Ochratoxin A (OTA) is a potent mycotoxin.

The gene cluster producing OTA is conserved over several species.

A consensus biosynthetic pathway is hypothesized from the OTA gene cluster.

Questions to Consider:
- Are these genes sufficient to produce OTA?
- Can we derivatize OTA for medicinal purposes?
- What is the mechanism of the halogenase and peptide synthetase steps?

Model to reconstitute the complete OTA biosynthetic pathway:

- **Aspergillus carbonarius**
  - **Polyketide Synthase (PKS)**
  - **P450 Oxygenase**
  - **Non-ribosomal Peptide Synthetase (NRPS)**
  - **Halogenase**
  - **DNA extraction**
  - **Genomic DNA**
  - **Polymerase Chain Reaction (PCR)**
  - **Yeast homologous recombination + auxotroph selection**
  - **Gene fragments**
  - **Fully assembled plasmid**
  - **Plasmid extraction + E. coli replication + Antibiotic selection**
  - **Expression in model organism (A. nidulans)**
  - **Increased plasmid concentration**
  - **Enzyme/small molecule characterization + analysis**

**Partial Reconstitution of OTA was achieved**

**Conclusions**
- We have confirmed the role of the PKS and p450 oxygenase enzymes in the OTA biosynthesis.
- The current proposed pathway fails at the non-ribosomal peptide synthetase step.
- The unusual substrates needed to make the peptide bond in OTA biosynthesis suggests a misannotation of the pathway or an unusually promiscuous enzyme.

**Future Directions**
- Achieve complete reconstitution of the OTA biosynthetic pathway.
- Characterize the NRPS and halogenase steps to justify interesting activity.
- Investigate an in vitro enzyme-catalyzed synthesis of OTA.
- Investigate possible medicinal derivatives of OTA.