How growth and size are controlled during animal development is an important question in biology. Several families of conserved cell–cell signaling pathways (e.g., Wnts, Hedgehog, Notch, BMPs, and receptor tyrosine kinase signaling) regulate organ size by controlling cell growth, proliferation, and survival (1). In addition, environmental factors such as nutrients, oxygen, and temperature influence tissue and organismal growth during development (1). The conserved target of rapamycin (TOR) kinase is perhaps the best-understood nutrient-dependent regulator of cell metabolism and growth in animals (2). The complex signaling network that couples extracellular nutrients to the activation of TOR has been extensively studied in simple systems such as yeast and in vitro cultured cells (3). However, it is studies in model organisms, most notably Drosophila, that have begun to reveal the role for nutrient/TOR signaling in the control of tissue and organismal growth (4). During a 4-d period of growth, Drosophila larvae increase in mass ~200-fold. This growth is dependent on nutrition—in particular, dietary protein. Because neither pupae nor adults grow, final body size is determined by both the rate of larval growth and the duration of the larval period (5). TOR signaling regulates body size by modulating these parameters of growth. For example, TOR signaling is cell-autonomously required for nutrition-dependent growth in all larval tissues (6, 7); loss of TOR function in individual cells or tissues leads to a subsequent reduction in cell size or tissue mass (6, 7). Importantly, modulation of TOR activity in specific tissues can also influence overall body size by exerting non–cell-autonomous, humoral effects on organismal growth and developmental timing (8, 9). For example, TOR signaling in the fat body couples dietary nutrients to systemic insulin signaling and body growth (9). Thus, in nutrient-rich conditions, amino acid import into fat cells promotes TOR activity, leading to the relay of a secreted factor(s) that triggers the release of several Drosophila insulin-like peptides (dILPs) from neurosecretory cells (NSC) in the brain (9). These dILPs then circulate throughout the animal and promote growth in all tissues. Similarly, TOR signaling in the prothoracic gland influences body size by modulating the release of the insect steroid hormone ecdysone, which controls the timing of pupation, and hence the duration of the larval period (8).

An important, but unresolved, issue concerns the identity of the key downstream effectors of nutrient/TOR-dependent animal growth. Stimulation of protein synthesis has been widely proposed as a growth-promoting output of the TOR pathway, based largely on studies in yeast and mammalian cell culture (2, 3, 10). The prevailing model is that nutrient/TOR signaling promotes protein synthesis via regulation of mRNA translation initiation. In particular, phosphorylation and inhibition of 4E-binding protein (4E-BP), a conserved repressor of eukaryotic initiation factor 4E (eIF4E), is thought to mediate many of TOR’s effects on translation (3, 10). However, genetic experiments in flies, worms, and mice suggest that 4E-BP has limited effects on tissue and organismal growth (11). Another mechanism by which TOR controls protein synthesis is by regulating the abundance of small noncoding RNAs, such as tRNA and 5S rRNA (12, 13). These RNAs are transcribed by RNA polymerase III (Pol III) and are essential for ribosome synthesis and mRNA translation (14). Recent reports in yeast and mammalian cell culture showed that nutrients and TOR signaling promote tRNA synthesis by inhibiting Maf1, a conserved repressor of Pol III-dependent tRNA transcription (15–17). However, the significance of the regulation of tRNA synthesis by Maf1 in the context of cell, tissue, and organismal growth during animal development has not been explored.

Here we identify a role for Drosophila Maf1 (dMaf1) as a regulator of nutrient-dependent growth and development. Importantly, we show that dMaf1 influences growth primarily in a non–cell-autonomous manner. During normal growth, when nutrients are abundant, inhibition of dMaf1 specifically in the fat body promotes tRNA synthesis, leading to increased organismal growth by stimulating systemic insulin signaling. Significantly, we identify a single transcript, the initiator methionine tRNA (tRNA_{Met}^\text{Met}) as the primary trigger of organismal growth in this context. 

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Results

Inhibition of dMaf1 Increases tRNA Synthesis, Accelerates Development, and Augments Body Size. We first confirmed that Drosophila Maf1 (dMaf1, CG40196) functions as a repressor of tRNA synthesis in vivo in larvae. Ubiquitous expression of a UAS-dMaf1 RNAi construct in larvae throughout development using the daughterless-GAL4 (da>) driver led to a reduction in dMaf1 protein levels (Fig. S1A) and a global increase in tRNA levels compared with controls (Fig. 1A). This result is consistent with a repressor function for dMaf1. Other Pol III-dependent transcripts were unaffected, no line alone, and the increase in tRNA synthesis was reversed by another independent insertion of the da>fl driver led to a reduction in dMaf1 protein levels (Fig. 1B). Because body size is determined by growth during the larval stage (5), pupal volume measurements provide an accurate index of both larval growth and adult body size. We observed a significant increase in pupal volume in da>dMaf1 RNAi pupae compared with controls (Fig. 1C and D). This increase in size was reproduced in another independent insertion of the dMaf1 RNAi transgene, as well as in two independent insertions of a dMaf1 RNAi transgene targeting an overlapping, but smaller, region of dMaf1 (Fig. S1E). Moreover, the increase in body size was reversed by overexpression of dMaf1, further confirming the specificity of the dMaf1 RNAi transgene (Fig. S1F). Overexpression of dMaf1 alone did not, however, have a dominant effect on tRNA synthesis or body size (Fig. S2). Thus, specific inhibition of dMaf1 is sufficient to augment tRNA levels and to promote an increase in organismal growth and the rate of development.

Elevated Synthesis of Single tRNA (Initiator Methionine) Is Sufficient to Drive Growth. As a repressor of tRNA synthesis, dMaf1 regulates the synthesis of all tRNAs. Any of these transcripts individually or in combination could potentially mediate the observed increase in larval growth rate and body size upon dMaf1 inhibition. A previous report, however, demonstrated that elevated levels of tRNA(Met) alone enhanced proliferation in cultured mammalian fibroblasts (18). We therefore hypothesized that levels of tRNA(Met) may be the limiting factor for the stimulation of growth and body size following inhibition of dMaf1. To test this hypothesis we made transgenic flies with a single P-element insertion, where the P element contains one extra copy of the tRNA(Met) gene, which we will refer to as P(tRNA(Met)). Levels of tRNA(Met); but not other tRNAs, were significantly elevated in P(tRNA(Met)) larvae (Fig. 2A). Strikingly, when we measured the development of these larvae we saw an accelerated growth rate and increased final size (Fig. 2B–D). We observed similar increases in growth in two independent P(tRNA(Met)) transgenic lines (Fig. S3A and B). In contrast, no increase in growth was observed in transgenic flies carrying a P element with an extra copy of other tRNAs, such as tRNA elongator methionine (P(tRNA(Met))), tRNA arginine (P(tRNA(Arg)))) (Fig. 2D and Fig. S3C), or tRNA alanine (P(tRNA(Ala))) (Fig. S3C). Thus, increased synthesis of only one tRNA, tRNA(Met), can phenocopy the effects of dMaf1 inhibition to drive growth and development in vivo.

Inhibition of dMaf1 and Elevated tRNA(Met) Synthesis Promotes Growth by Stimulating Protein Synthesis. It is remarkable that simply increasing the levels of a single tRNA can enhance organismal growth. However, tRNA(Met) plays a unique role among tRNAs in eukaryotic cells. It is assembled into a ternary complex along with eIF2 and GTP (19). This complex then associates with the 40S ribosome and other eIFs to trigger ribosome scanning of mRNAs and to initiate translation, and so it is possible that

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Fig. 1. Loss of dMaf1 increases growth rate and body size. (A) Levels of tRNA(Met), tRNA(Met), and tRNA(Met) were significantly elevated in da>dMaf1 RNAi larvae compared with da+ controls when measured by quantitative real-time PCR (P = 7.3 × 10⁻⁹; 0.0002 and 0.001, respectively, Student’s t test). Data were normalized to β-tubulin. (B) Time to pupation in da>dMaf1 RNAi larvae was decreased by 24 h compared with da+ controls (n > 200). (C and D) Pupal volume in da>dMaf1 RNAi flies was significantly greater than da+ controls (P = 2 × 10⁻¹²; Student’s t test, n = 58). (Scale bar: 1 mm.) Error bars represent SEM.

Fig. 2. Increased synthesis of tRNA(Met) stimulates growth. (A) Levels of tRNA(Met) and not other tRNAs were significantly elevated in P(tRNA(Met)) larvae compared with w¹¹¹⁸ controls when measured by quantitative real-time PCR (P = 0.0002; 0.36, and 0.21, respectively, Student’s t test). Data were normalized to β-tubulin. (B) The time to pupation in P(tRNA(Met)) larvae was decreased compared with w¹¹¹⁸ controls (n > 200). (C) Body size of P(tRNA(Met)) pupae is larger than controls. (Scale bar: 1 mm.) (D) Volume of P(tRNA(Met)) pupae (P = 1.3 × 10⁻⁹), not P(tRNA(Met)) or P(tRNA(Ala)) (P = 0.18 and 0.07, respectively), was significantly greater than w¹¹¹⁸ controls (Student’s t test, n = 90). Error bars represent SEM. N.D., not significantly different.
Inhibiting dMaf1 has a limited effect on cell-autonomous growth. A previous study showed that the stimulation of protein synthesis promoted the proliferation of cultured mammalian cells (18). Thus, the increase in body size caused by inhibition of dMaf1 may have resulted from cell-autonomous increases in cell size and/or cell number in all developing larval tissues.

We therefore explored whether stimulating tRNA synthesis could mediate cell-autonomous effects on growth in mitotic (imaginal discs, central nervous system) and/or polyploid (muscle, fat body, gut) tissues in the developing larvae. We first examined cell size and proliferation in the adult wing, which develops from the larval wing imaginal disc, a mitotic tissue. Because tRNA^{Met} alone is not sufficient to control protein synthesis and growth during animal development.

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**Nutrient Availability Inhibits dMaf1 in the Fat Body to Promote tRNA Synthesis and Stimulate Organismal Growth.** The larval fat body functions as both a sensor of nutritional status and an endocrine organ (21). In nutrient-rich conditions, the fat body signals to the brain to stimulate dILP release and promote systemic insulin signaling and growth (9). A major trigger for this fat-to-brain signal is nutrient/TOR signaling and protein synthesis in fat cells (22). We therefore hypothesized that the organismal effects of dMaf1 inhibition may arise from loss of dMaf1 function specifically in the fat body. In particular, we explored whether nutrient availability might stimulate systemic growth by inhibiting dMaf1 in fat cells to promote tRNA synthesis. We first found that levels of tRNA synthesis in the fat body of feeding larvae were significantly higher than in fat bodies from larvae starved of amino acids for 24 h (Fig. 4A). Levels of tRNA synthesis were also decreased upon starvation in the muscle (Fig. S6A). Furthermore, we found that in feeding larvae, silencing of dMaf1 specifically in the fat body. In particular, we explored whether nutrient availability might stimulate systemic growth by inhibiting dMaf1 in fat cells to promote tRNA synthesis. We first found that levels of tRNA synthesis in the fat body of feeding larvae were significantly higher than in fat bodies from larvae starved of amino acids for 24 h (Fig. 4A). Levels of tRNA synthesis were also decreased upon starvation in the muscle (Fig. S6A). Furthermore, we found that in feeding larvae, silencing of dMaf1 specifically in the fat body.

**Fig. 3. Elevated tRNA synthesis stimulates mRNA translation.** (A) Total protein per larva was significantly increased in P(tRNA^{Met}) larvae compared with w^{1118} controls when normalized for DNA (P = 0.008; Student’s t test). (B) Representative polysome profiles from w^{1118} (black trace) and P(tRNA^{Met}) larvae (green trace). Polysome peaks (arrowheads) in P(tRNA^{Met}) larvae were higher compared with controls, suggesting translation was increased, and percent of total RNA contained in the polysome fraction was significantly increased in P(tRNA^{Met}) larvae (P = 0.043; Student’s t test; see Fig. S4A for graph). Increased size in (C) da > dMaf1 RNAi (P = 6.1 × 10^{-13}; Student’s t test) and (D) P(tRNA^{Met}) pupae (P = 6.3 × 10^{-14}; Student’s t test) was suppressed when heterozygous for a null allele of ribosomal protein S3 (RPS3; P = 0.44 and 0.115, respectively, Student’s t test, n > 80). Error bars represent SEM.

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RNAi larvae was unaffected following 48 h of rapamycin treatment (Fig. 4C). These data demonstrate that nutrient/TOR signaling normally promotes tRNA synthesis in the fat body via dMaf1 inhibition. We therefore asked whether genetic inhibition of dMaf1 in the fat body could influence organismal growth and final size. When we expressed the dMaf1 RNAi transgene using cg-GAL4 (cg+), we phenocopied the increased larval growth rate and body size caused by a ubiquitous increase in tRNA synthesis. Thus, cg>dMaf1 RNAi larvae progressed from egg laying to pupation ~16 h faster than control larvae (Fig. 4D). Similar results were obtained using a second fat body-specific GAL4 driver, ppl-GAL4 (Fig. 4E), but not with other tissue-specific drivers (Fig. 4F and G and Fig. S7A and B). We also found that pupal volume was significantly increased in cg>dMaf1 RNAi animals compared with controls (Fig. S7C). Together, these results support our hypothesis that when nutrients are abundant, amino acid uptake into the fat body stimulates tRNA synthesis and consequently organismal growth via inhibition of dMaf1.

**Loss of dMaf1 in the Fat Body Stimulates Systemic Insulin Signaling.** Previous reports have shown that fat body-mediated effects on organismal growth occur via stimulation of systemic insulin signaling (9, 22). We therefore tested whether inhibition of dMaf1 in the fat body led to alterations in systemic insulin signaling. dILP 2 and dILP 5 are two brain-derived growth-promoting dILPs whose expression and release from the NSCs is increased by the fat-to-brain signal (9, 23, 24). When we expressed dMaf1 RNAi specifically in the fat body (cg>dMaf1 RNAi), we found elevated transcript levels of both dILP2 and dILP5 in peripheral tissues of these larvae compared with controls (Fig. 5A and B). This increase in dILP2 mRNA is due at least in part to increased transcript levels in the brain (Fig. S7D). When insulin signaling is high, the PI3K-Akt pathway inhibits the FOXO transcription factor, leading to reduced levels of FOXO transcriptional targets, such as Drosophila insulin receptor (dInR) (25). Therefore,
measuring dhnR mRNA levels is used as a readout of insulin signaling (9, 22). We found that dhnR transcripts were significantly decreased in peripheral tissues of cg-dMaf1 RNAi larvae compared with control larvae (Fig. 5C), suggesting an increase in insulin-Pi3K signaling. Importantly, we observed similar increases in both dILP levels and systemic insulin signaling in P(tRNAiMet) transgenic larvae (Fig. 5 D and E). Finally, we found the increased body size in da-Maf1 RNAi and P(tRNAiMet) flies observed was dependent on insulin signaling because, in both cases, the phenotype was suppressed in pupae heterozygous for either a null allele of dhnR or for a chromosomal deficiency that deletes the dlp-2, -3, and -5 genes (Fig. 5 F and G). Together these data support a model in which nutrient-dependent inhibition of dMaf1 in the fat body can promote systemic growth and body size by increasing both dILP expression and peripheral insulin signaling (Fig. 5H).

Discussion

In most animal model systems, TOR signaling couples nutrition to organismal growth. Nevertheless, the key metabolic effectors of TOR remain unclear. In our study, we identify the repression of dMaf1 as a downstream function of nutrients/TOR in the control of tissue and organismal growth. We demonstrate that nutrients promote tRNA synthesis during development by inhibiting dMaf1. This result is consistent with previous observations in yeast and cell culture experiments, showing that nutrient/TOR signaling directly inhibits Maf1 to promote tRNA synthesis (15–17). By investigating the significance of this repression in a developing animal, however, we define a role for dMaf1 as a repressor of tissue and organismal growth. Furthermore, we showed that simply increasing the synthesis of a single tRNA, tRNAiMet, could phenocopy the effect of dMaf1 inhibition. Based on these findings, we therefore propose a model of growth control in which nutrient/TOR signaling inhibits dMaf1 to stimulate tRNAiMet synthesis, ultimately driving development and promoting organismal growth (Fig. 5H).

The effect of elevated tRNAiMet synthesis on body size was associated with increased mRNA translation and was reversed by genetically reducing protein synthesis. As discussed in the introduction, the prevailing view is that inhibition of 4E-BP is a key target of nutrient/TOR signaling in the control of mRNA translation and growth. 4E-BP normally functions to inhibit eIF4E-mediated translation initiation, in turn controlling the rate of mRNA translation on body size. A growing literature suggests, however, that modulating protein synthesis has selective effects on mRNA translation. For example, in both yeast and mammalian cells, alterations in nutrient/TOR signaling lead to differential mRNA translation of genes important for growth, stress responses, and aging (33, 34). We therefore propose that the main consequence of increased tRNAiMet synthesis in the fat body is enhanced translation of specific mRNAs, ultimately leading to increased fat-to-brain signaling and organismal growth. The nature of the secreted factor(s) that signals from the fat to brain is unclear. However, perhaps either the factor itself (if it is a peptide) or genes required to synthesize or transport it is developmentally regulated by nutrient-dependent signaling in the fat body.

As well as increasing body size, dMaf1 inhibition and increased tRNAiMet synthesis also accelerated development. In insects, release of the steroid hormone ecdysone from the prothoracic gland (PG) is the primary regulator of developmental timing. Premature release of ecdysone accelerates progression through larval stages, leading to precocious pupation and smaller-sized adults (35–37). In contrast, delayed ecdysone release slows progression through larval stages, leading to late pupation and larger adults (36, 37). Dietary nutrition is an important regulator of ecdysone release and, consequently, developmental timing (8, 35–37). For example, several studies have demonstrated that increased P13K and TOR signaling, two key effectors of dietary nutrients, within the PG can stimulate the release of ecdysone (35, 36). Conversely, reducing P13K or TOR signaling in the PG delays ecdysone release and pupation (8, 36, 37). Finally, a recent study showed that increased expression of dILP2 in neurosecretory cells led to premature release of ecdysone and a significant acceleration of development (38). We therefore propose that inhibition of dMaf1 in the fat body accelerates development through effects on systemic insulin. This increase in insulin signaling would stimulate growth in all tissues and activate P13K signaling in the PG to trigger ecdysone release and precocious pupation. Together these effects promote both an overall increase in larval growth rate coupled with a shortening of the period of larval development.
In conclusion, our study has identified inhibition of tRNA synthesis by dMaf1 as a limiting factor for nutrition/TOR-induced tissue and organismal growth. These findings have several interesting implications for human biology. For example, deregulation of tRNA synthesis is observed in nearly all tumors (14). Our data, in combination with the previous study in mammalian organismal growth. These contexts, such as cancer, diabetes, and aging (10, 39–41), future studies on the regulation of tRNA synthesis may provide valuable insights into disease processes and progression.

Methods

**Fly Strains.** The following fly stocks were used: w1118, UAS-GFP, en-GAL4, yw; dinoF173M.5b, da-GAL4, c-gal-GAL4, ppi-GAL4, ey-GAL4, dMEf2-GAL4, elav-GAL4, dlp2-GAL4, F(3L)lp2–3,5TMTM5b, RPS31TM6B, Tu, Hsu, hsflp125C/+, and w1118;act >CD2-GAL4.

11. Teleman AA, Chen YW, Cohen SM (2005) 4E-BP functions as a metabolic brake used to tune translation and systemic insulin signaling. Because deregulation of both of these processes is a common occurrence in many pathological contexts, such as cancer, diabetes, and aging (10, 39–41), future studies on the regulation of tRNA synthesis may provide valuable insights into disease processes and progression.

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Pupal Volume. Pupal volume was calculated as described in Delanoue et al. (22).

Quantitative RT-PCR. Total RNA was extracted from larval tissues using TRIzol (Invitrogen), and cDNA was synthesized using SuperScript II (Invitrogen) according to manufacturer’s instructions. Additional details of experimental procedures are provided in SI Methods.

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Supporting Information

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SI Methods

Egg Collection. Adult flies laid eggs on grape juice agar plates supplemented with yeast paste for 4 h at 25 °C. After 24 h the plates were cleared of larvae, and larvae hatching within 4 h were placed in food vials in groups of 50 larvae.

Transgenic Flies. DNA from P-element–based transformation vectors containing relevant inserts were sent to Best Gene Inc. for injection into w[1118] Drosophila embryos. Lines containing single P-element insertions were made homozygous and crossed to the indicated drivers (UAS-dMaf1 RNAi, UAS-HA-Maf1) or used directly in experiments (tRNA flies). Thus, P(tRNA Met), P(tRNA Arg), P(tRNA Ala), and P(tRNA Arg) flies used in experiments are homozygous for a single P-element insertion (where each P element contains one copy of the indicated tRNA).

Pupation Rates. The number of pupae per vial was counted every 12 h. The mean number of pupae was calculated per time point for each genotype by taking the average of several vials.

Starvation. Larvae were collected for starvation 72 h after egg laying (AEL) and starved in 20% sucrose in PBS for 24 h.

Rapamycin Treatment. Larvae were collected for treatment 48 h after egg laying (AEL) and removed the supernatant without disturbing the top layer of fat. A total of 400 μL of 0.025 mM solution in DMSO for a further 48 h before dissection. Larvae were fed rapamycin or DMSO in food consisting of 3 g Formula 4-24 Instant Drosophila Medium (21.32 g instant media added to 71.52 mL dH2O; Carolina Biological Supply Co.) added to 1 g autoclaved yeast (in water).

Flow Cytometry. FACS analysis was performed as described previously (1).

Cell Size and Number Counts. The number of cells in the posterior adult wing was estimated by counting the number of wing hairs (one per cell) in a fixed area. This number was multiplied by the number of times the fixed region fit into the total compartment area. Cell size in the adult wing was estimated by dividing the compartment area by the number of cells. Fat body cell area was measured with the Zeiss Axiovision software. We determined whether loss of dMaf1 gave fat cells a growth advantage by taking the area of individual GFP+ (dMaf1 RNAi) cells and divided it by the area of a neighboring cell two cell diameters away.

Larval Protein Content. Five groups of 10 larvae were homogenized in 150 μL of lysis buffer (20 mM Hepes (pH 7.8), 450 mM NaCl, 25% glycerol, 50 mM NaF, 0.2 mM EDTA, 0.5% Triton X-100, 1 mM PMSF, 1 mM DTT, 1× Complete protease inhibitor mixture, and 1× PhosSTOP phosphatase inhibitor tablets [Roche]). Protein concentration was measured using a Bradford Assay, and five technical replicates per group of 10 larvae were performed.

Larval DNA Content. Genomic DNA was extracted from five groups of 10 larvae as previously described (2).

Quantitative RT-PCR. Total RNA was extracted, and cDNA was synthesized from larval tissues as described previously (2). Quantitative RT-PCR (qRT-PCR) experiments (SYBR Green PCR mix, MyIQ PCR machine; BioRad Laboratories) were performed using specific primer pairs (sequences available upon request). For qRT-PCR on RNA extracted from whole larvae, four groups of 10 larvae per genotype were collected 72 h AEL. Although data presented is normalized to β-tubulin, when the data were normalized to other genes the results were not changed. For qRT-PCR on RNA extracted from larval carcasses devoid of fat tissue, four groups of eight carcasses per genotype were dissected and collected into TRIzol at 96 h AEL. For qRT-PCR on RNA extracted from larval CNS, four groups of eight CNS per genotype were dissected and collected into TRIzol at 72 h AEL. For qRT-PCR on RNA extracted from fat tissue, four groups of eight fat bodies per genotype were dissected and collected into TRIzol at 96 h AEL. Data were normalized to β-tubulin. All qRT-PCR experiments were repeated a minimum of three times. Reference genes (e.g., β-tubulin) were chosen because transcript levels were unchanged in response to a variety of manipulations (3). All data were analyzed by Student’s t tests.

DNA Cloning. dMaf1 RNAi was amplified from cDNA (LD17963) using the primers ACACAACAGTGCCATCAGCA and GT-TTCACCCGCTGATAGAG. Thus, and cloned into pUAST. A second dMaf1 RNAi fragment, targeting an overlapping, but smaller, region of dMaf1, was amplified using the primers ACACAACAGTGCCATCAGCA and CATAGACCTGCAATGAC, and cloned into pUAST. The tRNA Met, tRNA Ala, tRNA Arg, and tRNA Met genomic fragments were amplified using the following primers: CAGTACCCGTCACACAGT and GAATAGCTGATCCTGATAG (tRNA Met); ACTTTCACTACGCCCGAAC- AGCG and TCCAAAGTGTAGAAGGCG (tRNA Met); GTCCCG- TCACAATGAAAAT and TCTTCCGACCTGAGC (tRNA Met); and CGTGGCCACTCTTTGAAAC and CGAAC- ATATGTTTTTCTACAGT (tRNA Met), and cloned into NolI sites in pCasper4 (4). dMaf1 overexpression constructs were amplified from LD17963 with flanking sequences for insertion into the vector pTHW (5′ Ha tag) from the Drosophila Gateway Vector collection plasmids (T. Murphy, Carnegie Institution of Washington, Washington, DC) using the following primers: GGGGACACATTTGATCAAAAAAGACAGCCTCGGCCGGC- CCCCTTCTACCATGAGCTCTTCAGTGAATGG and GGGGCACCTTGTACAGGAACTGGTGTCACATCTAC- ACATTTCCTTCTTTACAGG.

Polysome Gradient Centrifugation. Larvae (0.25 mL) were collected 72 h AEL and washed several times in double-distilled H2O to remove food debris. Larvae were lysed in 3 vol of polysome lysis buffer (25 mM Tris (pH 7.4), 10 mM MgCl2, 250 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.5 mM DTT, 100 mM cycloheximide, 1 mg/mL heparin, 1× Complete mini protease inhibitor mixture (Roche), 2.5 mM PMSF, 5 mM sodium fluoride, 1 mM sodium orthovanadate, and 200 U/mL Ribolock RNase inhibitor (Fermentas)]. To identify polysomal fractions, ribosomes were dissociated by lysing larvae in buffer as described above without MgCl2, and with the addition of 5 mM EDTA. Cleared lysates were prepared by centrifuging total larval lysate for 10 min at 15,000 × g and removing the supernatant without disturbing the top layer of fat. A total of 400 μg total RNA was loaded onto a 15–45% sucrose gradient (wt/wt) in polysome buffer [25 mM Tris (pH 7.4), 10 mM MgCl2, 250 mM NaCl, 1 mg/mL heparin, 100 μg/mL cycloheximide] made in 11-mL polyallomer tubes (Beckman) and spun in a Beckman Coulter Optima L-90k Ultracentrifuge for 150 min at 37,000 rpm (Beckman Coulter rotor SW-41). Polysome profiles were ob-
tained by running the gradient through a continuous UV detector (ISCO UA6) at a wavelength of 254 nm to give a trace (the polysome profile) showing the OD-corresponding total RNA, from the top of the gradient (ribosome subunits) to the bottom (polyribosome-loaded mRNA). Profiles are representative of traces obtained from three independent experiments.

**Polysome Quantification.** Polysome content was quantified by measuring the total area under the curve, as well as the area under the polysome portion of the curve for matched sets of control w^1118^ and P{tRNA^Met^} samples using ImageJ software (5). The percent of total RNA associated with the polysome fraction was calculated by dividing the area under the polysome curve by the total area under the curve and multiplying by 100%. The increase in polysome content was calculated based on data from six independent experiments.

**Western Blotting.** Samples were prepared for SDS/PAGE and immunoblotting by homogenizing groups of 10 larvae 72 h AEL in 60 μL 4× protein sample buffer using a motorized pestle. A total of 20 μL of sample was loaded into each well. SDS/PAGE and immunoblotting were performed as described in Marshall et al. (6). Anti-dMaf1, anti-β-tubulin (E7; DSHB), and anti-HA (6E2; Cell Signaling) were used at 1:1,000. HRP-conjugated secondaries were used at 1:10,000.

**dMaf1 Antibody Production.** A peptide, corresponding to amino acids 75–87 of dMaf1 (NNSQSGDEGITLC), was synthesized by University of Calgary Peptide Synthesis Service. The peptide was conjugated to keyhole limpet hemocyanin (KLH) using the Inject Maleimide-Activated Mariculture KLH Kit (Pierce Protein Research Products, Thermo Scientific) according to manufacturer’s instructions and then used for immunization. The antibody was affinity purified using the SulfoLink Immobilization Kit (Pierce Protein Research Products, Thermo Scientific) according to manufacturer’s instructions.

**Microscopy and Computing.** Images were obtained on a Zeiss Discovery.V8 stereomicroscope (pupal volume) or Zeiss Observer.Z1 (fat body pictures). Brightness and contrast were adjusted using Adobe Photoshop CS4.

Fig. S1. Several independent dMaf1 RNAi lines increase body size and can be rescued by overexpression of dMaf1. (A) Immunoblotting of larval extracts shows a decrease in dMaf1 protein levels following ubiquitous expression of the dMaf1 RNAi transgene. β-tubulin was used as a loading control. (B) Loss of dMaf1 had no effect on transcript levels of 5S rRNA (P = 0.37), as measured by quantitative real-time PCR. Data were normalized to β-tubulin. (C) UAS-dMaf1 RNAi line has no effect on tRNA\textsubscript{Met}, tRNA\textsubscript{Ile}, or 5S rRNA synthesis in the absence of a GAL4 driver. (D) Ubiquitous overexpression of dMaf1 (UAS-HA-dMaf1-5M) reversed the increase in tRNA synthesis observed upon inhibition of dMaf1. Synthesis of tRNA\textsubscript{Met} was significantly lower than larvae expressing the dMaf1 RNAi transgene alone, and not significantly different from da++ larvae (P = 0.00019 and 0.42, respectively, Student’s t test). (E) A significant increase in body size was observed with an independent insertion of the UAS-dMaf1 RNAi transgene, as well as in two other UAS-dMaf1 RNAi lines directed against an overlapping, but smaller, region of the dMaf1 locus (P = 4.1 × 10\textsuperscript{-9}, 1.2 × 10\textsuperscript{-10}, and 4 × 10\textsuperscript{-5}, respectively, Student’s t test). (F) Ubiquitous overexpression of a UAS-dMaf1 transgene rescued the increase in body size observed upon inhibition of dMaf1. Pupae coexpressing the dMaf1 RNAi and dMaf1 overexpression transgenes were not significantly larger than da++ control pupae (P = 0.23, Student’s t test). Overexpression of a dMaf1 transgene had no effect on body size (P = 0.27, Student’s t test). n > 90 for all pupal volume measurements. Error bars represent SEM. N.D., not significantly different.
Fig. 52. Overexpression of dMaf1 has no effect on tRNA synthesis or body size. (A) Immunoblotting of larval extracts with anti-dMaf1 (to detect endogenous dMaf1 protein) and anti-HA (to detect HA-tagged overexpressed protein) confirms dMaf1 overexpression. β-tubulin was used as a loading control. (B) Overexpression of either of two independent insertions of the dMaf1 overexpression transgene had no significant effect on transcript levels of tRNA\textsubscript{Met}, tRNA\textsubscript{Ile}, or tRNA\textsubscript{Leu} (\(P = 0.15\) and 0.27 (tRNA\textsubscript{Met}), \(P = 0.23\) and 0.30 (tRNA\textsubscript{Ile}), and \(P = 0.07\) and 0.26 (tRNA\textsubscript{Leu})), respectively, Student’s \(t\) test. (C) Overexpression of any of three independent insertions of the dMaf1 transgene had no effect on body size. (D) Overexpression of the dMaf1 transgene specifically in the fat body had no effect on body size (\(P = 0.24\), Student’s \(t\) test). Error bars represent SEM. N.D., not significantly different.

Fig. 53. Elevated levels of tRNA\textsubscript{Met}, and not other tRNAs, stimulate growth. (A and B) Pupal volume was significantly greater in two further independent transgenic lines with an extra copy of tRNA\textsubscript{Met}: \(P(\text{tRNA\textsubscript{Met}}\text{-3Mb}) = 6.32 \times 10^{-7}\) and \(P(\text{tRNA\textsubscript{Met}}\text{-1M}) = 8.5 \times 10^{-17}\). (C) Body size was unchanged in pupae with an extra copy of tRNA\textsubscript{Met}, tRNA\textsubscript{Ala}, and tRNA\textsubscript{Arg}. (Scale bar: 1 mm.) Error bars represent SEM.
Fig. S4. Elevated tRNA\textsuperscript{iMet} synthesis promotes mRNA translation. (A) Percent of total RNA in the polysome fraction is significantly increased in P(tRNA\textsuperscript{iMet}) larvae compared with w\textsuperscript{1118} control larvae. (B) Polysome profile from w\textsuperscript{1118} larvae lysed using normal Mg\textsuperscript{2+}-containing buffer (dashed gray line). Polysome profile from w\textsuperscript{1118} larvae lysed in buffer lacking Mg\textsuperscript{2+} (black line), which disassembles ribosomes into 40S and 60S subunits.

Fig. S5. tRNA\textsuperscript{synthetase} stimulates growth via a cell-autonomous mechanism. (A) Loss of dMaf1 in the posterior compartment of the wing (en\textgreater dMaf1 RNAi) significantly increases the posterior:anterior (P:A) ratio (\(P = 0.0014\), Student’s t test; \(n > 30\)). (B) Cell number in the posterior wing was significantly increased upon loss of dMaf1 (\(P = 0.000161\), Student’s t test; \(n > 30\)). (C) Cell area was not significantly different (N.D.) in en\textgreater dMaf1 RNAi wings compared with en\textgreater controls (\(P = 0.38\), Student’s t test). (D) en\textgreater dMaf1 RNAi cells were not different in size from control en\textgreater cells when cell volume was measured by flow cytometry. (E) Mosaic expression of dMaf1 RNAi (GFP\textsuperscript{+} cells) in the fat body showed no increase in cell size (\(P = 0.113\); \(n > 90\) per genotype). dMaf1 RNAi-expressing cells are marked by presence of GFP (arrowheads). (F) Mosaic expression of dMaf1 RNAi (GFP\textsuperscript{+} cells) in the fat body showed no increase in cell size. The ratio of a GFP\textsuperscript{+} cell to a neighboring GFP\textsuperscript{−} cell was measured in a control cross (act\textgreater +, genotype hs\textsuperscript{fip},actin\textgreater CD2\textgreater GAL4;CD2\textgreater UAS-GFP) and compared with the ratio in experimental cross (act\textgreater dMaf1 RNAi, genotype hs\textsuperscript{fip},actin\textgreater CD2\textgreater GAL4;dMaf1 RNAi/UAS-GFP). The dMaf1 RNAi-expressing cells were not significantly larger (N.D.) than their neighbor cells (\(P = 0.113\); \(n > 90\)). Error bars represent SEM.
Fig. S6. Loss of dMaf1 in the fat body bypasses starvation-induced decrease in tRNA synthesis. (A) Levels of tRNA<sup>Met</sup>, tRNA<sup>Leu</sup>, and tRNA<sup>Ile</sup> are significantly decreased in the muscle following starvation (P = 0.0004, 0.0001, and 0.001, respectively, Student’s t test). (B) Levels of tRNA<sup>Met</sup> and tRNA<sup>Leu</sup> were significantly elevated in cg>dMaf1 RNAi larvae compared with cg> controls (P = 0.015 and P = 0.022, respectively, Student’s t test). Levels of tRNA<sup>Met</sup> and tRNA<sup>Leu</sup> remained significantly elevated in cg>dMaf1 RNAi larvae compared with cg> controls following 24-h starvation in 20% sucrose in PBS (3 × 10<sup>−5</sup> and P = 4 × 10<sup>−6</sup>, respectively, Student’s t test). (C) Levels of tRNA<sup>Met</sup> and tRNA<sup>Leu</sup> were significantly elevated in cg>dMaf1 RNAi larvae compared with cg> controls with UAS-dMaf1 RNAi transgenic line directed against an overlapping, but smaller, region of dMaf1 (P = 0.024 and P = 0.027, respectively, Student’s t test). Levels of tRNA<sup>Met</sup> and tRNA<sup>Leu</sup> remained significantly elevated in cg>dMaf1 RNAi larvae compared with cg> controls following 24-h starvation in 20% sucrose in PBS (2.7 × 10<sup>−5</sup> and P = 2.4 × 10<sup>−5</sup>, respectively, Student’s t test), and were not significantly different from tRNA levels in cg>dMaf1 RNAi larvae in fed conditions (P = 0.37 and P = 0.48 respectively, Student’s t test). Error bars represent SEM.

Fig. S7. Loss of dMaf1 in eye or insulin-producing cells does not affect rate of larval growth. (A and B) The time to pupation was not different from controls in larvae with (A) eye- or (B) insulin-producing cell-specific expression of dMaf1 RNAi (n > 500 for both GAL4 drivers). (C) Pupal volume in cg>dMaf1 RNAi animals was significantly greater than controls (P = 0.0068, Student’s t test). (D) Expression of dilp2 mRNA was significantly increased in the CNS in cg>dMaf1 RNAi larvae compared with cg> control larvae (P = 0.004, Student’s t test). Error bars represent SEM.