Department of Biological Sciences Seminar Blog
Seminar Date: 3/17/17
Speaker: Dr. Stylianos Fakas, Alabama A&M University
Title: *"Regulation of phosphatidic acid phosphatase activity in the oleaginous yeast Yarrow lipolytic"*

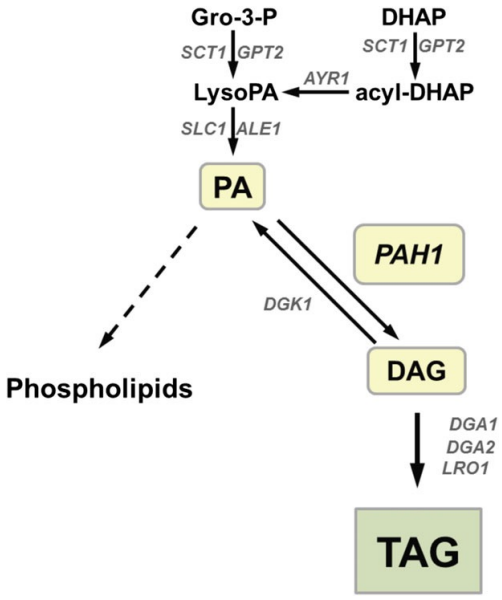
TAG!! You're it!! Increasing the production of triacylglycerol in oleaginous yeast and sparking a revolution in biofuels???

By: Alexiy Nikiforov

The concept of biofuels (any fuel acquired directly from living matter) has been around for a long time. They are an alternative to fossil fuels and have been typically produced from the sugars, starches, and oils found in plants. Despite being a cleaner source of fuel, there are still many challenges facing the use of plants as biofuels including the fuel efficiency, the financial cost, and the environmental impact. One scientist currently exploring an alternative to using plants as biofuels is Dr. Stylianos Fakas, Assistant Professor in the Department of Food & Animal Science at Alabama A&M University. Hailing originally from Greece, Dr. Fakas gave a fascinating talk at Duquesne University about his current research during the Spring 2017 Seminar Series.

In a nutshell, Dr. Fakas is currently researching how to increase the production of triacylglycerol (TAG) in an oleaginous yeast *Yarrowia lipolytic*a (YL). Oleaginous refers to a class microorganism that can produce and store a large amount of lipids in their cells. These lipids are stored in the form of TAG. In these cells, TAG is used partially as an energy storage and partially as a precursor to making fatty acids (2). From a technological standpoint, TAG is what is harvested for producing biofuels (2). But how can you ever produce enough TAG in these oleaginous yeast cells? The answer comes from understanding and regulating the set

of enzymes that controls TAG synthesis: phosphatidic acid phosphatase (PAP).



The PAP enzymes were first characterized in *Saccharomyces cerevisiae* (SC) yeast but because SC are non-oleaginous, this research must be conducted in an effective oleaginous yeast model. *Yarrowia lipolytic*a (YL) step right up (1). Wildtype YL can accumulate 40% of their mass in lipids and certain genetically engineered strains can go up to 90% lipid content. Such lipid accumulation can be stimulated by growing these yeast on nitrogen depleted media. This prevents the cells from synthesizing amino acids and properly dividing, but if you provide a carbon source, they will continue to accumulate lipids. In SC, phosphatidic acid (PA) can be used to make phospholipids or converted to diacylglycerol (DAG) by PAH1 (1). PAH1 is the main phosphatidic acid phosphatase (PAP) in SC and a homolog has been bound in YL. DAG is then further converted to triacylglycerol (TAG) which is the final product of the pathway.

Previous research has shown that PAP activity is regulated by the growth phase in SC and this same regulation was attempted in YL by Dr. Fakas' lab (1). These growth phases can be generally broken down into lag phase (cells preparing to divide), exponential phase (cells doubling every cycle), and stationary phase (cells no longer dividing). In SC, most of the cells lipids are manufactured in stationary phase and SC PAP activity is therefore higher in stationary phase (1). But in YL, it was discovered the opposite was true. There appeared to be more YL PAP activity in exponential phase than in stationary phase (1). This lead to further inquiry into the enzymes that make up SC & YL PAP. It turns out that

both SC & YL PAP are comprised of two magnesium dependent and two magnesium independent enzymes (1). So, the next thing they tested was the addition 1mM MgCl2 to the growth media. As expected, there was significantly more YL PAP activity with the addition of magnesium than the control (1). Another key component to understand how to regulate YL PAP is to understand where it is localized in the cell. In SC, PAP has been shown to be localized at the plasma membrane because PAP typically resides in the cytosol but the substrate for this enzyme is in the plasma membrane (1). The same localization signal was confirmed for YL PAP after cells were fractionated into membrane and cytosolic fractions, yielding more PAP activity in the membrane fraction than the cytosolic fraction (1).

In the last part of Dr. Fakas' seminar, he presented new data about another factor that regulates YL PAP activity, high vs low glucose media. As mentioned previously, without a nitrogen source in the media, YL will divide at a much slower rate, but will continue to accumulate lipids if there is a carbon source. The experiments they performed showed that greater lipid accumulation was triggered in high glucose media after day 2 as compared to low glucose media, which only initially had lipid accumulation. With high glucose media, YL PAP activity increased after day 2 and there was more YL PAP activity in the membrane fraction than the cytosolic fraction after a follow-up experiment. This data leads to the following research questions that Dr. Fakas' lab will attempt to answer: what enzymes are responsible for the increase of PAP activity in the presence of high glucose? What is the role of these enzymes in the accumulation of lipids? What is the mechanism of the regulation? The data presented in this seminar by Dr. Fakas is the first step in understand how to regulate YL PAP activity in the hope of increase YL TAG accumulation in YL. There may be more questions than answers at this moment, but these are questions worth answering.

References

(1) Hardman, D., Mcfalls, D., Fakas, S., Characterization of phosphatidic acid phosphatase activity in the oleaginous yeast Yarrowia lipolytica and its role in lipid biosynthesis. Yeast, 2016, DOI: 10.1002/yea.3216

(2) Fakas, S., Lipid biosynthesis in yeasts: A comparison of the lipid biosynthetic pathway between the model non-oleaginous yeast Saccharomyces cerevisiae and the model oleaginous yeast Yarrowia lipolytica. Engineering in Life Sciences, 2016, DOI: 10.1002/elsc.201600040