Protein Kinase D Is Dispensable for Development and Survival of Drosophila melanogaster

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ABSTRACT
Members of the Protein Kinase D (PKD) family are involved in numerous cellular processes in mammals, including cell survival after oxidative stress, polarized transport of Golgi vesicles, as well as cell migration and invasion. PKD proteins belong to the PKC/CAMK class of serine/threonine kinases, and transmit diacylglycerol-regulated signals. Whereas three PKD isoforms are known in mammals, Drosophila melanogaster contains a single PKD homolog. Previous analyses using overexpression and RNAi studies indicated likewise multi-facetted roles for Drosophila PKD, including the regulation of secretory transport and actin-cytoskeletal dynamics. Recently, involvement in growth regulation has been proposed based on the hypomorphic dPKDH allele. We have generated PKD null alleles that are homozygous viable without apparent phenotype. They largely match control flies regarding fertility, developmental timing and weight. Males, but not females, are slightly shorter lived and starvation sensitive. Furthermore, migration of pole cells in embryos and border cells in oocytes appears normal. PKD mutants tolerate heat, cold and osmotic stress like the control but are sensitive to oxidative stress, conforming to the described role for mammalian PKDs. A candidate screen to identify functionally redundant kinases uncovered genetic interactions of PKD with Pkc, sqa and Drak mutants, further supporting the role of PKD in oxidative stress response, and suggesting its involvement in starvation induced autophagy and regulation of cytoskeletal dynamics. Overall, PKD appears dispensable for fly development and survival presumably due to redundancy, but influences environmental responses.

KEYWORDS
Drosophila melanogaster
Protein kinase D
PKD null mutant
oxidative stress response
redundancy

INTRODUCTION
Protein Kinase D (PKD) isoforms are serine/threonine kinases of the Protein Kinase C family typified by a long N-terminal regulatory region followed by a catalytic kinase domain (Figure 1A). The regulatory region contains two cysteine-rich domains that bind to diacylglycerol and phorbol esters, and a Pleckstrin-homology module, that auto-inhibits the catalytic domain (Figure 1A) (reviewed in Fu and Rubin 2011). Once recruited to the membrane by diacylglycerol, PKD is activated by members of the PKC family through the phosphorylation of two serine residues in the activation loop of the kinase domain (Fu and Rubin 2011). Based on sequence similarity of the kinase domain, PKD has been classified as member of the Ca2+/Calmodulin-dependent serine/threonine protein kinases (CAMK) (Fu and Rubin 2011, Olayioye et al. 2013).

Three PKD isoforms exist in mammals, where they are involved in various processes, including the regulation of a cell survival response upon oxidative stress, cell proliferation, cell differentiation, cell motility and invasion, as well as secretory transport from the trans-Golgi compartment to the plasma membrane (reviewed in Fu and Rubin 2011, Olayioye et al. 2013, Cobbaut and Van Lint 2018). The extent of overlap in their biological function is still a matter of debate, but it is evident that all three act as multi-functional kinases with a major role in structural integrity and function of the Golgi complex as well as in the regulation of actin-cytoskeletal dynamics (Fu and Rubin 2011, Olayioye et al. 2013). Interestingly, a knock-out mutation in the murine PKD1 gene allows normal mouse development and fertility, albeit...
affecting oxidative stress response in embryonic fibroblasts (Zhang et al. 2015). Whereas this result may be taken as indication for functional redundancy of the three PKD isoforms, it may also be interpreted as a highly (perhaps cell type) specific, non-lethal function of the respective kinase. Alternatively, only laboratory, non-stressed conditions may allow normal development (Zhang et al. 2015). *Drosophila melanogaster* harbors a single PKD homolog with a similarity of 67% and identity of nearly 60% to any of the three human PKD kinases (Maier et al. 2006). Accordingly, *Drosophila* has been used as an *in vivo* model to study the biological roles of PKD. The *Drosophila* PKD gene is broadly expressed throughout development. Whereas PKD mRNA is uniform in imaginal tissues, it strongly accumulates in ectodermal derivatives in the late embryo, detected for example in the epithelia of the epidermis, the salivary glands, the hind- and the foregut (Maier et al. 2006). A ubiquitously expressed PKD-GFP fusion protein was present in the cytosol and along cell membranes, and in the trans-Golgi compartment of secretory tissues like the salivary glands (Maier et al. 2006). The biological function of PKD in the fly was assessed by the overexpression of presumptive activated and dominant negative isoforms of PKD, as well as by RNA interference experiments (Maier et al. 2006, Maier et al. 2007, Nagel et al. 2010). Whereas the overexpression of a dominant negative PKD isoform interfered with pattern formation in the wing, the activated PKD-SE isoform affected development more broadly (Maier et al. 2007). RNAi-mediated knockdown of PKD activity effected tissue loss primarily through apoptosis. Interestingly, a light-dependent degeneration of the adult retina was observed in flies overexpressing activated PKD-SE (Maier et al. 2007). Being a typical consequence of rhodopsin maturation or trafficking defects (Colley et al. 1993), this phenotype points to a role of PKD in secretory transport and in cytoskeletal dynamics (Maier et al. 2007).
The latter aspect was corroborated by the finding that *Drosophila* PKD, like its mammalian counterpart PKD1, impacts actin remodelling by the regulation of coflin activity through its phosphatase Slingshot (Barišić et al. 2011). Accordingly, accumulation of F-actin and phosphorylated coflin was likewise observed in cell clones either overexpressing activated PKD-SE or lacking Slingshot (Nagel et al. 2010, Barišić et al. 2011). These investigations provided evidence for a possible role of *Drosophila* PKD in cell motility by modulating actin dynamics (Barišić et al. 2011). Recently, a role of *Drosophila* PKD in the secretion of insulin like peptide ILP2 was uncovered, thereby influencing metabolism and growth of the developing animal (Ashe et al. 2018). In sum, *Drosophila* PKD appears to be a multifunctional kinase like its mammalian homologs (Fu and Rubin 2011, Olayioye et al. 2013).

In order to address the role of *Drosophila* PKD in further detail, we generated null alleles by ends-out directed homologous recombination (PKD\(^{26}\), PKD\(^{26}\)). Both alleles turned out to be homozygous viable without apparent phenotypes. With regard to developmental timing, life span, fertility, weight and fat content, the PKD null mutants were largely within the range of the control. Whereas the PKD null mutants tolerated various stress factors, sensitivity toward oxidative stress was uncovered. In a candidate kinase screen, we obtained evidence for redundant kinase function regarding oxidative stress response, starvation induced autophagy and regulation of cytoskeletal dynamics. Overall, our data indicate that PKD is largely dispensable for development and survival of *Drosophila* but is required for combattting oxidative stress.

### MATERIALS AND METHODS

#### Generation and confirmation of PKD mutant alleles

To generate PKD null mutants, we followed the ‘ends-out’ homologous recombination protocol developed by Gong and Golic (2003). To this end genomic fragments covering 5’ and 3’ regions of the locus were PCR-isolated from lambda phage ED clones (Maier et al. 1992): a 5 kb genomic Acc65I/AscI fragment starting within the first intron and covering the second coding exon (Phi; Figure 1B), and a 3.4 kb Acc65I/Sphi fragment overlapping the downstream CG18600 locus (Phr; Figure 1B). Primers included restriction enzyme target sites for cloning (bold):

- PKDph1UP 5’ GGG ACC GCA ATA TGC CGC TGT TAT TTA TTG ATC AAT 3’
- PKDph2LP 5’ GGC GCG CCT TAC GAC TGG TGG TCA GCA GAA CCT ACC ACG GTG ACA GCG A 3’
- PKDPhrUP 5’ GGG ACC GGA GGA ATT CTG TAT GAG CAG TA 3’
- PKDPhrLP 5’ GCA TCG CCA AAA ACG CGC GCA CAT TTA CAA C 3’

Fragments were cloned into pW25 transformation vector using compatible restriction sites (Gong and Golic 2003) to generate transgenic starter line T15-2 (second chromosomal insertion) by classical P-element mediated germ line transformation of y\(^{+}\) w653;f flies (Rubin and Spradling 1982). Homologous recombination was performed as described before (Gong and Golic 2003, Fischer et al. 2015), starting with 592 G1 single-crosses resulting in the line PKD\(^{26}\). The inserted white marker gene was excised with help of I-Cre exactly as described before (Rong et al. 2002) to yield allele PKD\(^{24}\).

Homologous recombination at the PKD locus at 92E was confirmed for PKD\(^{26}\) by chromosome in situ hybridization, by Southern blotting as described earlier (Preiss et al. 1988), and by PCR with primer pairs P1/P2, P3/4, P5/6, P5/7 and P6/8. Loss of mRNA expression in PKD\(^{26}\) was confirmed by in situ hybridization on whole mount embryos according to standard protocols (Tautz and Pfeifle 1989) and by RT-PCR. The breakpoint fragment generated by PCR from PKD\(^{24}\) total DNA with primer pair P5/P6 was sequence confirmed. The following primers were used (position is schematically shown in Figure 1B):

- P1 M-UP 5’ TCG AGT CCT CGG TGG AGA CGA 3’
- P2 M-LP 5’ CTC CGA GAT GCC GAC CCT CAA 3’
- P3 5’ RT-UP 5’ GGC GGT CAGCAC GAT TTC CA 3’
- P4 5’ RT-LP 5’ AGC GTT CCC GCT ATC ATG GAG 3’

To generate the second coding exon (Phl; Figure 1B), and a 3.4 kb Acc65I/Sphi fragment overlapping the downstream CG18600 locus (Phr; Figure 1B). Primers included restriction enzyme target sites for cloning (bold)

- PKDph1UP 5’ GGG ACC GCA ATA TGC CGC TGT TAT TTA TTG ATC AAT 3’
- PKDph2LP 5’ GGC GCG CCT TAC GAC TGG TGG TCA GCA GAA CCT ACC ACG GTG ACA GCG A 3’
- PKDPhrUP 5’ GGG ACC GGA GGA ATT CTG TAT GAG CAG TA 3’
- PKDPhrLP 5’ GCA TCG CCA AAA ACG CGC GCA CAT TTA CAA C 3’

Polytene chromosomes from salivary glands of PKD\(^{26}\) homozygous mutant third instar larvae were prepared for in situ hybridization as outlined in Ashburner (1989). The probe, labeled with DIG-dUTP by random priming (Tautz and Pfeifle 1989), was generated using CaSpeR-vector as template as it contains the white\(^{+}\) minigene (Pirrotta 1988), using the DIG DNA Labeling and Detection kit (Roche; Merck). Hybridization was as outlined in Ashburner (1989), and detection according to the manufacturer’s protocol. In situ hybridization on whole mount embryos was as described earlier (Tautz and Pfeifle 1989; Maier et al. 2006). Embryos from y\(^{+}\) w653 control flies and PKD\(^{26}\) mutant flies were collected overnight. As probe, we used PKD cDNA (pOT GH26429) (Maier et al. 2006), labeled with DIG-dUTP as above.

#### Transcriptional analysis by RT-PCR

Poly(A)\(^{+}\) RNA was isolated with the PolyAtract System 1000 kit (Promega Mannheim, Germany) from 25 PKD\(^{26}\), PKD\(^{24}\) and y\(^{+}\) w653 male flies each according the supplier’s protocol. cDNA was produced with qScriber cDNA Synthesis Kit (highQu, Kraichtalg, Germany) according to the manufacturer’s protocol. Amplification was with primer pairs P1/P2 and P3/P4, both overlapping introns: Genomic DNA should yield a 352 bp and a 1662 bp amplicon, respectively, whereas 278 bp and 282 bp are expected from cDNA. Tubulin 56D primers served as positive control (see primer list above). Absence of genomic DNA was tested in a non-RT control. NEB quick-load 100 bp DNA ladder was used as size standard.

Real time qRT-PCR was conducted as outlined in Praxenthaler et al. (2017) using Blue S’Green qPCR kit (Biozym, Hessisch-Oldendorf, Germany) on 10 ng of cDNA from PKD\(^{26}\), PKD\(^{24}\) and OreR flies in 10μl end volume using MIC magnetic induction cycler (bms, Pots Point, Australia) including target, no-template and non-RT controls. As internal references for PKD or SOD expression, βTub56D and tbp were used. Primers were selected from the DRSC FlyPrimer bank (Hu et al. 2013): SOD1, PP70435; SOD2, PP70435; tbp, PP1556. Primers for Tub56D and PKD (P1/P2) are listed above. Relative quantification of three biological and two technical replicates was performed with miccPCR software Version 2.6.5 based on REST\(^{+}\) taking target efficiency into account (Pfaffl et al. 2002).
Fly stocks

Information on strains is available at https://flybase.org. Crosses, combinations and recombinations were performed with standard genetic techniques. Double mutants were confirmed by PCR. The following stocks were used: Oregon R (OreR) and y w 7232 (BL6599), w 118B either isogenic line BL5905 or BL6326, Canton-S (CS), p38a1 (BL8822), bsk1 (BL3088), aPKC 86403 (BL10622), Drak 6508876c (Bellen et al. 2004), par-1 1608231 (BL10615), Pckcl 101809 (BL18258), sgu 01512 (BL18446), Strn- Mlk c 02860 (BL11089), Df(2R)Exel6065, Df(1) Exel6227, Df(1)Exel6236, Df(2L)Exel7077 (Parks et al. 2004).

Phenotypic analyses

Flies were raised under non-crowded conditions on standard agar-corn-molasses food (per liter 18g dry yeast, 10g soy flour, 22g molasses, 80g malt extract, 80g cornmeal, 6.25ml propionic acid, 8g agar-agar) at constant 25° and 78% humidity. Analyses were performed on one to five days old flies. To investigate developmental timing, offspring from parallel inter se crosses of 5 females and 3 males each was counted at days 8 to 18 (Wang et al. 2003, Fischer et al. 2015). To determine fertility, 4-8 virgin females (one to four days old) were kept in a vial with wild type males for three days, and then put on fresh food for 4-5 days at 18°; the number of offspring was recorded and calculated per female per day on food. For longevity experiments, animals separated by sex at the day of eclosion were transferred in batches of 25-30 to fresh food every third day; dead animals were recorded daily (Clancy et al. 2001, Wang et al. 2003, Tettweiler et al. 2005). High sucrose medium contained additional 10% sucrose (Wang and Clark 1995; Magwere et al. 2004). Using a precision balance adult weight was determined in batches of five animals 1-2 days after hatching (Fischer et al. 2015). Relative fat content was determined as percentage of dry weight as outlined in Vermeulen et al. (2006). The larval floating test was applied on fully fed, third instar wandering larvae in 8%, 10% and 12% sucrose in PBS, respectively (Reis et al. 2010; Fischer et al. 2015). Resistance to starvation was recorded either in the absence of food (wet starvation) or absence of all (dry starvation). To this end, sexed flies (2-3 days old) were starved in batches of 25 in empty vials (dry starvation) or in vials supplemented daily with a wet filter paper (wet starvation); dead animals were recorded regularly (Craig et al. 2004; Wang et al. 2004). Thermotolerance of flies, collected 2 days after eclosion, was tested in dry heat, wet heat and cold. Survival was determined by incubating flies in batches of 25-30 at 37° in an incubator on pre-warmed food for 2-3 hr (dry heat), or alternatively by submerging vials in a 37° degree water bath and recording dead animals regularly (Craig et al. 2004, Nielsen et al. 2005). To test cold sensitivity, two days old animals were cooled to 4° for 2 hr and recovery time until walking was recorded (Nielsen et al. 2005). To generate oxidantive stress, 20 males 3-5 days old were first starved for four hours on 1% agarose/PBS medium, and then transferred to vials with filter paper soaked with a solution of 20 mM paraquat in 5% sucrose at 25° in the dark; dead flies were recorded twice a day, and live flies then transferred to a fresh paraquat containing vial (Clancy et al. 2001; Wang et al. 2003). Osmotic stress was applied by rearing flies on food containing additional 0.5 M NaCl, death toll was recorded daily (Craig et al. 2004).

Border cell migration was studied by staining ovaries with rhodamine-coupled phalloidin (Molecular Probes, Eugene OR, USA) as outlined before (Nagel et al. 2012), analyzed by confocal microscopy using a BioRad MRC 1024 coupled to a Zeiss Axiohot and LaserSharp 2000 imaging software (Carl Zeiss AG, Oberkochen, Germany). Pole cells were stained in embryos using anti-vasa antibodies (developed by A. C. Spradling and D. Williams, obtained from the Developmental Studies Hybridoma Bank (DSHB) developed under the auspices of the NICHD and maintained by the Univ. of Iowa, Dept of Biology, Iowa, USA) as outlined before (Hay et al. 1988). Goat secondary antibody coupled to alkaline phosphatase was obtained from Jackson Immuno-Research Laboratories (Dianova, Hamburg, Germany). Microphotographs of chromosomes, embryos, larvae or adults were taken with a Pixera ES120 digital camera (Optronics) coupled to a Zeiss Axiohot or to a Leica Wild M3C stereomicroscope using the Pixera Viewfinder Version 2.0 software. Figures were assembled using Corel Photo Paint, Corel Draw, Exel, and BoxPlotR software. Statistical significance of probes was determined by ANOVA two-tailed test for multiple comparisons using Dunnet’s approach with raw p-values: \( P > 0.05 \) (not significant); \( * P < 0.05; ** P < 0.01; *** P < 0.001 \).

Data availability

PKD mutant strains are available upon request. Supplemental data comprise 4 Supplemental Figures S1 to S4 in one file and one Supplemental Table S1. Figure S1 shows the complete chromosome spread of Figure 1D. Figure S2 contains the comparison of the developmental timing of PKD mutants and several additional controls. Figure S3 contains the comparison of the lifespan of PKD mutants and several additional controls. Figure S4 contains the comparison of the starvation resistance of PKD mutants and several additional controls. Table S1 contains details on the kinase mutants used in the candidate screen. Supplemental material available at FigShare: https://doi.org/10.25387/g3.7667078.

RESULTS

Generation of PKD null mutant alleles by ends-out homologous recombination

The Drosophila PKD locus has been mapped to the right arm of chromosome 3 at position 91A2 (https://flybase.org). In order to generate specific PKD mutant alleles, we employed the technique of ends-out homologous recombination (Figure 1B, C) (Gong and Golic 2003). In allelic PKD–, all relevant coding regions of PKD were replaced by the white-Gene used for selection, i.e., the regulatory and the catalytic domain of the kinase (Figure 1A-C). Replacement of the PKD locus by the white-Gene in PKD– was confirmed by chromosomal in situ hybridization (Figure 1D, supplemental Figure S1). In allele PKD–, the white Gene was deleted by Cre 1-mediated recombination as outlined before (Rong et al. 2002). The resultant deleton was confirmed by sequence analysis of a PCR amplifying overlapping the breakpoint (Figure 1B, C). Absence of PKD transcripts was verified by in situ hybridization on whole mount PKD– embryos (Figure 1E) and by RT-PCR (Figure 1F) and qRT-PCR for both alleles, respectively.

PKD null mutants are homozygous viable without apparent phenotype

Based on our earlier studies we expected loss of PKD to affect growth and perhaps cause lethality (Maier et al. 2007). Yet, homozygous PKD null mutants were viable without apparent phenotype with respect to size or the overall pattern of the external organs (Figure 2A). Moreover, developmental timing, i.e., emergence of adult flies (Figure 2B) was similar to y w 118B Canton-S and notably Oregon R was observed (Figure 2, supplemental Figure S2). Still, PKD– mutants were within the normal range, and neither pupae
Lifespan of male and female control
ses. Statistical analysis was performed with a two-tailed ANOVA test relative to TM3 long-
er than the control is shown as fraction of the total (given in the respective legend). Whereas
relative fat content compared to
Moreover, OreR males, but not the females, had a signi-
but not compared to
which were, however, of lower weight compared to the wild type OreR
3B). The speci-
(Figure 3C): here the
life span also differed remarkably between several control strains
heterozygous siblings (Figure 2C). As observed for
mutant males, in contrast to that of the
PKD26 mutants lie in between the two control strains. In addi-
tion, we tested sensitivity of PKD mutants toward starvation under wet
conditions (Figure 3D) and dry conditions (Figure S4): males were slightly more
PKD mutants did not differ from
y1 w6723 for the
PKD26 mutants were more similar to the OreR wild type. We conclude
that the PKD mutants lie in between the two control strains. In addi-
tion, we tested sensitivity of PKD mutants toward starvation under wet
sugar diet (Magwere et al. 2004, Al Saud et al. 2015) was also observed
for the PKD26 mutant. When flies were raised on high sucrose, life span of
PKD26 mutants did not differ from y1 w6723 control, irrespective of
sex (Figure 3E).

PKD null mutants are sensitive for oxidative stress
As PKD is apparently not strictly required for fly development, life span
and fertility, we wondered whether this kinase might be involved in stress
regulation. We assayed sensitivity of PKD26 mutants toward a variety of
nor adults developed particularly slower than the controls (Figure 2,
supplemental Figure S2). We also addressed female fertility, which was
indistinguishable between the homozygous PKD mutants and their
heterozygous siblings (Figure 2C). As observed for fly hatching time,
the life span also differed remarkably between several control strains
(Figure 2D, D’; supplemental Figure S3). It was shortest in PKD26
mutant males, in contrast to that of the PKD26 females that lived longer
than the y1 w6723 control (Figure 2D, D’; supplemental Figure S3).

As it was recently suggested that PKD may play a role in weight
control and fat homeostasis (Ashe et al. 2018), we measured body
weight and fat content of our mutants (Figure 3A, B). Overall, there
was not a great difference between PKD26 mutants and y1 w6723 flies,
which were, however, of lower weight compared to the wild type OreR
but not compared to y1 w6723 used for further control (Figure 3A, B).
Moreover, OreR males, but not the females, had a significantly lower
relative fat content compared to PKD26 mutants and y1 w6723 (Figure
3B). The specific larval weight was measured in a buoyancy assay
(Figure 3C): here the PKD26 mutants matched the y1 w6723 control,
PKD26 mutants resisted the application of heat in a dry oven or in a water bath similar to controls (Figure 4A, B), whereas p38a1 mutants were more and bsk1 mutants slightly less sensitive, as described in the literature (Wang et al. 2003, Craig et al. 2004). Moreover, no particular cold sensitivity was observed (Figure 4C). Likewise, PKD26 flies tolerated osmotic stress similar to the control (Figure 4D). Oxidative stress, however, was less tolerated by the PKD null mutant alleles compared to the y1 w67c23 and OreR controls (Figure 4E). This is in line with the described role of mammalian PKDs to protect cells from oxidative stress mediated apoptosis. Here, PKD is involved in mitochondrial ROS detoxification by driving the expression of Manganese superoxide dismutase (MnSOD) (Storz 2007; Cobbaut and Van Lint 2018). To address a likewise role for Drosophila PKD, expression of superoxide dismutase was measured by quantitative RT-PCR in the two mutant PKD alleles. We addressed both, the expression of MnSOD (SOD2) as well as of Cu/ZnSOD (SOD1) that act in mitochondria and the cytosol, respectively, to detoxify the cell from superoxide released from mitochondria. In the absence of PKD, SOD1 and SOD2 levels were very similar those of the OreR control: a slight but not significant decrease was observed for SOD1 expression (0.72–0.87 fold), whereas expression of SOD2 was slightly increased (1.35–1.38 fold) (Figure 4F). Apparently, SOD regulation in Drosophila is largely independent of PKD activity in unstressed conditions.

Figure 3 Influence of PKD on body weight and starvation resistance. (A) BoxPlot representation of the fresh weight of animals of the given genotype weighed in batches of five given in mg. The left panel shows the weight range of males, the right panel that of females. (B) The relative fat content of male and female flies of the given genotype was determined on 25-30 animals per experiment relative to the dry weight. PKD26 mutants are not different from y1 w67c23 control, whereas OreR males have a significantly larger body weight, and hence, a relatively low fat content. BoxPlots in (A) and (B) depict medians as Center lines; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range; outliers are represented by dots. Sample points are given for each genotype in parentheses. Statistical analysis was performed with a two-tailed ANOVA test relative to y1 w67c23 using Dunnet’s approach with *** P < 0.001 and ** P < 0.01. (C) Larval specific weight was determined in a floating assay with sucrose of different density. Number of floating larvae is indicated for 12% sucrose (blue, 7 experiments with 10 larvae each) and 10% sucrose (orange, 8 experiments with 10 larvae each). At 8% sucrose, all larvae sank (70 animals tested per genotype). Note that PKD26 resembles y1 w67c23 control, and PKDcl4 the OreR control. (D) Sensitivity to starvation stress was measured as survival of male and female flies on wet filter paper over time; dead flies were counted regularly. Whereas males of different genotype were similar, female OreR flies were highly resistant and female y1 w67c23 very sensitive toward starvation (4-6 experiments each; the total number of tested animals is given in the legend for each genotype). (E) Lifespan was determined on medium with normal and high sucrose content (+s). High sucrose medium shortened life span of PKD26 mutants as well as y1 w67c23 control irrespective of sex to similar values.
Migration of border cells and pole cells appears normal in PKD mutants

Human PKDs are pivotal to cell motility by regulating cytoskeletal dynamics. Specifically, human PKDs act as negative regulator of Slingshot-phosphatase, thereby influencing cofilin availability and actin filament de/polymerization (reviewed in Olayioye et al. 2013). We have shown before that overexpression of active Drosophila PKD-SE negatively regulates Slingshot activity, suggesting a likewise involvement of PKD in cytoskeletal dynamics in the fly (Nagel et al. 2010, Barisić et al. 2011). Accordingly, we might expect an impact of a loss of Drosophila PKD on cell migration. We studied two well-characterized processes of cell migration during Drosophila development. First, we monitored migration of border cells during oogenesis. Border cells are specifically determined follicle cells that actively migrate from the anterior tip of the follicle in between the nurse cells to the anterior border of the oocyte (reviewed in Montell 2006). The timing of the migration can be followed by the columnar follicle cells that retract in parallel toward the posterior of the oocyte (reviewed in Montell 2006). No difference was seen in border cell migration behavior between PKD144 and control (Figure 5). Second, we studied pole cell migration during embryogenesis. Pole cells are the primordial germ cells of Drosophila. They arise at the posterior of the embryo and migrate through the posterior midgut and along the germ band to populate the gonadal mesoderm (Hay et al. 1988; reviewed in Montell 2006). This process appears normal in PKD mutant embryos that moreover, display the same number of pole cells than the wild type (Figure 6). We conclude that PKD is not required for these two processes of cell migration in Drosophila. As the overexpression of an activated isoform PKD-SE can influence the cytoskeleton (Nagel et al. 2010, Barisić et al. 2011), the most likely explanation for a lack of migration defects is the presence of redundant kinases that adopt the function of PKD in its absence.

A small candidate screen on potentially redundant kinases

The fact that a loss of Drosophila PKD appears to be without major phenotypic consequences suggests that it may act redundantly to some other kinase, most likely members of the family of PKC or CAMK kinases. Candidates located on the first or second chromosome were selected by the availability of mutant alleles, as shown in Supplemental Table S1, thereby covering most of the Drosophila PKC family members (four out of five), and about one quarter of all CAMK family members (7 of 30) (Morrison et al. 2000). The mutants were combined with PKD144 to record survival rate of the offspring. The fraction of balanced vs. homozygous PKD144 mutants was determined in the heterozygous background of the relevant kinase mutant. Survival rate of PKD144 homozygotes was reduced to 70–80% in a sqh“0121” and Pkcdelta“04408” background.
heterozygous background (Table 1). A likewise increased mortality of the PKD\textsuperscript{cl4} homozygotes was observed when either Pkdel90408 or Drak\textsuperscript{BG00876} were homozygous in addition (Table 1). Unfortunately, double mutants could only be tested for these two, as most of the kinase candidate mutants are homozygous lethal. As most kinase mutants are fully recessive, however, heterozygotes are unlikely to be influenced by a loss of PKD activity. Pronounced synthetic lethality, as we might have expected if one of the kinases required PKD to supplement its activity, was not observed.

**DISCUSSION**

We have generated specific knock out alleles of Drosophila PKD, which to our surprise were homozygous viable without apparent phenotype. Based on our own previous experiments, we expected conspicuous phenotypes, resulting for example from defects in the cytoskeleton or in protein secretion (Maier et al. 2007; Nagel et al. 2010; Barisic et al. 2011). Lack of drastic phenotypes, however, suggests redundancy: presumably other serine-threonine protein kinases of the PKC/CAMK family act in place of PKD. Our tentative candidate kinase screen indeed uncovered three kinases, Drak, Sqa and Pkc\textdagger, that may be linked with PKD activity, since respective hypomorphic alleles impeded fly viability in the absence of PKD (Table 1). The former two belong to the CAMK and the latter to the PKC family of kinases (Morrison et al. 2000). These results are rather intriguing in light of the known roles for mammalian PKDs in oxidative stress response and in the regulation of cell motility and invasion (Fu and Rubin 2011, Cobbat and Van Lint 2018, Olayioye et al. 2013).

It is well known that mammalian PKDs oppose the apoptotic effects of oxidative stress in a variety of cells (reviewed in Storz 2007; Cobbat and Van Lint 2018). In this process, PKD1 is activated by PKC\textdagger in response to phospholipase D activation at the mitochondrial membrane. Consequently, active PKD1 inhibits mitochondrial depolarization and decreases the release of cytochrome C, thereby protecting cells from apoptosis, and more generally from oxidative damage. In addition, PKD1 mediates expression of MnSOD, the superoxide dismutase that detoxifies the cell from superoxides released from the mitochondria. Altogether, PKD1 activity results in pro-survival signals in oxidative stress (reviewed in Storz 2007; Cobbat and Van Lint 2018). We have observed that PKD\textsuperscript{cl4} mutants display an increased sensitivity toward oxidative stress (Figure 4E), whereas in unstressed conditions transcription levels of MnSOD and Cu/ZnSOD were similar to wild type (Figure 4F). In addition, downregulating the activity of the Drosophila PKD\textdagger homolog strongly increased the mortality of PKD\textsuperscript{cl4} homozygotes (Table 1). Together, these data strongly support a likewise protective role for Drosophila PKD in combating oxidative stress in the fly.

In mammals, PKDs play an important role in actin cytoskeletal dynamics via the regulation of the phosphatase Slingshot (reviewed in Olayioye et al. 2013). Using overexpression experiments, we have shown earlier that Drosophila PKD likewise affects Slingshot activity, and consequently the dynamics of filamentous actin turnover (Barisic et al. 2011, Nagel et al. 2010). However, the complete absence of PKD had no apparent influence on the migration of border or pole cells, indicating redundancy for PKD activity in the context of cell motility. A possible candidate for a redundant kinase might be Drak, which has been involved in actomyosin contractility and dynamics (Neubueser and Hipfner 2010, Chougule et al. 2016). Our work shows a genetic interaction between PKD and Drak, since a downregulation of Drak activity in the hypomorphic allele Drak\textsuperscript{BG00876} increased mortality of PKD\textsuperscript{cl4} mutants considerably (Table 1). We thus conclude that Drak and PKD may act together in the regulation of cell motility.

RNAi mediated knock down of PKD activity affected cell growth and differentiation (Maier et al. 2007). In accordance with these data, Ashie and co-workers reported growth defects resulting from PKD depletion (Ashie et al. 2018). Moreover, a specific role for PKD in the release of Drosophila insulin like peptide ILP2 was reported, explaining the reduced weight as well as starvation sensitivity of the dPKD\textsuperscript{cl4} allele used in their study. The mutant dPKD\textsuperscript{cl4} allele has a 70% reduced PKD mRNA level in third instar larvae (Ashie et al. 2018). One might have expected an even stronger phenotype in the complete absence of PKD, which

![Figure 5](image.png)

Figure 5. Border cell migration. (A-A’) Sketch of border cell migration at stages 9 to 10 during Drosophila oogenesis. Early in stage 9, a cluster of border cells dispatches from the anterior tip of the follicle (A, red arrow), to migrate in between the large, polyploid nurse cells (A’) to finally reach the anterior of the oocyte in stage 10 (A”). At the same time, the columnar follicle cells retract posteriorly; they are a useful marker for orderly migration (read arrowheads). Border cells are depicted in red, and highlighted by a red frame and a red arrow. (B-B”) Cell outlines were visualized with phalloidin staining (black). Confocal images taken from the respective stages of control (B-B”) and PKD\textsuperscript{cl4} mutant females (C-C”) are shown inverse for better visibility.

Border cells are marked by red frame and arrow; columnar follicle cell margins by read arrowheads, respectively, as in (A-A’). The migratory behavior of border cells appears not different between the two genotypes.
however, was not observed. PKD null mutants were largely normal with respect to growth and weight. How can we explain the different results obtained from the null alleles PKD26 and PKDcl4 compared to dPKDH?

First, dPKDH might rather match a null allele based on the low residual PKD expression levels. In this case, the null alleles may not show dramatically stronger phenotypes. The observed differences may then be attributed to external factors, i.e., rearing conditions (food composition, relative humidity, temperature etc.) or internal factors, i.e., genetic background. The latter appears more likely, as we have seen a high variance among various control strains regarding several tested parameters (Figures 2, 3, S2-S4). Still, none of the control strains displayed such strong and specific defects as the dPKDH allele in Ashe et al. (2018), which was developmentally retarded and underweight. We therefore favor the possibility of a second site hit in the dPKDH allele that influences its phenotype. PKD null mutants are evidently without or very little phenotype on their own, presumably due to the function of redundant kinase(s). Mutation in (any) one of these kinases is without conspicuous phenotype, at least when heterozygous. The absence of PKD, however, may uncover a growth defect in such a mutant, if the respective kinase is involved in the regulation for example of TOR signaling activity. A rescue of this growth defect by addition of PKD - for example by ubiquitous overexpression like Act\textsuperscript{dPKD} (Ashe et al. 2018) - is to be expected, since in this case, PKD can take over the redundant function. A possible candidate for such a redundant kinase

![Figure 6 Pole cell migration. (A) Embryos were stained with anti-vasa antibodies to mark the pole cells. Anterior is to the left, embryonic stages are indicated (st5-st15). Pole cells originate at the posterior pole (stage 5), and migrate through the posterior midgut (stage 8) to move posteriorly during the following stages (9-14), before they conglomerate with mesodermal cells (stage 15) to form the round gonadal anlagen in the embryo. No apparent differences are seen between the control Oregon R (OreR, upper row) and PKDcl4 mutants (lower row). (B) Moreover, the number of pole cells was not significantly different between the two PKD alleles, PKD26 and PKDcl4, and the control OreR. Center lines of the BoxPlots show the medians; box limits indicate the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles; whiskers extend 1.5 times the interquartile range; dot represents outlier. Number of data points is given in parentheses for each genotype.](image)

### Table 1 Candidate kinase screen Expected fraction of homozygous PKD\textsuperscript{cl4} animals inferred from the number heterozygous PKD\textsuperscript{cl4} siblings in a heterozygous background of the respective candidate kinase mutant is given in percent %. The final inter se cross was of the following genotype: M or Y/FM7; PKD\textsuperscript{cl4}/TM3Sb and M/CyO; PKD\textsuperscript{cl4}/TM6B, respectively, with M representing mutant allele or deletion

| Kinase                                      | allele/deletion | n\textsuperscript{a} | % PKD expected\textsuperscript{b} |
|---------------------------------------------|-----------------|-----------------------|-----------------------------------|
| atypical protein kinase C (aPKC)            | aPKC\textsuperscript{K04403} / CyO | 46                    | 117.2%                            |
| Inactivation no afterpotential C (inaC)      | both contained within: |                        |                                   |
| Protein C kinase 53E (PKC53E)               | Df(2R)Exel6065/ CyO | 75                    | 112.5%                            |
| Protein kinase C \delta(Pkc\delta)          | Pkcdelta\textsuperscript{O04408} / FM7 | 84\textsuperscript{c} | 84.7%                             |
| Ca\textsuperscript{2+}/calmodulin dependent protein kinase family (CAMK) | Pkcdelta\textsuperscript{O04408} / FM7 | 271                   | 85.3%                             |
| Kinase                                      | allele/deletion | n\textsuperscript{a} | % PKD expected\textsuperscript{b} |
| AMP-activated protein kinase \alpha subunit (AMPK\alpha) | Df(1)Exel6227 / FM7 | 120                   | 107.7%                            |
| Death associated protein kinase related (Drak) | Drak\textsuperscript{BG00876} | 167\textsuperscript{c} | 80.0%                             |
| Ioki (lok)                                  | Df(2L)Exel7077 / CyO | 221                   | 138.6%                            |
| MAP kinase activated protein-kinase-2 (MK2) | Df(1)Exel6236 / FM7 | 203                   | 94.2%                             |
| par-1 (par-1)                               | par-1\textsuperscript{G06323} / CyO | 39                    | 139.1%                            |
| spaghetti-squash activator (sqa)            | sqa\textsuperscript{F05132} / CyO | 151                   | 69.6%                             |
| Stretchin-Mlck (Strn-MLCK) Myosin light chain kinase | Strn-Mlck\textsuperscript{O24860} / CyO | 127                   | 134.2%                            |

\textsuperscript{a}, total number of offspring analyzed.

\textsuperscript{b}, expected number of homozygous PKD\textsuperscript{cl4} animals was determined from the number of the heterozygous siblings.

\textsuperscript{c}, allele is homozygous viable and was tested in homozygosis.
might be spaghetti squash activator (sqa), which encodes a novel myosin light chain kinase with a role in starvation induced autophagy and the regulation of TOR signaling activity (Tang et al. 2011, Findlay et al. 2007). The sqaDf1712 allele is fully recessive, and the heterozygotes are without apparent phenotype. Still, the sqaDf1712 heterozygous background caused a dramatic lowered survival rate of less than 70% of PKD+/+ flies (Table 1). It is hence conceivable that PKD plays indeed a role in growth regulation, however, overlaps functionally with other kinases in this process, for example with Sqa. Overall, our results suggest that presumably PKD’s absence can be replaced in the respective context by one or several other kinases with overlapping function. For example, Pkcα has not yet been assigned a specific role, as the mutants are viable without phenotype. Still, the mutant allele PkcdeltaaDf marked-ly impedes fly viability in the absence of PKD activity, strongly indicating fundamental redundancy. For technical reasons, we could test only a small subset of the Drosophila CAMK family. We expect overlap of PKD with further protein kinases not yet included in this test.

To date the functions of PKDs have been studied primarily in model cell-culture systems, and only few data exist on PKDs’ functions in the context of normal cells and tissues of intact organisms (Fu and Rubin 2011). Three models have been studied by now, D. melanogaster (this work), C. elegans and the mouse. Null mutants in either of the two PKD isoforms from C. elegans, named DPK-1 and DPK-2, are viable. Animals lacking DPK-1 display locomotory defects, whereas DPK-2 mutants impede adult life span by affecting stress and innate immunity responses (Feng et al. 2006, Feng et al. 2007, Ren et al. 2009). PKD1 mutant mice are viable and fertile, but appear semilethals with only half of the expected offspring. Embryonic fibroblasts derived from these mice are highly susceptible to apoptosis induced by oxidative stress or by starvation, which is mediated by PKCε activity (Zhang et al. 2015). In these cells, PKD1 is the key regulator in determining the threshold of mitochondrial depolarization affecting Bcl2-Bax fractions, implicating a role for PKD1 during aging and nutrient deprivation (Zhang et al. 2015). As neither PKD2 nor PKD3 can compensate the loss of PKD1, viability of the PKD1 mutants is most likely not explained by redundancy with the other PKD isoforms. Rather oxidative stress responses may be tissue specific, or defects are observed only when animals are subjected to non-laboratory, stressful conditions (Zhang et al. 2015). This work shows that Drosophila PKD null alleles are viable and fertile, however, are sensitive toward oxidative stress. Moreover, we uncovered genetic interactions of Drosophila PKD with three kinases PKCε, Drak and Sqa. Most likely overlapping functions exist between PKD and other kinases of the PKC/CAMK family, explaining the lack of apparent phenotypes in the null mutants. Whether the overlap is restricted to these three kinases or extends to a larger kinase family, and whether it applies only to certain tissues or processes, will require further investigation in the future.

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LITERATURE CITED
Al Saud, S. N., A. C. Summerfield, and N. Alic, 2015 Ablation of insulin-producing cells prevents obesity but not premature mortality caused by a high-sugar diet in Drosophila. Proc. R. Soc. B. 282: 20141720. https://doi.org/10.1098/rspb.2014.1720

Ashburner, M., 1989, pp. 37–49 in Drosophila: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

Ashe, S., V. Malhotra, and P. Raghu, 2018 Protein kinase D regulates metabolism and growth by controlling secretion of insulin like peptide. Dev. Biol. 434: 175–185. https://doi.org/10.1016/j.ydbio.2017.12.008

Barišić, S., A. C. Nagel, M. Franz-Wachtel, B. Macek, A. Preiss et al., 2011 Phosphorylation of Ser 402 impedes phosphatase activity of slingshot 1. EMBO Rep. 12: 527–533. https://doi.org/10.1038/embor.2011.53

Bellen, H. J., R. W. Levis, G. Liao, Y. He, J. W. Carlson et al., 2004 The BDGBP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics 167: 761–781. https://doi.org/10.1534/genetics.104.026427

Chougule, A. B., M. C. Hastert, and J. H. Thomas, 2016 Drak is required for actomyosin organization during Drosophila cellularization. G3 (Bethesda) 6: 819–828. https://doi.org/10.1534/g3.115.026401

Clancy, D. J., D. Gems, L. G. Harshman, S. Oldham, H. Stocker et al., 2001 Extension of life-span by loss of CHICO, a Drosophila insulin receptor substate protein. Science 292: 104–106. https://doi.org/10.1126/science.1057991

Colley, N. J., J. A. Cassill, E. K. Baker, and C. S. Zuker, 1995 Defective intracellular transport is the molecular basis of rhodopsin-dependent dominant retinal degeneration. Proc. Natl. Acad. Sci. USA 92: 3070–3074. https://doi.org/10.1073/pnas.92.7.3070

Craig, C. R., J. L. Fink, Y. Yagi, Y. T. Ip, and R. L. Cagan, 2004 A Drosophila p38 orthologue is required for environmental stress response. EMBO Rep. 5: 1058–1063. https://doi.org/10.1038/sj.embor.7400282

Cobbaum, M., and J. Van Lint, 2018 Function and regulation of Protein Kinase D in oxidative stress: a tale of isoforms. Oxid. Med. Cell. Longev. 2018: 2138502. https://doi.org/10.1155/2018/2138502

Feng, H., M. Ren, S. L. Wu, D. H. Hall, and C. S. Rubin, 2006 Characterization of a novel protein kinase D: Caenorhabditis elegans DPK-1 is activated by translocation-phosphorylation and regulates movement and growth in vivo. J. Biol. Chem. 281: 17801–17814. https://doi.org/10.1074/jbc.M511899200

Feng, H., M. Ren, L. Chen, and C. S. Rubin, 2007 Properties, regulation, and in vivo functions of a novel protein kinase D: Caenorhabditis elegans DPK-2 links diacylglycerol second messenger to the regulation of stress responses and life span. J. Biol. Chem. 282: 31273–31288. https://doi.org/10.1074/jbc.M701532200

Findlay, G. L., L. Yan, J. Procter, V. Mieulet, and R. F. Lamb, 2007 A MAP4 kinase related to Ste20 is a nutrient-sensitive regulator of mTOR signalling. Biochem. J. 403: 13–20. https://doi.org/10.1042/BJ20061881

Fischer, P., M. R. La Rosa, A. Schulz, A. Preiss, and A. C. Nagel, 2015 Cyclin G functions as a positive regulator of growth and metabolism in Drosophila. PLoS Genet. 11: e1005440. https://doi.org/10.1371/journal.pgen.1005440

Fu, Y., and C. S. Rubin, 2011 Protein kinase D: coupling extracellular stimuli to the regulation of cell physiology. EMBO Rep. 12: 785–796. https://doi.org/10.1038/embor.2011.135

Gong, W. J., and K. G. Golic, 2003 Ends-out, or replacement, gene targeting in Drosophila. Proc. Natl. Acad. Sci. USA 100: 2566–2561. https://doi.org/10.1073/pnas.0353820100

Hay, B., L. Ackerman, S. Barbel, L. Y. Jan, and Y. N. Jan, 1988 Identification of a component of Drosophila polar granules. Development 103: 625–640.

Hu, Y., R. Sopko, F. Foos, C. Kelley, I. Flockhart et al., 2013 FlyPrimerBank: an online database for Drosophila melanogaster gene expression analysis and knock-down evaluation of RNAi reagents. G3 (Bethesda) 3: 1607–1616. https://doi.org/10.1534/g3.113.007021

Lindsay, D., and G. G. Zimm, 1992 The Genome of Drosophila melanogaster. Academic Press, San Diego.

Magwere, T., T. Chapman, and L. Partridge, 2004 Sex differences in the effect of dietary restriction on life span and mortality rates in female and male Drosophila melanogaster. J. Gerontol. A Biol. Sci. Med. Sci. 59: B3–B9. https://doi.org/10.1093/gerona/59.1.B3

Maier, D., G. Stumm, K. Kuhn, and A. Preiss, 1992 Hairless, a Drosophila gene involved in neural development, encodes a novel, serine rich protein. Mech. Dev. 38: 143–156. https://doi.org/10.1016/0925-4773(92)90008-6
Parks, A. L., K. R. Cook, M. Belvin, N. A. Dompe, R. Fawcett, V. Pirrotta, et al., 1988 Vectors for P-mediated transformation in Drosophila melanogaster. BMC Dev. Biol. 7: 74. https://doi.org/10.1186/1471-213X-7-74

Montell, D., 2006 The social lives of migrating cells in Drosophila. Curr. Opin. Genet. Dev. 16: 374–383. https://doi.org/10.1016/j.gde.2006.06.010

Morrison, D. K., M. S. Murakami, and V. Cleghon, 2000 Protein kinases and phosphatases in the Drosophila genome. J. Cell Biol. 150: F57–F62. https://doi.org/10.1083/jcb.150.2.F57

Nagel, A. C., J. Schmid, J. S. Auer, A. Preiss, and D. Maier, 2010 Constitutively active Protein Kinase D acts as negative regulator of the Slingshot- phosphatase in Drosophila. Hereditas 147: 237–242. https://doi.org/10.1111/j.1601-5223.2010.02200.x

Nagel, A. C., J. Szwedzinska, P. Fischer, D. Maier, I. Woch et al., 2012 Dorso-ventral axis formation of the Drosophila oocyte requires Cyclin G. Hereditas 149: 186–196. https://doi.org/10.1111/j.1601-5223.2012.02273.x

Neubueser, D., and D. R. Hipfner, 2010 Overlapping roles of Drosophila Drak and Rho kinases in epithelial tissue morphogenesis. Mol. Biol. Cell 21: 2869–2879. https://doi.org/10.1091/mbc.e10-04-0328

Nielsen, M. M., J. Overgaard, J. G. Sørensen, M. Holmstrup, J. Justesen et al., 2005 Role of HSF activation for resistance to heat, cold and high-temperature knock-down. J. Insect Physiol. 51: 1320–1329. https://doi.org/10.1016/j.jinsphys.2005.08.002

Olajoye, M. A., S. Barisić, and A. Hausser, 2013 Multi-level control of actin dynamics by protein kinase D. Cell. Signal. 25: 1739–1747. https://doi.org/10.1016/j.cellsig.2013.04.010

Parks, A. L., K. R. Cook, M. Belvin, N. A. Dompe, R. Fawcett et al., 2004 Systematic generation of high-resolution deletion coverage of the Drosophila melanogaster genome. Nat. Genet. 36: 288–292. https://doi.org/10.1038/ng1312

Pfaffl, M. W., G. W. Horgan, and L. Dempfle, 2002 Relative expression software tool (REST) for a group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 30: e36. https://doi.org/10.1093/nar/30.9.e36

Pirrotta, V., 1988 Vectors for P-mediated transformation in Drosophila, pp. 437–456 in Vectors: A Molecular Cloning Vectors and Their Uses, edited by Rodriguez, R. L., and D. T. Denhardt, Butterworth, Stoneham. https://doi.org/10.1016/B978-0-409-90042-2.50028-3

Praxenthaler, H., A. C. Nagel, A. Schulz, M. Zimmermann, M. Meier et al., 2017 Hairless-deficient Suppressors of Hairless alleles reveal Su(H) protein levels are dependent on complex formation with Hairless. PLoS Genet. 13: e1006774. https://doi.org/10.1371/journal.pgen.1006774

Preiss, A., D. A. Hartley, and S. Artavanis-Tsakonas, 1988 The molecular genetics of Enhancer of split, a gene required for embryonic neural development in Drosophila. EMBO J. 7: 3917–3927. https://doi.org/10.1002/j.1460-2075.1988.tb03278.x

Reis, T., M. R. Van Gilst, and J. K. Hariharan, 2010 A buoyancy-based screen of Drosophila larvae for fat storage mutants reveals a role for Sir2 in coupling fat storage to nutrient availability. PLoS Genet. 6: e1001206. https://doi.org/10.1371/journal.pgen.1001206

Ren, M., H. Feng, Y. Fu, M. Land, and C. S. Rubin, 2009 Protein kinase D is an essential regulator of C. elegans innate immunity. Immunity 30: 521–532. https://doi.org/10.1016/j.immuni.2009.03.007

Rong, Y. S., W. Titen, H. B. Xie, M. M. Golic, M. Bastiani et al., 2002 Targeted mutagenesis by homologous recombination in D. melanogaster. Genes Dev. 16: 1568–1581. https://doi.org/10.1101/gad.986602

Rubin, G. M., and A. C. Spradling, 1982 Genetic transformation of Drosophila with transposable element vectors. Science 218: 348–353. https://doi.org/10.1126/science.6289436

Storz, P., 2007 Mitochondrial ROS – radical detoxification, mediated by protein kinase D. Trends Cell Biol. 17: 13–18. https://doi.org/10.1016/j.tcb.2006.11.003

Tang, H. W., Y. B. Wang, S. L. Wang, M. H. Wu, S. Y. Lin et al., 2011 Atg-1 mediated myosin II activation regulates autophagosome formation during starvation induced autophagy. EMBO J. 30: 636–651. https://doi.org/10.1038/emboj.2010.338

Tautz, D., and C. Pfeifle, 1989 A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. Chromosoma 98: 81–85. https://doi.org/10.1007/BF00291041

Tettweiler, G., M. Miron, M. Jenkins, N. Sonenberg, and P. F. Lasko, 2005 Starvation and oxidative stress resistance in Drosophila are mediated through the eIF4E-binding protein, d4E-BP. Genes Dev. 19: 1840–1843. https://doi.org/10.1101/gad.1311805

Vermeulen, C. J., L. Van de Zande, and R. Bijlsma, 2006 Developmental and age specific effects of selection on divergent virgin life span on fat content and starvation resistance in Drosophila melanogaster. J. Insect Physiol. 52: 910–919. https://doi.org/10.1016/j.jinsphys.2006.05.014

Wang, L., and A. G. Clark, 1995 Physiological genetics of the response to a high-sucrose diet by Drosophila melanogaster. Biochem. Genet. 33: 149–165. https://doi.org/10.1007/BF00554727

Wang, M. C., D. Bohmann, and H. Jasper, 2003 JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. Dev. Cell 5: 811–816. https://doi.org/10.1016/S1534-5807(03)00323-X

Wang, H. D., P. Kazemi-Esfarjani, and S. Benzer, 2004 Multiple-stress analysis for isolation of Drosophila longevity genes. Proc. Natl. Acad. Sci. USA 101: 12610–12615. https://doi.org/10.1073/pnas.0404648101

Zhang, T., P. Sell, U. Braun, and M. Leitges, 2015 PKD1 protein is involved in reactive oxygen species-mediated mitochondrial depolarization in cooperation with Protein Kinase Cβ. J. Biol. Chem. 290: 10472–10485. Erratum: 16877. https://doi.org/10.1074/jbc.M114.619148

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