**Criteria for selecting candidates** (script: interesting\_genes.Rmd)

***IIS QTL interval genes:*** (from script: genes\_in\_QTL.Rmd)

* 215 DEGs identified under BCIs that are in both treatment effect DEGs and interaction effect DEGs (LRT, qval<0.05) (all\_diffExpr\_underQTL.csv).
* This set had 139 unique genes (unique\_geneList.csv).
* 12 of these are located within 100 Kb of eQTL peak markers: Ahcy, CG40486, Non1, prel, ced-6, CG33774, Wnt2, CG1882, Dgat2, mus205, CG12822, CG10357. Converted coordinates to v6

**Modules from WGCNA** (from script: wgcnaCoex.Rmd)

* WGNCA was done on batch-corrected (limma) and rlog transformed exprs data (rlogtrt\_batchCor.csv).
* Identified 22 modules including grey with 45 genes.

**Genes from GSEA analysis using GAGE** (script: gsea\_GO.Rmd)

* Set up KEEG and GO gene sets using org.Dm.eg.db database
* Used treatment DEGs (2475 genes, DEGs\_lrt.treatment\_0.05.csv)
* Converted IDs to entrez and run GAGE on each diet in each tissue
* Identified 4 pathways qval<0.05:

pathway                         stat.mean            q.val          contrast

dme01100 Metabolic pathways                  5.378229     2.939188e-06     FC\_DRHS\_B

dme01200 Carbon metabolism                  3.310645     2.263392e-02     FC\_DRHS\_B

dme00190 Oxidative phosphorylation              2.946144     4.523595e-02     FC\_DRHS\_B

dme04141 Protein processing in endoplasmic reticulum      2.828623     4.523595e-02     FC\_DRHS\_B

* Obtained genes for these pathways from the db
* Performed GO on same 2475 DEGs
* Identified 43 terms at qva<0.05, and 4 terms at 0.01:
  + "GO:0044281 small molecule metabolic process"
  + "GO:0007154 cell communication"
  + "GO:0023052 signaling"
  + GO:0007165 signal transduction"
* Fetched genes for these 4 GO terms

Alternative: select a few based on biology or use all 43 GO terms

**Genes from known pathways - FB precomputed file** fbgn\_annotation\_ID\_fb\_2019\_02.tsv

* Fetched 110 genes in the FOXO signaling pathway, and 5 sirtuin genes known in *D. melanogaster*.
  + 109 of these found in modules
  + Identified 16 genes from this list that are DE: (Sirt2, Diap1, beta'COP, CG5059, Gycbeta1008, DOR, TBPH, hid,p38c, Ilp5, alphaCOP, AMPKalpha, Rheb, Cdk2, arm, Atg1) Which modules?
  + Zero of QTL genes
* Fetched 52 Toll pathway genes.
  + 49 genes recovered in modules. (List not used further (toll1)).
  + 12 DEGs are Toll pathway genes (toll3: PGRP-SC1a, spz, Gprk2, tub, dgrn, grass, wek, Spn88Ea, senju, spirit, Hrs, Herc4). Which modules?
* Fetched 55 IIS pathway genes.
  + 55 recovered in modules
  + Zero among QTL genes
  + 6 DEGs are IIS pathway genes (iis3: Ilp5, tgo, Rheb, slmb, Myc, lin-28). Which modules?

**16 FOXO, 12 Toll, 6 IIS DEGs, i.e. 32 unique genes considered candidates**

Gene summaries – FlyBase

lin-28

The gene lin-28 is referred to in FlyBase by the symbol Dmel\lin-28 (CG17334, FBgn0035626). It is a SO0000010:protein\_coding\_gene from Drosophila melanogaster. It has one annotated transcript and one polypeptide. Gene sequence location is 3L:5654681..5657384. It has the cytological map location 64E1. Protein features are: Cold shock domain; Cold-shock protein, DNA-binding; Nucleic acid-binding, OB-fold; Zinc finger, CCHC-type; Zinc finger, CCHC-type superfamily. Its molecular function is described by: zinc ion binding; mRNA binding. It is involved in the biological process described with: positive regulation of insulin receptor signaling pathway; oogenesis; symmetric stem cell division; negative regulation of pre-miRNA processing; positive regulation of stem cell proliferation. 22 alleles are reported. The phenotypes of these alleles manifest in: abdominal temporary eclosion muscle DA; female germline stem cell; male abdominal 5 muscle; nurse cell; egg chamber; eye; intestinal stem cell of posterior adult midgut epithelium; intestinal stem cell; abdominal dorsal muscle. The phenotypic classes of alleles include: phenotype; increased mortality; visible; increased mortality during development. Summary of modENCODE Temporal Expression Profile: Temporal profile ranges from a peak of high expression to a trough of extremely low expression. Peak expression observed within 06-18 hour embryonic stages.

Stratoulias et al., 2014, PLoS ONE 9(6): e101141

Lin-28 Regulates Oogenesis and Muscle Formation in Drosophila melanogaster.

Stem cells switch between asymmetric and symmetric division to expand in number as tissues grow during development and in response to environmental changes. The stem cell intrinsic proteins controlling this switch are largely unknown, but one candidate is the Lin-28 pluripotency factor. A conserved RNA-binding protein that is downregulated in most animals as they develop from embryos to adults, Lin-28 persists in populations of adult stem cells. Its function in these cells has not been previously characterized. Here, we report that Lin-28 is highly enriched in adult intestinal stem cells in the Drosophila intestine. lin-28 null mutants are homozygous viable but display defects in this population of cells, which fail to undergo a characteristic food-triggered expansion in number and have reduced rates of symmetric division as well as reduced insulin signaling. Immunoprecipitation of Lin-28-bound mRNAs identified Insulin-like Receptor (InR), forced expression of which completely rescues lin-28-associated defects in intestinal stem cell number and division pattern. Furthermore, this stem cell activity of lin-28 is independent of one well-known lin-28 target, the microRNA let-7, which has limited expression in the intestinal epithelium. These results identify Lin-28 as a stem cell intrinsic factor that boosts insulin signaling in intestinal progenitor cells and promotes their symmetric division in response to nutrients, defining a mechanism through which Lin-28 controls the adult stem cell division patterns that underlie tissue homeostasis and regeneration.

Chen, C.H., Luhur, A., Sokol, N. (2015). Lin-28 promotes symmetric stem cell division and drives adaptive growth in the adult Drosophila intestine.  [Development 142(20): 3478--3487](http://dx.doi.org/10.1242/dev.127951)

Myc

Genetic studies in Drosophila melanogaster reveal an important role for Myc in controlling growth. Similar studies have also shown how components of the insulin and target of rapamycin (TOR) pathways are key regulators of growth. Despite a few suggestions that Myc transcriptional activity lies downstream of these pathways, a molecular mechanism linking these signaling pathways to Myc has not been clearly described. Using biochemical and genetic approaches we tried to identify novel mechanisms that control Myc activity upon activation of insulin and TOR signaling pathways.Our biochemical studies show that insulin induces Myc protein accumulation in Drosophila S2 cells, which correlates with a decrease in the activity of glycogen synthase kinase 3-beta (GSK3β ) a kinase that is responsible for Myc protein degradation. Induction of Myc by insulin is inhibited by the presence of the TOR inhibitor rapamycin, suggesting that insulin-induced Myc protein accumulation depends on the activation of TOR complex 1. Treatment with amino acids that directly activate the TOR pathway results in Myc protein accumulation, which also depends on the ability of S6K kinase to inhibit GSK3β activity. Myc upregulation by insulin and TOR pathways is a mechanism conserved in cells from the wing imaginal disc, where expression of Dp110 and Rheb also induces Myc protein accumulation, while inhibition of insulin and TOR pathways result in the opposite effect. Our functional analysis, aimed at quantifying the relative contribution of Myc to ommatidial growth downstream of insulin and TOR pathways, revealed that Myc activity is necessary to sustain the proliferation of cells from the ommatidia upon Dp110 expression, while its contribution downstream of TOR is significant to control the size of the ommatidia. Our study presents novel evidence that Myc activity acts downstream of insulin and TOR pathways to control growth in Drosophila. At the biochemical level we found that both these pathways converge at GSK3β to control Myc protein stability, while our genetic analysis shows that insulin and TOR pathways have different requirements for Myc activity during development of the eye, suggesting that Myc might be differentially induced by these pathways during growth or proliferation of cells that make up the ommatidia.

Parisi, F., Riccardo, S., Daniel, M., Saqcena, M., Kundu, N., Pession, A., Grifoni, D., Stocker, H., Tabak, E., Bellosta, P. (2011). Drosophila insulin and target of rapamycin (TOR) pathways regulate GSK3 beta activity to control Myc stability and determine Myc expression in vivo.  [BMC Biol. 9(): 65.](http://dx.doi.org/10.1186/1741-7007-9-65)

Drosophila dMyc (dMyc) is known for its role in cell-autonomous regulation of growth. Here we address its role in the fat body (FB), a metabolic tissue that functions as a sensor of circulating nutrients to control the release of Drosophila Insulin-like peptides (Dilps) from the brain influencing growth and development. Our results show that expression of dMyc in the FB affects development and animal size. Expression of dMyc, but not of CycD/cdk4 or Rheb, in the FB diminishes the ability to retain Drosophila Insulin-like peptide-2 (DILP2) in the brain during starvation, suggesting that expression of dMyc mimics the signal that remotely controls the release of Dilps into the hemolymph. dMyc also affects glucose metabolism and increases the transcription of Glucose-transporter-1 mRNA, and of Hexokinase and Pyruvate-Kinase mRNAs, key regulators of glycolysis. These animals are able to counteract the increased levels of circulating trehalose induced by a high sugar diet leading to the conclusion that dMyc activity in the FB promotes glucose disposal. dMyc expression induces cell autonomous accumulation of triglycerides, which correlates with increased levels of Fatty Acid Synthase and Acetyl CoA Carboxylase mRNAs, enzymes responsible for lipid synthesis. We also found the expression of Stearoyl-CoA desaturase, Desat1 mRNA significantly higher in FB overexpressing dMyc. Desat1 is an enzyme that is necessary for monosaturation and production of fatty acids, and its reduction affects dMyc's ability to induce fat storage and resistance to animal survival. In conclusion, here we present novel evidences for dMyc function in the Drosophila FB in controlling systemic growth. We discovered that dMyc expression triggers cell autonomous mechanisms that control glucose and lipid metabolism to favor the storage of nutrients (lipids and sugars). In addition, the regulation of Desat1 controls the synthesis of triglycerides in FB and this may affect the humoral signal that controls DILP2 release in the brain.

Parisi, F., Riccardo, S., Zola, S., Lora, C., Grifoni, D., Brown, L.M., Bellosta, P. (2013). dMyc expression in the fat body affects DILP2 release and increases the expression of the fat desaturase Desat1 resulting in organismal growth.  [Dev. Biol. 379(1): 64--75.](http://dx.doi.org/10.1016/j.ydbio.2013.04.008)

Tgo

The gene tango is referred to in FlyBase by the symbol Dmel\tgo (CG11987, FBgn0264075). It is a SO0000010:protein\_coding\_gene from Drosophila melanogaster. It has 2 annotated transcripts and 2 polypeptides (1 unique). Gene sequence location is 3R:9016773..9020022. It has the cytological map location 85C2. Protein features are: Helix-loop-helix DNA-binding domain superfamily; Myc-type, basic helix-loop-helix (bHLH) domain; Nuclear translocator; PAS domain; PAS domain superfamily; PAS fold. Its molecular function is described by: myosin binding; sequence-specific DNA binding; protein heterodimerization activity; DNA-binding transcription activator activity, RNA polymerase II-specific; DNA-binding transcription factor activity, RNA polymerase II-specific; DNA-binding transcription factor activity. It is involved in the biological process described with 14 unique terms, many of which group under: central nervous system development; limb development; positive regulation of cellular metabolic process; regulation of anatomical structure morphogenesis; response to decreased oxygen levels; transmembrane receptor protein tyrosine kinase signaling pathway; post-embryonic animal organ morphogenesis; photoreceptor cell differentiation; multicellular organismal reproductive process; negative regulation of cellular metabolic process; brain development. 32 alleles are reported. The phenotypes of these alleles manifest in: somatic precursor cell; plasma membrane bounded cell projection; larval outer optic anlage; female organism; adult gut; cell; perineurium; chaeta; maxillary palpus; cell part; tarsal segment 3; epithelium; adult gnathal segment. The phenotypic classes of alleles include: increased mortality; neuroanatomy defective; phenotype; lethal; cell migration defective; visible; short lived.

Doronkin, S., Djagaeva, I., Nagle, M.E., Reiter, L.T., Seagroves, T.N. (2010). Dose-dependent modulation of HIF-1alpha/sima controls the rate of cell migration and invasion in Drosophila ovary border cells.  [Oncogene 29(8): 1123--1134.](http://dx.doi.org/10.1038/onc.2009.407)

Dekanty, A., Lavista-Llanos, S., Irisarri, M., Oldham, S., Wappner, P. (2005). The insulin-PI3K/TOR pathway induces a HIF-dependent transcriptional response in Drosophila by promoting nuclear localization of HIF-alpha/Sima.  [J. Cell Sci. 118(23): 5431--5441.](http://dx.doi.org/10.1242/jcs.02648)