Effects of Drosophila Ribosomal Protein S6 Kinase on Wing Growth

M. J. Stewart
University of Arkansas at Monticello, stewartm@uamont.edu

J. L. Hunt
North Dakota State University

Follow this and additional works at: http://scholarworks.uark.edu/jaas

Part of the Biology Commons

Recommended Citation
Available at: http://scholarworks.uark.edu/jaas/vol66/iss1/25
Effects of *Drosophila* Ribosomal Protein S6 Kinase on Wing Growth

M.J. Stewart¹ ² and J.L. Hunt¹

¹School of Mathematical and Natural Sciences, University of Arkansas at Monticello, Monticello, AR 71656  
²Department of Biological Sciences, North Dakota State University, Fargo, ND 58105.

¹Correspondence: stewartm@uamont.edu

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.
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<sup>STDE</sup>, S<sub>118</sub> and T<sub>422</sub> were changed to aspartic acid (D) or glutamic acid (E), respectively. To make dS6K<sup>TE</sup>, T<sub>398</sub> was changed to E. In a third activated variant called dS6K<sup>STDETE</sup>, T<sub>398</sub>, S<sub>418</sub>, and T<sub>422</sub> were changed to acidic amino acids. To make a dominant negative variant called dS6K<sup>KQ</sup>, a lysine (K<sub>109</sub>) 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<sup>KQ</sup> 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 en>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 w<sup>1118</sup> virgin females or with twenty virgin females of each of the genotypes that follow: UAS-dS6K<sup>WT-4</sup>/CyO (II), UAS-dS6K<sup>WT-5</sup>/UAS-dS6K<sup>WT-5</sup> (III), UAS-dS6K<sup>STDE-2</sup>/UAS-dS6K<sup>TE-2</sup> (III), UAS-dS6K<sup>TE-4</sup>/UAS-dS6K<sup>TE-4</sup> (III), UAS-dS6K<sup>STDE-2</sup>/UAS-dS6K<sup>STDE-2</sup> (III), UAS-dS6K<sup>STDE-4</sup>/TM3, Sb<sup>1</sup> (III), UAS-dS6K<sup>STDETE-8A</sup>/CyO (II), UAS-dS6K<sup>STDETE-8A</sup>/CyO (II), UAS-dS6K<sup>KQ-4</sup>/CyO (II), UAS-dS6K<sup>KQ-4</sup>/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<sup>WT-4</sup> (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 ddH<sub>2</sub>O. All crosses and egg collections for wing measurements were performed at 25°C and at the same time.

---

**Journal of the Arkansas Academy of Science, Vol. 66, 2012**

142
Wing mount preparations and wing analysis

Control F$_1$ females of the genotype w$^{118}$/y w; en>Gal4/+ or F$_1$ w$^{118}$/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 90º 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 90º angle to vein 5 and intersected the midpoint of the junction of the posterior crossvein 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’ Λ = 0.007, $F = 24.95$, $p < 0.001$. The partial $\eta^2$ 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$^{STDTE-8A}$ 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$^{STDTE}$ and dS6K$^{STDTE-8A}$ 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$^{KQ}$ 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$^{TE-4}$,
dS6^KSTDE-2 and dS6^KSTDETE-74H, we found significant decreases in the size of the anterior wing compartments (Table 1). In the case of dS6^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 dS6^K lines, 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 \( \mu \text{m}^2 \) boxes for trichome root counts were placed as shown. acv = anterior crossvein. pcv = posterior crossvein.

**Ectopic dS6^K expression changes cell size**

To determine whether the dS6^K induced changes in compartment areas were caused by changes in cell size, we counted trichome (sensory hair) roots in 10,000 \( \mu \text{m}^2 \) 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 dS6^K alleles in all dS6^K^WT lines and all activated dS6^K lines caused significant decreases in posterior trichome density and thus increases in posterior compartment cell size. Expression of the dominant negative dS6^K^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 dS6^KSTDE-2 and dS6^KSTDETE-7, but not with the other dS6^K lines (Table 2).

**Ectopic dS6^K 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 dS6^K transgenes were expressed in the posterior wing compartment. To do this, we averaged the number of cells in the 10,000 \( \mu \text{m}^2 \) 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/dS6^KSTDETE-8A fly and (C) an en>Gal4/dS6^KQ-2 fly. All panels are the same magnification and the size bar in (C) is 50 \( \mu \text{m} \).
Effects of Drosophila Ribosomal Protein S6 Kinase on Wing Growth

**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 KQ < 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 μm² rather than measurements of cell volume in μm³ 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\textsuperscript{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\textsuperscript{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\textsuperscript{WT-5} (but not dS6K\textsuperscript{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\textsuperscript{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 \textit{w}\textsuperscript{1118}/\textit{y w}; \textit{en}>\textit{GAL4}/+ females and \textit{w}\textsuperscript{1118}/\textit{y w} females with the indicated dS6K transgene and one copy of the \textit{en}>\textit{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. \textit{1}Wing 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). \textit{2}Percent change in compartment area relative to controls.

<table>
<thead>
<tr>
<th>\textit{Drosophila} line</th>
<th>\textit{1}Compartment area x \textit{10}^{5} \text{\mu m}^{2} (sd)</th>
<th>\textit{2}Percent change in compartment area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior</td>
<td>Posterior</td>
</tr>
<tr>
<td>Control</td>
<td>4.87 (0.09)</td>
<td>8.27 (0.17)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{WT-4}</td>
<td>5.00 (0.08)</td>
<td>8.74 (0.18)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{WT-5}</td>
<td>4.72 (0.15)</td>
<td>8.88 (0.32)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{TE-2}</td>
<td>4.70 (0.15)</td>
<td>8.83 (0.28)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{TE-4}</td>
<td>4.70 (0.08)</td>
<td>8.79 (0.23)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{STDE-2}</td>
<td>4.63 (0.10)</td>
<td>8.82 (0.17)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{STDE-4}</td>
<td>4.76 (0.08)</td>
<td>8.60 (0.10)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{STDETEA}</td>
<td>4.79 (0.11)</td>
<td>9.05 (0.24)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{STDETE74H}</td>
<td>4.47 (0.06)</td>
<td>8.94 (0.10)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{KQ4}</td>
<td>4.97 (0.10)</td>
<td>7.73 (0.24)</td>
</tr>
<tr>
<td>dS6K\textsuperscript{KQ6}</td>
<td>5.07 (0.07)</td>
<td>7.83 (0.11)</td>
</tr>
</tbody>
</table>

ANOVA $F$ 30.12 44.64
ANOVA $p$ <0.001 <0.001
Partial $\eta^2$ 0.503 0.797
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 μm² areas shown in Fig. 1 (standard deviations are in parentheses).

2Reciprocal of cell density.

3Calculated by multiplying the anterior or posterior cell densities per μm² (this table) by the values of the anterior or posterior compartment areas shown in Table 1.

<table>
<thead>
<tr>
<th>Drosophila line</th>
<th>Anterior Compartment</th>
<th>Posterior Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>¹Cell density per μm²</td>
<td>²Area per cell (x 10⁻³ μm²)</td>
</tr>
<tr>
<td>Control</td>
<td>5.87 (0.02)</td>
<td>170.36</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>5.93 (0.17)</td>
<td>168.63</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>5.98 (0.10)</td>
<td>167.22</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>6.00 (0.17)</td>
<td>166.67</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>6.15 (0.19)</td>
<td>162.60</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>5.90 (0.15)</td>
<td>169.49</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>6.06 (0.19)</td>
<td>165.02</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>6.42 (0.20)</td>
<td>155.76</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>5.82 (0.12)</td>
<td>171.82</td>
</tr>
<tr>
<td>dS6K⁸¹⁸</td>
<td>5.77 (0.19)</td>
<td>173.31</td>
</tr>
</tbody>
</table>

ANOVA F         | 11.66                 | <0.001                 | 0.376   | 98.37   | <0.001 | 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 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.

Acknowledgements

This work was supported by a grant from the NSF to MJS (MCB-0077618) and by ND EPSCoR through NSF grant OSR-9452892.

Literature Cited


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.