

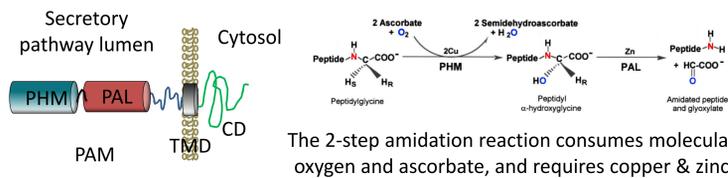
Chlamydomonas Secretes Amidated Peptides During Mating

Raj Luxmi¹, Dhivya Kumar², Crysten E. Blaby-Haas³, Richard E. Mains¹, Stephen M. King² and Betty A. Eipper^{1,2}

Departments of ¹Neuroscience and ²Molecular Biology and Biophysics, University of Connecticut Health Center, Farmington, CT, USA and ²Brookhaven National Laboratory, Upton, NY, USA.

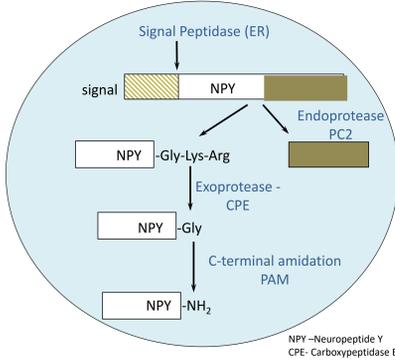
Introduction

Chlamydomonas Peptidylglycine α -Amidating Monooxygenase (PAM) shares many features with mammalian PAM

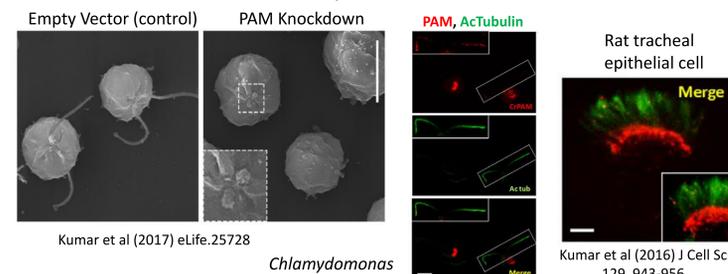


Peptide Processing: ER → Golgi → Secretory Granules

In neurons and endocrine cells, peptide precursors are cleaved by subtilisin-like endoproteases as the newly synthesized proteins exit the Golgi complex. Luminal pH drops as secretory granules mature, activating many of the peptide processing enzymes. Mature peptides can be stored in secretory granules for many days, and released in response to specific stimuli.

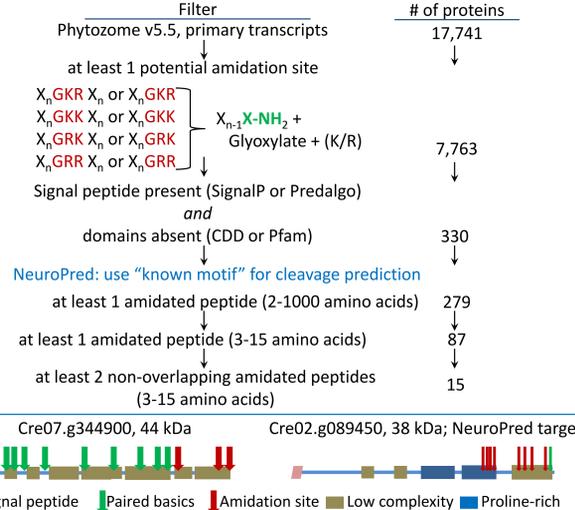


Reducing CrPAM expression produced cells unable to assemble cilia that extended beyond the transition zone



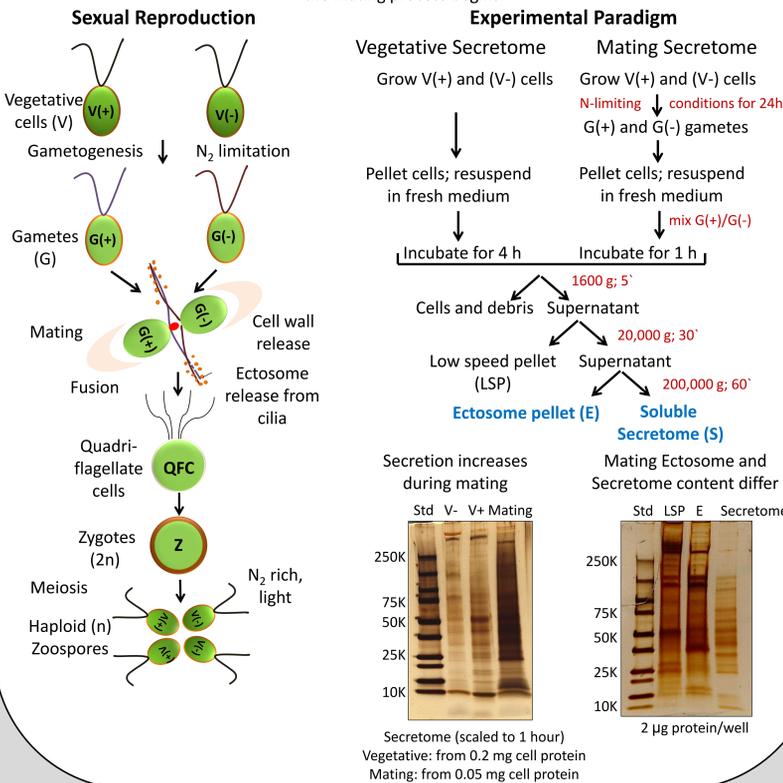
CrPAM, like rodent PAM, localizes to the Golgi. The fact that CrPAM also localizes to the ciliary membrane led to identification of PAM in mammalian motile and primary cilia.

Bioinformatic identification of *Chlamydomonas* proteins resembling classical prepropeptides

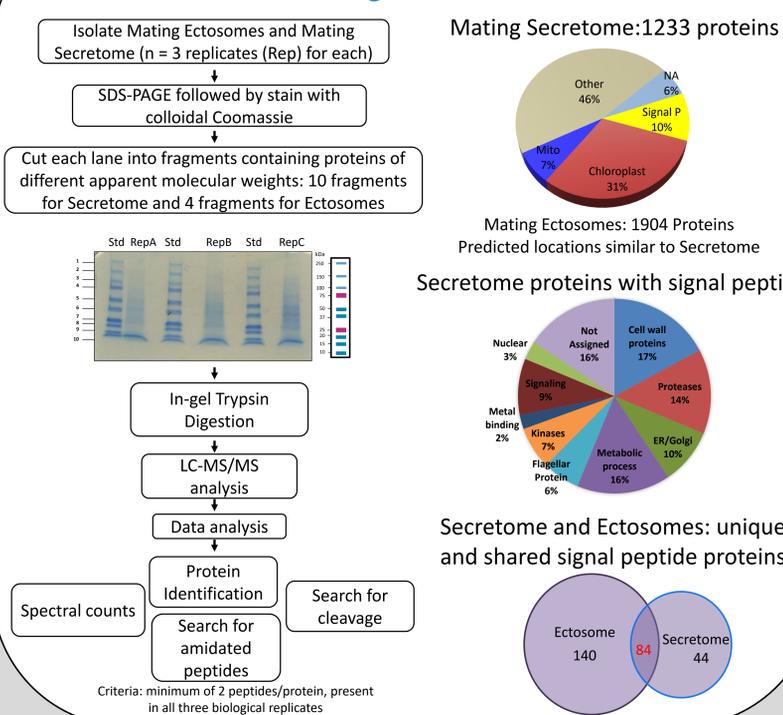


Analysis of the *Chlamydomonas* secretome

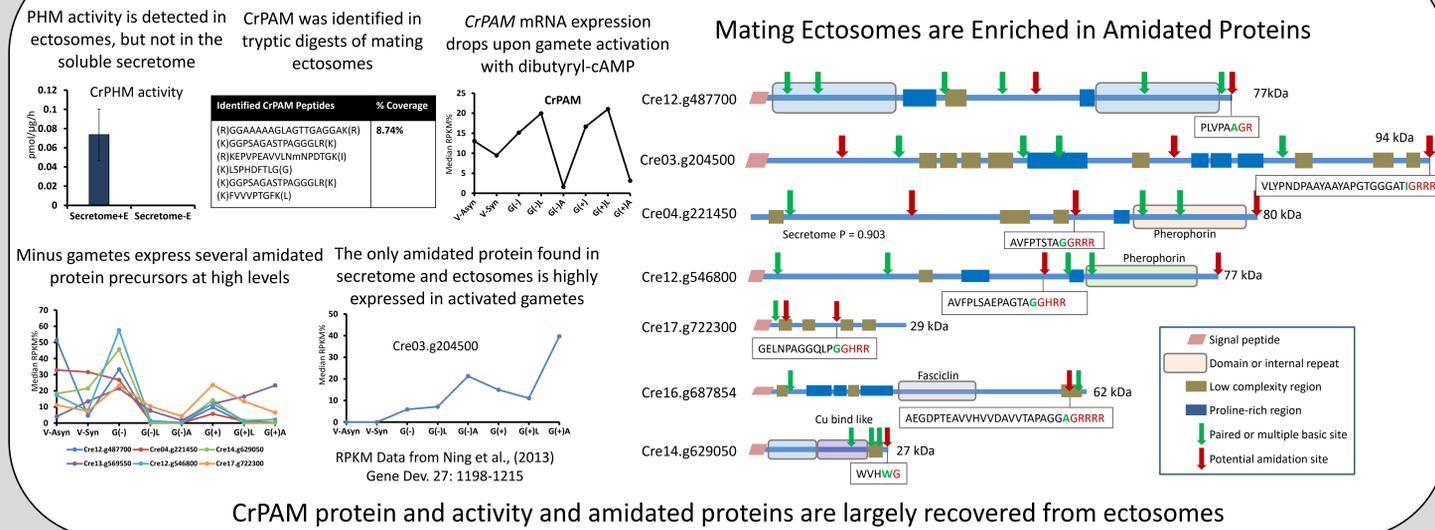
Chlamydomonas secrete cell wall components, lytic enzymes, enzymes involved in nutrient capture and metal-binding proteins. Neuropeptides are known to play key roles in reproduction and nutrient sensing and expression of CrPAM mRNA peaks in gametes (Ning et al., 2013, Genes Dev. 27: 1198-1215). Therefore, we focused on secretion events that occur when (+) and (-) gametes are mixed and the mating process begins.



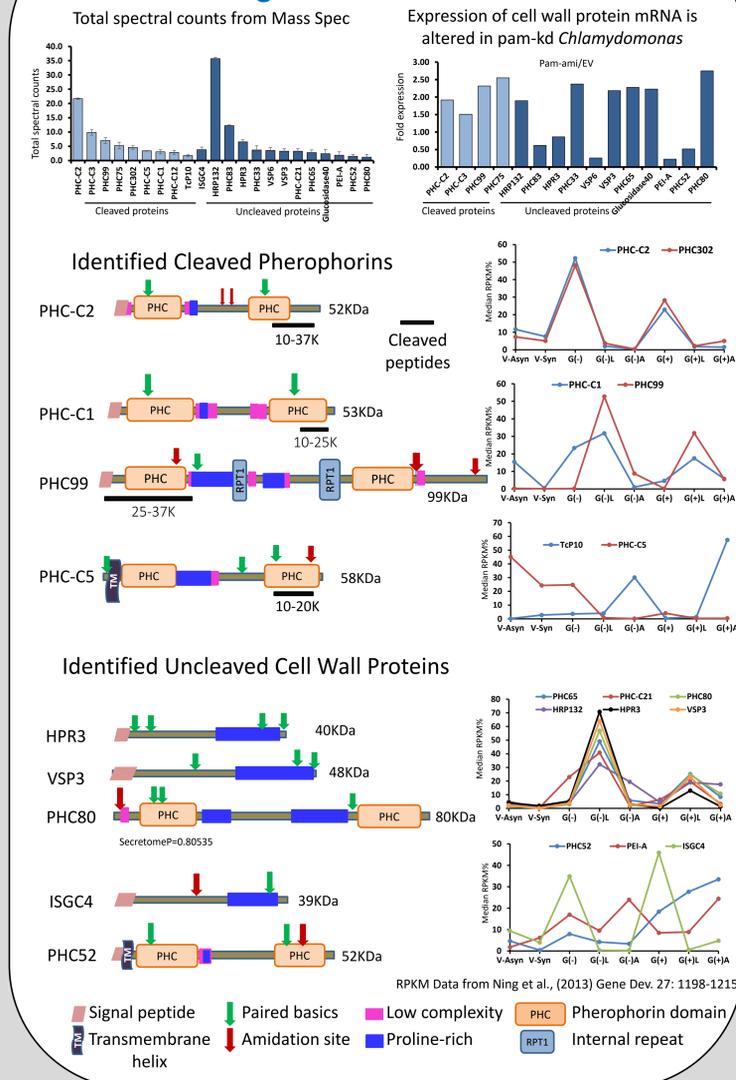
Proteins identified in mating secretome and mating ectosomes



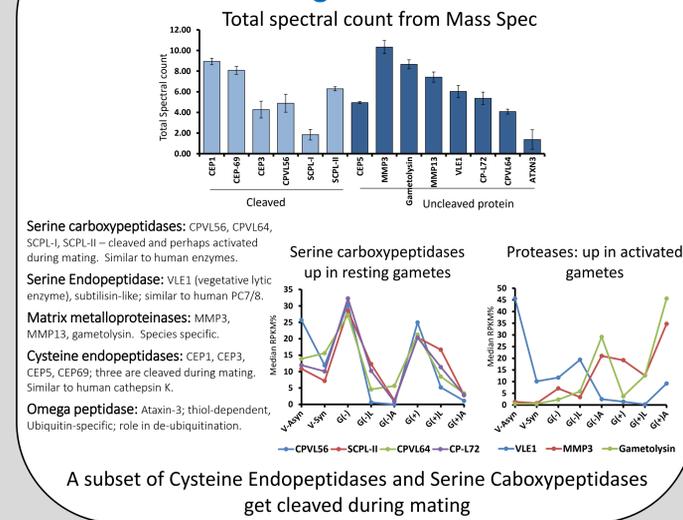
Chlamydomonas synthesizes amidated peptides



A subset of the cell wall proteins in the mating secretome is cleaved



Proteases are a major component of the mating secretome



Conclusions

- V(+) and V(-) vegetative secretomes differ from each other and from mating secretome.
 - Mating secretome contains more protein than vegetative secretome.
 - Mating ectosomes, not mating secretome, contain CrPAM.
 - Amidated proteins were identified in mating ectosomes; many have C-terminal amidation sites.
 - Transcripts encoding amidated proteins are most highly expressed in minus gametes.
 - Chlamydomonas* genome encodes multiple proteins that resemble preneuropeptides, but their amidated products were not identified in mating ectosomes or in the mating secretome.
 - Several pherophorins recovered in the mating secretome are cleaved, generating N- and C-terminal fragments. In *Volvox*, a C-terminal pherophorin fragment serves as the sexual inducer.
 - The mating secretome contains multiple endo- and exo-proteases.
- Supported by grants DK032949 (to BAE) and GM051293 (to SMK) from the National Institutes of Health.