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ANALYSIS OF EYES SHUT (EYS) DURING INTESTINAL REGENERATION OF *DROSOPHILA MELANOGASTER*

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Introduction:

The intestine is a self-renewing organ under constant stress. It is the first line of defense against toxins and bacteria and enables digestion and nutrient absorption. Its homeostasis (controlled regulation) plays a critical role in maintenance of organ function and prevention of tumor generation.

Eyes-shut (eys) is an important extracellular protein conserved from Drosophila to humans that plays a role in the eye and the nervous system formation and function (Cook et al., 2008) (Husain et al., 2006). While the role of eys is well known in these organs, it has never been studied in the intestine. Normally, eys is not expressed in the intestine. However, data from Dr. Bruce Edgar's lab has shown that after intestinal stress, eys' RNA is upregulated, suggesting a possible function in stress-induced intestinal regeneration. Hence, we investigated its role in the intestine together with rumi and promin (prom), known partner of eys in the eye. Rumi O-glucosylates eys to regulate its secretion and stability, while prom is a transmembrane receptor that binds to eys to promote cellular integrity (Gurudev et al., 2014) (Cook et al., 2008).

Methods and Materials:

In order to discover the possible function of eys and its partners in the Drosophila gut, I have performed several experiments together with Dr. Pénalva, the post-doc working on this project and directly supervising me at the bench. First, I confirmed upregulation of eys in the Drosophila gut post-stress treatment both by RT-qPCR and immunofluorescence. Second, I analyzed rumi expression by RT-qPCR. Third, I performed knockdown (KD) experiments of eys and rumi in order to determine whether they are required for ISC regeneration. Finally, I tested whether eys is sufficient for cellular regeneration.

Results:

When compared to a negative control of sucrose, in response to intestinal challenge, eys is upregulated from both oxidative stress, detergent feeding, and bacterial infection. Each stress saw a logarithmic fold induction greater than 1, with P.e. infection resulting in a statistically significant 17.34 fold increase in mRNA expression. Since eys is stress-responsive, it could have a role in intestinal regeneration.

While the expression of eys RNA in unchallenged conditions is extremely low and increases after stress, rumi shows stronger basal levels of expression but is not upregulated after stress. It seems as if stress has no significant effect on RNA levels of rumi as qPCR results show no statistically significant changes. Thus, rumi is not regulated at the RNA level in the intestine but could still regulate eys when eys is expressed.

In KD experiments, the amount of ISC proliferation was scored using phospho-histone 3, a marker of mitosis. When compared to the control of the same genetic background, ISC proliferation is decreased in response to P.e., Ecc15, SDS, and Bleo in the midgut expressing eys RNAi specifically in the ECs. However, it was not decreased in response to H2O2. Since ISC response is visibly decreased in 4/5 stresses when eys expression is reduced, it can be determined that proper eys function is required for intestinal regeneration.

I also observed that the knockdown of rumi also decreased the stem cell proliferation level in response to all stresses tested. This shows that rumi is also required for intestinal regeneration.

When compared to a control line, overexpression does not affect the number of pH3+ divisions after 2, 5, or 7 days. This suggests that increasing eys level in the ECs is not sufficient to trigger ISC proliferation. It is possible that eys activity is not sufficient or eys is not stable and/or secreted in absence of stress.

Conclusion:
We have shown that eys and rumi are required for proper intestinal homeostasis. However, eys is not sufficient to produce an intestinal response when simply overexpressed. Further studies are needed to determine if rumi is sufficient to induce intestinal stress-response when overexpressed and if prom is required/sufficient for intestinal regeneration. The investigation into eys and its partners is crucial to understanding cellular regeneration of the intestine and could help devise new therapeutic strategies for gastrointestinal diseases or cancers.

References:
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