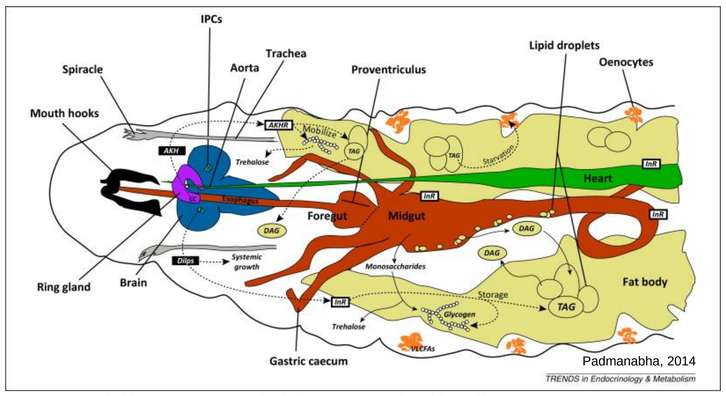
# RNA-binding protein Alan shepard regulates fat storage homeostasis

Claire Gillette, Kelsey Hazegh, Tânia Reis
University of Colorado Anschutz Medical Campus | Department of Medicine | Division of Endocrinology, Metabolism, and Diabetes | Molecular Biology Program | Aurora, CO, USA

## Abstract

Metabolism is an integrated, multi-organ process, and is best studied within the context of the whole organism. Mounting evidence points to an important yet poorly understood role for genetic background in the control of organismal fat levels. The RNA-binding protein Alan shepard (*shep*) has no characterized role in metabolism. We used tissue-specific RNAi to determine in which organs *shep* is required for regulation of organismal fat. We find that knockdown of *shep* in the brain phenocopies the high-fat phenotype of the mutant and drives changes in the complex metabolic behaviors of feeding and activity. Additionally, knockdown of *shep* in the fat body results in a lean phenotype accompanied with a modest increase in activity. Shep's genomic locus codes for 8 mRNA and 6 protein isoforms. Thus far, we have shown that fat body-specific overexpression of the Shep-RE isoform increases organismal fat levels, suggesting a positive associate between Shep-RE expression and the fat body's propensity to store fat. We show that the RNA Recognition Motifs are necessary for the metabolic function of Shep-RE. We further find that Shep transcript and protein levels are regulated in a nutrient-dependent manner. As the nutritional content of the diet increases, *shep* mRNA and protein expression decreases in the fat body. This suggests that *shep* is regulated by a nutrient sensing pathway. Our work is now focused on determining the role of shep isoforms in different organs in regulating overall organismal energy metabolism, focusing here on Shep function in the fat body.




## *Drosophila* as a model for metabolic disease

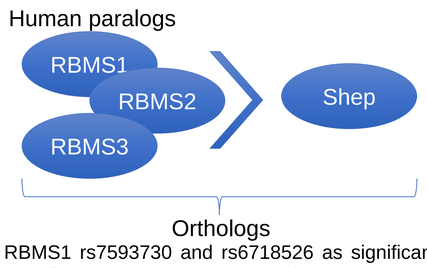
Basic metabolic pathways are highly conserved; glycolysis is very similar from yeast to humans. *Drosophila* are an advantageous model because they share many metabolic organs with mammals– brain, gut, and fat body (FB; glycogen and TGs storage organ). They also share many complex inter-tissue signaling pathways such as the gut-brain axis and neuropeptide/signal peptide signaling between metabolic organs. For example, *Drosophila* have Insulin-like peptides (dILPs) and AKH (glucagon homolog) to regulate circulating sugars.

## Forward genetic screen identified RNA-binding protein Alan shepard (Shep) as obesity predisposing gene

| Mutant gene | Buoyancy score | Molecular function |
|---|---|---|
| *shep* | +++ | mRNA binding |

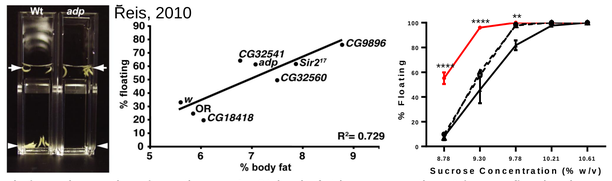
Reis, 2010

## Human ortholog of Shep (RBMS) is implicated in Type 2 Diabetes Risk



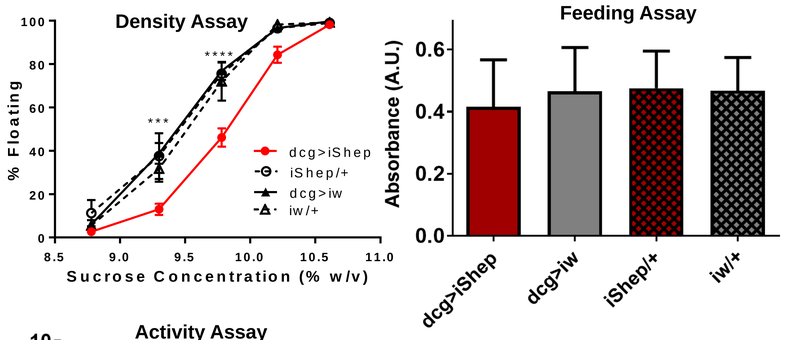

GWAS identified RBMS1 rs7593730 and rs6718526 as significantly associated with T2D risk (Campbell, 2012 and Kazakova, 2018).

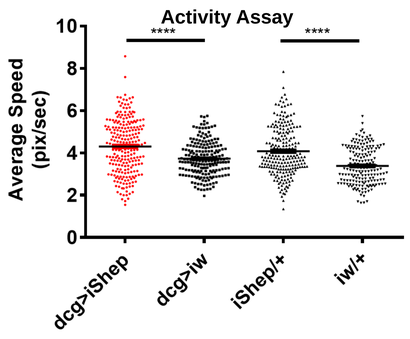
## Using the density assay to assess fat storage




Fat tissue is less dense than lean tissue. As % body fat increases, the % larvae floating increases. In the last graph the red-line genotype is comparatively more fat than the black-line genotypes and controls.

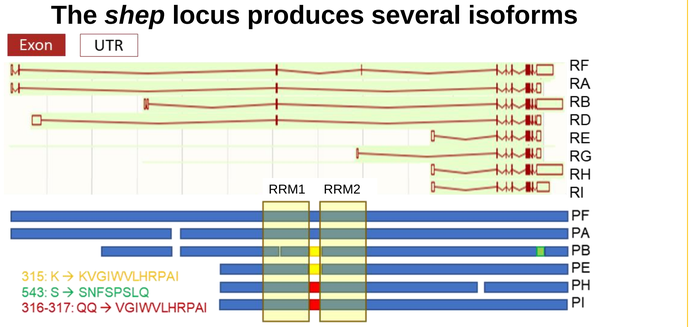
## FB-specific *shep*-RNAi drives a lean phenotype that is independent of behavioral changes



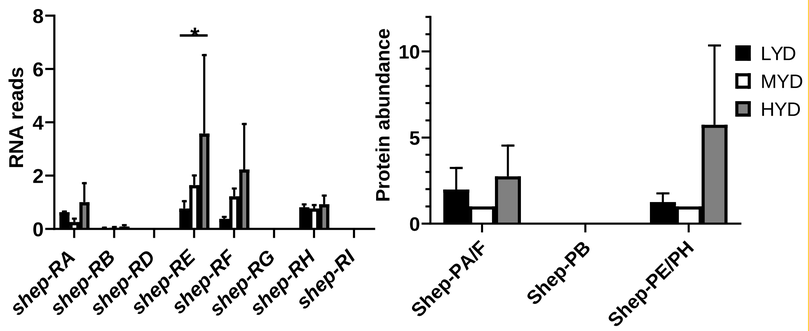

***shep* knockdown in the fat body drives a lean phenotype**. Third instar larvae with FB-specific *shep*-RNAi expression were analyzed using the density assay. (Density Assay: 2-way ANOVA analysis, *p<0.05, n=8.) Feeding Assay: unpaired student T-Test, *p<0.05, n=4. Activity Assay: one-way ANOVA analysis, *p<0.05, n=4)

## The *shep* locus produces several isoforms



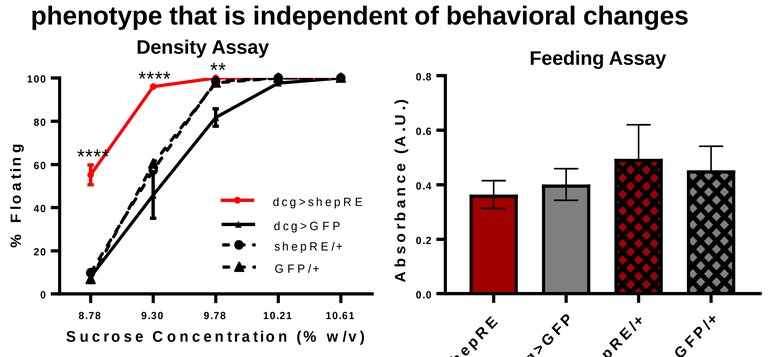

Shep has 8 mRNA and 6 protein isoforms. We hypothesize that fat storage is regulated by one (or more) isoforms. It has two highly conserved RNA Recognition Motifs (RRMs).

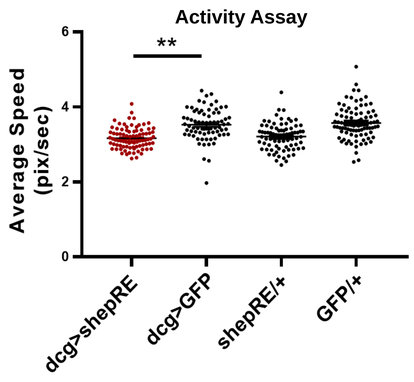
## Diet modulates FB *shep* isoform expression. Shep-PE is the most responsive isoform to diet challenge and expression increases with increased nutrient content.




**FB *shep* isoform expression changes with diet challenge.** RNAseq of dissected fat bodies from $w^{1118}$ wandering third in-star larvae reared on Low-Yeast Diet (LYD), Medium-Yeast Diet (MYD), and High-Yeast Diet (HYD). (Two-way ANOVA analysis, n=3, *p<0.05). Western blot of isolated fat bodies from $w^{1118}$ wandering third-instar larvae. aShep antibody and size separation identify three broad classes of Shep bands: Shep-PA/PF, Shep-PB, and Shep-PE/PH. (Western imaged using Odyssey Li-Cor and analyzed using ImageStudio Lite. 2-Way ANOVA Analysis, n=3).

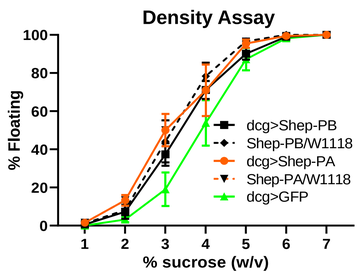
## FB-specific ShepRE overexpression induces a fat phenotype that is independent of behavioral changes



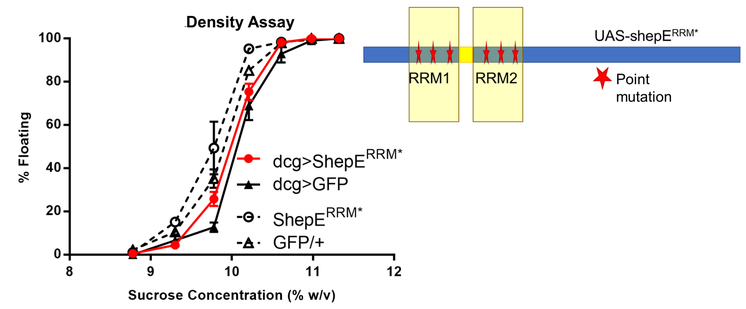

**ShepRE overexpression in the fat body drives a fat phenotype**. Third instar larvae with FB-specific ShepRE expression were analyzed using the density assay. (Density Assay: 2-way ANOVA analysis, *p<0.05, n=8.) Feeding Assay: unpaired student T-Test, *p<0.05, n=4. Activity Assay: one-way ANOVA analysis, *p<0.05, n=4)

## Shep-PA and Shep-PB lack fat storage phenotypes

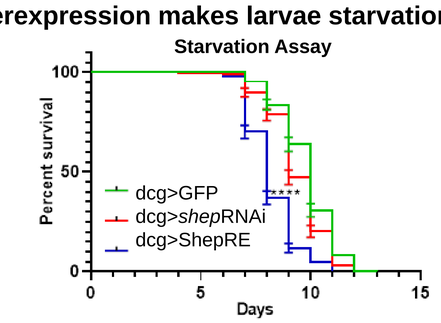



Overexpression of Shep-PA and Shep-PB is the same as their corresponding genetic background control, and more fat than the overexpression control.. This intermediate phenotype suggests Shep-PA and Shep-PB are not the isoforms that regulate Shep's FB-specific metabolic function. (Preliminary data)

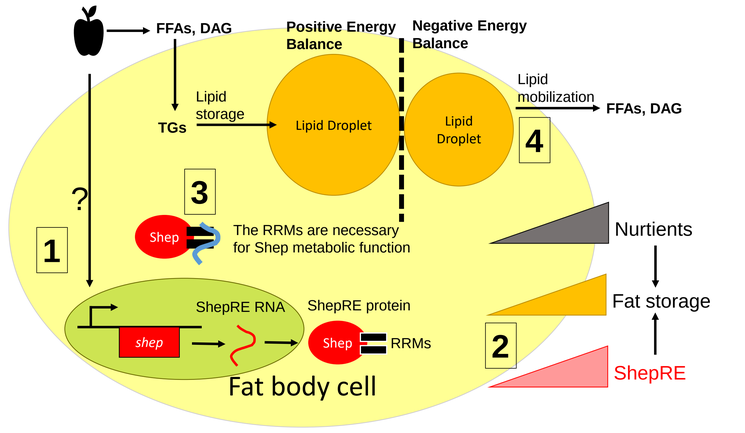
## RNA-binding is necessary for ShepRE fat storage regulation. We hypothesize that ShepRE regulates lipid regulating proteins post-transcriptionally.




## ShepRE overexpression makes larvae starvation sensitive




Larvae were kept on a starvation media. Shep-RE overexpression dies a mean 20% faster than overexpression control. We hypothesize that this could be due to impaired ability to liberate stored lipids (****p<0.0001, log-rank).




Shep was identified as obesity predisposing gene in a forward genetic screen (Reis, 2010). We showed that FB-specific *shep* depletion caused a lean phenotype. Additionally, *shep* mRNA/protein expression increases as nutrient content of diet increases. The ShepRE isoform changes the most upon diet challenge. FB-specific overexpression of ShepRE drives a fat phenotype. This phenotype requires the RRMs.

### Conclusions on Shep function within the fay body:

1. **As nutrient content of diet increases, ShepRE expression increases**
2. **ShepRE overexpression has a fat phenotype independent of behavior**
3. **The RRMs are necessary for ShepRE fat regulatory function**
4. **ShepRE overexpression makes larvae starvation sensitive, we hypothesize due to impaired ability to liberate stored fat**

## Acknowledgements and References

We would like to thank Dahong Chen and Elissa Lei (NIDDK, Maryland) for generously sharing their UAS-Shep *Drosophila* lines. We would like to thank Dr. Kathleen Beckingham (Rice University) for generously sharing her Rb-α-Shep and gp-α -Shep antibodies. We would like to acknowledge the University of Colorado Molecular Biology PhD training program, CU-AMC Department of Endocrinology, Diabetes, and Metabolism, and the CU-AMC RNA Bioscience Initiative for their support.

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