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Report

A Role for S6 Kinase and Serotonin in Postmating Dietary Switch and Balance of Nutrients in *D. melanogaster*

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Summary

Balancing intake of diverse nutrients is important for organismal growth, reproduction, and survival. A shift in an organism's optimal diet due to changes in nutritional requirements after developmental or environmental changes is referred to as dietary switch and has been observed in several species [1]. Here we demonstrate that female *Drosophila melanogaster* also undergo a dietary switch following mating that leads to an increased preference for yeast, the major source of protein in their diet. We also demonstrate that S6 kinase (S6K) and serotonin production are involved in the postmating dietary switch. To further investigate the ability of *D. melanogaster* to balance nutrient intake, we examined the dietary preferences of adult flies following deprivation of yeast or sucrose. We observe that following conditioning on a diet deficient in either carbohydrates or yeast, *D. melanogaster* show a strong preference for the deficient nutrient. Furthermore, flies with activated *dS6K* or flies fed a serotonin precursor exhibit enhanced preference for yeast in this assay. Our results suggest that TOR signaling and serotonin may play an important role in maintaining nutrient balance in *D. melanogaster*. These studies may contribute to our understanding of metabolic disorders such as obesity and diabetes [2].

Results and Discussion

In order to maintain adequate nutritional status, organisms not only have to consume sufficient nutrients to meet energy needs but also need to select a balanced diet from foods that vary widely in nutritional quality. Furthermore, in organisms ranging from insects to mammals, the required nutritional balance can be different depending on developmental stage or changes in the environment [3, 4]. These distinct nutrient requirements can be satisfied by appropriately selecting from the various types of nutrients available to the organism [3, 4].

In order to test the ability of adult *Drosophila melanogaster* to select different types of nutrients, we developed a nutrient preference assay. In this assay, flies were given the freedom to choose two incomplete yet complementary diets in a sealed chamber. One food option consisted of a dietary mix lacking yeast extract (the major source of protein in the diet), whereas the other option consisted of a dietary mix lacking sucrose (the major source of carbohydrate in the diet). To measure ingestion of yeast, we gave flies a choice between the two food options wherein the food containing yeast but no sucrose contained a radiolabeled tracer. To measure sucrose ingestion, we gave a separate population of flies a choice between the two foods wherein the food containing sucrose but no yeast contained a radiolabeled tracer. This allowed the measurement of yeast and sucrose intake in the same strain of flies, albeit in separate experiments (for more details, see Experimental Procedures and Figure S1 available online). Using the radiolabeled food, we examined whether both male and female flies ingested an optimal diet by consuming the supplied dietary mixes in a nonrandom fashion. The nutrient preference assay demonstrated a significant sex-specific difference in food choice, with females eating a much larger amount of yeast than males and males consuming more sucrose relative to females (Figure 1A). This disparity in preferred nutrients between males and females is expected, because females have a greater need for protein and other nutrients in yeast extract to effect egg production [5, 6]. Mating in female *D. melanogaster* leads to significant behavioral and physiological changes, including increased feeding [7], enhanced egg production, and decreased longevity [8]. Therefore, we examined whether the mating status of female flies is important for the increased preference for yeast observed in female flies over male flies. A nutrient preference assay on mated and nonmated flies showed that mated females chose to consume significantly more yeast than their virgin counterparts (Figure 1B). These findings are consistent with the idea that mated female flies need to consume more protein to meet their increased investment in reproductive output [6, 7, 9].

It has been shown that the target of rapamycin (TOR) pathway plays a conserved role in nutrient sensing and growth in multiple species [10]. Essential amino acids activate the TOR pathway after being transported into the cell [10]. S6 kinase (S6K) is a target of TOR that, in the presence of amino acids, is activated via phosphorylation and mediates downstream effects on enhancing mRNA translation and growth [10]. Given the role of the TOR pathway in nutrient sensing, we tested the possibility that *S6K* may modulate nutrient choice at the organismal level. We examined the role of *S6K* in postmating dietary switch by using different forms of *dS6K*: wild-type, dominant-negative, and constitutively active. The dominant-negative form was previously generated by replacing a lysine with glutamine in the ATP binding site (*UAS-S6K^{DN}*), and the constitutively active form by replacing multiple phosphorylation sites of S6K with acidic amino acids (*UAS-S6K^{ACT}*) [11]. Overexpression of the dominant-negative form has previously been shown to reduce growth, whereas the constitutively active form enhances growth [11]. We specifically examined the changes in nutrient preference of flies overexpressing these different forms of *dS6K* in neuronal and fat tissues. Fat and neuronal tissues were chosen because they play a key role in sensing nutrient status and mediating physiological changes in the organism. Flies overexpressing the constitutively active *dS6K* via a pan-neuronal Gal4 enhancer trap, *appl*-GAL4, demonstrated an increase in yeast consumption for the virgin female population (Figure 2A).

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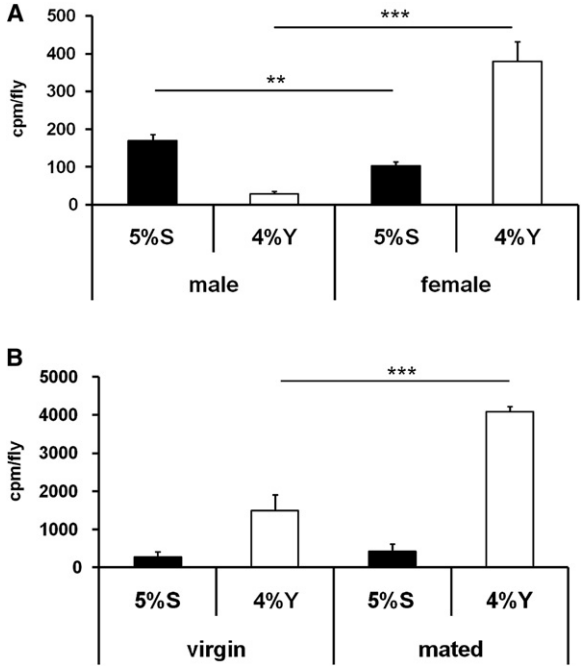

Figure 1. Sex- and Mating-Dependent Differences in Nutrient Preference for Sucrose or Yeast in *D. melanogaster*

To measure ingestion of yeast, we gave flies a choice between two food options wherein only food deficient in sucrose but containing 4% yeast extract (4%Y; white bars) contained a radiolabeled tracer. To measure ingestion of sucrose, we gave flies a choice between two food options wherein only food deficient in yeast but containing 5% sucrose (5%S; black bars) contained a radiolabeled tracer. Radioactivity (cpm) of [α-^{32}P]dCTP-labeled food ingested per fly in 24 hr is shown (mean ± standard deviation of four replicate samples of 15–20 flies each). **p < 0.01, ***p < 0.001. See also Figure S1.

(A) Results of nutrient preference assay demonstrating the differences in dietary preferences of males and females in the *w1118* strain.

(B) Differences in dietary consumption of yeast and sucrose in virgin and mated females in the *w1118* strain.

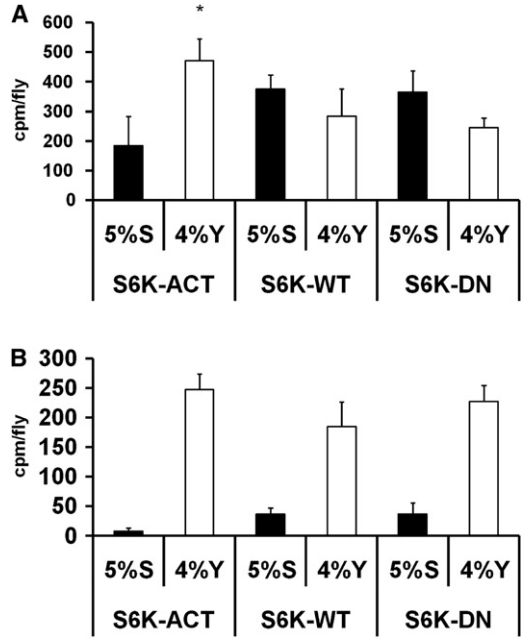

Figure 2. Effect of *dS6K* on Nutrient Preference in Virgin and Mated *D. melanogaster*

Nutrient preference for sucrose (black bars) and yeast (white bars) was measured upon *dS6K* manipulation. Radioactivity (cpm) of [α-^{32}P]dCTP-labeled food ingested per fly in 24 hr is shown (mean ± standard deviation of four replicate samples of 15–20 flies each). **p < 0.01. See also Figure S2.

(A) Virgin female flies overexpressing constitutively active (S6K-ACT), wild-type (S6K-WT), or dominant-negative (S6K-DN) forms of *dS6K* in neurons driven by the pan-neuronal *appl*-GAL4 enhancer trap were tested for sucrose or yeast ingestion. The genotypes tested were *appl-GAL4/+;UAS-S6K^{STDETE}/+;+/+* (S6K-ACT), *appl-GAL4/+;UAS-S6K^{WT}/+;+/+* (S6K-WT), and *appl-GAL4/+;UAS-S6K^{KQ}/+;+/+* (S6K-DN). Virgin S6K-ACT flies were found to ingest significantly more yeast compared to the other two strains as calculated by one-way analysis of variance (ANOVA) (p < 0.003).

(B) As in (A), except mated female flies were used for the experiment.

However, modulation of *dS6K* in mated females did not show a significant change in nutrient preference (Figure 2B). The manipulation of *dS6K* via *appl*-GAL4 is likely to alter gene expression during both development and adulthood such that changes in *dS6K* activity during development cannot be ruled out as a contributing factor in the above experiments. Modulation of *dS6K* in the fat body did not lead to any significant changes in yeast consumption (data not shown). To further assess the role of *dS6K* in postmating dietary switch, we tested the possibility that mated flies have increased *dS6K* phosphorylation. However, the levels of S6K phosphorylation measured in fly heads with an antibody that recognizes the phosphorylated form of S6K did not show any significant differences between virgin and mated flies (Figure S2). Although these experiments do not support a role for *dS6K* activation leading to the increased preference for yeast in mated flies, it is possible that *dS6K* activation in specific neurons may mediate this change but was not detectable in our experiments with whole fly heads. It has previously been shown that neuronal modulation of *dS6K* plays an important role in mediating responses to hunger [12]. Our results show that, in addition, *dS6K* activation in neuronal cells enhances ingestion of food with a higher ratio of protein to sugar in unmated flies.

Serotonin has previously been implicated in changing the protein/carbohydrate ratio of ingested nutrients in animals such as cockroaches [13] and rats [14]. To determine the role of serotonin in postmating dietary switch in *D. melanogaster*, we examined whether serotonin levels are altered upon modulation of *dS6K*. Increased neuronal *dS6K* activation led to increased serotonin production (Figure 3A). However, no significant increase in serotonin levels was observed in fly heads upon mating (Figure S3A). To assess the relationship between *dS6K* and serotonin in our paradigm, we examined whether addition of the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP) to the food would alter S6K activity. 5-HTP has previously been used to study the effects of serotonin in *D. melanogaster* [15]. We observed that flies exposed to 5-HTP showed an increase in serotonin levels in vivo (Figure S3A). Addition of 5-HTP to fly food or *Drosophila* embryonic S2 cells failed to affect S6K phosphorylation as measured by an antibody that recognizes phosphorylated S6K (data not shown).

To assess the role of serotonin in influencing nutrient choices, we analyzed the nutrient preference of flies conditioned on food containing 5-HTP. Virgin flies exposed to a diet containing 5-HTP showed a significant increase in yeast preference compared to control virgin females on a normal diet (Figure 3B). No significant differences of 5-HTP treatment