Protein-Protein interactions in Drosophila germ cell development

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Introduction
During germ cell formation in Drosophila embryos, cells migrate from their site of origin to the site of organ formation. In particular, germ cells need to migrate long distances from their site of origin to the site where ovaries and testicles develop. It was found that this cell migration is guided by a chemical signaling pathway. Cells which diverge from the migration route are destroyed by apoptosis. Dr. Coffman’s group at Iowa State University has identified mutations that impair the destruction of cells that have strayed from their migration path. One of these mutations affects the function of a G-protein coupled receptor, Tre1. In this study we look at physical interactions of the Tre1 protein with ten candidate proteins.

Methods
We used the yeast split-ubiquitin system to detect protein interactions (Fig. 2). In this system, two hybrid proteins (termed bait and prey) reconstitute a functional ubiquitin molecule – but only if they interact. Reconstitution of ubiquitin leads to cleavage of the bait by a ubiquitin-specific protease, with subsequent release of an activating transcription factor into the nucleus. As a result, the transcription of two reporter genes begins and the indicator yeast can grow on media lacking adenine and histidine.

Results
A typical result is shown in Figure 4. Growth of transformants on medium lacking leucine and tryptophan indicates that the cells contain both bait and prey plasmids. Growth on medium lacking leucine, tryptophan and histidine indicates activation of the HIS3 reporter gene. Growth on medium lacking leucine, tryptophan, histidine and adenine indicates activation of both the HIS3 and Ade2 reporter genes and is the most stringent measure of interaction.

Discussion
We have identified interactions between the G-protein coupled receptor Tre1 and two, possibly three, candidate proteins. Candidates 3 and 10 are members of a protein phosphatase family that have been previously implicated in germ cell migration, which makes our result particularly valuable. Adding to the picture is the fact that the phenotypes of mutations in genes #3 and 10 closely resemble treI mutant phenotypes. All of the above results indicate that these proteins function in the same pathway.