Unexpected Role for Dosage Compensation in the Control of Dauer Arrest, Insulin-Like Signaling, and FoxO Transcription Factor Activity in *Caenorhabditis elegans*

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ABSTRACT During embryogenesis, an essential process known as dosage compensation is initiated to equalize gene expression from sex chromosomes. Although much is known about how dosage compensation is established, the consequences of modulating the stability of dosage compensation postembryonically are not known. Here we define a role for the *Caenorhabditis elegans* dosage compensation complex (DCC) in the regulation of DAF-2 insulin-like signaling. In a screen for dauer regulatory genes that control the activity of the FoxO transcription factor DAF-16, we isolated three mutant alleles of *dpy-21*, which encodes a conserved DCC component. Knockdown of multiple DCC components in hermaphrodite and male animals indicates that the dauer suppression phenotype of *dpy-21* mutants is due to a defect in dosage compensation *per se*. In *dpy-21* mutants, expression of several X-linked genes that promote dauer bypass is elevated, including four genes encoding components of the DAF-2 insulin-like pathway that antagonize DAF-16/FoxO activity. Accordingly, *dpy-21* mutation reduced the expression of DAF-16/FoxO target genes by promoting the exclusion of DAF-16/FoxO from nuclei. Thus, dosage compensation enhances dauer arrest by repressing X-linked genes that promote reproductive development through the inhibition of DAF-16/FoxO nuclear translocation. This work is the first to establish a specific postembryonic function for dosage compensation in any organism. The influence of dosage compensation on dauer arrest, a larval developmental fate governed by the integration of multiple environmental inputs and signaling outputs, suggests that the dosage compensation machinery may respond to external cues by modulating signaling pathways through chromosome-wide regulation of gene expression.

IN the nematode *Caenorhabditis elegans*, the DAF-2 insulin-like growth factor receptor (IGFR) ortholog promotes reproductive development and aging by inhibiting the FoxO transcription factor DAF-16 through the AGE-1 phosphoinositide 3-kinase (PI3K) and the conserved kinases PDK-1, AKT-1, and AKT-2 (Fielenbach and Antebi 2008; Kenyon 2010). *daf-2* mutants were first isolated in genetic screens for dauer-constitutive mutants (Riddle *et al.* 1981). In replete environments, hatched embryos develop reproductively by traversing four larval stages (L1–L4) prior to adulthood. Under conditions of increased population density, reduced food availability, or elevated temperature, L1 larvae enter a distinct developmental pathway that culminates in arrest as an alternative, long-lived, morphologically distinct third larval stage known as dauer (Riddle 1988). *daf-2/IGFR*, *age-1/PI3K*, *pdk-1*, and *akt-1* loss-of-function mutants all have dauer-constitutive phenotypes; *i.e.*, they undergo dauer arrest under conditions in which wild-type animals develop reproductively (Riddle *et al.* 1981; Vowels and Thomas 1992; Gottlieb and Ruvkun 1994; Morris *et al.* 1996; Kimura *et al.* 1997; Paradis *et al.* 1999; Ailion and Thomas 2003). The dauer-constitutive phenotype of these mutants requires DAF-16/FoxO, as loss of *daf-16/FoxO* func-

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tion fully suppresses dauer arrest in *daf-2/IGFR*, *age-1/PI3K*, *pdk-1*, and *akt-1* mutants (Vowels and Thomas 1992 ; Gottlieb and Ruvkun 1994; Larsen *et al.* 1995; Paradis *et al.* 1999; Ailion and Thomas 2003). Taken together, these data indicate that the DAF-2/AGE-1/PDK-1/AKT-1 pathway promotes reproductive development by inhibiting DAF-16/FoxO.

Two other conserved signaling pathways play important roles in dauer regulation. The transforming growth factor-β (TGFβ)-like ligand DAF-7 (Ren *et al.* 1996) inhibits dauer arrest in parallel to the DAF-2/IGFR pathway by signaling through the type I TGFβ receptor homolog DAF-1 (Georgi *et al.* 1990) and the type II receptor homolog DAF-4 (Estevez *et al.* 1993) to regulate the SnoN homolog DAF-5 (Da Graca *et al.* 2004; Tewari *et al.* 2004) and the SMAD homologs DAF-3, DAF-8, and DAF-14 (Patterson *et al.* 1997; Inoue and Thomas 2000; Park *et al.* 2010). Downstream of the DAF-2/IGFR and DAF-7/TGFβ pathways, a hormone biosynthetic pathway consisting of DAF-36 (Rottiers *et al.* 2006), DHS-16 (Wollam *et al.* 2012), and DAF-9 (Gerisch *et al.* 2001; Jia *et al.* 2002) makes Δ^7-dafachronic acid (Δ^7-DA), a steroid ligand that prevents dauer arrest by binding to the DAF-12 nuclear receptor (Motola *et al.* 2006).

Although insulin- and insulin-like ligand-induced inhibition of FoxO transcription factors through nuclear export and cytoplasmic sequestration is a well-established mechanism of FoxO regulation, nuclear translocation is not sufficient to fully activate FoxO (Lin *et al.* 2001; Tsai *et al.* 2003). In *C. elegans*, the EAK proteins comprise a conserved pathway that acts in parallel to AKT-1 to control the activity of nuclear DAF-16/FoxO (Williams *et al.* 2010). *eak* mutations, while causing a weak dauer-constitutive phenotype in isolation, strongly enhance the dauer-constitutive phenotype caused by *akt-1* mutations (Hu *et al.* 2006; Zhang *et al.* 2008; Alam *et al.* 2010; Dumas *et al.* 2010). EAK-7, which is likely the most downstream component of the EAK pathway, is a conserved protein of unknown function that is expressed in the same tissues as DAF-16/FoxO. Although EAK-7 likely regulates the nuclear pool of DAF-16/FoxO, it is situated at the plasma membrane (Alam *et al.* 2010), suggesting that it controls DAF-16/FoxO activity via unknown intermediary molecules.

We conducted a genetic screen to identify new FoxO regulators that may mediate EAK-7 action. Herein we describe our initial findings, which reveal an unexpected role for dosage compensation in controlling dauer arrest, insulin-like signaling, and FoxO transcription factor activity.

Materials and Methods

C. elegans strains and maintenance

The following strains were used in this study: N2 Bristol, CB4856 (Wicks *et al.* 2001), TJ356 [DAF-16::GFP(*zIs356*) IV] (Henderson and Johnson 2001), BQ1 *akt-1(mg306)* V (Hu *et al.* 2006), RB759 *akt-1(ok525)* V (Hertweck *et al.* 2004), VC204 *akt-2(ok393)* X (Hertweck *et al.* 2004), DR40 *daf-1(m40)* IV (Georgi *et al.* 1990), DR1572 *daf-2 (e1368)* III (Kimura *et al.* 1997), CB1393 *daf-8(e1393)* I (Park *et al.* 2010), AA86 *daf-12(rh61rh411)* X (Antebi *et al.* 2000), DR77 *daf-14(m77)* IV (Inoue and Thomas 2000), CF1038 *daf-16(mu86)* I (Lin *et al.* 1997), AA292 *daf-36 (k114)* V (Rottiers *et al.* 2006), CB428 *dpy-21(e428)* V (Yonker and Meyer 2003), and TY148 *dpy-28(y1)* III (Meyer and Casson 1986). The following mutant alleles were used: *daf-9(dh6)* and *daf-9(k182)* (Gerisch *et al.* 2001), *eak-7 (tm3188)* (Alam *et al.* 2010), and *sdc-2(y46)* (Nusbaum and Meyer 1989). Double, triple, and quadruple mutant animals were constructed by conventional methods. Strains generated in this study are listed in Supporting Information, Table S4. Animals were maintained on nematode growth media (NGM) plates seeded with *Escherichia coli* OP50.

Suppressor of eak-7;akt-1 (seak) screen

eak-7(tm3188) and *akt-1(ok525)* both harbor deletion mutations that are likely to be null alleles (Hertweck *et al.* 2004; Alam *et al.* 2010); these alleles were chosen to minimize the chances of isolating informational suppressors such as tRNA anticodon mutants or mutants defective in nonsense-mediated mRNA decay. *eak-7(tm3188);akt-1 (ok525)* double mutant animals were mutagenized at the mid-L4 larval stage using *N*-ethyl-*N*-nitrosourea (ENU) at a concentration of 0.5 mM in M9 buffer for 4 hr at room temperature, with gentle agitation (De Stasio and Dorman 2001). Mutagenized animals were plated on NGM plates and were allowed to recover overnight at 20°. After recovery, three P_0 animals were plated on each of 14 plates and allowed to lay eggs for 6 hr at 20°. After egg lay, P_0 animals were removed, and F_1 animals were grown to adulthood at 20°. Ten gravid F_1 animals were plated on each of 60 plates and allowed to lay eggs for 6 hr at 20°. After egg lay, F_1 animals were removed, and plates containing F_2 generation eggs were shifted to 25°. After 60 hr at 25°, all non-dauer F_2 animals were picked, pooled on one plate corresponding to their F_1 plate of origin, and grown to adulthood. Once gravid, these F_2 dauer bypassors were singled to new plates, with up to eight animals singled per F_1 plate of origin. A total of 330 F_2 dauer bypassors were singled, approximately one third of which were sterile. Egg lays followed by dauer bypassor selection at 25° were repeated with this generation, and strains that showed highly penetrant dauer suppression in four subsequent generations were pursued further. In the case of multiple suppressed strains deriving from the same F_1 plate of origin, only one strain was pursued to exclude the possibility of isolating sibling mutant animals. This selection scheme resulted in the isolation of 16 independent mutant strains with a verified *eak-7;akt-1* dauer suppression phenotype. Approximately 1200 haploid genomes were screened.

Whole-genome sequencing

Genomic DNA was isolated from each of the 16 *seak* strains and the nonmutagenized *eak-7;akt-1* double-mutant strain by phenol-chloroform extraction and subjected to whole-

genome sequencing using the Illumina HiSeq2000 platform. Approximately 30–40× genome coverage was obtained using 100-nucleotide paired-end reads.

Sequence analysis

Sequence reads were mapped to the WormBase reference genome build WS220 using the short-read aligner BWA (Li and Durbin 2009). Single-nucleotide variants (SNVs) were identified with the help of the SAMtools toolbox (Li *et al.* 2009). Each SNV was annotated with a custom-made Perl script and gene information available from WormBase v. WS220. Sequences of the *seak* mutant strains were compared to that of the nonmutagenized *eak-7;akt-1* parental strain. All nonsynonymous changes and predicted splice junction mutations were considered for subsequent analysis. A total of 664 such SNVs were identified among the 16 *seak* strains.

Mapping

Single-nucleotide polymorphism mapping was performed using the polymorphic Hawaiian CB4856 strain and a set of 48 primer pairs distributed throughout the genome (eight per chromosome) that flank *Dra*I restriction site polymorphisms (Davis *et al.* 2005). Since the mutagenesis was performed on *eak-7;akt-1* double-mutant animals, we constructed a recombinant inbred strain for mapping (BQ29 *dpIr1* [N2 → CB4856, *eak-7(tm3188)*] IV; [N2 → CB4856, *akt-1(ok525)*] V). BQ29 was generated by outcrossing the original *eak-7;akt-1* double mutant 10 times with CB4856 and identifying *eak-7;akt-1* double-mutant recombinants harboring CB4856 polymorphisms at sites flanking *eak-7* and *akt-1*. BQ29 animals arrested as dauers to the same extent as *eak-7(tm3188);akt-1(ok525)* double mutants in the N2 Bristol background, indicating that the CB4856 background does not strongly influence dauer arrest in *eak-7;akt-1* double-mutant animals.

Mapping was performed by crossing *seak* mutant hermaphrodites (*eak-7;akt-1;seak*) with BQ29 males and isolating F_2 dauers and bypassors. After confirming the dauer-constitutive and suppressor phenotypes in the following generation, F_2 dauers and bypassors were pooled and assayed for all 48 *Dra*I SNPs (Davis *et al.* 2005).

Dauer arrest assays

Dauer arrest assays were performed at the indicated temperatures in Percival I-36NL incubators (Percival Scientific, Inc., Perry, IA) as described (Hu *et al.* 2006). In assays involving *daf-9(k182)* and *daf-36(k114)* mutants, supplemental cholesterol was not added to the NGM assay plates (Gerisch *et al.* 2001; Rottiers *et al.* 2006). In assays involving *daf-9 (dh6)* mutants, animals were propagated on NGM plates supplemented with 10 nM Δ^7-DA to rescue the constitutive dauer phenotype of *daf-9(dh6)* (Sharma *et al.* 2009). Gravid animals raised on Δ^7-DA were transferred to NGM plates without Δ^7-DA and allowed to lay eggs for the dauer assay. Raw data and statistical analysis of all dauer assays are presented in Table S1.

RNAi

Feeding RNAi was performed using variations of standard procedures (Kamath *et al.* 2001). For dauer assays, NGM plates containing 5 mM IPTG and 25 μg/ml carbenicillin were seeded with 500 μl of overnight culture of *E. coli* HT115 harboring either control L4440 vector or indicated RNAi plasmid. Gravid animals cultured on NGM plates seeded with *E. coli* OP50 were picked to assay plates for 6-hr egg lays at 20°. Dauers were scored after progeny had been incubated at the assay temperature for 48–60 hr.

Male dauer assay

eak-7;akt-1 males were crossed with *eak-7;akt-1* hermaphrodites. Mated hermaphrodites were picked for egg lays on RNAi plates. The dauer arrest phenotype of male and hermaphrodite cross progeny was scored. The sex of dauers was determined by allowing the animals to recover to adulthood.

RNA preparation and real-time quantitative PCR

A total of 100–200 adult animals were allowed to lay eggs for 6 hr at 20° on NGM plates seeded with *E. coli* OP50. After egg lay, adults were removed and eggs were shifted to 25°. L2 larvae were harvested 24 hr later, washed twice in M9 buffer, and then frozen in TRIzol reagent (Invitrogen, catalog no. 15596-026). RNA was extracted using TRIzol, cleaned using the RNeasy Mini Kit (Qiagen, catalog no. 74104), and treated with DNAse (Qiagen, RNase-Free DNase Set, catalog no. 79254). mRNA was reverse transcribed using the SuperScript First-Strand Synthesis System for RT–PCR and oligo(dT) primers (Invitrogen, catalog no. 11904-018). cDNA, 10 ng, was used as template for real-time quantitative PCR amplification using the Absolute Blue QPCR SYBR Green Mix (Thermo Scientific, catalog no. AB-4166/B) in a 15-μl reaction volume. Reactions were run on an Eppendorf realplex Mastercycler. Primer sequences are presented in Table S2. Relative expression levels of each gene were determined using the $\Delta\Delta 2C_t$ method (Nolan *et al.* 2006). Gene expression levels were normalized relative to actin (*act-1*) in the same sample, and then relative to the levels of the same gene in the control sample (wild-type/N2 Bristol or *eak-7;akt-1* siblings, as indicated). Measurements were performed in triplicate, with three or four biological replicates for each condition.

DAF-16A:GFP localization

daf-16(mu86);zIs356;akt-1(mg306) animals (*akt-1* mutants harboring a DAF-16A::GFP transgene as the only source of DAF-16) were cultured for two generations on *dpy-21* RNAi or vector control at 20°. L2 or L3 larvae were mounted on glass slides using a thin pad of 2% agarose and 10 mM sodium azide to paralyze the animals. Images were captured using an Olympus BX61 epifluorescence compound microscope equipped with a Hamamatsu ORCA ER camera and Slidebook 4.0.1 digital microscopy software (Intelligent Imaging Innovations) and processed using ImageJ software. Images were captured within 20 min of mounting animals on slides to prevent variations in DAF-16A::GFP localization

due to prolonged stress induced by mounting. Images were then blinded and animals sorted into categories 1–5 (Figure S2) based on the extent of nuclear localization of DAF-16A::GFP: 1 indicates that all cells have only cytoplasmic GFP, and 5 indicates that all cells have exclusively nuclear GFP.

Results

A genetic screen for novel DAF-16/FoxO regulators

We pursued a genetic approach to identify molecules that mediate EAK-7 regulation of DAF-16/FoxO. The strong dauer-constitutive phenotype of *eak-7;akt-1* double mutants is fully suppressed by *daf-16/FoxO* loss-of-function mutations (Alam *et al.* 2010). We reasoned that screening for loss-of-function mutations that suppress the dauer-constitutive phenotype of *eak-7;akt-1* double mutants might identify genes encoding novel DAF-16/FoxO activators. Therefore, we mutagenized *eak-7(null);akt-1(null)* double mutant animals with ENU and screened for rare F_2 **s**uppressors of the ***e**ak-7;**ak**t-1* dauer-constitutive phenotype (*seak* mutants). Sixteen independent mutant strains were validated as bonafide *seak* mutants based on consistent high penetrance of the *seak* phenotype in subsequent generations.

To identify molecular lesions in these mutant strains, we isolated and sequenced genomic DNA isolated from non-outcrossed *seak* mutants and the original nonmutagenized *eak-7;akt-1* double mutant. Subsequently, each *seak* mutant was subjected to low-resolution single nucleotide polymorphism (SNP) mapping with the BQ29 recombinant inbred strain (Wicks *et al.* 2001; Davis *et al.* 2005).

dpy-21 inactivation suppresses dauer arrest in eak-7;akt-1 double mutants

Three independent *seak* strains harbored distinct nonsynonymous mutations in the conserved gene *dpy-21* (alleles *dp253*, *dp579* and *dp381*; Figure 1A). Each allele is predicted to be a missense mutation affecting a conserved residue in the conserved C-terminal region of DPY-21 (Figure 1A; Yonker and Meyer 2003). Low-resolution SNP mapping indicated that the genomic region containing *dpy-21* is linked to the dauer suppression phenotype in each of these three strains. *dpy-21* RNAi also suppressed the dauer-constitutive phenotype of *eak-7;akt-1* double mutants (Figure 1B). Moreover, the *dpy-21(e428)* null allele [hereafter referred to as "*dpy-21(null)*"] (Yonker and Meyer 2003), as well as *dpy-21(dp253)*, isolated from other mutagenesis-induced SNVs present in the original *seak* strain, also strongly suppressed the dauer-constitutive phenotype of *eak-7;akt-1* double mutants (Figure 1C). Taken together, these data indicate that *dpy-21* inactivation suppresses the dauer-constitutive phenotype of *eak-7;akt-1* double mutants. Therefore, the three mutant alleles that emerged from our screen (Figure 1A) are likely *dpy-21* loss-of-function mutations.

DPY-21 is a general regulator of dauer arrest

To determine whether DPY-21 influences dauer arrest generally as opposed to specifically in the context of *eak-7* and

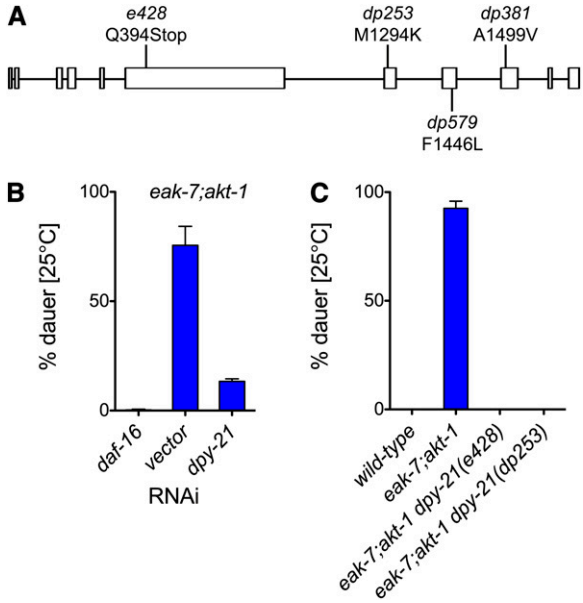

Figure 1 *dpy-21* inactivation suppresses the dauer-constitutive phenotype of *eak-7;akt-1* double mutants. (A) Genomic structure of the *dpy-21* locus and locations of the *e428* null allele and three alleles isolated in this study. Exons are denoted by boxes and introns by lines. (B and C) Effect of reduction of *dpy-21* activity on the dauer-constitutive phenotype of *eak-7;akt-1* double mutants at 25°. Error bars indicate SEM. Raw data and statistics are presented in Table S1. (B) *dpy-21* RNAi suppresses the dauer-constitutive phenotype of *eak-7;akt-1* mutants (13.4% mean dauer arrest in animals exposed to *dpy-21* RNAi compared to 75.6% in animals exposed to control vector, $P = 0.0004$ by two-sided *t*-test). Data are from one representative experiment with >250 animals assayed per condition. (C) *dpy-21(e428)* and *dpy-21(dp253)* suppress the dauer-constitutive phenotype of *eak-7;akt-1* mutants (0% mean dauer arrest in *eak-7;akt-1 dpy-21(e428)* triple mutants compared to 94.2% in *eak-7;akt-1* double mutants, $P < 0.0001$; 0% mean dauer arrest in *eak-7;akt-1 dpy-21(dp253)* triple mutants, $P < 0.0001$). Data are pooled from three replicate experiments with at least 1000 animals assayed per genotype.

akt-1 mutation, we determined the effect of reducing DPY-21 activity on dauer arrest in the background of other dauer-constitutive mutations using both *dpy-21* RNAi and *dpy-21 (null)* genetic mutation. Dauer arrest is controlled by three major signal transduction pathways; in addition to the DAF-2/IGFR pathway, the DAF-7 TGFβ-like pathway and the DAF-9 DA pathway also promote reproductive development by inhibiting dauer arrest (Fielenbach and Antebi 2008). We tested the effect of *dpy-21* inactivation on dauer arrest in animals harboring mutations in each of these three pathways that confer a dauer-constitutive phenotype.

Reducing the activity of *dpy-21* suppressed dauer arrest in animals with reduced DAF-2/IGFR signaling. *dpy-21* RNAi modestly but significantly suppressed the 25° dauer-constitutive phenotype of animals harboring *daf-2(e1368)*, which contains a missense mutation in the ligand-binding domain of DAF-2/IGFR (Kimura *et al.* 1997) (Figure 2A), and *dpy-21(null)* strongly suppressed *daf-2(e1368)* dauer arrest (Figure 2B). Additionally, *dpy-21(null)* suppressed the 27° dauer arrest phenotype of *akt-1(ok525)*, a likely null allele harboring a 1251-bp deletion eliminating the kinase

domain (Hertweck *et al.* 2004) (Figure S1A). These data indicate that DPY-21 promotes dauer arrest in the context of reduced DAF-2/IGFR signaling.

In the TGFβ-like pathway, a ligand (DAF-7, Ren *et al.* 1996) and its receptors [the type I and type II TGFβ receptor-like molecules DAF-1 (Georgi *et al.* 1990) and DAF-4 (Estevez *et al.* 1993), respectively] promote reproductive development by regulating the activity of three SMAD-like molecules [DAF-3 (Patterson *et al.* 1997), DAF-8 (Park *et al.* 2010), and DAF-14 (Inoue and Thomas 2000)] and a Sno transcription factor homolog [DAF-5 (Da Graca *et al.* 2004; Tewari *et al.* 2004)]. *dpy-21* inactivation also suppresses the dauer-constitutive phenotypes of mutants in the DAF-7/TGFβ-like pathway. Reducing *dpy-21* activity modestly suppresses dauer arrest caused by the *daf-1(m40)* and *daf-14(m77)* nonsense mutations (Georgi *et al.* 1990; Inoue and Thomas 2000) (Figures 2, C and D), indicating that DPY-21 also promotes dauer arrest in the context of reduced DAF-7 TGFβ-like signaling. The effect of *dpy-21* knockdown on the dauer-constitutive phenotype caused by the *daf-8(e1393)* missense allele is unclear; *dpy-21* RNAi suppresses *daf-8(e1393)* dauer arrest, but *dpy-21(null)* slightly enhances *daf-8(e1393)* dauer arrest (Figure S1B).

In the dafachronic acid (DA) pathway, multiple biosynthetic enzymes [DAF-36 (Rottiers *et al.* 2006), DHS-16 (Wollam *et al.* 2012), and DAF-9 (Gerisch *et al.* 2001; Jia *et al.* 2002)] participate in the biosynthesis of Δ^7-DA, a steroid ligand that promotes reproductive development by binding to the DAF-12 nuclear receptor (Motola *et al.* 2006). DAF-9 and DAF-12 act downstream of the DAF-2/IGFR and DAF-7/ TGFβ-like pathways to control dauer arrest (Gerisch *et al.* 2001; Jia *et al.* 2002; Gerisch and Antebi 2004; Mak and Ruvkun 2004). We tested the effect of *dpy-21* inactivation on the dauer-constitutive phenotype of *daf-9(dh6)* animals, which harbor a null mutation *in daf-9* that completely abrogates DA biosynthesis (Gerisch *et al.* 2001; Motola *et al.* 2006) [hereafter referred to as "*daf-9(null)*"]. Neither *dpy-21* RNAi (Figure 2E) nor *dpy-21(null)* (Figure 2F) influenced the dauer-constitutive phenotype of *daf-9(null)* mutants at 25°. In contrast, *dpy-21(null)* modestly suppressed the 27° dauer-constitutive phenotypes caused by the hypomorphic *daf-9(k182)* mutation (Gerisch *et al.* 2001) and the *daf-36(k114)* null mutation (Rottiers *et al.* 2006), which reduces the biosynthesis of dafachronic acids (Wollam *et al.* 2011) (Figure S1C).

Overall, these data indicate that DPY-21 is important for dauer arrest in the context of reduced DAF-2/IGFR signaling and plays a modest role in controlling DAF-7/TGFβ-like and perhaps DA hormonal signaling.

Dosage compensation influences dauer arrest

DPY-21 was originally identified as one of 10 components of the *C. elegans* dosage compensation complex (DCC) (Yonker and Meyer 2003). This multiprotein complex mediates dosage compensation by assembling on both hermaphrodite X chromosomes to reduce X-linked gene expression by ~50%, thereby equating X-linked gene expression between XX hermaphrodites and XO males (Meyer 2010). Based on its established role in dosage compensation, we sought to determine whether the suppression of dauer arrest by DPY-21 inactivation was a consequence of reduced dosage compensation *per se* as opposed to impairment of a DPY-21 activity that is independent of dosage compensation. To test this, we determined the effect of RNAi inactivation of each of the nine other DCC components on the dauer-constitutive phenotype of *eak-7;akt-1* double mutants. Strikingly, we found that reducing the activity of most DCC components by RNAi suppresses the dauer-constitutive phenotype of *eak-7;akt-1* double mutants (Figure 3A). It is noteworthy that, whereas loss-of-function mutations in most DCC components results in lethality (Plenefisch *et al.* 1989), RNAi-based inactivation of individual DCC components RNAi for multiple generations is not lethal (data not shown), suggesting that RNAi directed against these genes does not completely abrogate their function. Thus, the differential penetrance of dauer suppression observed after RNAi-based knockdown of DCC components could be a consequence of variable efficacy of RNAi against each targeted gene.

To further evaluate the role of other DCC components in dauer regulation, we reduced the activity of two DCC components via genetic mutation. *y1* is a temperature-sensitive missense allele of *dpy-28* (Plenefisch *et al.* 1989), which encodes a core DCC subunit (Tsai *et al.* 2008), and *y46* is a weak allele of *sdc-2* (Nusbaum and Meyer 1989), which encodes a hermaphrodite-specific protein that targets the DCC to X chromosomes (Dawes *et al.* 1999). In accordance with our RNAi-based findings, both *dpy-28(y1)* and *sdc-2(y46)* completely suppress the dauer-constitutive phenotype of *eak-7;akt-1* double mutant animals (Figures 3B and 3C, respectively). Together, these results implicate the DCC itself in the control of dauer arrest.

Reducing DCC activity could suppress dauer arrest due to a reduction in dosage compensation. Alternatively, the dauer suppression phenotype could be a consequence of perturbing a novel, dosage-compensation-independent function of the DCC. To distinguish between these two possibilities, we took advantage of the observation that mutations that disrupt dosage compensation, while causing severe phenotypes in XX hermaphrodite animals, are phenotypically silent in XO male animals owing to the fact that the DCC does not repress X-linked gene expression in males (Meyer 2010). Therefore, we compared the effect of DCC component RNAi on the dauer-constitutive phenotype of *eak-7;akt-1* hermaphrodites and males. In contrast to what was observed in *eak-7;akt-1* hermaphrodites, DCC component RNAi had no effect on the dauer-constitutive phenotype of *eak-7;akt-1* male siblings. In comparison, *daf-16/FoxO* and *daf-12* RNAi fully suppressed dauer arrest in *eak-7;akt-1* hermaphrodites and males (Figure 3A). Because DCC-mediated repression does not occur in males, these data suggest that the DCC controls dauer arrest through dosage compensation *per se*.

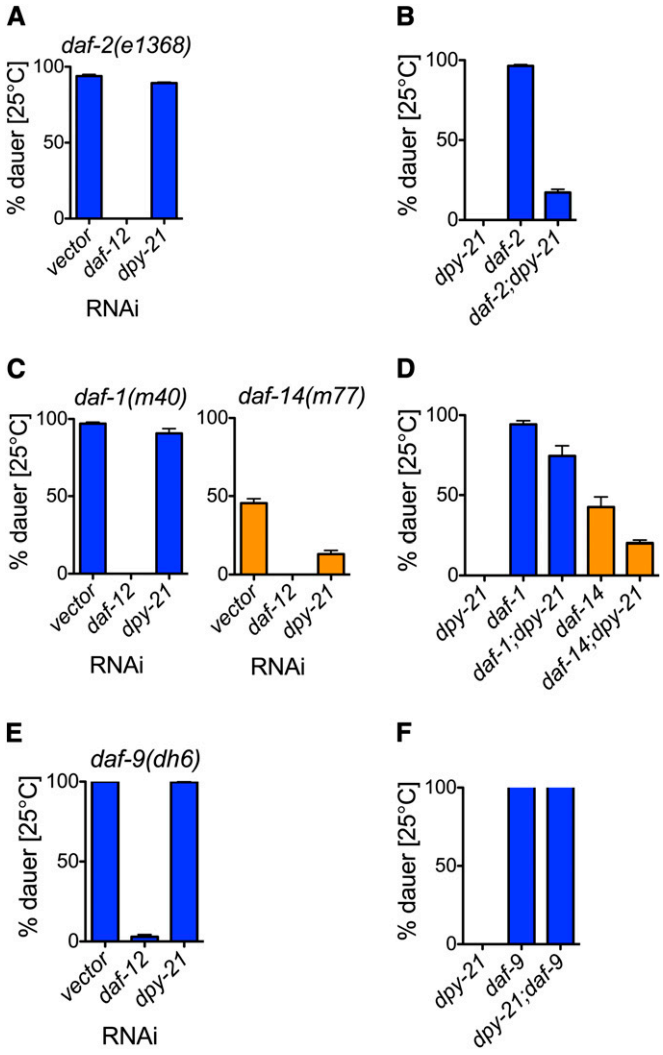

Figure 2 DPY-21 is a general regulator of dauer arrest. Effects of reducing *dpy-21* activity on the dauer-constitutive phenotypes of DAF-2/IGFR pathway, DAF-7/TGFβ-like pathway, and dafachronic acid pathway mutants are shown. (A) *dpy-21* RNAi suppresses the dauer-constitutive phenotype of *daf-2(e1368)* mutants (91.7% mean dauer arrest in animals exposed to *dpy-21* RNAi compared to 95.2% in animals exposed to control vector, $P = 0.0275$ by two-sided *t*-test). Data are pooled from three replicate experiments with at least 1000 animals assayed per genotype. (B) *dpy-21(null)* suppresses the dauer-constitutive phenotype of *daf-2(e1368)* mutants (17.2% mean dauer arrest in *daf-2;dpy-21* compared to 96.5% in *daf-2*, $P < 0.0001$). Data are pooled from three replicate experiments with at least 1300 animals assayed per genotype. (C) Influence of *dpy-21* RNAi on the dauer-constitutive phenotype of mutants with reduced DAF-7/TGFβ-like pathway signaling (91.0% mean dauer arrest in *daf-1(m40)* mutant animals exposed to control vector compared to 89.3% in animals exposed to *dpy-21* RNAi, $P = 0.7064$; 16.0% mean dauer arrest in *daf-14(m77)* mutant animals exposed to *dpy-21* RNAi compared to 33.8% in animals exposed to control vector, $P = 0.0321$). Data are pooled from two replicate experiments with at least 550 animals assayed per condition. (D) *dpy-21(null)* suppresses the dauer-constitutive phenotype of *daf-1(m40)* and *daf-14(m77)* mutants (74.5% mean dauer arrest in *daf-1;dpy-21* compared to 94.3% in *daf-1*, $P = 0.01$; 20.0% mean dauer arrest in *daf-14;dpy-21* compared to 42.5% in *daf-14*, $P = 0.0037$). Data are pooled from three replicate experiments with at least 750 animals assayed per genotype. (E and F) Neither *dpy-21* RNAi nor *dpy-21(null)* suppresses the dauer-constitutive phenotype of *daf-9(dh6)* mutant animals. (E) $P = 0.2153$ for *dpy-21* RNAi compared to vector control. Data are pooled from three replicate experiments with at least 800 animals per condition. (F) $P = 0.9503$ for *dpy-21(null);daf-9(dh6)* compared to *daf-9(dh6)*. Data are from one representative experiment with at least 250 animals per genotype. Error bars indicate SEM. Raw data and statistics are presented in Table S1.

X-linked dauer inhibitory genes are upregulated in dpy-21 mutants

Dosage compensation could potentially influence dauer arrest by controlling the expression of X-linked genes that normally function to inhibit dauer arrest. *C. elegans* has nine known X-linked dauer regulatory genes, most of which encode proteins that inhibit dauer arrest: *mrp-1* (Yabe *et al.* 2005), *daf-3* (Patterson *et al.* 1997), *pdk-1* (Paradis *et al.* 1999), *ncr-1* (Li *et al.* 2004), *daf-9* (Gerisch *et al.* 2001; Jia *et al.* 2002), *ist-1* (Wolkow *et al.* 2002), *daf-12* (Antebi *et al.* 2000), *ftt-2* (Li *et al.* 2007), and *akt-2* (Paradis and Ruvkun 1998). Six of these genes have previously been shown to be upregulated approximately twofold in embryos defective in dosage compensation (Jans *et al.* 2009) (Table S3). Notably, four of these genes, *ist-1*, *pdk-1*, *akt-2*, and *ftt-2*, encode components of the DAF-2/IGFR pathway, and the products of all four genes inhibit DAF-16/FoxO activity (Paradis *et al.* 1999; Paradis and Ruvkun 1998; Wolkow *et al.* 2002; Li *et al.* 2007).

We quantified expression of these nine dauer regulatory genes in *eak-7;akt-1 dpy-21* triple mutant animals and their *eak-7;akt-1* double-mutant siblings in animals raised at 25° for 24 hr after hatching (this time point corresponds to the L2 transition, following which animals commit to either dauer arrest or reproductive development). Expression of most of the X-linked dauer regulatory genes was increased approximately twofold in *eak-7;akt-1 dpy-21* triple mutants compared to *eak-7;akt-1* double-mutant siblings, consistent with their modulation by dosage compensation [Figure 4A, left, and Figure S3; (Jans *et al.* 2009)]. All four genes encoding DAF-2/IGFR pathway components that inhibit DAF-16/FoxO were upregulated in the context of *dpy-21* mutation (*ist-1*, 2.87-fold increase in *eak-7;akt-1 dpy-21* compared to *eak-7;akt-1*; *pdk-1*, increased 1.77-fold; *akt-2*, increased 3.46-fold; *ftt-2*, increased 1.57-fold). An increase in expression of these proteins would be predicted to inhibit DAF-16/FoxO activity by promoting its phosphorylation, nuclear export, and/or retention in the cytoplasm.

Strikingly, the expression of *daf-9*, the cytochrome P450 family member that catalyzes the final step in DA biosynthesis (Motola *et al.* 2006), was increased in the context of *dpy-21* mutation to a much greater extent than would be expected based on a reduction in dosage compensation. In four independent biological replicates, *daf-9* expression increased ~8- to 30-fold in *eak-7;akt-1 dpy-21* triple mutants compared to *eak-7;akt-1* siblings. This may be a consequence of suppression of the *eak-7;akt-1* dauer-constitutive phenotype by *dpy-21 (null)*, as hypodermal *daf-9* expression is induced in reproductively growing *daf-2* mutant larvae but repressed in *daf-2*

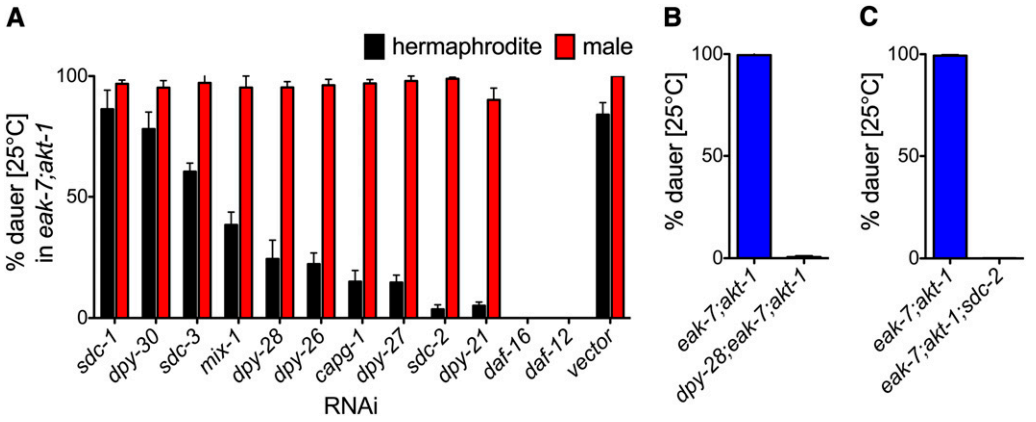

Figure 3 Dosage compensation influences dauer arrest. (A) Effect of RNAi of individual dosage compensation complex (DCC) components on the dauer-constitutive phenotype of *eak-7;akt-1* hermaphrodites (black bars) and males (red bars). RNAi of most DCC components suppresses the dauer-constitutive phenotype of *eak-7;akt-1* hermaphrodites ($P < 0.05$ for all DCC components except for *sdc-1* and *dpy-30*, which were not significantly different from vector, as calculated by one-way ANOVA). Dauer arrest in male animals subjected to DCC component RNAi was not statistically different from vector control for any RNAi clone tested. In contrast, *daf-16* and *daf-12* RNAi caused significant suppression of dauer arrest in both hermaphrodite and male animals ($P < 0.001$ for comparison between *daf-16* or *daf-12* RNAi and vector control). Data are from a single experiment, representative of three replicates, with 100–800 animals scored per RNAi condition, per sex. (B) *dpy-28(y1)* suppresses the dauer-constitutive phenotype of *eak-7;akt-1* double mutants (0.6% mean dauer arrest in *dpy-28;eak-7;akt-1* compared to 99.5% in *eak-7;akt-1*, $P < 0.0001$). Data are pooled from three replicate experiments with at least 200 animals assayed per genotype. (C) *sdc-2(y46)* suppresses the dauer-constitutive phenotype of *eak-7;akt-1* double mutants (0.1% mean dauer arrest in *eak-7;akt-1;sdc-2* compared to 99.3% in *eak-7;akt-1*, $P < 0.0001$). Data are pooled from three replicate experiments with at least 700 animals assayed per genotype. Error bars indicate SEM. Raw data and statistics are presented in Table S1.

mutant dauers (Gerisch and Antebi 2004). Notably, although many X-linked genes are upregulated approximately twofold in mutants defective in dosage compensation, the influence of the DCC on the expression of individual X-linked genes is highly variable (Jans *et al.* 2009).

To determine whether changes in the expression of autosomal dauer inhibitory genes contribute to suppression of *eak-7;akt-1* dauer arrest by *dpy-21* inactivation, we quantified the expression of 11 autosomal dauer inhibitory genes in *eak-7;akt-1 dpy-21* triple mutants and their *eak-7;akt-1* double mutant siblings (Figure 4A, right, and Figure S3). In contrast to X-linked dauer regulatory genes, expression of autosomal dauer inhibitory genes either did not change or increased only modestly in *eak-7;akt-1 dpy-21* triple mutants compared to *eak-7;akt-1* sibling controls (Figures 4A and Figure S3, compare left and right). This finding is consistent with a model whereby *dpy-21* mutation suppresses dauer arrest via the perturbation of dosage compensation.

DPY-21 promotes DAF-16/FoxO nuclear localization

Our genetic analysis suggests that DPY-21 controls dauer arrest primarily by influencing DAF-2/IGFR signaling (Figure 2). The upregulation of four X-linked genes encoding DAF-2/IGFR pathway components that inhibit DAF-16/FoxO activity in the context of *dpy-21* mutation (Figure 4A) is predicted to result in DAF-16/FoxO inhibition due to increased nuclear export and cytoplasmic sequestration of DAF-16/FoxO. If this is the major mechanism through which DPY-21 controls dauer arrest, then *dpy-21* inactivation should promote the nuclear export of DAF-16/FoxO. To test this model, we determined the effect of *dpy-21* RNAi on the subcellular localization of a functional DAF-16A::GFP fusion protein (Henderson and Johnson 2001b) in *daf-16 (null);akt-1(null)* double-mutant animals (Figures 4B and Figure S2). As previously shown (Zhang *et al.* 2008; Alam *et al.* 2010; Dumas *et al.* 2010), DAF-16A::GFP was nuclear in many *akt-1(null)* animals cultured on *E. coli* containing a control RNAi construct. Exposure of animals of the same genotype to *dpy-21* RNAi promoted the nuclear export and cytoplasmic retention of DAF-16A::GFP. Therefore, DPY-21 promotes the nuclear localization of DAF-16A::GFP.

DPY-21 activates DAF-16/FoxO

To determine the impact of *dpy-21* inactivation on DAF-16/FoxO activity in dauer regulation, we quantified the expression of the DAF-16/FoxO target genes *sod-3*, *mtl-1*, and *dod-3* (Murphy *et al.* 2003; Oh *et al.* 2006) in *eak-7;akt-1 dpy-21* triple mutants and their *eak-7;akt-1* double-mutant siblings grown at 25° for 24 hr after hatching. As previously shown (Alam *et al.* 2010), the expression of all three genes is increased in a *daf-16/FoxO*-dependent manner in *eak-7;akt-1* double mutants (Figure 4C). *dpy-21* null mutation strongly reduced the expression of at least two of the three genes in the *eak-7;akt-1* double mutant background, although not to the same extent as a *daf-16* null mutation. These results are consistent with a model whereby DPY-21 activates DAF-16/FoxO, as *dpy-21* mutation results in significant but incomplete inhibition of DAF-16/FoxO activity.

The X-linked gene akt-2 is required for suppression of the dauer-constitutive phenotype of eak-7;akt-1 double mutants by dpy-21 mutation

If suppression of the dauer-constitutive phenotype of *eak-7;akt-1* double mutants by *dpy-21* mutation (Figure 1C) is due to DAF-16/FoxO inhibition secondary to increases in the expression of the X-linked genes *ist-1*, *ftt-2*, *pdk-1*, and *akt-2* (Figure 4), then a reduction in the expression of *ist-1*, *ftt-2*, *pdk-1*, and/or *akt-2* in *eak-7;akt-1 dpy-21* triple

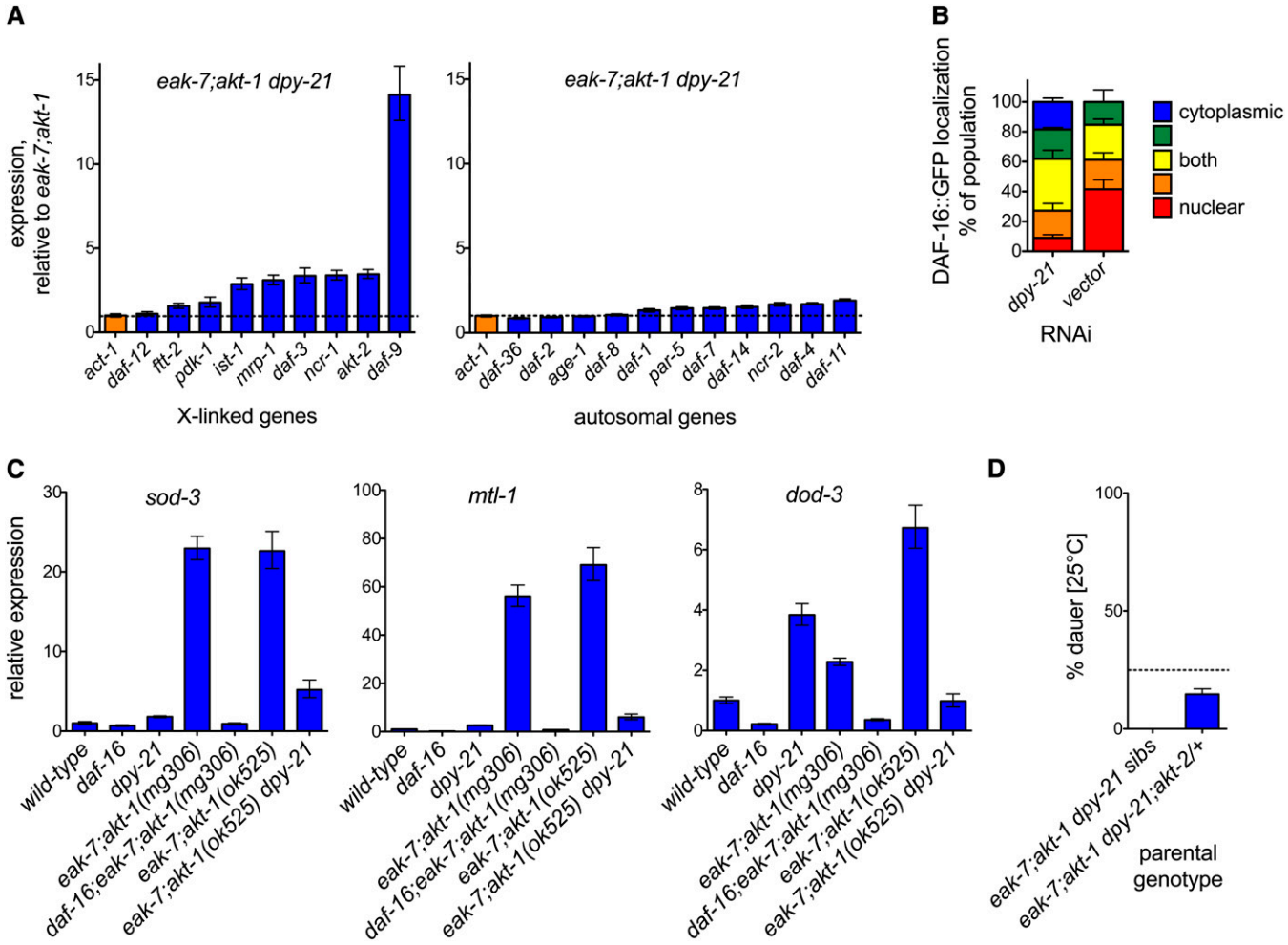

Figure 4 DPY-21 activates DAF-16/FoxO. (A) Effect of *dpy-21(null)* on the expression of X-linked and autosomal dauer inhibitory genes (blue bars) in larvae grown at 25° 24 hr after hatching. Data are normalized to expression levels of actin (*act-1*, orange bars), which is not X-linked. Expression in *eak-7;akt-1 dpy-21* relative to expression in *eak-7;akt-1* is shown. The dashed line indicates relative expression of one. Data are from a single representative experiment. Error bars indicate 95% confidence interval. Replicate data are presented in Figure S3. (B) DPY-21 promotes DAF-16/FoxO nuclear localization in an *akt-1(null)* background. Localization of DAF-16A::GFP was assessed in L2 or L3 *daf-16(null);akt-1(null)* double mutant animals after growth on *E. coli* harboring either control plasmid or *dpy-21* RNAi plasmid for two generations. DAF-16A::GFP localization was scored in a blinded fashion on a 1–5 cytoplasmic/nuclear scale: 1, all cells in the animal have completely cytoplasmic localization; 2, most cells in the animal have completely cytoplasmic localization; 3, cells have both nuclear and cytoplasmic localization; 4, most cells in the animal have completely nuclear localization; 5, all cells in the animal have completely nuclear localization. See Figure S2 for representative images. Data represent three replicate experiments of ~30 animals per condition. Error bars indicate SEM. (C) DPY-21 promotes DAF-16/FoxO target gene expression. *sod-3*, *mtl-1*, and *dod-3* expression were quantified in larvae grown at 25° 24 hr after hatching. Data are normalized to expression levels of actin (*act-1*) and expressed relative to wild-type N2 Bristol expression. *eak-7;akt-1(ok525)* animals are siblings of *eak-7;akt-1 dpy-21* animals. Data are from a single experiment. Error bars indicate 95% confidence interval. Replicate data are presented in Figure S4. (D) Reducing *akt-2* gene dosage induces dauer arrest in *eak-7;akt-1 dpy-21(null)* triple mutants. Dauer arrest was assayed in the progeny of *eak-7;akt-1 dpy-21(null);akt-2*/+ parents compared to *eak-7;akt-1 dpy-21(null)* sibling controls. Progeny of *eak-7;akt-1 dpy-21(null);akt-2*/+ parents exhibited 14.72% dauer arrest. The dashed line indicates 25%. Data are pooled from two replicate experiments with at least 500 animals assayed per parental genotype. Error bars indicate SEM. Raw data and statistics are presented in Table S1.

mutants should restore the dauer-constitutive phenotype. To test this, we determined the effect of reducing *akt-2* gene dosage on the dauer-constitutive phenotype of *eak-7;akt-1 dpy-21* triple mutants.

We assayed the progeny of *eak-7;akt-1 dpy-21* animals heterozygous for the *akt-2(ok393)* null mutation (*eak-7;akt-1 dpy-21;akt-2/*+) for dauer arrest at 25°. Approximately one-quarter of these animals should be *eak-7;akt-1 dpy-21* triple mutants that are wild type at the *akt-2* locus and should not undergo dauer arrest (Figure 1C). Half of the progeny should be *eak-7;akt-1 dpy-21;akt-2/*+, and the remaining quarter should be *eak-7;akt-1 dpy-21;akt-2* quadruple mutants. Therefore, any evidence of dauer arrest in the progeny of *eak-7;akt-1 dpy-21;akt-2/*+ animals would indicate that suppression of *eak-7;akt-1* dauer arrest by *dpy-21* mutation requires *akt-2*.

Slightly less than one-quarter of the progeny of *eak-7;akt-1 dpy-21;akt-2/*+ animals underwent dauer arrest at 25° (Figure 4D). These dauers did not recover after several days of incubation at 15°, suggesting that they were all *eak-7;*

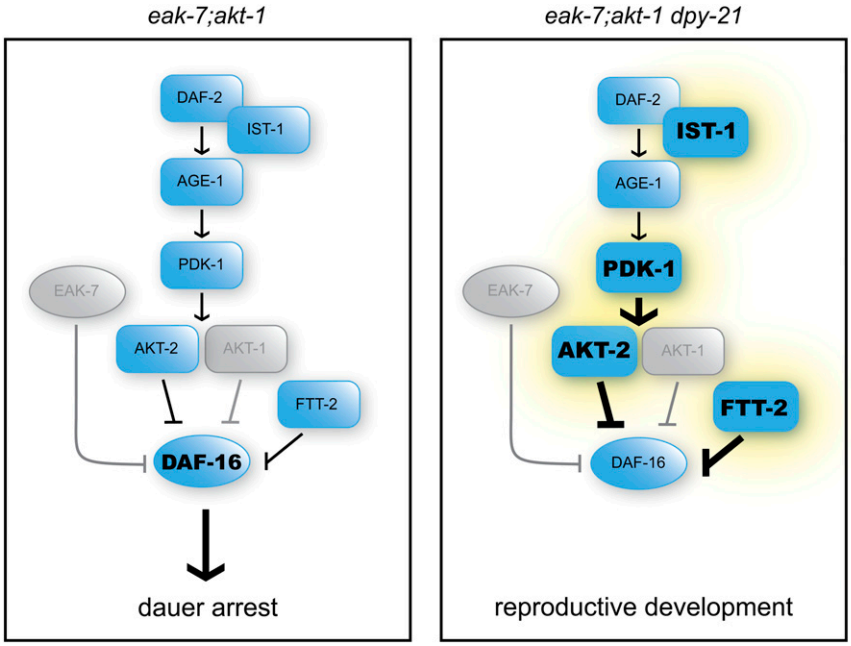

Figure 5 Model of DAF-2/IGFR pathway regulation by DPY-21. In *eak-7;akt-1* double mutant animals, activated DAF-16/FoxO promotes dauer arrest (left). In *eak-7;akt-1 dpy-21* triple mutant animals, increased expression of *ist-1*, *pdk-1*, *akt-2*, and *ftt-2* contributes to suppression of dauer arrest by promoting the inhibition of DAF-16/FoxO (right).

akt-1 dpy-21;akt-2 quadruple mutant animals. Therefore, *akt-2* activity is essential for the suppression of *eak-7;akt-1* dauer arrest by *dpy-21* null mutation. Reducing *akt-2* gene dosage by twofold appears to be insufficient to induce dauer arrest in this context.

Discussion

Although much is known about the molecular components of the dosage compensation machinery and its influence on X-linked gene expression in *C. elegans* and other organisms, the physiologic consequences of dosage compensation are poorly understood. We have discovered a new function for dosage compensation in the control of dauer arrest, DAF-2/IGFR signaling, and DAF-16/FoxO activity.

We have shown that *dpy-21* mutation suppresses dauer arrest due to a defect in dosage compensation (Figures 1, 2, and 3). Impaired compensation of X-linked gene expression results in the increased expression of dauer inhibitory genes, including four genes encoding DAF-2/IGFR signaling components that inhibit DAF-16/FoxO activity by promoting its nuclear export and cytoplasmic retention (Figure 4A). Accordingly, *dpy-21* inactivation induces the relocalization of DAF-16/FoxO from nuclei to cytoplasm (Figure 4B) and inhibits the expression of DAF-16/FoxO target genes (Figure 4C). *akt-2* is required for the suppression of *eak-7;akt-1* dauer arrest by *dpy-21* mutation (Figure 4D), indicating that *dpy-21* inactivation suppresses the *eak-7;akt-1* dauer-constitutive phenotype at least in part by promoting DAF-16/FoxO inhibition via AKT-2. Our results support a model in which DPY-21 (and presumably the DCC) controls dauer arrest and DAF-16/FoxO activity by modulating the expression of DAF-2/IGFR pathway components that influence the nucleocytoplasmic trafficking of DAF-16/FoxO (Figure 5). At this point, we cannot exclude the possibility that other X-linked and autosomal dauer inhibitory genes, the expression of some of which is increased in *dpy-21* mutants (Figure 4A), may contribute to the suppression of *eak-7;akt-1* dauer arrest by *dpy-21* inactivation. This could explain the modest suppressive effect of *dpy-21* inactivation on the dauer-constitutive phenotype of DAF-7/TGFβ-like pathway mutants (Figures 2C and 2D).

The establishment of a role for dosage compensation in the control of dauer arrest and insulin-like signaling may serve as a platform for investigations into other postembryonic processes that might be influenced by dosage compensation, whether dosage compensation is physiologically regulated, and whether it is dysregulated in human disease. Indeed, a recent study in mice suggests that sex chromosome dosage *per se* can influence metabolic phenotypes independently of gonadal sex (Chen *et al.* 2012). Furthermore, skewing of X chromosome inactivation increases with age in human females and is attenuated in cohorts of female centenarians (Gentilini *et al.* 2012), suggesting a correlation between dysregulation of dosage compensation and the aging process. To date, studies on dosage compensation have largely focused on the establishment of dosage compensation during embryogenesis; mechanisms governing the postembryonic stability of dosage compensation remain poorly understood. Further inquiry into the function and regulation of dosage compensation in postembryonic contexts has the potential to illuminate new functions for dosage compensation and provide insights into the pathogenesis of human disease.

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Unexpected Role for Dosage Compensation in the Control of Dauer Arrest, Insulin-Like Signaling, and FoxO Transcription Factor Activity in *Caenorhabditis elegans*

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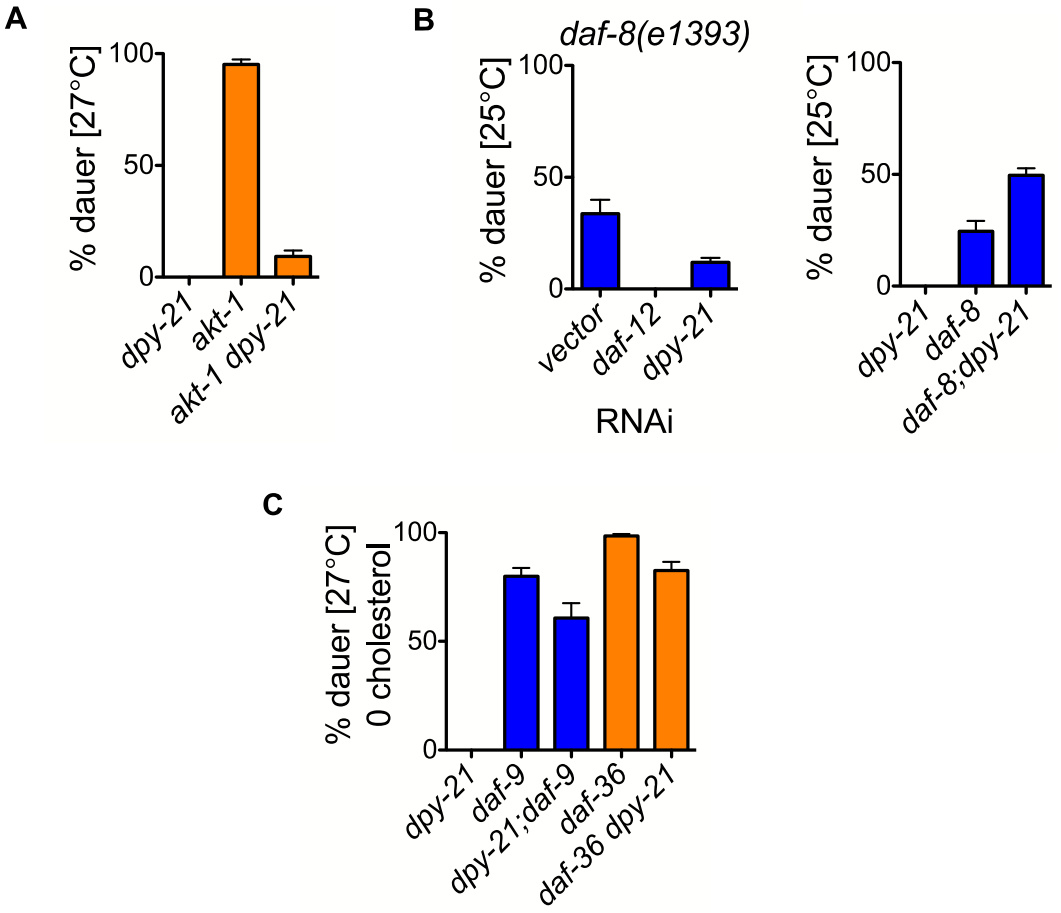

Figure S1 DPY-21 regulates dauer arrest. (A) *dpy-21(null)* suppresses the dauer-constitutive phenotype of *akt-1* mutant animals at 27° (6.3% dauer arrest in *akt-1 dpy-21* mutants compared to 92.5% arrest in *akt-1*, P <0.0001). Data are pooled from four replicate experiments with at least 700 animals scored per genotype. **(B)** *dpy-21* RNAi suppresses the dauer-constitutive phenotype of the TGFβ-like pathway mutant *daf-8(e1393)* (11.9% dauer arrest in *daf-8* mutants exposed to *dpy-21* RNAi compared to 33.7% arrest in animals exposed to control vector, p = 0.0289). Data are from a single representative experiment with at least 450 animals scored per condition. In contrast, *dpy-21(null)* enhances dauer arrest of *daf-8* mutant animals (49.6% dauer arrest in *daf-8; dpy-21* mutants compared to 24.5% arrest in *daf-8*, p = 0.0004). Data are pooled from three replicate experiments with at least 950 animals scored per genotype. **(C)** *dpy-21(null)* suppresses the dauer-constitutive phenotype of *daf-9(k182)* and *daf-36(k114)* mutants raised on plates without supplemental cholesterol at 27° (60.7% dauer arrest in *dpy-21;daf-9* mutants compared to 79.9% arrest in *daf-9*, p = 0.0269; 82.6% dauer arrest in *daf-36 dpy-21* mutants compared to 98.4% arrest in *daf-36*, p = 0.0004). Data are pooled from four replicate experiments with at least 450 animals scored per genotype. Error bars indicate SEM. Raw data and statistics are presented in Table S1.

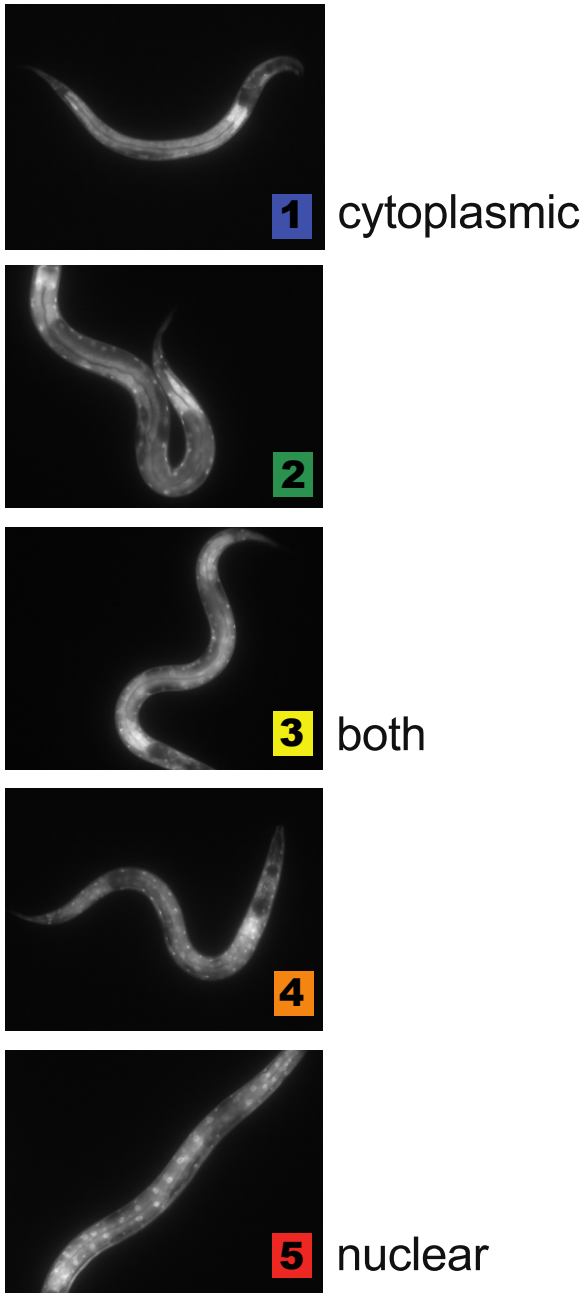

Figure S2 Representative images of animals exhibiting cytoplasmic and nuclear DAF-16A::GFP localization. The scale ranges from "1" (all cells have completely cytoplasmic localization) to "5" (all cells have completely nuclear localization).

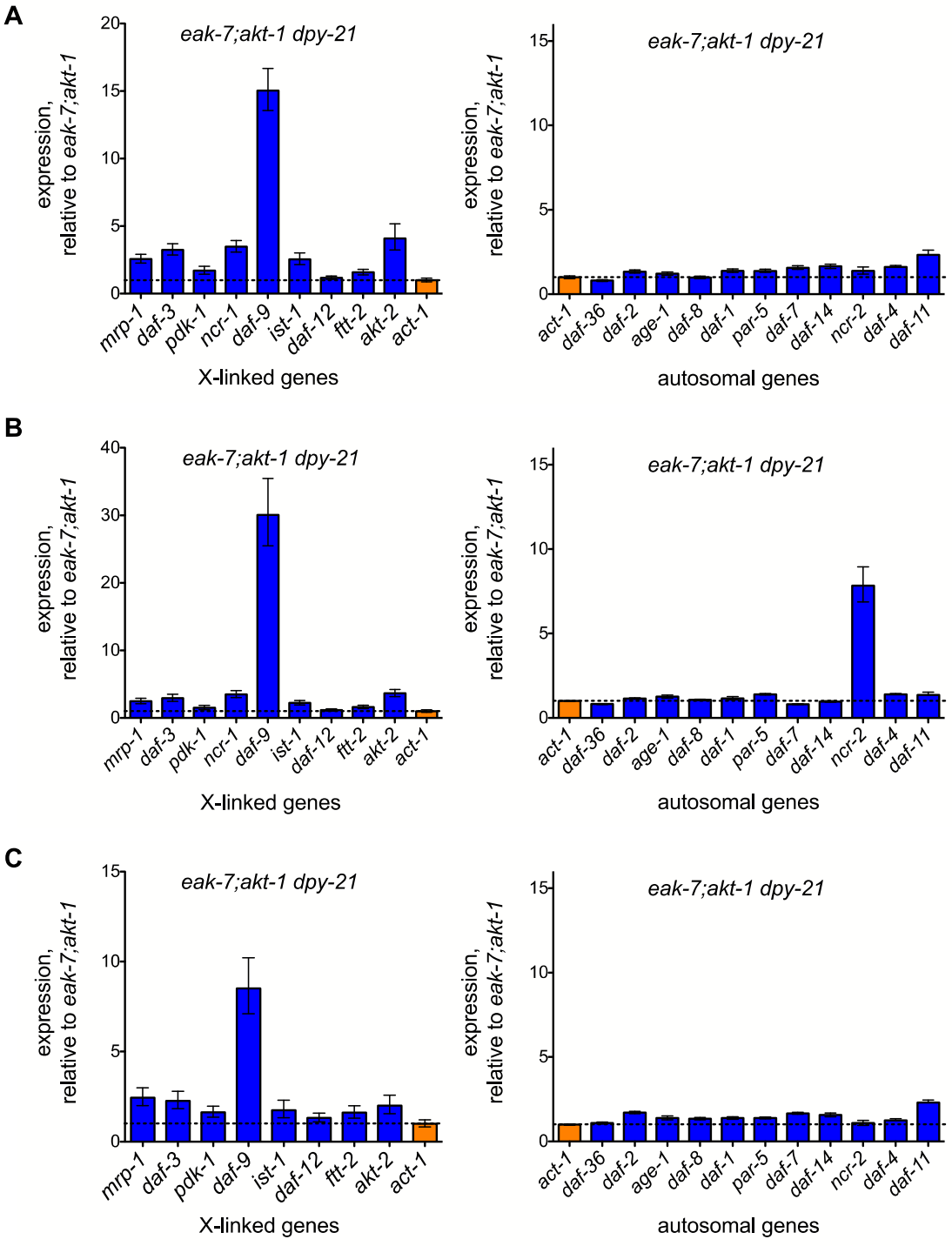

Figure S3 Biological replicates for the X-linked and autosomal gene expression experiment presented in Figure 4A. Data are normalized to actin (*act-1,* orange bar) and expressed relative to *eak-7;akt-1 dpy-21(wt)* expression. Dashed line indicates relative expression of one. Each row corresponds to one biological replicate, with X-linked and autosomal dauer inhibitory gene expression profiled from the same sample. Error bars indicate 95% confidence interval. The increase in *ncr-2* expression observed in the right panel of B. was not reproducible.

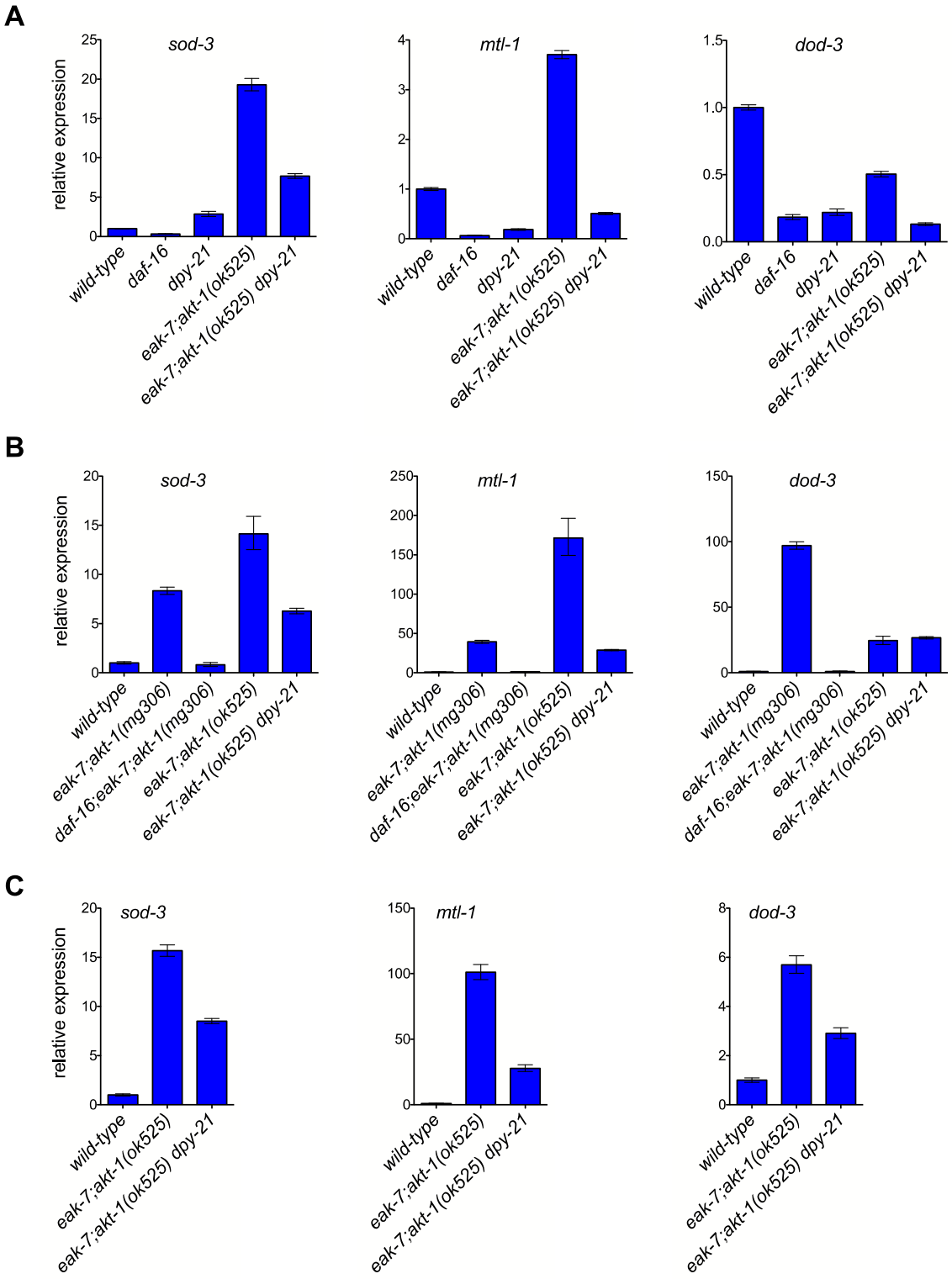

Figure S4 Biological replicates for the DAF-16/FoxO target gene expression experiment presented in Figure 4C. Data are normalized to actin (*act-1*) and expressed relative to wild-type N2 Bristol expression. *eak-7;akt-1(ok525)* animals are siblings of *eak-7;akt-1 dpy-21* animals. Data in each row are from a single biological replicate. Error bars indicate 95% confidence interval.

Table S1 Raw data and statistical analysis of all dauer assays

Table S1 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.149948/-/DC1.

Table S2 Primers used for real-time quantitative PCR

Target	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
sod-3	TATTAAGCGCGACTTCGGTTCCCT	CGTGCTCCCAAACGTCAATTCCAA
mtl-1	ATGGCTTGCAAGTGTGACTG	CACATTTGTCTCCGCACTTG
dod-3	AAAAAGCCATGTTCCCGAAT	GCTGCGAAAAGCAAGAAAAT
akt-2	TCGTGATATGAAACTCGAAAATTTGC	ATTCTGGTGTTCCGCAAAAGGTG
daf-12	AATGTTTAGAGTTCTTCGGTTTCTTC	T TCGTTCATCGGAGGATCAGAG
daf-3	AGCTGCGAAGGGAAGCAACA	CTCAATCCAAACTGGACAATCATG
daf-9	ATTCCCCACAAAACAATCGAAGAAT	GAGATTCAAACACGTTTGGATCG
ftt-2	ACAGAAACTCGGTTGTCGAGAAG	ATAGAAGAAGACAGAGAAGTTGAGAG
ist-1	ATTGAAGATAACAAAGTGACCAGAAG	TGCATGCTGAAGATCTTCTATTCG
mrp-1	CGACTGAATACGGTTATGGATAGT	CACAACATTTGCATCTTTTGCCATTG
ncr-1	GCGTGCGTTTTGTCGCCAAC	C AATCCCACCTTCCGCTTTAAG
pdk-1	TTTATACATACGCCCAACCGCGT	T TTCGATAGTCACCGAGTACCG
age-1	GGATCATTTGAAGAAAACCCTCTTC	TTTGGTAGACCATGATCCATTGAAG
daf-1	GTACTGTTAGATACCTTGCACC	CGGCAGAACATCTCCATCTTC
daf-11	TTGTTTCGGAGAAGCTGTTATATTAG	ACGACCAATTCCTTCAATAGTACG
daf-14	TTCAAATATCTCAAGCCAATCTTCTT	AGCTCGTAGTAAAATATGGTACAC
daf-2	CCGGTGCGAAGAACGGTG	CCCACGTAATATGAATAGCGTCC
daf-36	GGATGGAAAATGGGAAGTGAAATC	ACCATGCTGCTCTGCAACAAG
daf-4	AATCTCTAGACAAGTTCCCATTCT	CTCCTCCTTCTTATTCTGCTGA
daf-7	GCACACCACTTCAACTTGGC	GATCAGCTTGATGTAGTCGTAC
daf-8	ACATATCAAGACGTCTACTGTCTC	CATCGGATTATTCAAATGAGCCTG
ncr-2	CAATATGGCAATGTCTCTTGGAATC	TAGATCCATTACTGACAAGTGCAG
par-5	GGCTTACCAGGAGGCTCTTG	CAAGCGTGCTCTGGAGTGTTC
scd-2	ATAGAATCCAACTGCACGAACTG	CATATTGGACTCTTGCGAGATCT

Table S3 Dosage compensation of X-linked dauer regulatory genes.

Gene	Compensated?	Increase (fold) in *sdc-2* mutant	Increase (fold) in *dpy-27* mutant
mrp-1	Yes	1.5	1.8
daf-3	Yes	1.8	1.7
ncr-1	Yes	2.0	1.7
ist-1	Yes	2.1	1.8
daf-12	Yes	2.6	2.1
akt-2	Yes	2.8	3.1
pdk-1	Not listed		
daf-9	Not listed		
ftt-2	Not listed		

Adapted from Jans *et al.* 2009, Supplemental Table 4, Dosage-Compensated Genes and Supplemental Table 5, Non-Compensated Genes. “Not listed” indicates a gene that was not defined as dosage-compensated or non-compensated in the study.

Table S4 Strains generated in this study

Table S4 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.149948/-/DC1.