

Complete biosynthesis of opioids in yeast

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Introduction

- Opioids are drugs used in medicine
 - Pain management and palliative care
- Diverse market demands but so far the farming of opium poppies remains as the only source
- Yeast was engineered to produce selected opioid compounds (thebaine and hydrocodone starting from sugar)
- Result full opiate biosynthesis in yeast
- Only proof-of-principle and there is still a lot left before optimization and scale up can be achieved.

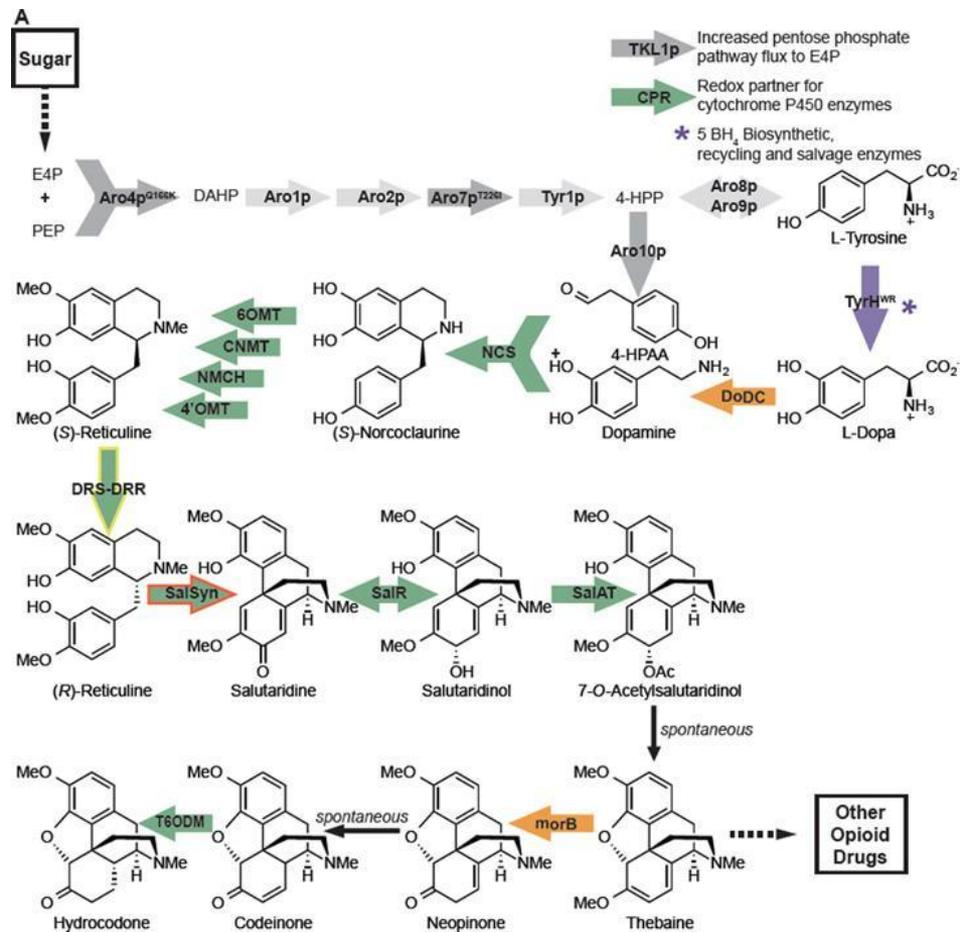


Why was this done?

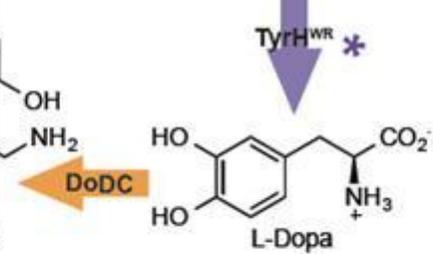
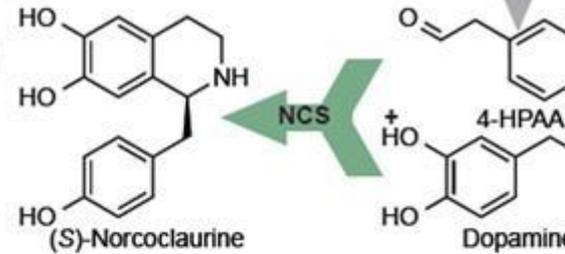
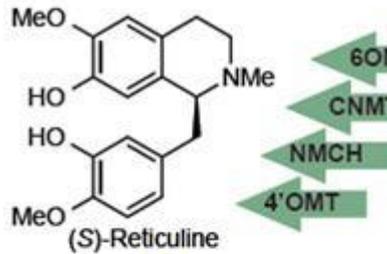
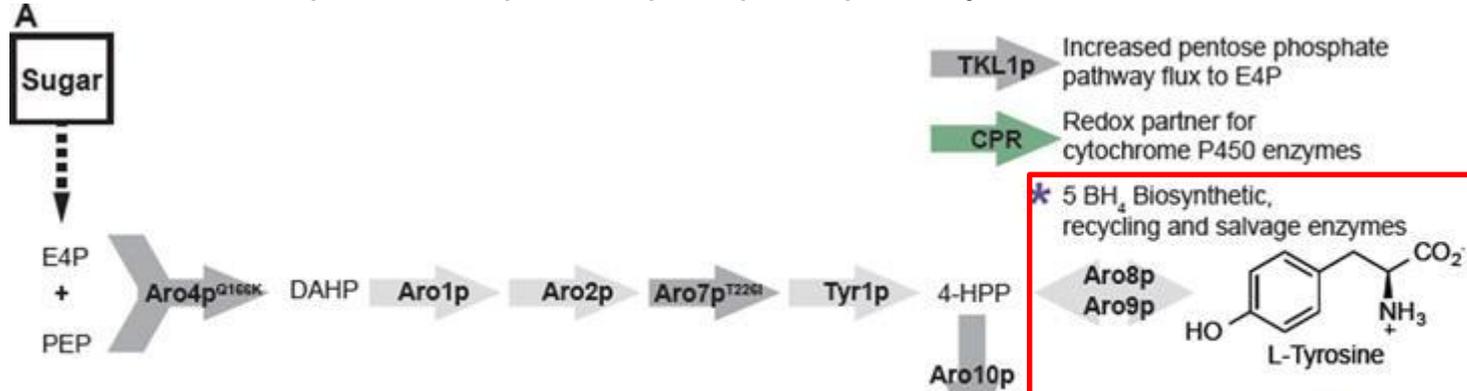
- In developing world there is a serious shortage of painkillers, estimated 5.5 billion people have “low to nonexistent access to treatment for moderate or severe pain”
- All natural opiates and semi-synthetic opioids are derived from the opium poppy
 - Around 100,000 hectares of opium poppy are cultivated annually to meet the medical and scientific demand
- Susceptible to environmental factors such as pests, disease and climate
 - instability and variability into supply chain -> growing pressure to diversify supply
- **To address these problems:** microbial based manufacturing process for opioids in baker's yeast
 - Industrial cultivation of baker's yeast occurs over days vs the annual cultivation of plants
 - The production process is not susceptible to external environmental factors
 - Could provide greater consistency in product composition and impurity profiles across batches



The opioid pathway methods and approaches



I. Over-expression of pentose phosphate pathway



Step 1: Creation of a S-reticuline synthesizing yeast strain

I. Over-expression of pentose phosphate pathway

- Increased activity of native proteins

II. Tyrosine conversion to L-Dopa

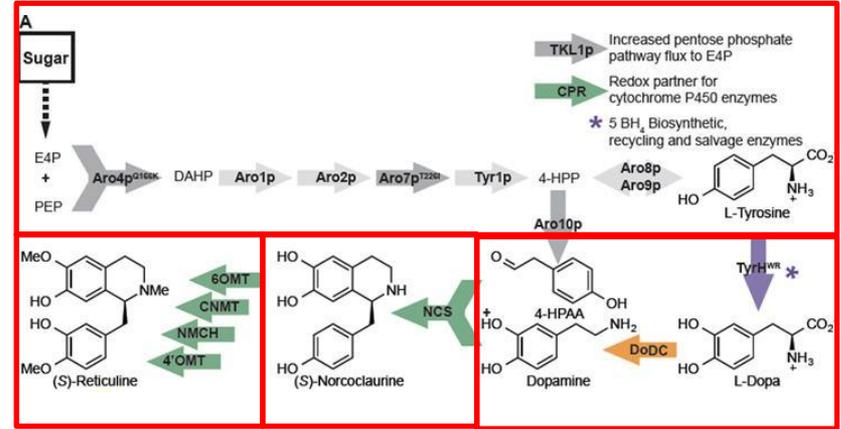
- With the help of redox factor BH₄ and 4 proteins from *Rattus norvegicus* (2 reductases, a synthase and a hydratase)

III. Synthetization of the BIA backbone

- This part of the pathway uses proteins from a (*R. norvegicus*), a plant (*C. japonica*) and a bacteria (*P. putida*).

IV. Synthetization of BIA branchpoint

- uses 5 plant proteins from *P. somniferum* (4x) and from *Eschscholzia californica* (1x)



Details of the S-reticuline producing yeast strain.

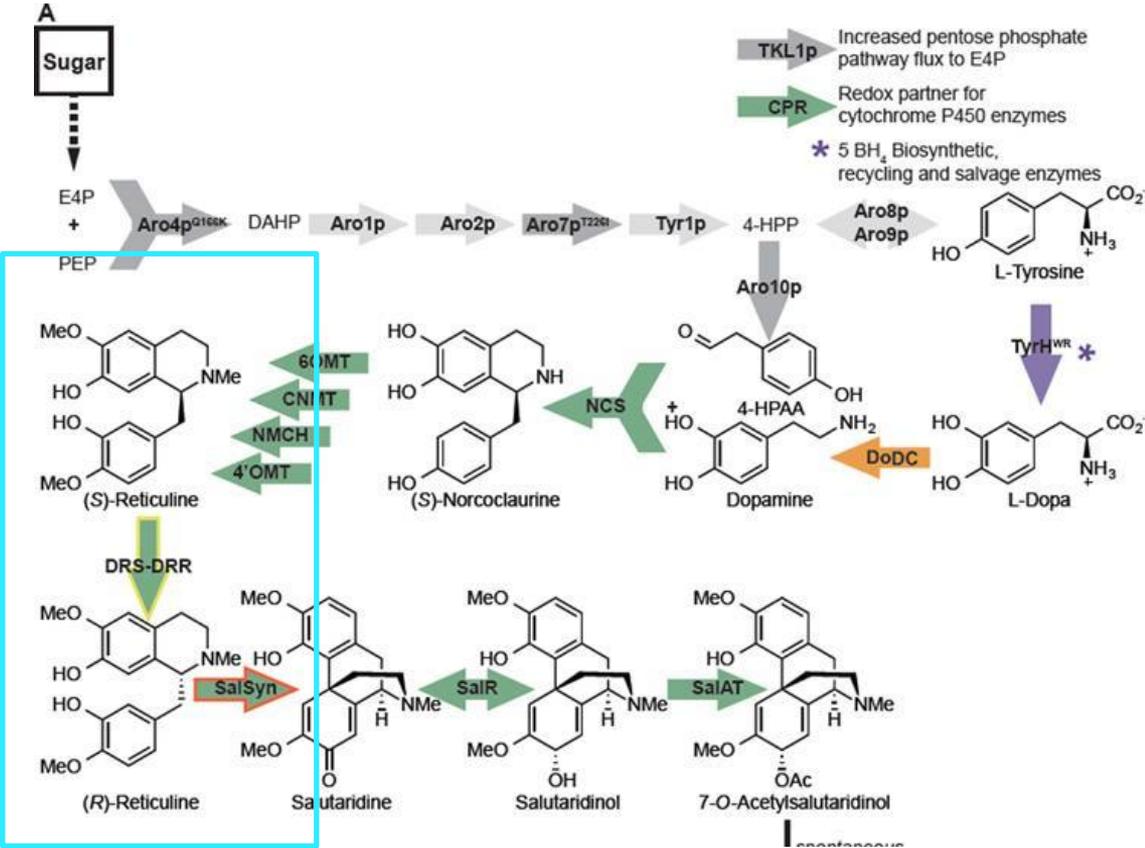
Step 2. Strain refinery

NCS creates exclusively S-enantiomer of Reticuline.

- Opium poppy has protein complexes that turn S \square R. **But how?**

- From literature: COR knock-out similar effect \square BLAST & Genbank \square similar sequences for transformation

\square Fusion protein **DRS-DRR**



Step 3. Thebaine production

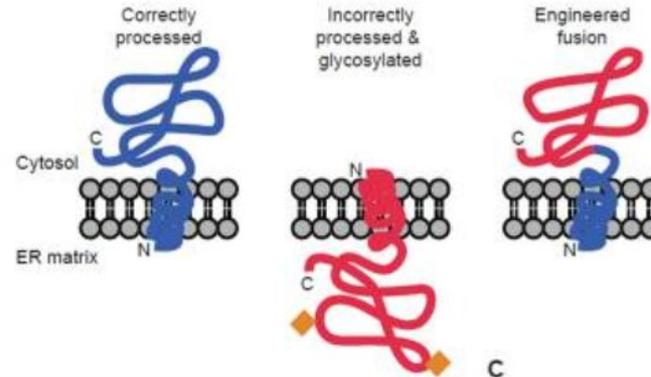
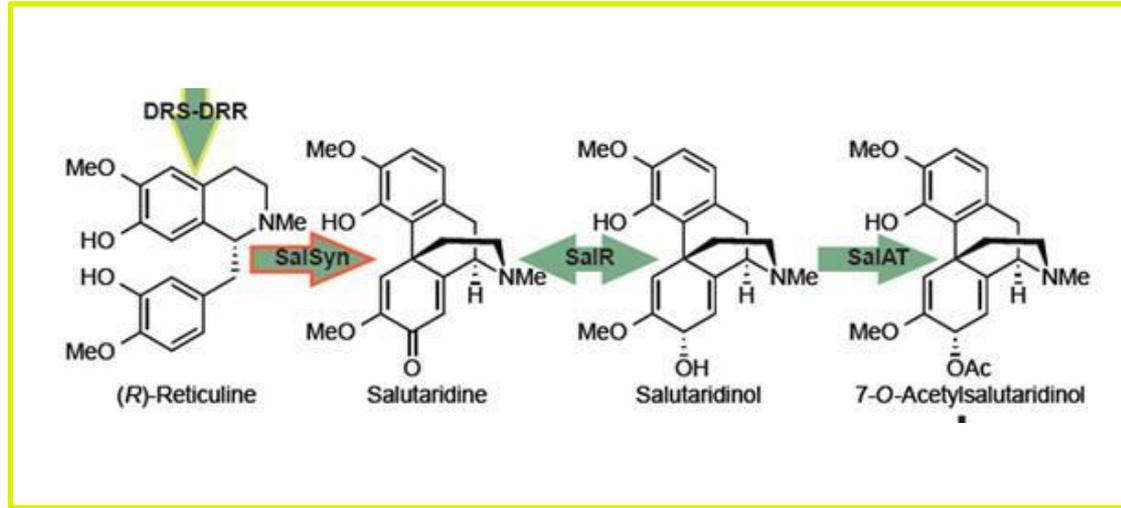
Encodes the expression of the best suited enzyme variants according to the study.

SalSyn (catalyzing agent, tobacco plant) turns **R-reticuline** into **Salutaridine**

- Problem: Yeast glycosylation decreases SalSyn activity
- Solution: Fusion protein with parts from a similar protein which do not require glycosylation at all

SalR (*P. Bracteatum*) and **SalAT** (*P. somniferum*)

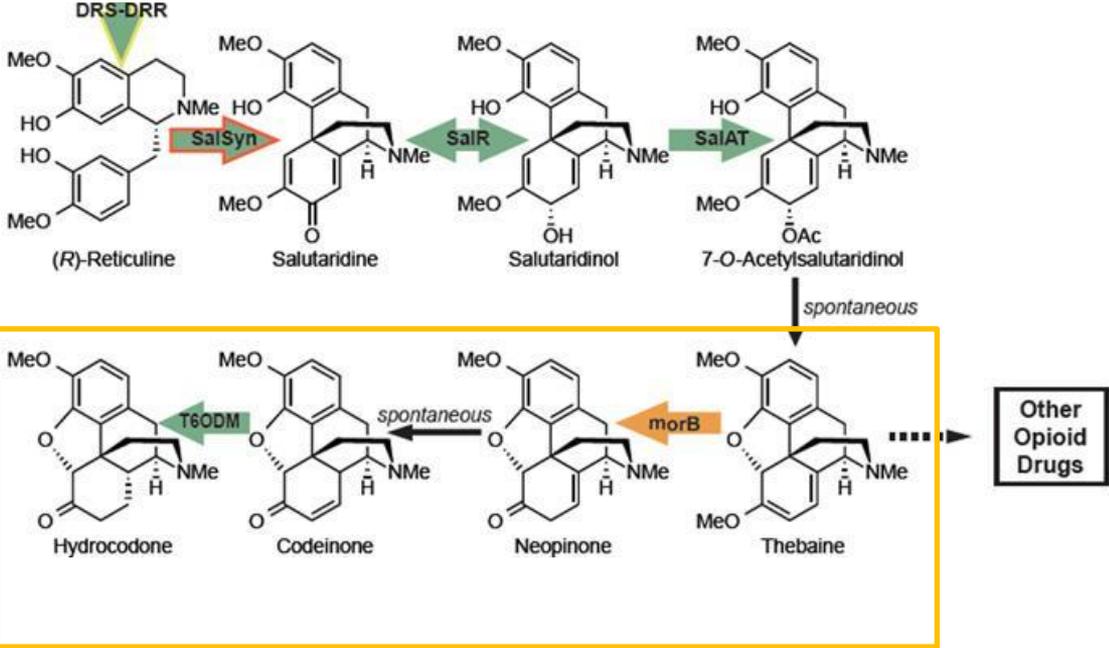
- **Further processing** of the compound, to get it closer to the thebaine structure
- From literature



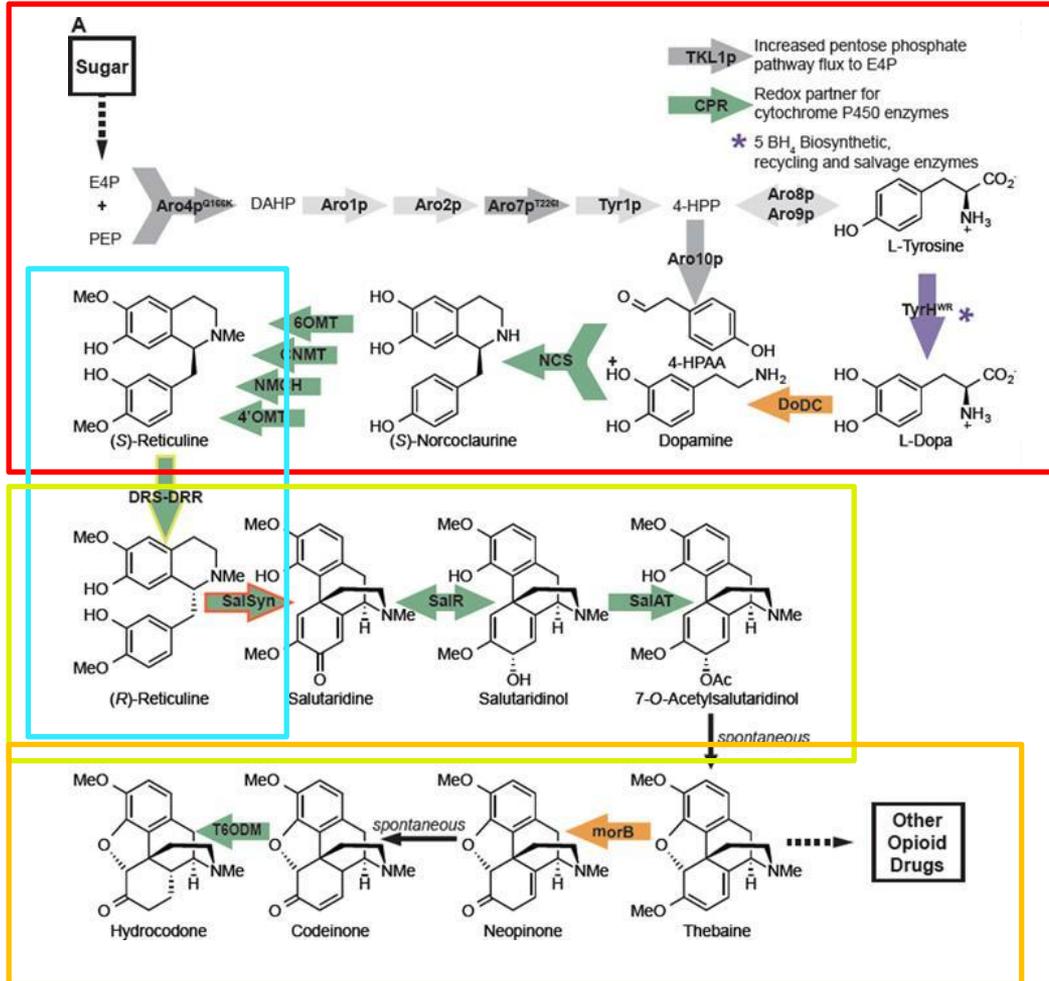
Step 4. Opioid synthetization

Synthetization of hydrocodone from thebaine:

- morB (reductase, *P. Putida M10*)
- and T6ODM (demethylase, *P. Somniferum*) from litterature



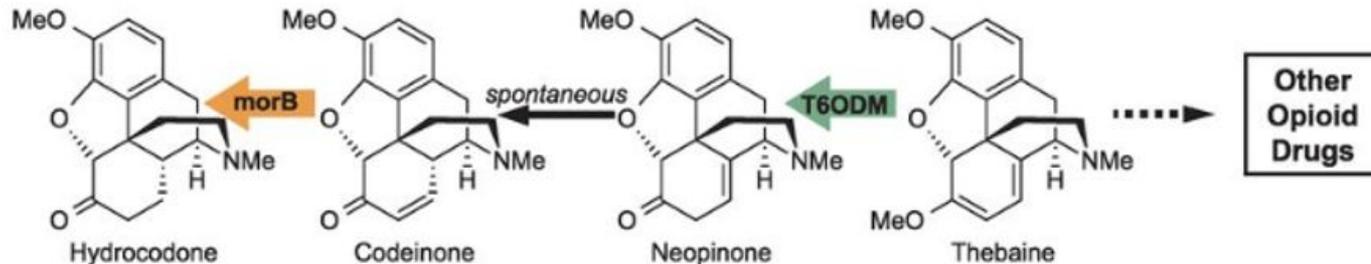
The complete pathway



Achievements

- **Successful biosynthesis of thebaine and hydrocodone**

- ⇒ Complex molecules from simple sugars
- ⇒ Important precursor to the opioid painkillers such as morphine and codeine
- ⇒ Potential alternative route to produce morphine and codeine that's not dependent on opium poppy cultivation



Limitations

- **Complex biosynthesis pathway**

⇒ Enzymatic reactions are difficult to engineer and optimize

- **Low yield of the final products**

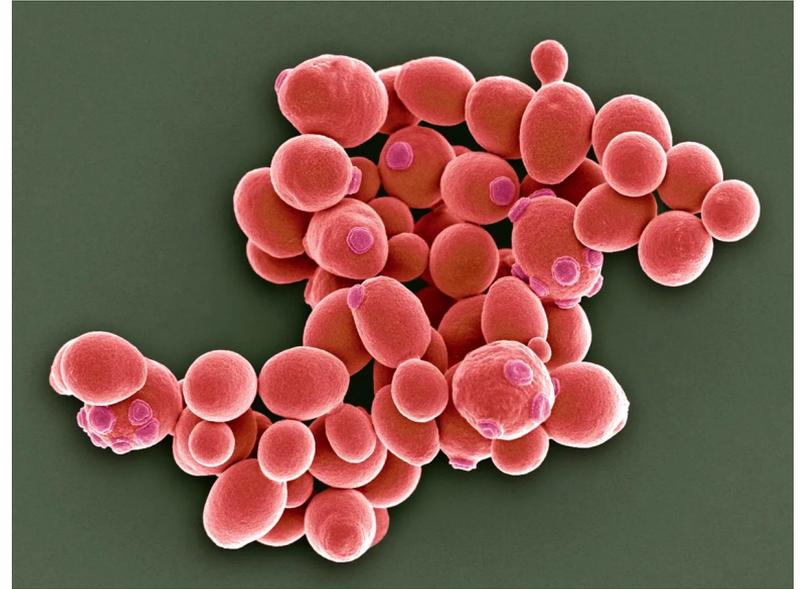
⊘ Concern about the illegal abuse of these engineered yeast strains



Path forward

Producing opioids in yeast is an important path forward because:

- Presents a safer production method
- Could potentially be more cost effective
- Potential environmental benefits: reduced land, water and pesticide use
- Could provide a more ethical alternative for opioid production
- Highlights the possibility of using yeast as chassis for production of many other complex chemicals and materials
- Opens the route for the development of better opioid based medications



Thank you!