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Effects of *Drosophila* Ribosomal Protein S6 Kinase on Wing Growth

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Abstract

In multicellular animals, organ size, cell size and total organism size are regulated by signaling through the insulin receptor and TOR signaling pathways. The ribosomal protein S6 kinase is a key component of these pathways. It has been shown that mice or *Drosophila* lacking this kinase have a reduced body size that is associated with a decrease in cell size. Ectopic expression of activated or dominant negative transgenic variants of the *Drosophila* homolog of ribosomal S6 kinase (*dS6K*) has been shown to cause phenotypes that are consistent with a role for *dS6K* in growth, but whether the phenotypes were due to changes in cell size, cell number or other causes has not been shown. Here we show that ectopic expression of *dS6K* transgenes in the posterior wing compartment alters compartment size primarily by changes in cell size.

Introduction

The size that an organism, organ or tissue attains can be due to cell growth (defined here as an increase in cell mass or size) or to changes in cell number due to cell division or cell death. In most cases, cell growth and division are coupled and cells divide only after attaining sufficient mass. However, experiments in yeast, fruit flies and mammals have shown that under some circumstances, growth and division are separable processes and cell growth can occur in the absence of cell division (Johnston 1998, Neufeld et al. 1998, Volarevic et al. 2000).

In multicellular organisms such as fruit flies (*Drosophila melanogaster*) and mammals, organism size and organ size are regulated by signaling pathways that involve the protein kinase TOR (target of rapamycin) and the insulin receptor (InR). Signaling through TOR, which is regulated by nutrient and energy availability, is integrated with InR signaling to converge on downstream effectors to control cell size and number, and thus control organ and organism size (Hay and Sonenberg 2004, Guertin et al. 2006). One

effector that lies on the TOR and InR pathways is the ribosomal protein S6 kinase 1 (S6K1), which has been proposed to affect growth by phosphorylating ribosomal protein S6 (RpS6) although the biological effects of RpS6 phosphorylation are not completely understood (Magnuson et al. 2012). Additionally, S6K1 may affect cell growth through other mechanisms, including indirect effects on UBF, a nucleolar transcription factor for genes that code for the 43S RNA precursor of the 18S, 5.8S and 28S ribosomal RNAs (Hannan et al. 2003).

Mice and *Drosophila* that lack the *S6K1* gene are small, indicating a role for S6K1 in organism growth. Previous work showed that most flies homozygous for a null allele of *dS6K*, the *Drosophila* *S6K1* homolog, died during development while the few that did survive to adulthood were only about one-half the size of wild type flies (Montagne et al. 1999). The small size was due to a decrease in cell size rather than changes in cell number. In experiments with mice, Shima et al. (1998) showed that mice homozygous for a knockout of *S6K1* were approximately 15-20% smaller than wild type mice at birth. However, in *S6K1* knockout mice, RpS6 phosphorylation was not impaired, leading Shima et al. (1998) to identify a second kinase in mice, called S6K2, that can phosphorylate RpS6. Studies using cells derived from *S6K1* and *S6K2* double knockout mice showed that an absence of *S6K1*, but not *S6K2*, caused reduced size of mouse myotubes and myoblasts (Ohanna et al. 2005). Thus, it seems that *dS6K* and S6K1 affect growth by affecting cell size rather than cell proliferation.

In studies to determine how mammalian S6K1 is activated, it was shown that phosphorylation of multiple serine and threonine residues in S6K1 is important for its activation. Phosphorylation of S6K1 proceeds in a sequential manner that is thought to lead to full S6K1 activation (Pullen and Thomas 1997). Additionally, a lysine in the ATP binding site of S6K1 was shown to be important for activity. Mutation of this lysine to glutamine results in a kinase-dead S6K1 which, when ectopically expressed in cells, acts as a dominant negative to inhibit the endogenous S6K1

(von Manteuffel et al. 1997).

Many of the key amino acids important for S6K1 activity are conserved in dS6K (Stewart et al. 1996), an observation that we used to design dS6K variants predicted to have altered activity (Barcelo and Stewart 2002). Three activated dS6K variants were made in which serine (S) and threonine (T) residues, conserved with S6K1, were changed to acidic amino acids to mimic the effects of phosphorylation. For the activated variant called *dS6K^{STDE}*, S₄₁₈ and T₄₂₂ were changed to aspartic acid (D) or glutamic acid (E), respectively. To make *dS6K^{TE}*, T₃₉₈ was changed to E. In a third activated variant called *dS6K^{STDETE}*, T₃₉₈, S₄₁₈, and T₄₂₂ were changed to acidic amino acids. To make a dominant negative variant called *dS6K^{KQ}*, a lysine (K₁₀₉) in the ATP-binding pocket of dS6K was changed to glutamine (Q) (Barcelo and Stewart 2002).

The *Drosophila* wing has a dorsal compartment and a ventral compartment, with each compartment made of a single epithelial layer. Previously, we used the *GAL4-UAS* system (Brand and Perrimon 1993) and the *apterous>GAL4* driver to drive expression of the *dS6K* transgene variants (described above) specifically in the dorsal wing compartment (Barcelo and Stewart 2002). Expressing wild type or activated *dS6K* variants in the dorsal wing compartment caused adult wings to bend downward. Conversely, expressing dominant-negative *dS6K^{KQ}* in the dorsal wing compartment caused adult wings to bend upward. While these results showed that expression of *dS6K* variants altered the curvature of the wing, they did not assess how wing curvature was changed and thus did not address the cellular function of *dS6K*. Wing curvature could be altered through changes in cell size, cell number or both. Alternatively, since mammalian S6K1 has been shown to be associated with the cytoskeleton (Berven et al. 2004) and mutations in *Drosophila* genes coding for cytoskeleton or cytoskeleton-associated components can change wing curvature (Hughes et al. 2010; Thomas et al. 1998), it is possible that ectopic *dS6K* expression in the dorsal wing layer could cause wing curvature by altering cytoskeletal function.

It has been shown that overexpressing modified variants of signaling components can be used to identify genes that act in a signaling pathway (Huang and Rubin 2000) and that many *Drosophila* genes are conserved in humans (Fortini et al. 2000). As the signaling pathways that lead to human S6K1 activation are important for normal cellular processes as well as processes that go awry in pathologies such as cancer, diabetes, organ hypertrophy and obesity (Gibbons et al.

2009, Magnuson et al. 2012), the *dS6K* transgenes we developed may be powerful tools for understanding signaling networks that lead to dS6K and S6K1 activation. Thus, it is important to determine if overexpressing modified *dS6K* transgene variants affects growth by altering cell size or cell number. Here we have addressed this issue by using the *engrailed>GAL4* (*en>GAL4*) driver to drive expression of *UAS-dS6K* variants in the dorsal and ventral layers of the posterior wing compartment. This resulted in flat wings that we could mount on microscope slides to obtain cell size measurements and cell number estimates. We show that ectopic *dS6K* expression affects compartment growth primarily through changes in cell size.

Materials and Methods

Crosses and *Drosophila* handling

Twenty *en>Gal4* males were mated with twenty *w¹¹¹⁸* virgin females or with twenty virgin females of each of the genotypes that follow: *UAS-dS6K^{WT-4}/CyO* (II), *UAS-dS6K^{WT-5}/UAS-dS6K^{WT-5}* (III), *UAS-dS6K^{TE-2}/UAS-dS6K^{TE-2}* (III), *UAS-dS6K^{TE-4}/UAS-dS6K^{TE-4}* (III), *UAS-dS6K^{STDE-2}/UAS-dS6K^{STDE-2}* (III), *UAS-dS6K^{STDE-4/TM3, Sb¹}* (III), *UAS-dS6K^{STDETE-8A}/CyO* (II), *UAS-dS6K^{STDETE74H}/CyO* (II), *UAS-dS6K^{KQ-4}/CyO* (II), *UAS-dS6K^{KQ-6}/CyO* (II). For each *dS6K* transgenic line, the superscript term indicates the *UAS-dS6K* allele (as a cDNA), the superscript number indicates the particular independent transgenic line and the Roman numeral in parentheses indicates the chromosome on which the transgene is inserted. For example, the designation *UAS-dS6K^{WT-4}* (II) indicates that the transgene is a *UAS*-linked wild-type *dS6K* cDNA, is inserted on chromosome two and was isolated as an independent transformant that we called line 4. In our experiments, we used two independent lines of each *dS6K* transgene.

Crosses were made in vials and transferred to egg laying containers for four-hour egg collections. Embryos were aged for ~27 hours and 50 first instar larvae were placed into fresh vials with standard cornmeal/molasses *Drosophila* food that was supplemented with 200µl of a yeast paste made with 0.43 g of yeast per ml of ddH₂O. All crosses and egg collections for wing measurements were performed at 25°C and at the same time.

Wing mount preparations and wing analysis

Control F₁ females of the genotype $w^{1118}/y\ w$; $en>Gal4/+$ or F₁ $w^{1118}/y\ w$ females that inherited the $en>Gal4$ driver and a *dS6K* allele were aged for four to five days in fresh food vials before wings were mounted on microscope slides with Permount (Fischer Scientific). One wing per female was used. Photographs were taken with a SPOT digital camera on an Olympus BX60 compound microscope. Whole wings were photographed at a magnification of 4X. Wing areas used for trichome root counts were photographed at a magnification of 20X. The digital images of the wing compartment areas shown in Fig. 1 were measured with Scion Image 4.0.2.

Because each cell in the adult wing contains a single trichome, counting the number of trichome roots in a defined intervein area can be used as a way to determine cell density and to calculate cell area (Leevers et al. 1996, Montagne et al. 1999, Robertson 1959, Verdu et al. 1999). In addition, because wing cells are completely flattened, cell area rather than cell volume is a measure of cell size (Robertson 1959). Additionally, the number of cells per compartment can be estimated by multiplying the compartment area in μm^2 by the cell density/ μm^2 of a defined region. Cell density was measured by counting trichome roots on the dorsal wing surface inside 10,000 μm^2 boxes positioned as shown in Fig. 1. The regions used for trichome counts were chosen based on two criteria. First, the intervein region had to be large enough to accommodate the placement of a 10,000 μm^2 box on digital photographs of the region. Second, the intervein region had to have one or more landmarks that would allow us to reproducibly place a 10,000 μm^2 box in the same position on photographs of independent wings. Box *i* was placed with one edge parallel to vein two and a second edge aligned with a line that was drawn at a 90° angle to vein two and intersected the midpoint of the junction of the anterior crossvein and vein three. Boxes *ii* and *iii* were aligned parallel to vein three or four, respectively, with one corner just proximal to the wing margin. Box *iv* was aligned parallel with vein four and the corner of the box was positioned at the intersection of vein four and the posterior crossvein. Box *v* was aligned with one edge parallel to vein five and a second edge aligned with a line that was drawn at a 90° angle to vein 5 and intersected the midpoint of the junction of the posterior cross vein and vein five.

Statistical analysis

Compartment areas and cell areas in anterior and posterior wing compartments for each of the *dS6K* alleles, as well as controls, were compared using the one-way MANOVA procedure of SPSS 16 (Green and Salkind 2008). Analyses of variances (ANOVA) on each independent variable were conducted as follow-up tests to the MANOVA. Using the Bonferroni method, each ANOVA was tested at the $\alpha = 0.0125$ level. A post-hoc Tukey HSD test was used to assess differences in effects of alleles.

Significant differences were found among the *dS6K* variants for anterior and posterior compartment areas and for cell areas, Wilks' $\Lambda = 0.007$, $F = 24.95$, $p < 0.001$. The partial η^2 for the MANOVA was 0.713, indicating a strong effect on compartment areas and on cell areas. Each of the ANOVAs was significant (all $p < 0.001$). Specific results for each ANOVA and for the post-hoc Tukey HSD are in Tables 1 and 2.

Results***Ectopic dS6K expression alters compartment size***

To determine whether *dS6K* expression in the posterior wing compartment alters compartment size, we measured wing areas as shown in Fig. 1. As compared to control wings, there were statistically significant changes in the posterior compartment area of wings for at least one transgenic line of each of the *dS6K* transgenes. Expression of the dominant-negative *dS6K* in both transgenic lines for this allele, *dS6K^{KQ-4}* and *dS6K^{KQ-5}*, resulted in a significant decrease in posterior compartment size as compared to control wings (Table 1). The difference in wing size can be seen in Fig. 2, which shows wings from control flies and flies in which *dS6K^{STDETE-8A}* or *dS6K^{KQ-4}* were expressed in the posterior wing compartment.

Next, we compared the posterior compartment areas between and among the different *dS6K* transgenic alleles. Posterior wing compartment areas of flies carrying the transgenic alleles *dS6K^{WT}*, *dS6K^{TE}*, *dS6K^{STDE}* and *dS6K^{STDETE}* clustered together statistically (Table 1). However, the posterior compartment area in wings from flies expressing *dS6K^{KQ}* was significantly smaller than the same region of wings from flies expressing *dS6K^{WT}* or *dS6K* activated alleles (Table 1).

To determine whether *dS6K* expression in the posterior wing compartment caused compensatory effects in the anterior wing compartment, we measured anterior compartment areas. For *dS6K^{TE-2}*, *dS6K^{TE-4}*,

$dS6K^{STDE-2}$ and $dS6K^{STDE-74H}$, we found significant decreases in the size of the anterior wing compartments (Table 1). In the case of $dS6K^{KQ-6}$, in which transgene expression in the posterior compartment caused decreased size, there was a compensatory increase in the anterior compartment (Table 1). The percent changes in posterior and anterior wing compartments, for all $dS6K$ transgenes, are summarized in Fig. 3B.

Figure 1. Shown is a control $w^{1118}/y\ w; en>Gal4/+$ adult wing, marked to show the areas that we analyzed. Longitudinal veins are marked with numerals 1, 2, 3, 4 or 5. The posterior compartment was measured as the area bounded by the dashed heavy line, but with the anterior limit defined by longitudinal vein 4. The anterior compartment was measured as the area bounded by the dashed heavy line, but with the posterior limit defined by longitudinal vein 3. The approximate position of the anterior/posterior compartment boundary, which is not associated with morphological markers, is shown as a thin dashed line just anterior to crossvein 4. The 10,000 μm^2 boxes for trichome root counts were placed as shown. acv = anterior crossvein. pcv = posterior crossvein.

Ectopic $dS6K$ expression changes cell size

To determine whether the $dS6K$ induced changes in compartment areas were caused by changes in cell size, we counted trichome (sensory hair) roots in 10,000 μm^2 areas of the wing as a way to determine cell size (see the materials and methods section). This analysis shows that in comparison to control wings, expression of $dS6K$ alleles in all $dS6K^{WT}$ lines and all activated $dS6K$ lines caused significant decreases in posterior trichome density and thus increases in posterior compartment cell size. Expression of the dominant negative $dS6K^{KQ}$ transgene decreased cell size in the posterior compartment (Table 2 and Fig. 3A). Even though $en>GAL4$ drives UAS -transgene expression in the posterior compartment, we did observe significant decreases in the size of cells in the

anterior compartment with $dS6K^{STDE-2}$ and $dS6K^{STDE-7}$, but not with the other $dS6K$ lines (Table 2).

Ectopic $dS6K$ expression causes small effects on cell number

We estimated the number of cells in the posterior and anterior wing compartments of wings from control flies and of wings in which the $dS6K$ transgenes were expressed in the posterior wing compartment. To do this, we averaged the number of cells in the 10,000 μm^2 boxes in the posterior or anterior compartment and multiplied that number by the posterior or anterior compartment area, respectively. The values that we obtained for estimated cell numbers are shown in Table 2. The percent change in cell numbers in wing compartments for each of the transgenic lines, relative to cell numbers of controls, is shown in Table 2.

Figure 2. Shown are wings from (A) a $w^{1118}/y\ w; en>GAL4/+$ control fly, (B) an $en>GAL4/dS6K^{STDE-8A}$ fly and (C) an $en>GAL4/dS6K^{KQ-4}$ fly. All panels are the same magnification and the size bar in (C) is 50 μm .

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Figure 3. (A) The data from Table 2 was used to calculate the percent change in cells per μm^2 in the anterior and posterior wing compartments of flies expressing *dS6K* transgenes in the posterior wing compartment. The percent change in cells per μm^2 was calculated relative to the cells per μm^2 of controls. (B) The data from Table 1 was used to calculate the percent change in wing compartment areas in wings in which *dS6K* transgenes were expressed in the posterior compartment. The percent change in compartment area was calculated relative to the compartment area of control wings."

Discussion

To address how *dS6K* transgene expression affects growth, we used *en>GAL4* to overexpress *UAS-linked dS6K* transgene variants in the posterior wing compartment. Because this did not cause wings to bend as did expressing *dS6K* transgenes with *ap>GAL4* (Barcelo and Stewart 2002), we were able to prepare flat mount wing preparations that could be used to measure *dS6K* transgene effects on compartment and cell size. Consistent with the observation that a null mutation of the endogenous *dS6K* alters organism growth through changes in cell size (Montagne et al. 1999), *dS6K* transgenes exert their effects on wing compartment growth primarily through changes in cell size.

The posterior compartment area changes and posterior cell size changes that we observed are consistent with the predicted activities of the *dS6K* variants. Expressing *dS6K*^{WT} or any of the activated *dS6K* variants in the posterior wing compartment increased posterior compartment size and cell size. Conversely, expressing dominant negative *dS6K*^{KQ} in the posterior wing compartment decreased posterior compartment size and cell size (Table 1 and Fig. 3). To test if *dS6K*^{KQ} expression reduces size by inhibiting endogenous *dS6K* or by altering activity of upstream signaling components, a future experiment could

compare the effects of *dS6K*^{KQ} expression with the effects of using RNAi to deplete endogenous *dS6K*.

Because mammalian S6K1 phosphorylation and activation proceeds in a sequential manner (Pullen and Thomas 1997), it could be expected that expression of the activated *dS6K* alleles would cause a graded series of size increases. In this case, the expected order from least growth increase to most, would be *dS6K*^{WT} < *dS6K*^{TE} < *dS6K*^{STDE} < *dS6K*^{STDETE}. However, we did not observe this trend. If the *dS6K* protein is phosphorylated and activated in a sequential manner, it is possible that we did not detect a graded series of size increases because of transgene insertion position-dependent effects that cause different levels of ectopic *dS6K* expression. Such position-dependent effects could explain the observation that, while expression of *dS6K*^{WT} and all *dS6K* activated variants increased posterior compartment area, the increase was not statistically significant for *dS6K*^{WT-5} and *dS6K*^{STDE-4}.

Our data shows that expression of *dS6K* transgenes causes changes in the total size of the posterior wing compartment and that this is mediated by changes in cell area, which we used as a measure of cell size. Because wild-type wing cells are completely flattened, measurements of cell surface area in μm^2 rather than measurements of cell volume in μm^3 can be used to determine cell size (Robertson 1959). Since we measured cell surface area rather than cell volume, we

cannot discount the possibility that expression of the dS6K variants unexpectedly altered cell volume and thickness. While it is possible that small changes in cell number may have contributed to the changes in posterior wing compartment size, our data suggests that the major effects of ectopic dS6K expression are on cell size. The estimated changes in cell numbers are considerably smaller than the changes in cell size that we were able to determine by direct measurement (see Table 2). For example, expression of dS6K^{WT-5} caused a 0.80% increase in cell number, which is considerably smaller than the 6.02% increase in cell size that we observed. Additionally, our estimates of cell number show that cell numbers decrease in the posterior compartment in the case of expressing dS6K^{WT-4} and all of the activated dS6K alleles, an effect that would not cause increased compartment area size.

Although GAL4 expression from the *en>GAL4* driver is reported to occur only in the posterior wing

compartment (Diaz-Benjumea and Cohen 1993), we observed that when we used this driver to express dS6K^{WT-5} (but not dS6K^{WT-4}) as well as all of the activated dS6K variants in the posterior compartment, the posterior compartment size increased while the anterior compartment size decreased. Likewise, we observed that when the posterior compartment area decreased as a result of dS6K^{KQ} expression in the posterior compartment, the size of the anterior compartment increased. It may be that in response to a change in posterior compartment size, the anterior compartment responds by compensating in an attempt to regulate overall wing size. This idea is supported by data reported by others in which compensatory changes in wing areas were observed upon overexpression or mosaic analysis of several genes that have a role in controlling cell size (Resino and García-Bellido 2004).

Table 1. Mean anterior and posterior compartment areas from wings of control *w¹¹¹⁸/y w*; *en>GAL4/+* females and *w¹¹¹⁸/y w* females with the indicated dS6K transgene and one copy of the *en>GAL4* driver. ANOVA statistics for measurements of anterior and posterior compartments of wings are given. Compartment area values with the same letter superscript were not significantly different from each other. ¹Wing compartment areas as shown in Fig. 1 were measured using Scion Image 4.0.2 and are presented as mean values (standard deviations in parentheses). ²Percent change in compartment area relative to controls.

<i>Drosophila</i> line	¹ Compartment area x 10 ⁵ μm ² (sd)		² Percent change in compartment area	
	Anterior	Posterior	Anterior	Posterior
Control	^{cd} 4.87 (0.09)	^g 8.27 (0.17)		
dS6K ^{WT-4}	^{de} 5.00 (0.08)	^{hij} 8.74 (0.18)	+2.67%	+5.68%
dS6K ^{WT-5}	^{bc} 4.72 (0.15)	^{gh} 8.88 (0.32)	-3.08%	+7.38%
dS6K ^{TE-2}	^b 4.70 (0.15)	^{hij} 8.83 (0.28)	-3.49%	+6.77%
dS6K ^{TE-4}	^b 4.70 (0.08)	^{hij} 8.79 (0.23)	-3.49%	+6.29%
dS6K ^{STDE-2}	^{ab} 4.63 (0.10)	^{hij} 8.82 (0.17)	-4.93%	+6.65%
dS6K ^{STDE-4}	^{bc} 4.76 (0.08)	^{ghi} 8.60 (0.10)	-2.26%	+3.99%
dS6K ^{STDETE8A}	^{bc} 4.79 (0.11)	^j 9.05 (0.24)	-1.64%	+9.43%
dS6K ^{STDETE74H}	^a 4.47 (0.06)	^{ij} 8.94 (0.10)	-8.21%	+8.10%
dS6K ^{KQ4}	^{de} 4.97 (0.10)	^f 7.73 (0.24)	+2.05%	-6.55%
dS6K ^{KQ6}	^e 5.07 (0.07)	^f 7.83 (0.11)	+4.11%	-5.32%
ANOVA <i>F</i>	30.12	44.64		
ANOVA <i>p</i>	<0.001	<0.001		
Partial η ²	0.503	0.797		

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Table 2. Cell densities in anterior and posterior compartments. Mean cell densities from anterior and posterior areas were analyzed on the same wings as were analyzed for Table 1. ANOVA statistics are given. Anterior compartment values with the same letter superscript were not significantly different from each other. Posterior compartment values with the same letter superscript were not significantly different from each other. ¹Cell density was calculated by counting trichome roots on the dorsal wing surface in the 10,000 μm^2 areas shown in Fig. 1 (standard deviations are in parentheses).

² Reciprocal of cell density.

³ Calculated by multiplying the anterior or posterior cell densities per μm^2 (this table) by the values of the anterior or posterior compartment areas shown in Table 1.

<i>Drosophila</i> line	Anterior Compartment				Posterior Compartment					
	¹ Cell density per μm^2	² Area per cell ($\times 10^{-3}$ μm^2)	Change in area per cell	³ Estimated number of cells in compartment	Change in number of cells	¹ Cell density per μm^2	² Area per cell ($\times 10^{-3}$ μm^2)	Change in area per cell	³ Estimated number of cells in compartment	Change in number of cells
Control	^{ab} 5.87 (0.02)	170.36		2858.69		ⁱ 6.69 (0.12)	149.48		5532.63	
<i>dS6K^{HT-4}</i>	^{abc} 5.93 (0.19)	168.63	-1.02%	2965.00	+3.72%	^{gh} 6.31 (0.12)	158.48	+6.02%	5514.94	-0.32%
<i>dS6K^{HT-5}</i>	^{abc} 5.93 (0.17)	168.63	-1.02%	2798.96	-2.09%	^{gh} 6.28 (0.23)	159.24	+6.53%	5576.64	+0.80%
<i>dS6K^{TE-2}</i>	^{abc} 5.98 (0.10)	167.22	-1.84%	2810.60	-1.68%	^{fg} 6.07 (0.17)	164.74	+10.21%	5359.81	-3.12%
<i>dS6K^{TE-4}</i>	^{abc} 6.00 (0.17)	166.67	-2.17%	2820.00	-1.35%	^{fgh} 6.07 (0.13)	164.74	+10.21%	5335.53	-3.56%
<i>dS6K^{STDE-2}</i>	^{cd} 6.15 (0.19)	162.60	-4.56%	2847.45	-0.39%	^f 5.98 (0.16)	167.22	+11.87%	5274.36	-4.67%
<i>dS6K^{STDE-4}</i>	^{abc} 5.90 (0.15)	169.49	-0.51%	2808.40	-1.76%	^h 6.31 (0.09)	158.48	+6.02%	5426.60	-1.92%
<i>dS6K^{STDETE8A}</i>	^{bc} 6.06 (0.19)	165.02	-3.13%	2902.74	+1.54%	^{fgh} 6.10 (0.15)	163.93	+9.67%	5520.50	-0.22%
<i>dS6K^{STDETE74H}</i>	^d 6.42 (0.20)	155.76	-8.57%	2869.74	+0.39%	^{fgh} 6.10 (0.14)	163.93	+9.67%	5453.40	-1.43%
<i>dS6K^{KQ4}</i>	^{ab} 5.82 (0.12)	171.82	+0.86%	2892.54	+1.18%	^j 7.27 (0.12)	137.55	-7.98%	5619.71	+1.57%
<i>dS6K^{KQ6}</i>	^a 5.77 (0.19)	173.31	+1.73%	2925.39	+2.33%	^j 7.22 (0.13)	138.50	-7.35%	5653.26	-2.18%
ANOVA <i>F</i>	11.66					98.37				
ANOVA <i>p</i>	<0.001					<0.001				
Partial η^2	0.376					0.884				

Conclusions

Our results support the hypothesis that a major role of dS6K is to regulate cell size. Overexpression of the dS6K variants alters compartment area primarily by changing cell size. Each of the dS6K variants affects compartment and cell size in the predicted manner, with wild type and activated dS6K variants increasing size while the dominant negative dS6K^{KQ} decreases size. In the case of most of the dS6K variants, the size changes are statistically significant. Our work showing that dS6K expression alters cell size should be of value for additional studies to investigate signaling networks involving dS6K.

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Literature Cited

- Barcelo H** and **MJ Stewart**. 2002. Altering *Drosophila* S6 kinase activity is consistent with a role for S6 kinase in growth. *Genesis* 34:83-85.
- Berven LA**, **FS Willard** and **MF Crouch**. 2004. Role of the p70(S6K) pathway in regulating the actin cytoskeleton and cell migration. *Experimental Cell Research* 296:183-195.
- Brand AH** and **N Perrimon**. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401-415.
- Diaz-Benjumea FJ** and **SM Cohen**. 1993. Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* 75:741-752.
- Fortini ME**, **MP Skupski**, **MS Boguski** and **IK Hariharan**. 2000. A survey of human disease gene counterparts in the *Drosophila* genome. *Journal of Cell Biology* 150:F23-F30.
- Gibbons JJ**, **RT Abraham** and **K Yu**. 2009. Mammalian target of rapamycin: discovery of rapamycin reveals a signaling pathway important for normal and cancer cell growth. *Seminars in Oncology* 36:S3-S17.
- Green SB** and **NJ Salkind**. 2008. Using SPSS for Windows and Macintosh. Upper Saddle River: Pearson Prentice Hall. p 1-453.
- Guertin DA**, **KVP Guntur**, **GW Bell**, **CC Thoreen** and **DM Sabatini**. 2006. Functional genomics identifies TOR-regulated genes that control growth and division. *Current Biology* 16:958-970.
- Hannan KM**, **Y Brandenburger**, **A Jenkins**, **K Sharkey**, **A Cavanaugh**, **L Rothblum**, **T Moss**, **G Poortinga**, **GA McArthur**, **RB Pearson** and **RD Hannan**. 2003. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxyl-terminal activation domain of the nucleolar transcription factor UBF. *Molecular and Cellular Biology* 23:8862-8877.
- Hay N** and **N Sonenberg**. 2004. Upstream and downstream of mTOR. *Genes and Development* 18:1926-1945.
- Huang AM** and **GM Rubin**. 2000. A misexpression screen identifies genes that can modulate RAS1 pathway signaling in *Drosophila melanogaster*. *Genetics* 156:1219-1230.
- Hughes SC**, **E Formstecher** and **RG Fehon**. 2010. Sip1, the *Drosophila* orthologue of EBP50/NHERF1, functions with the sterile 20 family kinase Slik to regulate Moesin activity. *Journal of Cell Sciences* 123:1099-1107.
- Johnston LA**. 1998. Uncoupling growth from the cell cycle. *Bioessays* 20:283-286.
- Leevers SF**, **D Weinkove**, **LK MacDougall**, **E Hafen** and **MD Waterfield**. 1996. The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth. *European Molecular Biology Organization Journal* 15:6584-6594.
- Magnuson B**, **B Ekim** and **DC Fingar**. 2012. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochemical Journal* 441:1-21.
- Montagne J**, **MJ Stewart**, **H Stocker**, **E Hafen**, **SC Kozma** and **G Thomas**. 1999. *Drosophila* S6 kinase: a regulator of cell size. *Science* 285:2126-2129.
- Neufeld TP**, **AF de la Cruz**, **LA Johnston** and **BA Edgar**. 1998. Coordination of growth and cell division in the *Drosophila* wing. *Cell* 93:1183-1193.
- Ohanna M**, **AK Sobering**, **T Lapointe**, **L Lorenzo**, **C Praud**, **E Petroulakis**, **N Sonenberg**, **PA Kelly**, **A Sotiropoulos** and **M Pende**. 2005. Atrophy of S6K1(-/-) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nature Cell Biology* 7:286-294.

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- Pullen N and G Thomas.** 1997. The modular phosphorylation and activation of p70^{s6k}. Federation of European Biochemical Societies Letters 410:78-82.
- Resino J and A García-Bellido.** 2004. *Drosophila* genetic variants that change cell size and rate of proliferation affect cell communication and hence patterning. Mechanisms of Development 121:351-364.
- Robertson, FW.** 1959. Studies in quantitative inheritance. Xii. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. Genetics 44:869-896.
- Shima H, M Pende, Y Chen, S Fumagalli, G Thomas and SC Kozma.** 1998. Disruption of the p70(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. European Molecular Biology Organization Journal 17:6649-6659.
- Stewart MJ, COA Berry, F Zilberman, G Thomas and SC Kozma.** 1996. The *Drosophila* p70^{s6k} homolog displays conserved regulatory motifs and rapamycin sensitivity. Proceedings of the National Academy of Sciences U.S.A. 93:10791-10796.
- Thomas GH, DC Zarnescu, AE Juedes, MA Bales, A Londergan, CC Korte and DP Kiehart.** 1998. *Drosophila* B_{Heavy}-spectrin is essential for development and contributes to specific cell fates in the eye. Development 125:2125-2134.
- Verdu J, MA Buratovich, EL Wilder and MJ Birnbaum.** 1999. Cell-autonomous regulation of cell and organ growth in *Drosophila* by Atk/PKB. Nature Cell Biology 1:500-506.
- von Manteuffel SB, PB Dennis, N Pullen, AC Gingras, N Sonenberg and G Thomas.** 1997. The insulin-induced signalling pathway leading to S6 and initiation factor 4E binding protein 1 phosphorylation bifurcates at a rapamycin-sensitive point immediately upstream of p70s6k. Molecular and Cellular Biology 17:5426-5436.
- Volarevic S, MJ Stewart, B Ledermann, F Zilberman, L Terracciano, E Montini, M Grompe, SC Kozma and G Thomas.** 2000. Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. Science 388:2045-2047.