GALDAR: a genetically encoded galactose sensor for visualizing sugar metabolism in vivo

SAKIZLI, UGURCAN

(Degree)
博士（医学）

(Date of Degree)
2024-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲第8974号

(URL)
https://hdl.handle.net/20.500.14094/0100492483

（課程博士関係）

学位論文の内容要 旨

GALDAR: a genetically encoded galactose sensor for visualizing sugar metabolism *in vivo*

GALDAR: *in vivo* で糖代謝をイメージングするための遺伝学的にエンコードされたガラクトースセンサーの開発

Graduate School of Medicine

Division of Developmental and Regenerative Medicine

Ichiro Hiratani

SAKIZLI UGURCAN

Summary

Sugar metabolism plays a key role in sustaining life throughout all domains of life. Even though the dynamics of sugar metabolism is well understood at cellular level, there is a gap in our understanding of it at organismal level. Galactose, a hexose stereoisomer of glucose, is transported with the same transporters as glucose and can be utilized as an energy source through glycolysis pathway after going through Leloir pathway. It also has a role in glycosylation, a post-translational protein modification. Defects of galactose metabolism therefore are detrimental in most organisms.

In this study, we transgenically implemented the galactose sensing mechanism of yeast into *Drosophila* and developed a genetically encoded sensor for *in vivo* galactose detection. We named the new sensor GALDAR (GALactose raDAR). The main proteins in the galactose sensing system of yeast are: Gal4, Gal80 and Gal3. Gal4 and Gal80 have been implemented to other organisms for gene expression regulation. This study provides the first example of the addition of Gal3 into a more complex model organism. Gal3 regulates gene expression by inhibiting the transcriptional repressor Gal80 only in the presence of intracellular galactose, allowing for gene expression of genes with Upstream Activating Sequences (UAS). Fly's normal laboratory diet does not contain galactose, which enabled us to assess cellular galactose amounts by simply feeding the flies with galactose.

We used a fluorescent protein as a readout for cellular galactose amounts and analyzed major metabolic organs of *Drosophila*. Importantly, absorptive cells in the midgut and muscles are positive for GALDAR, whereas intestinal stem cells and fat body cells remained negative. These negative results might be observed because of a higher galactose metabolism speed in these tissues. Therefore, we investigated the effects of galactose metabolism on the GALDAR signals. Using a homozygous mutant for the first enzyme in the Leloir pathway, Galk, we interrupted galactose catabolism and focused on galactose uptake only. In this condition we saw a broader GALDAR signal, which indicates that galactose is actively metabolized throughout the organism. In addition to galactose metabolism in the adult organism, we also observed a decrease in GALDAR signals as the fly larvae develop. We connected this observation with the increased galactose metabolism and decreased galactose amounts in the developing larvae. Lastly, we developed a newer version of GALDAR which can be used together with Gal4/UAS system, which is used in almost all studies using *Drosophila*. This new version of the sensor relies on galactose-dependent binding of Gal3 and Gal80. With this new version we were able detect physiological amounts of galactose in the midgut and fat body of *Drosophila* after feeding them with fig fruit.

In conclusion, we developed and characterized a novel genetically encoded galactose sensor, which will potentially be useful for understanding sugar metabolism at organismal level. In principle, the GALDAR system is not restricted to use in *Drosophila*, but it can be used in any eukaryotic system.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第3401号	氏　名	Sakizli Ugurcan
論文題目 Title of Dissertation	GALDAR: a genetically encoded galactose sensor for visualizing sugar metabolism *in vivo* GALDAR: *in vivo* で糖代謝をイメージングするための遺伝学的にエンコードされたガラクトースセンサーの開発		
審査委員 Examiner	主　査 Chief Examiner 土居敏智之 副　査 Vice-examiner 根本秀明 副　査 Vice-examiner 小川芽		

（要旨は１，０００字～２，０００字程度）

Introduction

Sugar metabolism plays a key role in sustaining life throughout all domains of life. Even though the dynamics of sugar metabolism is well understood at cellular level, there is a gap in its understanding at an organismal level. Galactose, a hexose stereoisomer of glucose, is transported with the same transporters as glucose and can be utilized as an energy source through glycolysis pathway after going through Leloir pathway. It also has a role in glycosylation, a post-translational protein modification. Defects of galactose metabolism therefore are detrimental in most organisms. In this study, cellular galactose levels of various tissues were detected *in vivo* using a novel genetically encoded galactose sensor, GALDAR (GALactose raDAR), which is based on yeast galactose sensing system. Importantly, GALDAR shows that intestinal stem cells of the midgut do not incorporate a detectable amount of galactose and there is a significant change in galactose metabolism during larval development.

Methods

In this study, the galactose sensing mechanism of yeast was transgenically implemented into *Drosophila* and a genetically encoded sensor for *in vivo* galactose detection was developed and named GALDAR. Laboratory fly food does not contain galactose, which makes it suitable for assessing cellular galactose amounts by feeding the flies with galactose supplemented food. GALDAR signals in fixed tissues were analyzed using confocal microscopy following feeding for 3 days. *Galk* expression levels were determined using qPCR. Galactose amounts in larval staged were analyzed with LC-MS/MS.

Results and Discussion

The main proteins in the galactose sensing system of yeast are: Gal4, Gal80 and Gal3. Gal4 and Gal80 have been implemented to other organisms for gene expression regulation. This study provides the first example of the utilization of Gal3 into a more complex model organism. Gal3 regulates gene expression by inhibiting the transcriptional repressor Gal80 only in the presence of intracellular galactose, allowing for Gal4 mediated expression of genes with Upstream Activating Sequences (UAS). A fluorescent protein was used as a readout for cellular galactose availability and major metabolic organs of *Drosophila* were analyzed. Importantly, absorptive cells in the midgut and muscles are positive for GALDAR, whereas intestinal stem cells and fat body cells remained negative. These negative results might be observed because of a higher galactose metabolism speed in these tissues. Therefore, the effects of galactose metabolism on the GALDAR signals were investigated. Using a

homozygous mutant for the first enzyme in the Leloir pathway, *Galk*, galactose catabolism was interrupted, allowing for focusing on galactose uptake only. In this condition a broader GALDAR signal was observed, which indicates that galactose is actively metabolized throughout the organism. In addition to galactose metabolism in the adult organism, GALDAR signals of the fly larvae were also investigated. GALDAR signals become weaker as the larva develops. This observation can be explained with the increased galactose metabolism and decreased galactose amounts in the developing larvae. In order to confirm this, *Galk* expression levels of larval fat body were quantified using qPCR. Consistently, galactose amounts are lower in the developed larvae. Lastly, a newer version of GALDAR was developed and characterized, which can be used together with commonly used Gal4/UAS system. This new version of the sensor relies on galactose-dependent binding of Gal3 and Gal80. With this new version detection physiological amounts of galactose in the midgut and fat body of *Drosophila* were possible even at a physiological amount, demonstrated by positive GALDAR signals after feeding the flies with fig fruit.

Conclusion and Discussion

In conclusion, a novel genetically encoded galactose sensor was developed and characterized, which will potentially be useful for understanding sugar metabolism at organismal level. In principle, the GALDAR system is not restricted to use in *Drosophila*, but it can be used in any eukaryotic system.

The candidate, having completed studies on metabolism of *Drosophila melanogaster*, with a specialty in galactose metabolism, and having advanced the field of knowledge in the area of sugar metabolism, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).