 (44% amino acid identity) and CG6094 (39% identity), though these are omitted from the list of canonical factors presented here. Both mammalian and fly genomes harbor single copy genes encoding the 2 mitochondrial recycling factors, mRRF1 and mRRF2 (Table 2). None of these factors have been characterized in flies to date.

Conclusions

Translation factors have been studied in *D. melanogaster* for some 30 years, with many studies taking advantage of the tractability of this organism to probe gene function through genetic and phenotypic analyses. However, most of these investigations have focused on one or only a few translation factors. Prior to this report, several factors had not been clearly identified at all and a comprehensive and up-to-date overview was lacking. This survey identifies the full set of canonical translation factors in *D. melanogaster* and provides a systematic nomenclature for flies that is consistent with that used more broadly. In addition, we have improved the accuracy and quality of GO annotations of these factors – these improvements will directly assist the many studies that rely on such annotations to interpret data and guide research. Finally, we have compared the well-characterized set of mammalian translation factors to that in flies, and provided an overview of the published literature on the fly factors to date. This analysis reveals an expansion of certain translation factors compared to mammals, including a set of 11 testis-specific factors, indicating that specialized translation machinery operates in the male germline of *D. melanogaster* (also see ref.[9]). (There is no evidence for a similar enrichment of specific translation factors in the female germline [Supplementary Table 2]).

Together, the information and annotation improvements reported herein will facilitate access to existing

information on translation factors in flies and will aid further discoveries in the field. There are 3 areas in particular that would benefit from further study. First, it is apparent that ~50% of translation factors (including the majority of mitochondrial translation factors) are uncharacterized at the molecular and genetic level in flies (Tables 1 and 2). The study of these factors in the experimentally tractable fly system is likely to reveal new features of their canonical roles in a multicellular organism. Second, there are now many examples across species of 'translation factors' having non-translational functions, including several examples in flies such as: eIF3e promoting the neddylation of cullins [69]; eIF3m effecting apoptosome activity [70,71]; a role for eEF5 in autophagy regulation [59]; and eEFγ regulating organelle transport along microtubules.[72] The fly is an ideal genetic system to discover and dissect such alternative roles. Finally, we are not aware of any fly models of human diseases associated with defective translation factors.[4-6] Given the high evolutionary conservation of these proteins, and the proven utility of modeling human disease in *D. melanogaster*,[73] including those associated with defects in other components of the translational machinery,[7] we hope and expect that this avenue of research will expand in the near future.

Disclosure of potential conflicts of interest

The authors declare no competing or financial interests.

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Author contributions

S.J.M. and P.L. performed the analysis, H.A reviewed and revised GO annotations, S.J.M. prepared the manuscript, P.L. edited the manuscript.

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