KNOCK OUT OF LIPOPROTEIN LIPASE 1 GENE REDUCES THE AMOUNTS OF OOCYTE LIPID DROPLETS IN MEDAKA *Oryzias latipes*

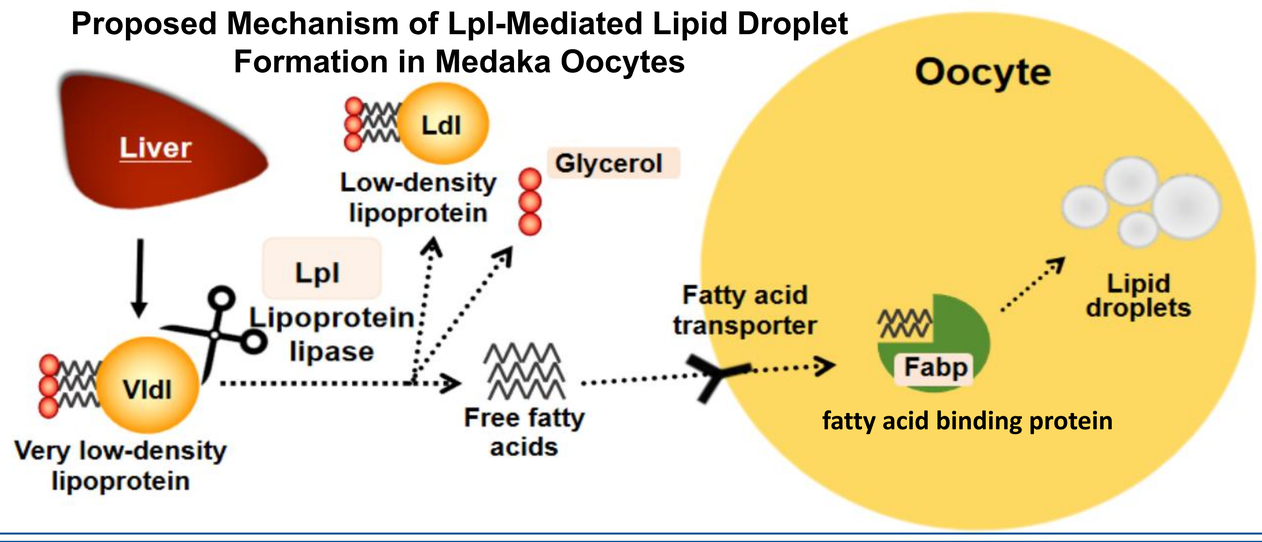
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Introduction

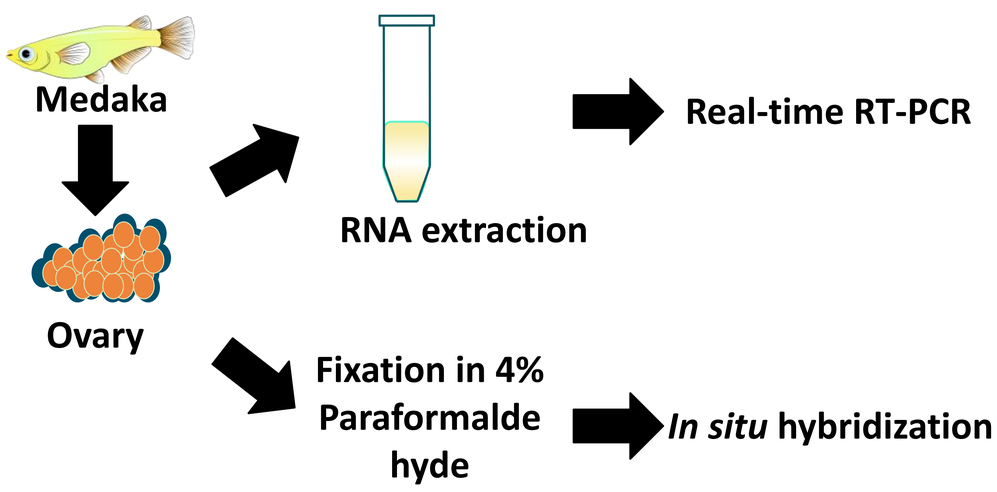
Lipoprotein lipase (Lpl) is a key enzyme that hydrolyzes triglycerides in circulating lipoproteins and releases free fatty acids for cellular uptake. In teleost fishes, very low density lipoprotein (Vldl) is considered as an important source of neutral lipids for oocytes, and Lpl has been proposed to mediate lipid droplet formation by supplying fatty acids to developing oocytes. Studies in several teleost species have shown that both ovarian Lpl activity and *lpl* expression increase in association with the progress of oocyte lipid droplet formation and/or oocyte growth. However, there is no direct evidence for the involvement of Lpl in oocyte lipid droplet formation to date. To address this issue, we investigated the role of *lpl1* on oocyte lipid droplet formation in medaka (*Oryzias latipes*) using a CRISPR/Cas9-mediated knockout approach.

Proposed Mechanism of Lpl-Mediated Lipid Droplet Formation in Medaka Oocytes

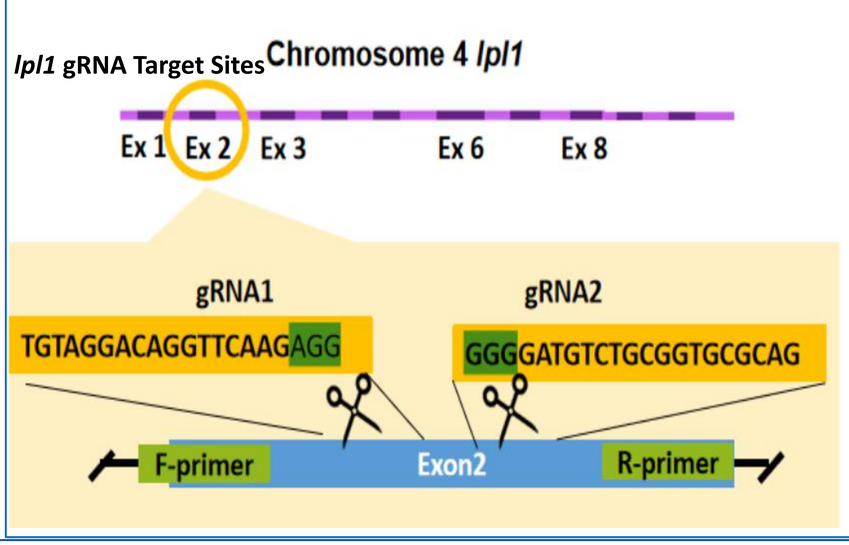


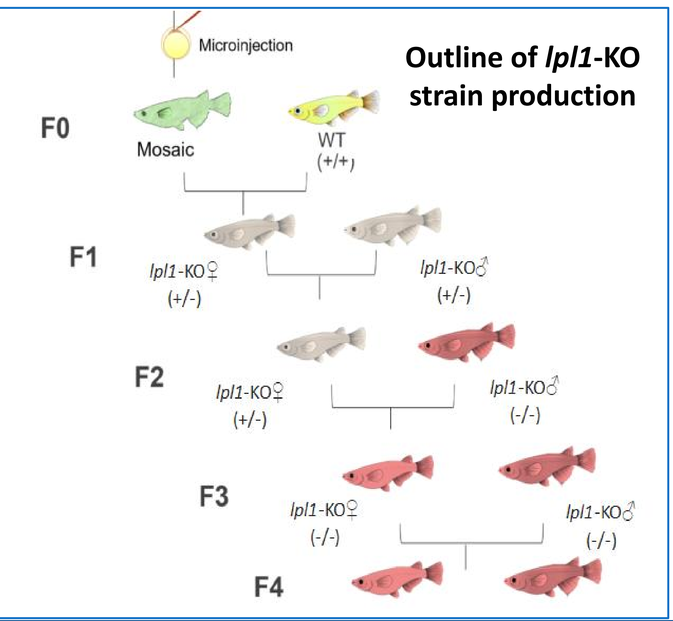
Materials and methods

Expression analysis of *lpl1* and *lpl2*



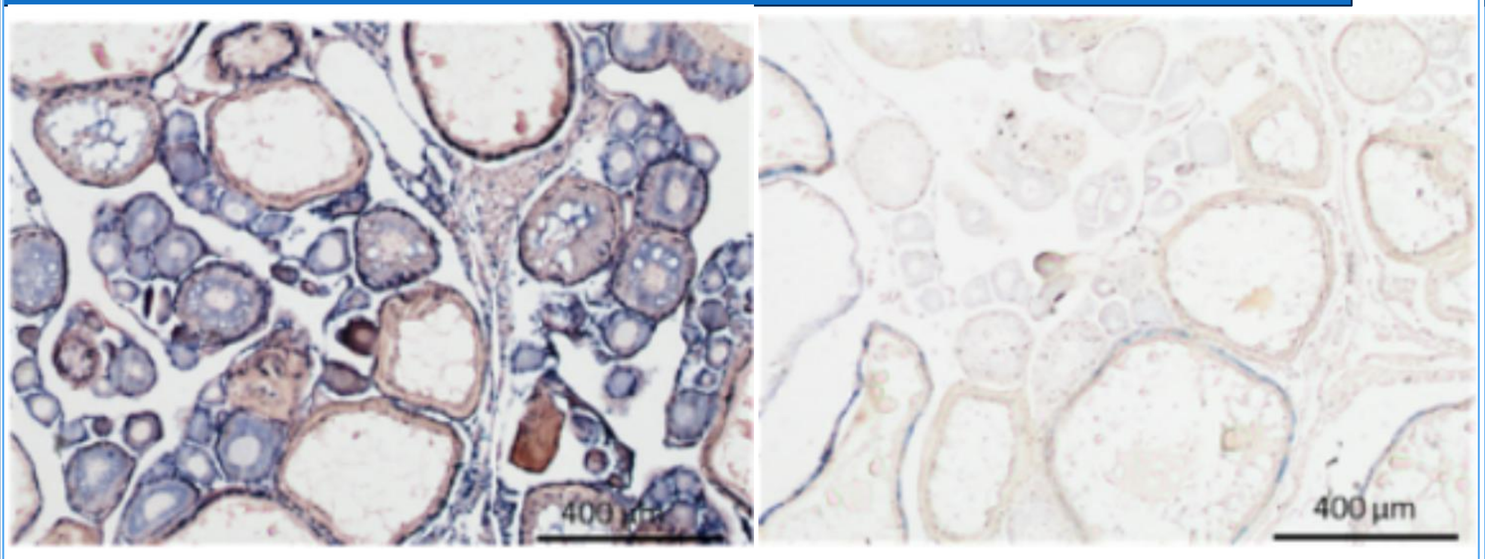
lpl1-KO system strategy





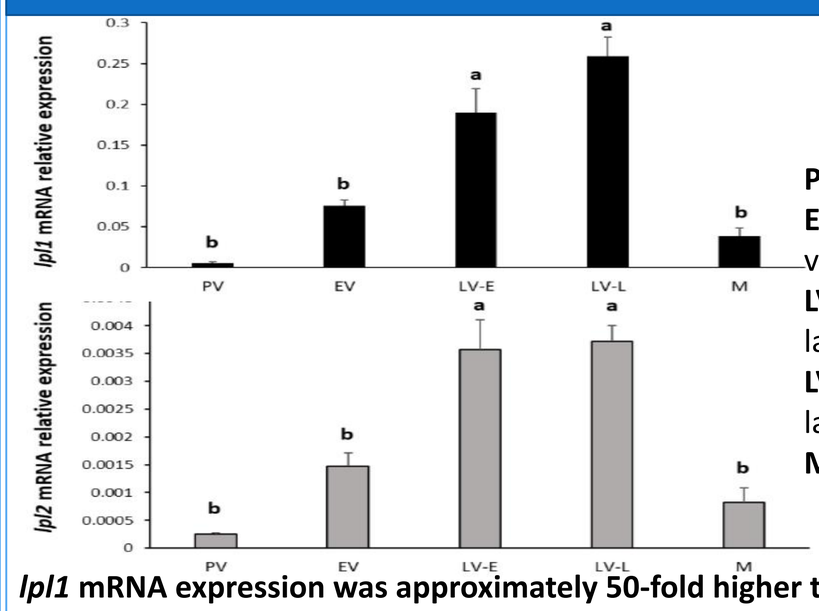
Result

Localization of *lpl1* (left) and *lpl2* (right) mRNA in the ovary during oocyte development



Strong signal of *lpl1* were observed in the ovarian follicle cells, whereas signal of *lpl2* were weak.

lpl1 and *lpl2* expression during oocyte development



PV: previtellogenesis
EV: early vitellogenesis
LV-E: early phase of late vitellogenesis
LV-L: late phase of late vitellogenesis
M: maturation

***lpl1* mRNA expression was approximately 50-fold higher than *lpl2* across all developmental stages.**

Mutation and amino acid analysis

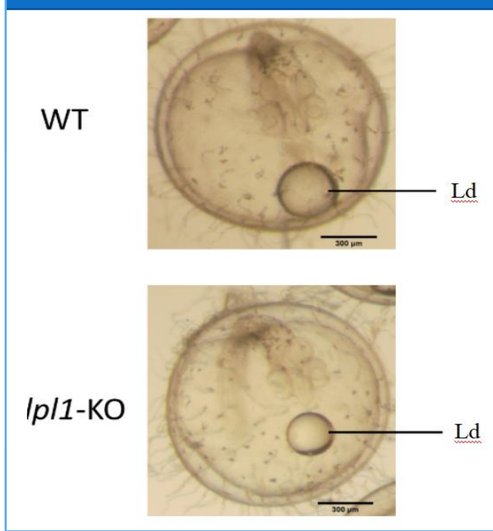
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WT      GATTACGCCGACAT-------CCTGTCCAAGTTCCTCCCTGCGCACCGCAGACATCCCCGAT  114
lpl1-ko GATTACGCCGTCTT-GGACGCCCTGTCCAAGTTCCTCCCTGCGCACCGCAGACATCCCCGAT  119
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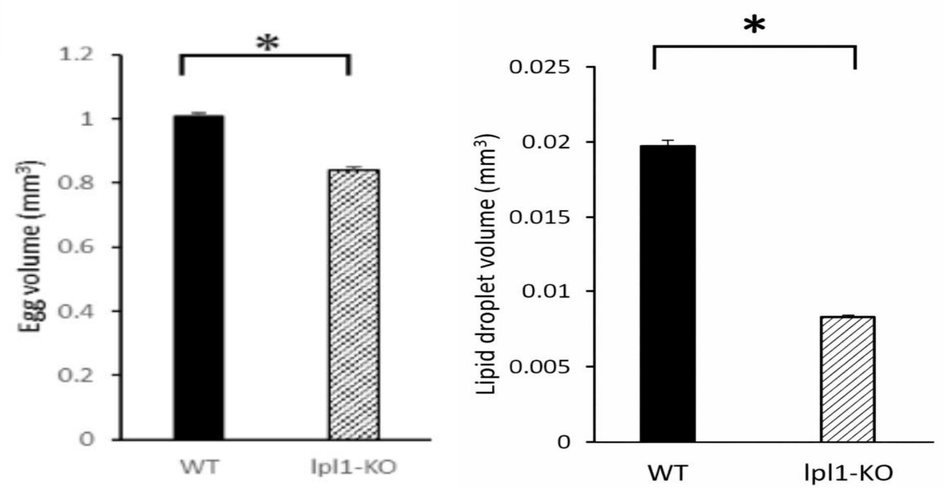
WT
MGKELLCFFTLWITLGNISATFSSDLESNTTSLFVNSTTTAAPLPTTTEWMTDYA
DILSKFSLRTADIPDEDMCYIQAGHPETIKDCQFNAEAQTFIVIHGWTVTGMFE
SWVPKLVSALYERVPNANVIVVDWLTRASQHYPTSASYTKLVGRDVAKFVTWL
QNELQLPWDRIHVLGYSLGAHVAGVAGHLTDNKISRITGLDPAGPSFEHADDQ
STLSRDDGQFVDVLHTNTRGSPDRSIGIQRPVGHIDIYPNGGTFQPGCDIQNTL
LGIASAGIKGLQNMDQLVKCSHERSIHLFIDSLVNTQHQSMAYRCNSKEAFNKG
VCLSCRKNRCNKLGYNINKVRTARSTKMYLKTRGMMPYKVFHYQVKAHFFSKS
EQSFTEQPMKISLFGTHGETEDIAFVLPELSCNNTVSFLITTDVDIGELMIVKLR
WEKDAILSWSDWWGSSKFHIRKLRIKSGETQSKVIFNAKEGEFADLVRGGEDA
VFVKSKEDNISRKERRMHRLKTEGSLFGKSDA*

lpl1-KO
MGKELLCFFTLWITLGNISATFSSDLESNTTSLFVNSTTTAAPLPTTTEWMTDYA
VLDACPSSPCAPQTSPMRTCATSKPASQKPSRTVSSTQKLRPSSSYTAGR*

This frameshift mutation is predicted to disrupt Lpl1 protein integrity and abolish its normal function.

Egg and lipid droplet phenotype





***lpl1*-KO eggs exhibited a 16.5% reduction in egg volume and a 56.7% reduction in lipid droplet volume compared with WT**

Conclusion

lpl1 was identified as the predominant *lpl* gene in the medaka ovary.
Functional disruption of *lpl1* reduced oocyte lipid droplet accumulation and egg size, demonstrating an essential role for Lpl1 in oocyte lipid droplet formation.