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Signaling Pathways for Triacylglycerol Lipid Droplet Formation

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Commentary

It is well established that lipid droplets form from the endoplasmic reticulum (ER) bilayer in organisms across the phylogenetic tree. What it less understood is how gene products combine actions to make this event occur. Since lipid droplets have a neutral lipid core, much effort has gone into understanding the roles of neutral lipid synthesizing enzymes in droplet biogenesis. In yeast, these include the phosphatidic acid phosphatase (Pah1), which converts phosphatidic acid (PA) to diacylglycerol (DAG), the acyl-CoA-diacylglycerol acyltransferase (Dga1), which converts diacylglycerol (DAG) to triacylglycerol (TAG), the phospholipid:diacylglycerol acyltransferse (Lro1/Plh1), which converts DAG and phospholipids to TAG, and the acyl-CoA sterol acyltransferases (Are1 and Are2), which convert sterols to sterol esters. Here, we focus on signaling pathways for TAG synthesis.

Of the above factors, the most progress has been made in understanding the signaling dynamics of Pah1. This work has been motivated by the crucial cellular role of Pah1 in determining whether PA is converted to phospholipids or whether it is stored in the form of DAG (and ultimately TAG). Because Pah1's action is so crucial, it is tightly regulated both spatially and temporally. Soluble Pah1 is localized to the cytosol where it is phosphorylated by Pho85-Pho80, Cdc28/Cdk1, protein kinases A and C, and protein kinase CKII (Figure 1) [1-3]. The effects of protein kinase C and protein kinase CKII on Pah1 activity are relatively modest. Target of rapamycin complex 1 (TORC1) has been shown to also phosphorylate and thus inhibit Pah1 [4]. The phosphorylation of Pah1 by TORC1 is particularly interesting because it depends on the presence of the Nem1-Spo7 complex, which is normally associated with the activation of Pah1 through dephosphorylated at the ER bilayer. Pah1 dephosphorylated by Nem1-Spo7 inserts into the ER membrane via its N-terminal amphipathic helix (Met-1 – Ile-18) [5]. Pah1 phosphorylated by Pho85-Pho80 shows the highest affinity for Nem1-Spo7 [6]. Once in the ER, Pah1 converts PA to DAG. Surprisingly the active form of Pah1 is rapidly degraded.

At this point, the signaling mechanisms of TAG production from DAG - ultimately causing lipid droplet formation - are less clear. It would be unusual if Dga1 and Lro1 are not modified post-translationally. Dga1 has one predicted phosphorylation site close to its N-terminus, but mutagenesis studies are lacking. However, it is possible that its activity is modulated by ubiquitination [7].

Recent work has shown that the mammalian ortholog of Dga1, DGAT2, is able to convert ceramide to acylceramide, which is subsequently stored in lipid droplets [8]. Thus, the functionality of this enzyme may be diverse and include sophisticated regulation. Even less is known about the post-translational modification of Lro1. Isolation of these two factors with potential bound co-factors and analysis of biochemical modifications will be helpful to complete the signaling picture that has started with Pah1.

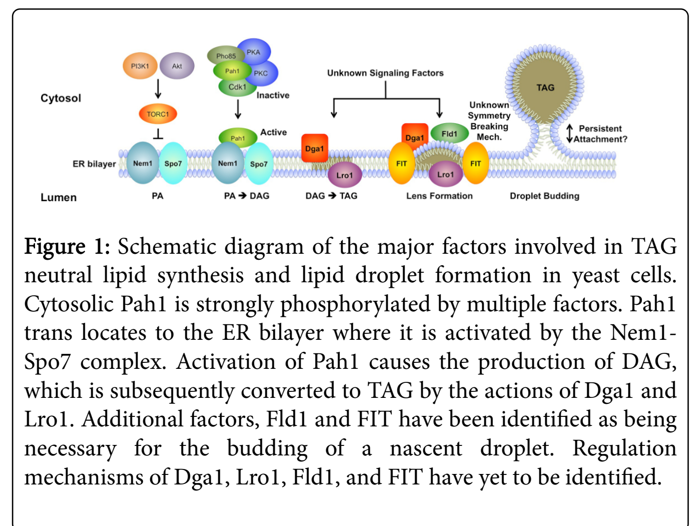

Figure 1: Schematic diagram of the major factors involved in TAG neutral lipid synthesis and lipid droplet formation in yeast cells. Cytosolic Pah1 is strongly phosphorylated by multiple factors. Pah1 trans locates to the ER bilayer where it is activated by the Nem1-Spo7 complex. Activation of Pah1 causes the production of DAG, which is subsequently converted to TAG by the actions of Dga1 and Lro1. Additional factors, Fld1 and FIT have been identified as being necessary for the budding of a nascent droplet. Regulation mechanisms of Dga1, Lro1, Fld1, and FIT have yet to be identified.

Much recent work has focused on seipin gene products. Seipin was implicated in lipid droplet formation when knockouts in budding yeast cells (fldΔ) showed unusual droplet morphologies [9]. The study of seipin homologues leads into a discussion of the elucidation of the budding mechanism of lipid droplet and away from the synthesis of particular neutral lipids. Yeast cells lacking Fld1 show delayed droplet biogenesis events with increased neutral lipid build up in the ER bilayer [10]. This points to, Fld1/seipin playing a role in the so-called ER "escape hatch" progression. The localization of Pah1 (and potentially Dga1 and Lro1) to seipin is an intriguing possibility for droplet formation into the cytosol. An additional crucial question that remains to be answered is how the neutral lipids produced by Pah1, Dga1, and Lro1 are guided into a forming droplet. That is, how are PA, DAG, and TAG partitioned in certain regions of the ER? Clues may be had in the function of fat storage inducing (FIT) proteins, which bind TAG and are localized to the ER. It has been recently shown that FIT proteins are required for proper budding of droplets from the ER in yeast cells [11]. It is possible that FIT may sequester TAG at precise locations in the ER membrane and thus be responsible for localized formation. Upstream factors driving FIT action are currently unknown.

The final step of lipid droplet biogenesis is the separation of the nascent droplet from the ER. Or not. It is still unclear if lipid droplets are indeed distinct organelles. High-resolution images of droplets have shown that they remain in close proximity to the ER and in many cases it is difficult to ascertain if they have actually separated [12]. Recent

evidence has shown that mature droplets can communicate with ER luminal proteins [13]. Of course, this increases the chance that droplets are docked to the ER; however, this phenomenon might be organism specific. It is still possible that unknown factors perform a pinching mechanism at a droplet neck, initiating the formation of an isolated organelle that will certainly continue to be a focal point of cell metabolic studies.

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