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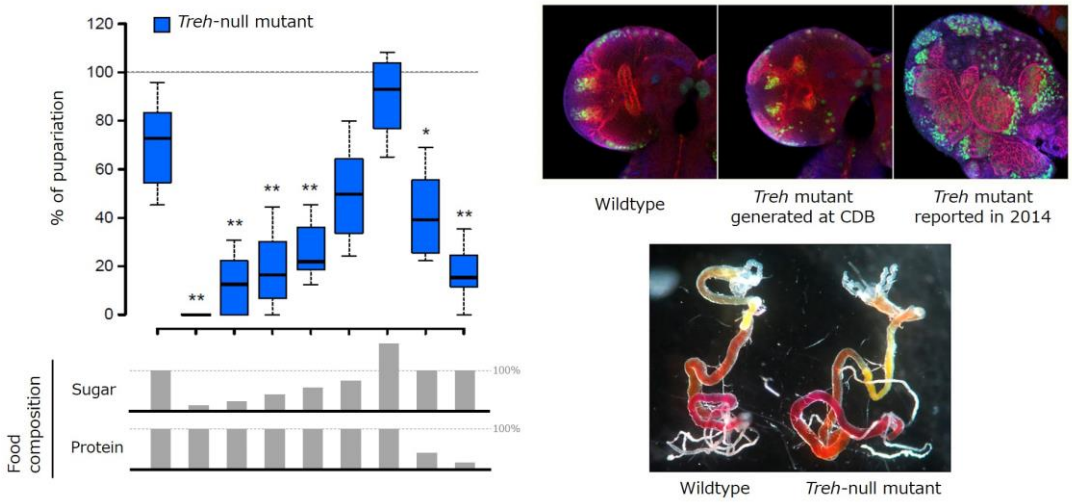
Trehalose metabolism essential for adaptation to changes in dietary conditions

June 06, 2017– In insects, the disaccharide sugar trehalose is the main sugar compound and energy source circulating in the hemolymph (insect equivalent of blood), and due to its chemical stability and inertness, is also thought to play a role in protecting organisms from environmental stresses such as desiccation and starvation. Trehalose is synthesized from glucose by trehalose-6-phosphate synthase (Tps1) and is broken down by trehalose-hydrolyzing enzyme, trehalase (Treh), regulation of these processes is important for maintaining metabolic homeostasis. Whereas mammals including humans cannot synthesize trehalose, they possess the Treh enzyme to break down trehalose that is ingested into the body. Treh is a highly conserved enzyme, being found in a range of organisms from bacteria to mammals, including humans, but much of its physiological functions remain unclear.

Now in a new study published in *Scientific Reports*, former research scientist Tetsuo Yasugi and his colleagues in the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) carried out a detailed comparison of previously reported *Drosophila* mutants carrying a mutation in the *Treh* gene, which is important in trehalose metabolism. Rearing the fly mutants under identical conditions, they found that larval lethality of *Treh* mutants are highly dependent on dietary conditions, requiring high levels of both sugar and protein. The team also revealed that under poor dietary conditions, lethality in *Treh* mutants is caused by overaccumulation of trehalose, not loss of Treh function.



Top right: Morphological phenotypes of optic lobe (brain). No abnormalities were observed in *Treh*-null mutant generated by the lab. Left: Food composition and the survival rates of mutants to pupal stages when reared under those food conditions. *Treh* mutants are sensitive to low sugar or low protein conditions. Bottom right: Gut of wildtype and *Treh*-null mutants. No major morphological or functional differences were observed.

The Laboratory for Growth Control Signaling previously generated several different *Drosophila* strains carrying a mutation in the *Treh* gene using the CRISPR/Cas9 system, and reported that loss of Treh function results in lethality at pupal stages and is also more susceptible to desiccation (see Science News: Sept. 15, 2016). Around the same time, two other groups—one in China in 2014 and the other in Germany in 2015—also independently generated *Treh* mutants, but reported phenotypes that appeared to differ from those observed by Nishimura's team, particularly in terms of the lethal stages. The *Treh* mutant reported in 2014, which carried a deletion or insertion within the gene, were lethal between larval and pupal stages, while the *Treh*-null mutants reported in 2015, which were generated using the TALEN system, showed lethality in early larval stages. Although in most cases, the *Treh* mutants did not develop to adult stages, it was unclear why the timing of lethal phases observed by each group differed. Yasugi and his colleagues thus decided to take a closer examination at the phenotypes of the reported mutant strains to find an answer.

The team obtained the *Treh* mutant strains from both groups, and bred them under identical experimental conditions. In addition to larval and pupal lethality, the *Treh* mutants reported in 2014

were described to display abnormalities of the optic lobe (brain), which was verified by Yasugi et al. However, they did not see the same abnormal disorganization in the optic lobe of their own CRISPR/Cas9-induced *Treh* mutants. Further analyses revealed that the *Treh* mutant reported in 2014 in fact also carried a mutation in the *lgl* gene locus, which is located at the ends of the chromosome on which the *Treh* gene is found, and backcross experiments demonstrated that it was in fact the mutation in *lgl*, not *Treh*, that caused abnormalities in the optic lobe.

The team next explored the differences in the timing of lethality among the mutant strains when bred under identical conditions. Surprisingly, the TALEN-induced *Treh*-null mutants, which reportedly displayed lethality during larval stages, were found to be capable of developing to pupal stages, similar to the CRISPR/Cas9-induced *Treh*-null strains. Furthermore, all mutant strains were discovered to have comparable trehalose and glucose levels—higher trehalose and reduced glucose levels than seen in wildtype. So why then were the TALEN-induced *Treh*-null mutants previously found to be lethal in larval stages?

The team hypothesized that diet (i.e. nutrient availability) was a possible underlying factor for differences in lethality stages. In the lab, *Drosophila* are normally reared on agar-cornmeal media, which contains a mixture of glucose (sugar), yeast (protein, etc.) and cornmeal (sugar, fat, etc.), but fly food recipes vary among groups and the quality of ingredients purchased from vendors may also show differences between lots. The lab previously demonstrated that survival rates in larval stages of *Tps1* mutants, which cannot produce trehalose, was sensitive to low sugar conditions but maintained stable survival rates under low protein conditions, supporting their idea that nutrient availability can affect lethality in mutants with problems linked to metabolism (see Science News: Feb. 9, 2015). When Yasugi et al. bred the *Treh* mutant strains under various dietary conditions, they found that low sugar or low protein conditions led to marked reduction in survival rates of *Treh* mutants in larval stages. They also generated *Tps1*, *Treh* double mutants which under low protein conditions could survive to pupal stages, with lethal effects of *Treh* mutants being mitigated by additional *Tps1* mutation. Together these results indicated the overaccumulation of trehalose due to inability to metabolise trehalose, not the loss of functional Treh, as the cause of larval lethality in *Treh* mutants. The team also confirmed that all *Treh* mutants exhibited normal feeding behavior, and relatively normal digestive and fat body (insect equivalent of the liver) functions, also ruling out possibility of these factors contributing to lethality under restricted diet conditions.

“Whereas food composition is not as critical when studying morphogenesis, we do need to be careful about food composition when looking at metabolism and growth effects, especially when using mutants with defects in metabolism-associated genes,” says Nishimura. “Trehalose is a non-reducing sugar, and as such, has properties that differ from reducing sugars such as glucose, which when accumulated can trigger hyperlipidemia or arteriosclerosis. Our present study however suggests that problems in trehalose metabolism can also lead to serious defects. Our next goal will be to reveal what exactly goes on when there is an overaccumulation of trehalose in the body.”

Science News
Trehalose important for maintaining body water homeostasis (Sept. 15, 2016)
http://www.cdb.riken.jp/en/news/2016/researches/0915_9554.html

When flies are low on sugar (Feb. 9, 2015)
http://www.cdb.riken.jp/en/news/2015/researches/0209_6022.html