151. The Path to Triacylglycerol (TAG) Obesity in the *sta6* Strain of *Chlamydomonas reinhardtii*

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Project Goals: When the *sta6* (starch-null) strain of the green microalga *Chlamydomonas reinhardtii* is nitrogen-starved in acetate and then "boosted" after 2 days with additional acetate, the cells become "obese" after 8 days, with TAG-filled lipid bodies filling their cytoplasm and chloroplasts (1). To assess the transcriptional correlates of this response, *sta6* and the starch-forming *cw15* strain were subjected to RNA-Seq analysis during the 2 days prior and 2 days post boost. During the 2 hr post boost, ~ 425 genes are up-regulated ≥2-fold and ~875 genes are down-regulated ≥2-fold in each strain. Our results indicate that the boost serves both to avert an autophagy program and to prolong the operation of key biochemical pathways that conserve nitrogen and shuttle carbon from acetate into storage lipid, the outcome being enhanced TAG accumulation, notably in *sta6*. Four genes -- encoding a diacylglycerol acyltransferase (*DGTT2*), a glycerol-3-P dehydrogenase (*GPD3*), and two candidate lipases (Cre03.g155250 and Cre17.g735600) - are selectively up- regulated in *sta6*, and are therefore candidates for future genetic engineering.

The Merchant/Pellegrini and Los Alamos laboratories recently generated and analyzed RNA-Seq transcriptomes of *cw15*, *sta6*, and several complemented *sta6* strains during two days of N-starvation (0→48h-N) (2). In collaboration with these groups, the Goodenough lab generated a second pair of transcriptomes using *cw15* and *sta6*, tracing 0→48h-N gene expression patterns under a different set of culture conditions and taking the time course out to 96h-N, with an intervening acetate boost. Analysis of these data was deeply informed by cross-comparisons with the Blaby et al. (2) data and the Boyle et al. data (3) on an N-starved wild-type strain.

By consolidating these data, it has been possible to identify "robust" biochemical pathways, like starch, fatty-acid, and TAG biosynthesis, wherein patterns of expression of the relevant genes are largely concordant regardless of genetic background or culture conditions, thereby calling attention to the few exceptional cases. Also identified are 21 "sensitive" genes, encoding products operating in several pathways, including the glyoxylate and Calvin Benson cycles, gluconeogenesis, and the pentose phosphate pathway, that are influenced by on-going carbon flux; their expression is coordinated but varies within strains and between conditions, suggesting that they play a role in monitoring and responding to N-depletion in particular biosynthetic/metabolic contexts. Thirteen of these "sensitive" genes are strongly responsive to the cell's acetate status.

The bulk rate of acetate depletion from the medium is not boost-enhanced, but evidence for a spike in acetate uptake is presented, and three candidate acetate permease-encoding genes in the *GPR1_FUN34_YaaH* superfamily are strongly boost-up-regulated.

A cohort of 64 autophagy-related genes is down-regulated by boost. We propose that this is linked to microscopic observations showing that non-boosted cells initiate an autophagocytic response at 48h-N, accompanied by diminished TAG accumulation, that is not initiated in boosted cells.

The four genes whose expression is specifically enhanced in *sta6* encode enzymes expected to play a role in lipid-body formation, where one of the candidate lipases -- Cre03.g155250 – is homologous to *PGD1*, recently shown to participate in TAG biosynthesis (4). Whether they specifically operate in the chloroplast to form chloroplast lipid bodies will be the subject of future experimentation. We further propose that the disruption of starch synthesis in *sta6* creates a glucose-6-P "backflow" that feeds into chloroplast lipid-body formation.

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