Uncovering functional roles in development for differentially expressed ribosomal protein eRpL22-like using a conditional gene knockout strategy

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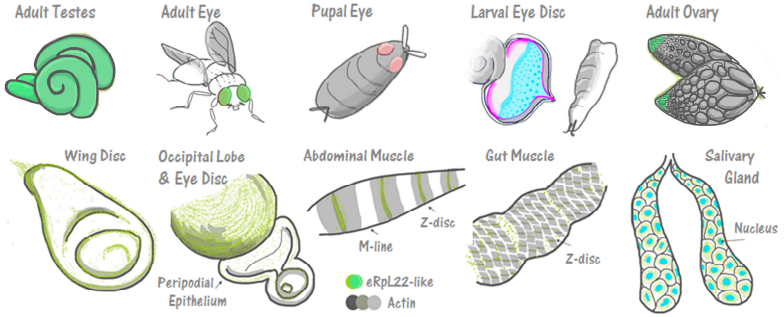
Ribosomes are molecular machines that translate mRNA into proteins and have a large (L) and small (S) subunit. Ribosomes themselves are made of ribosomal-RNA and a combination of several ribosomal proteins (RPs). The Ware Lab uses *Drosophila* to study two structurally divergent and developmentally essential paralogues: **eRpL22** (ubiquitously expressed) and **eRpL22-like** (tissue-specific expression across development) (1-6).



To elucidate roles for eRpL22-like, we characterized morphological defects in an **eRpL22-like heat-shock conditional knockout homozygote (CKO)**. Summarized **ovary-specific phenotypes** include: 1 → 7

Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs (also expressed within the ovary) involved in cell polarity, suggesting that paralogue-specific "specialized ribosomes" translate specific mRNAs. Therefore, we expect eRpL22-like CKO mutants to have reduced expression of the following genes with subsequent phenotypes:

(I) **Dally-like protein** (Dlp): knockdown (KD) causes over-proliferation/differentiation of follicle cells (FCs). Phenotypes include excess ovarian nurse cells (8). A Dlp homolog is associated with Omodysplasia-1 (9).

(II) **Belle**: KD delays Notch activation during oogenesis, preventing FC entry into endocycle. Phenotypes include immature FCs and stage-6 egg chamber (EC) arrest (10). A Belle homolog is associated with x-linked mental retardation (11).

(III) **Dacapo**: KD reduces Cyclin-E inhibition, which promotes FC mitosis. Phenotypes include immature cells with small nuclei in the ovary (12). Cyclin-E is also enriched on eRpL22-like polysomes - KD promotes precocious endocycle transition and results in variable defects in cell size and number within the ovary (13).

(IV) **Adaptor Protein complex 2, μ subunit**: KD results in misshapen FC multi-layering/ingression at EC A-P axes (14).

(V) **Par-1**: KD results in defective apical-basal membrane polarity and impaired border cell migration in the ovary (15, 16). A Par-1 homolog is associated with VIPB, a congenital vision disease (17).

(VI) **Kekkon-1**: a negative regulator of epidermal growth factor receptor (EGFR), enriched on eRpL22 polysomes. We have previously shown that eRpL22-like KD results in a compensatory increase in eRpL22 expression, suggesting that Kek-1 will be overexpressed (OE) in eRpL22-like CKO mutants. Kek-1 OE reduces EGFR activity, resulting in elongation and ventralization phenotypes in the ovary (18). Many human diseases result from EGFR signaling disruption (ex. RASopathies).

The disease-states presently associated with eRpL22 are limited to cancer susceptibility and T-cell abnormalities; no conditions are as yet associated with eRpL22-like (19). Immunohistochemical characterization of our eRpL22-like CKO mutant allows us to tease-apart individual ovarian phenotypes, elucidate putative extra-ribosomal functions of eRpL22 paralogues, and potentially reveal a novel model useful for studying specific human conditions arising from a spectrum of epithelial polarity defects. These preliminary data broaden the context for investigation of the role of eRpL22-like as an essential player across multiple developmental processes.