Exciting search for *Chlamydomonas* metacaspase interactors

The master project in the The Plant Catabolism Laboratory (https://www.slu.se/en/departments/molecular-sciences/research-groups/bozhkov).

Main subject: Biology Contact: yong.zou@slu.se

Caspases are **c**ysteine-dependent **asp**artate-directed prote**ases** found almost exclusively in animals, with a crucial role in programmed cell death (PCD) and inflammation among other fundamental processes. There are no direct sequence homologs of caspases in the genomes of organisms from other kingdoms of life than animals. However, they have distantly related class of proteases named metacaspases and sharing caspase fold and conserved catalytic dyad of Cysteine and Histidine (Uren *et al*., 2000). Notably, there are key biochemical differences between caspases and metacaspases. While caspases are active as Ca^{2+} independent dimers, active metacaspases are monomers and their activation usually requires millimolar concentrations of Ca^{2+}. Furthermore, caspases cleave their substrates after Aspartate, whereas metacaspases after Arginine or Lysine residues. Great efforts have been made in the last two decades to understand caspase regulation and function, but metacaspase research remains in its infancy. In most higher plants including *Arabidopsis* there are several members of the two major structural types (I and II) metacaspases, hampering understanding of non-redundant functions of individual family members. In contrast, haploid genome of green algae *Chlamydomonas reinhardtii* encodes only one member of either type of metacaspases, providing powerful paradigm for the metacaspase structure-function research.

Yeast two hybrid (Y2H) is a useful molecular tool to detect protein-protein interactions (PPI). The premise behind the test is the reconstitution of a functional transcription factor (TF) when two proteins or polypeptides can interact in genetically modified yeast strains. In Y2H, the TF is split into two separate parts, the DNA-binding domain (BD) and activating domain (AD), which are responsible for binding the upstream activating sequence (UAS) and initiating transcription of downstream reporter genes, respectively. The proteins of interest are fused to BD (referred as “bait”) and AD (referred as “prey”) separately. When interaction occurs, BD and AD are recruited in close proximity and a functional TF is reconstituted, promoting the expression of the reporter gene and then resulting in a specific phenotype, such as growth on a selective medium or color changes of the yeast colonies.

In this project, we plan to construct a Y2H library with catalytic active or catalytically dead mutant metacaspases as baits, and *Chlamydomonas* transcriptomes in different growth conditions as prey candidates. We aim to find proteins interacting with metacaspases, including their substrates. The preselected interaction partners will be re-validated using Y2H or other PPI methods (e.g. co-immunoprecipitation).

We are looking for a highly motivated student with a burning interest in laboratory work and passion for molecular biology.

You will acquire skills in:

1. Molecular techniques including protein extraction, Western blot, DNA/plasmid extraction, agarose gel electrophoresis, RNA extraction, qPCR
2. Chlamydomonas culture
3. Yeast two hybrid test and library construction