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Regulation of Mg^{+2}-Dependent Phosphatidic Acid Phosphatase Enzymes in the Yeast *Yarrowia lipolytica*

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Single-cell oils (SCOs) are considered major products capable of producing polyunsaturated fatty acids (PUFAs) that are essential for human nutrition. They are derived from microorganisms such as *Yarrowia lipolytica.* In this yeast, during the lipid biosynthesis,phosphatidic acid phosphatase (PAP) catalyzes the conversionof diacylglycerol (DAG) to triacylglycerol (TAG) in a reaction that depends on Mg^{+2}. In *Y. lipolytica*, the *PAH1* and *APP1* genes encode for Mg^{+2}-dependent PAP activity, but their regulation has not been studied in detail. In this work, we constructed a strain that lacks both *PAH1* and *APP1* (i.e., *pah1*Δ*app1*Δ) to examine the contribution of these enzymes to PAP activity and TAG biosynthesis. We grew the strains in high glucose media that favors the lipid accumulation and measured the PAP enzymatic activity during lipogenesis. The contribution of the genes was examined by comparing the PAP activity and lipid profiles between a wild type strain, a mutant strain that lacks *PAH1* (i.e., *pah1*Δ), and a mutant strain that lacks both *PAH1* and *APP1* (i.e., *pah1*Δ*app1*Δ). The results showed that 90% of the Mg^{+2}-dependent PAP activity is encoded by the *PAH1* gene, while *APP1* is only contributing 10% of that activity.Next, we examined the role that these enzymes play in TAG biosynthesis. The lack of *PAH1*alone (i.e., *pah1*Δ) resulted in a 20% reduction of the TAG levels, while the lack of *APP1* did not have a significant effect on TAG levels. These results showed that *APP1* does not contribute to TAG biosynthesis in *Y. lipolytica*.

Keywords: Single-cell oils, PUFAs, Triacylglycerols, Phosphatidic acid phosphatase, High glucose, PAP activity, Lipid profile, Yarrowia. lipolytica.