Mechanisms of cell-cell membrane fusion during yeast mating

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Cell membrane fusion is the first physical step initiating the conception of every human life. Astonishingly, we lack fundamental knowledge of the proteins which mediate gamete cells to fuse during fertilization in Fungi and vertebrates[1]. This ability for the membranes of two cells to fuse is central for the sexual life cycle. In our research group, we aim to identify and characterize the proteins that mediate and regulate cell fusion in the mating of baker's yeast, a simple eukaryotic model system for which the cell fusion machinery remains unidentified. We have tackled this problem starting from two different angles: 1) a proteomics-centered approach where we have identified new pheromone-regulated proteins using SILAC-based mass spectrometry, and 2) a functional approach making use of the yeast knockout collection to screen for mutants defective in cell fusion.

With the first approach, we have uncovered a protein called Mpo1p which has been previously reported to be involved in the phytosphingosine metabolic pathway leading to the production of odd-numbered fatty acids[2]. Knockouts of Mpo1p leads to a ~40% cell fusion defect, implicating that lipid organization and/or homeostasis may be important in the ability for plasma membranes to fuse. We have also identified a protein called Pun1p which is highly-regulated in response to mating pheromone and is recruited to the cell junction between two mating cells. Although no defect is detected upon deletion, Pun1p may have at least 4 additional homologs, and our preliminary analysis suggests that members of this putative family of proteins may be involved in the expansion of the fusion pore.

With the second approach, we have utilized of a novel flow-cytometry based fusion assay which makes use of the complementation of split-GFP fragments to quantitatively measure cell fusion[3]. Using synthetic gene array (SGA) technology to incorporate the GFP fragments into the yeast knockout library, we have identified >100 genes whose deletion results in a defect late in the mating pathway during cell fusion. Pending microscopic confirmation of the defects which is currently underway, we have preliminarily identified genes involved in ergosterol, chitin, mannoprotein, cell wall and sphingolipid biosynthesis/homeostasis, as well as genes involved in pheromone processing/signaling, trafficking, vacuolar acidification, and genes with unclear function (11.3% of total hits).

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