Dissecting Proteasomes in Microsporidia

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1. Abstract

Microsporidia are spore-forming, obligate intracellular parasites that infect a broad host of organisms including humans. In humans, microsporidia infections range in severity from asymptomatic to fatality, the latter of which is more common in immunocompromised individuals. There are no treatments available for microsporidiosis, but recent evidence has suggested that targeting the proteasome may have therapeutic value. The proteasome is a multi-subunit molecular machine that is responsible for degrading unwanted or unneeded proteins in the cells. Proteasome dysregulation drives many diseases including cancers, autoimmune diseases, and neurological disorders, and there are FDA-approved drugs to treat cancer. Microsporidia has undergone extensive reduction evolution leading to a highly divergent proteasome and are sensitive to treatment with proteasome inhibitors. Here we seek to understand how one subcomplex of the microsporidia differs from the human proteasome in order to develop new treatments for microsporidiosis. Although a continually increasing risk factor amongst individuals with HIV and not only, there is no cure or treatment for microsporidia. Attacking the proteasome in microsporidia, however, seems a promising way to come up with possible treatment or even a cure. Proteasomes are responsible for degradation of waste in the cell, meaning that disruption of such process can lead to increase of cell toxicity, bringing death to the affected cell.

2. The ubiquitin-proteasome system mediates >80% of regulated degradation in eukaryotes

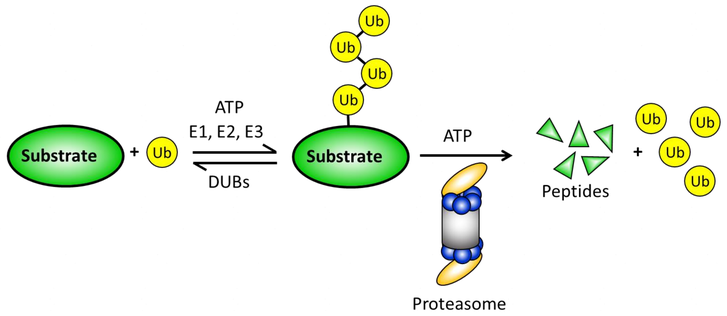


Figure 1 (Above): Substrates, such as misfolded proteins, are marked for degradation by the addition of a polyubiquitin tag. This process is coordinated by E1, E2, and E3 enzymes that activate ubiquitin (Ub) and conjugate Ub moieties to the substrate. The Ub-tagged substrate is directed to the proteasome where degradation occurs.

3. The 26S proteasome: one of nature's smallest recycling centers

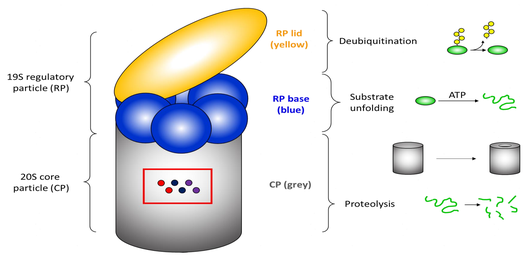
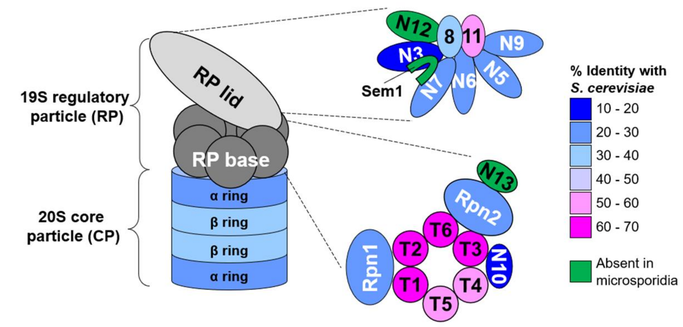


Figure 2 (Above): The proteasome performs the vast majority of regulated and quality control protein degradation in eukaryotes. The proteasome is extremely highly conserved, and consists of three main subcomplexes, the regulatory particle (RP) lid (yellow), the RP base (blue), and the core particle (grey). Incoming substrates are first engaged by the RP. The lid removes the substrate's polyubiquitin targeting signal (deubiquitination), whereas the base uses mechanical force derived from ATP hydrolysis to unfold the substrate and translocate it into the core particle, where it is cleaved into short peptides.

4. Ultrastructure and conservation of the microsporidia 26S proteasome



Adapted from Huntsman, E.M. et al. *Biomolecules* **2021** 11, 1600.

Figure 3 (Above): The microspordial proteasome has undergone extensive reduction evolution compared to the yeast proteasome. Some subunits that are completely absent (i.e. Rpn12) are absolutely required for yeast and human proteasome assembly. This highlights the potential to selectively targeting the microspordial proteasome while leaving the host proteasome unharmed.

5. Synthesis of the microsporidial base

Overview of steps:
1. Gibson Assembly
2. DNA isolation
3. Restriction digest

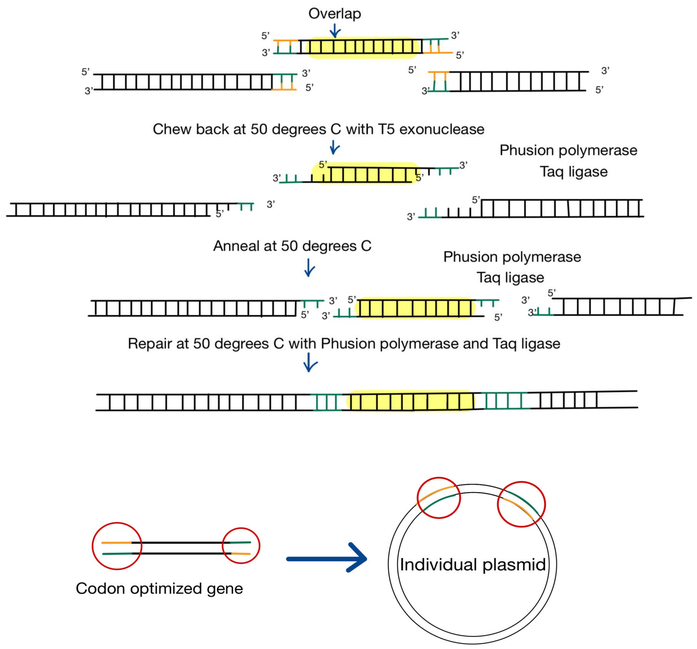


Figure 4a (Top): Illustration of Gibson assembly through the use of T5 Exonuclease, Phusion DNA, Taq Ligase and NAD+, all while in ISO buffer. Nine plasmids were constructed; one for each gene in the base subcomplex.
Figure 4b (Bottom): Simple illustration of the big picture

Components of restriction digest of 3226 plasmid, with the RPT 2 gene:
- Molecular biology grade dH2O
- 10x Buffer CutSmart Buffer
- 3226 DNA plasmid
- NcoI enzyme
- BamHI enzyme

Components of restriction digest of 3225 plasmid, with the RPT 1gene:
- Molecular biology grade dH2O
- 10x Buffer CutSmart Buffer
- 3225 DNA plasmid
- NcoI Enzyme
- BamHI enzyme
- CIP enzyme

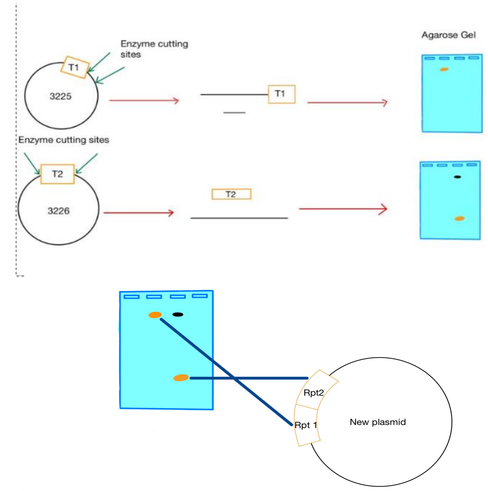


Figure 5 (Above): Illustration of Restriction Digest, and then insertion of noticed DNA genes into the same plasmid

6. Conclusions and Future Directions

- Using the same process as described above, each of the 6 Rpt base subunits will be inserted in the same plasmid.
- The same process will be performed for the 3 Rpn subunits of the base.
- These two plasmids will be introduced into bacterial cells. Protein will be induced and purified.
- With the purified subcomplex in hand, we can begin to assess its function and work towards obtaining its structure.

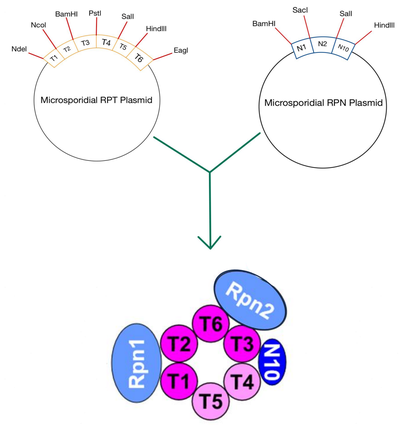


Figure 6 (Above): Fully synthesized RP base subcomplex of the microsporidial proteasome from the Rpn and Rpt plasmids.

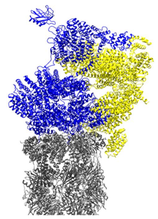


Figure 7 (Above): High-resolution structure of yeast proteasome

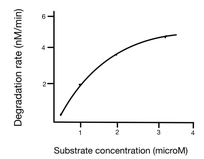


Figure 8 (Above): Effect of substrate concentration on degradation rate.

7. References

Huntsman EM, Cho RM, Kogan HV, McNamara-Bordewick NK, Tomko RJ Jr, Snow JW. Proteasome Inhibition Is an Effective Treatment Strategy for Microsporidia Infection in Honey Bees. Biomolecules. 2021 Oct 29;11(11):1600. doi: 10.3390/biom11111600. PMID: 34827599; PMCID: PMC8615682.