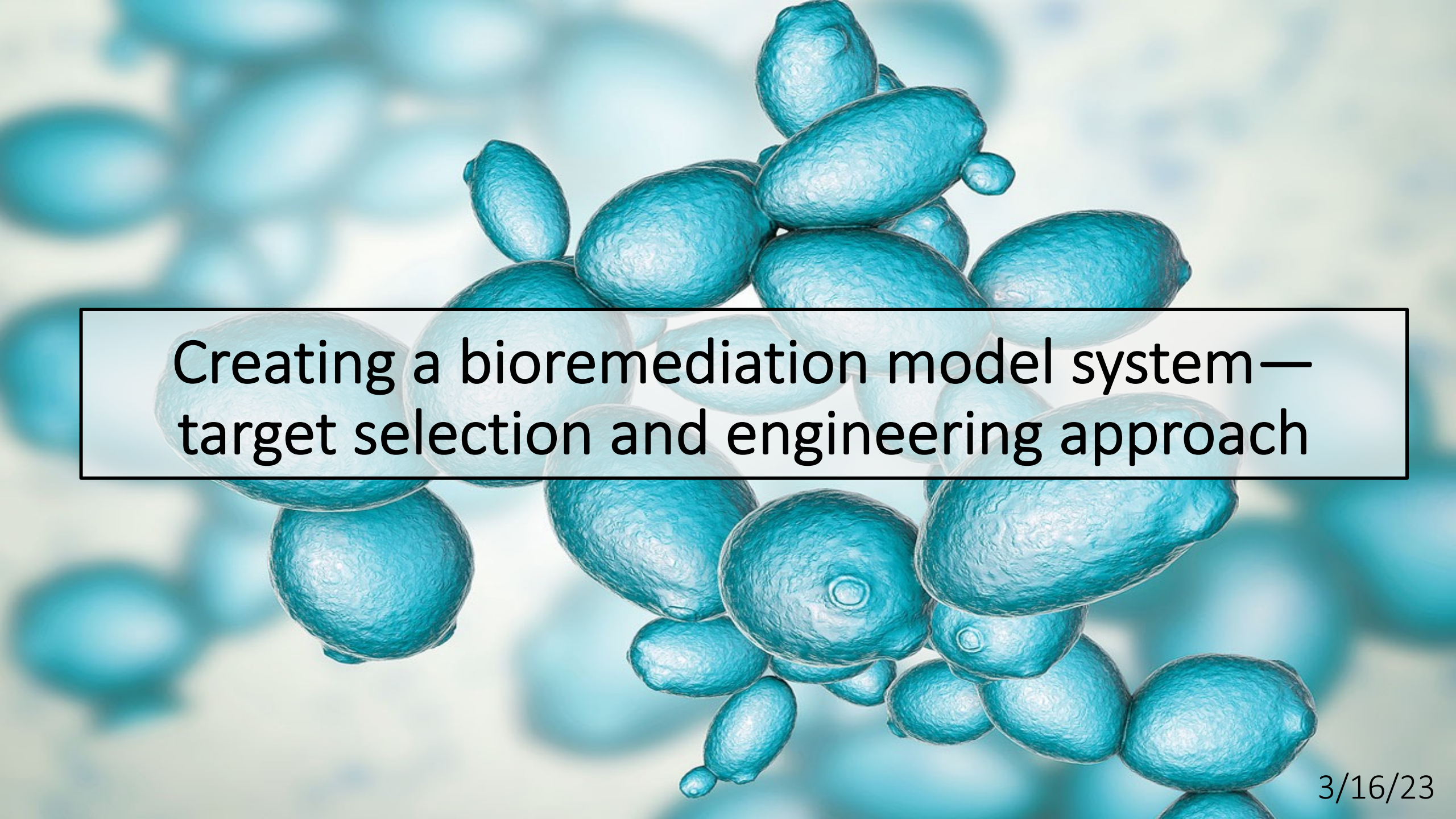


Creating a bioremediation model system—target selection and engineering approach

3/16/23

Module Outline

- M2D1: Environmental heavy metal contamination
- **M2D2**: Model system – target selection and engineering approach
- M2D3: Model system – choosing and modifying a chassis
- M2D4: Screening a system—high throughput vs functional screens
- M2D5: Analysis of elemental metals – laboratory and field approaches
- M2D6: Applying remediation strategies—advantages and pitfalls
- M2D7: Engineering a problem-specific bioremediation solution
- M2D8: Comm Lab

Intro

Design

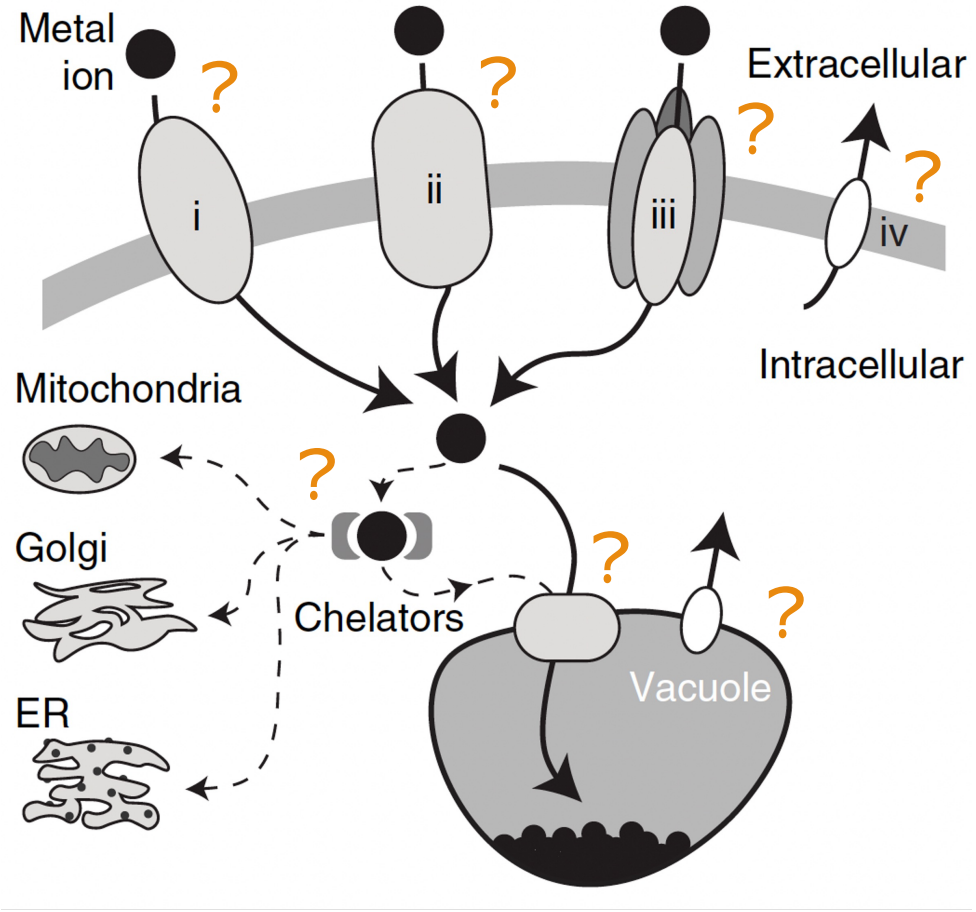
Test

Apply

Review

Overview of today's lecture

- Choosing a target
  - Conceptual pipeline for target selection
  - Fet4 as a target
  - Other options to consider in our model system
- Modifying a target
  - Genetic engineering as an approach
  - Exogenous expression vs genomic integration
  - Rational design vs random mutagenesis



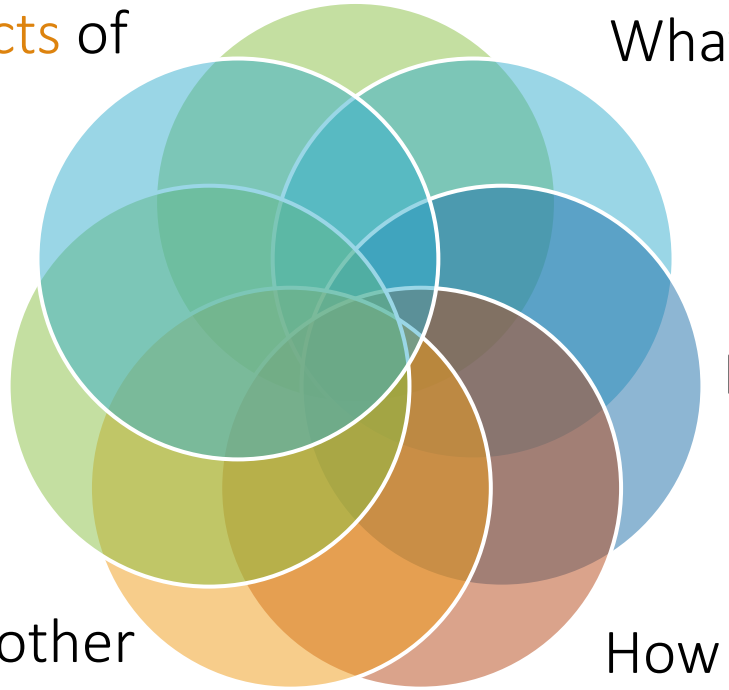
Considerations for choosing a target

Key questions in target selection

How well characterized is the target?

What are the downstream effects of modulating the target?

What is the subcellular distribution of the target?



How would modulating this target affect its native function?

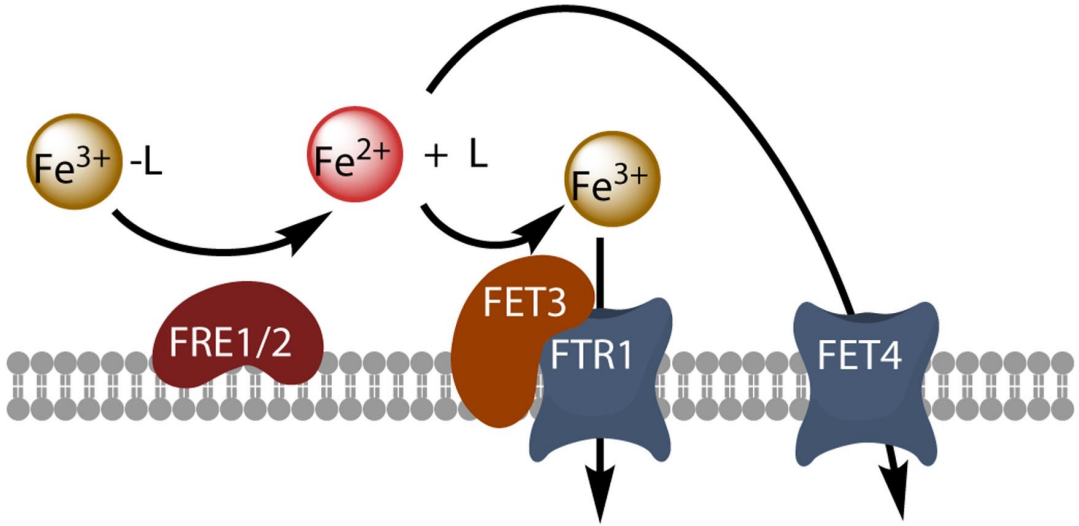
Does the target exist in multiple forms or species?

Does the target interact with other proteins and what are the consequences of those interactions?

How abundant is the target and under what conditions is it expressed?

How well characterized is the potential target?

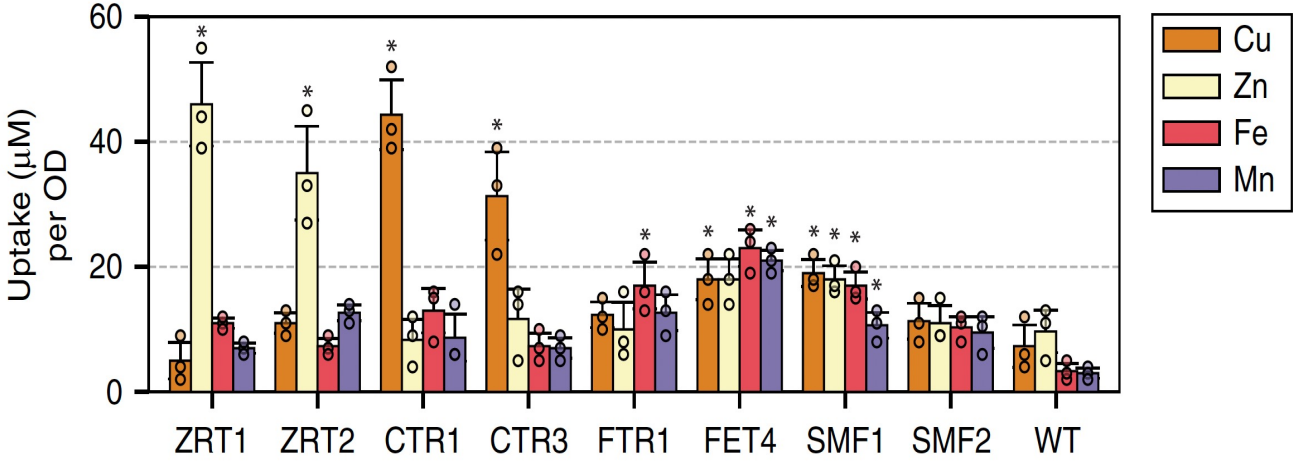
- Is the sequence known?
- Has the structure been solved?
- Is the interactome known?
  - Are there known ligands or protein-protein interactions?
- Is the biological function(s) known?

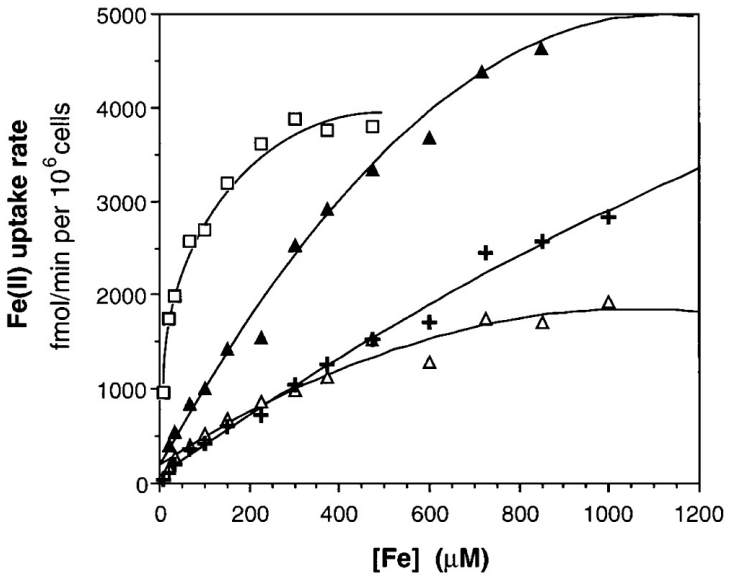


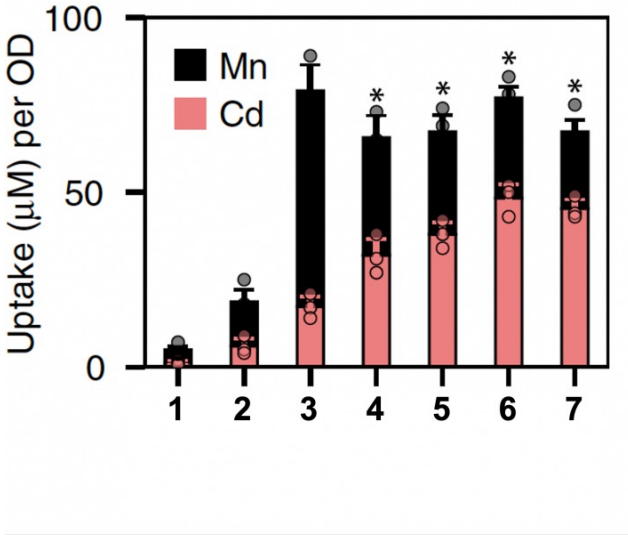
- Fet4: low affinity iron permease
- Sequence and subcellular localization known
- Structure is predicted
- Non-essential
- Expressed mostly under anaerobic conditions

What do we know about target interaction with our substrates?

- Fet4 takes up multiple types of divalent metals
- Fet4 knockout *S. cerevisiae* take up less cadmium than wild type
  - Endogenous Fet4 expression can be downregulated by cadmium
- The SMF1 transporter will take up more cadmium in response to a single point mutation
- Fet4 will change affinity for iron in response to a single point mutation

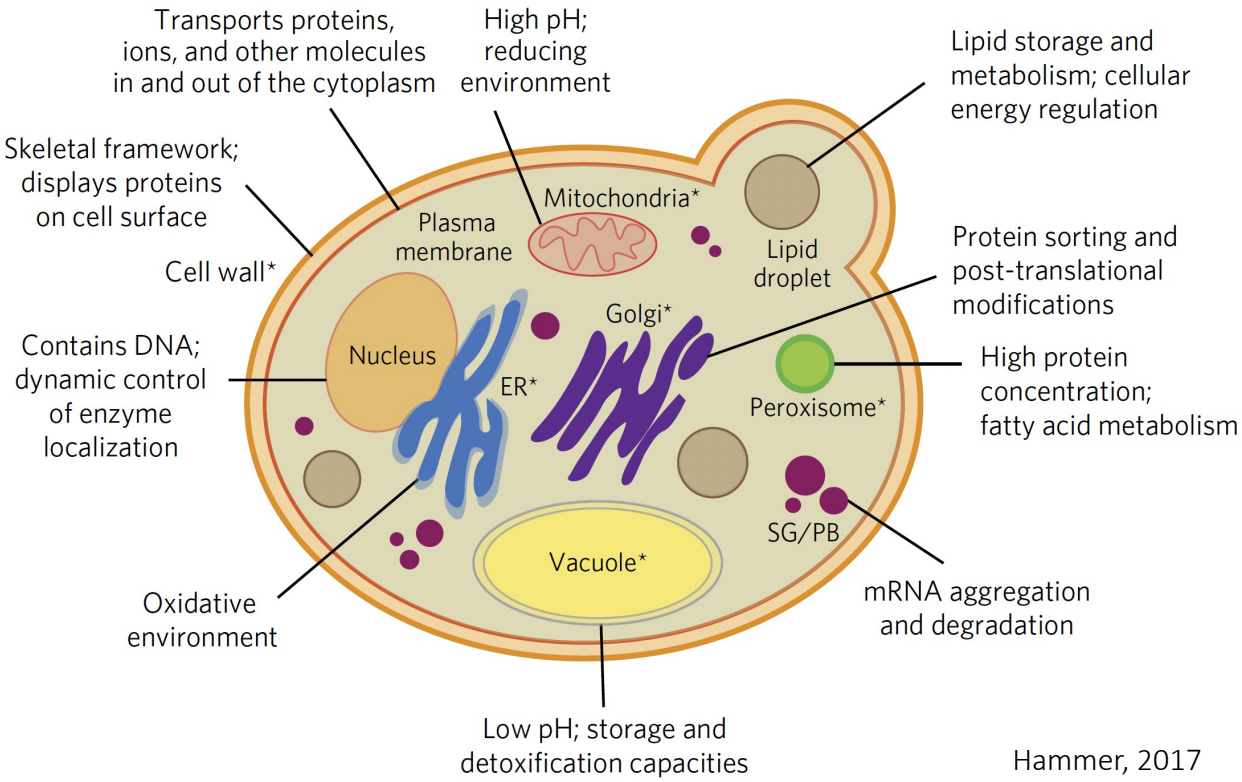






What is the subcellular distribution of the target?

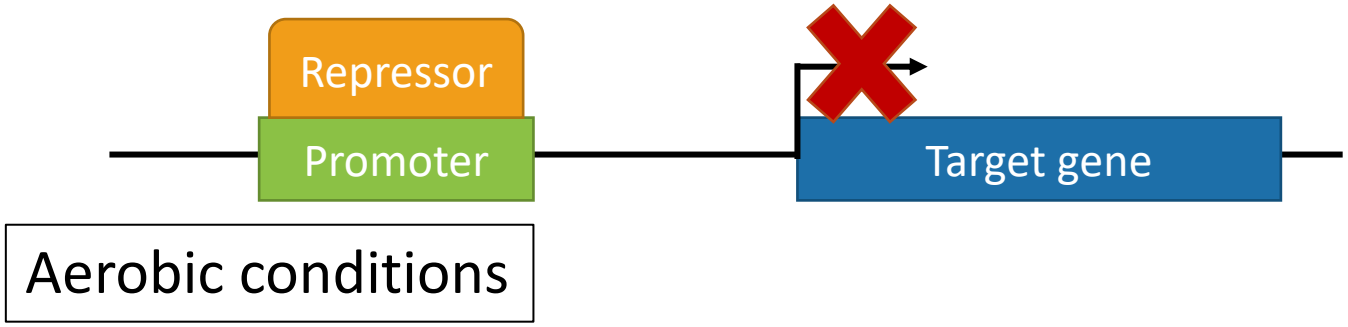
- Cytosolic vs. membrane bound
  - Transmembrane domains
- Cell surface vs. intracellular organelle
  - Localization sequences
- Localization is a key part of function
  - Provides insight into binding partners
- Localization-specific features can be points for manipulation or necessary to avoid

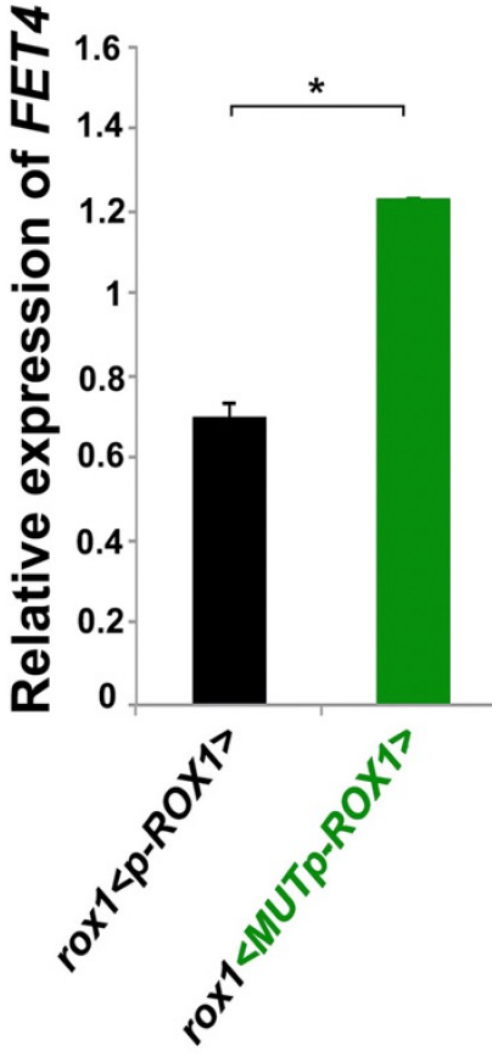


Hammer, 2017

How abundant is the target and under what conditions is it expressed?

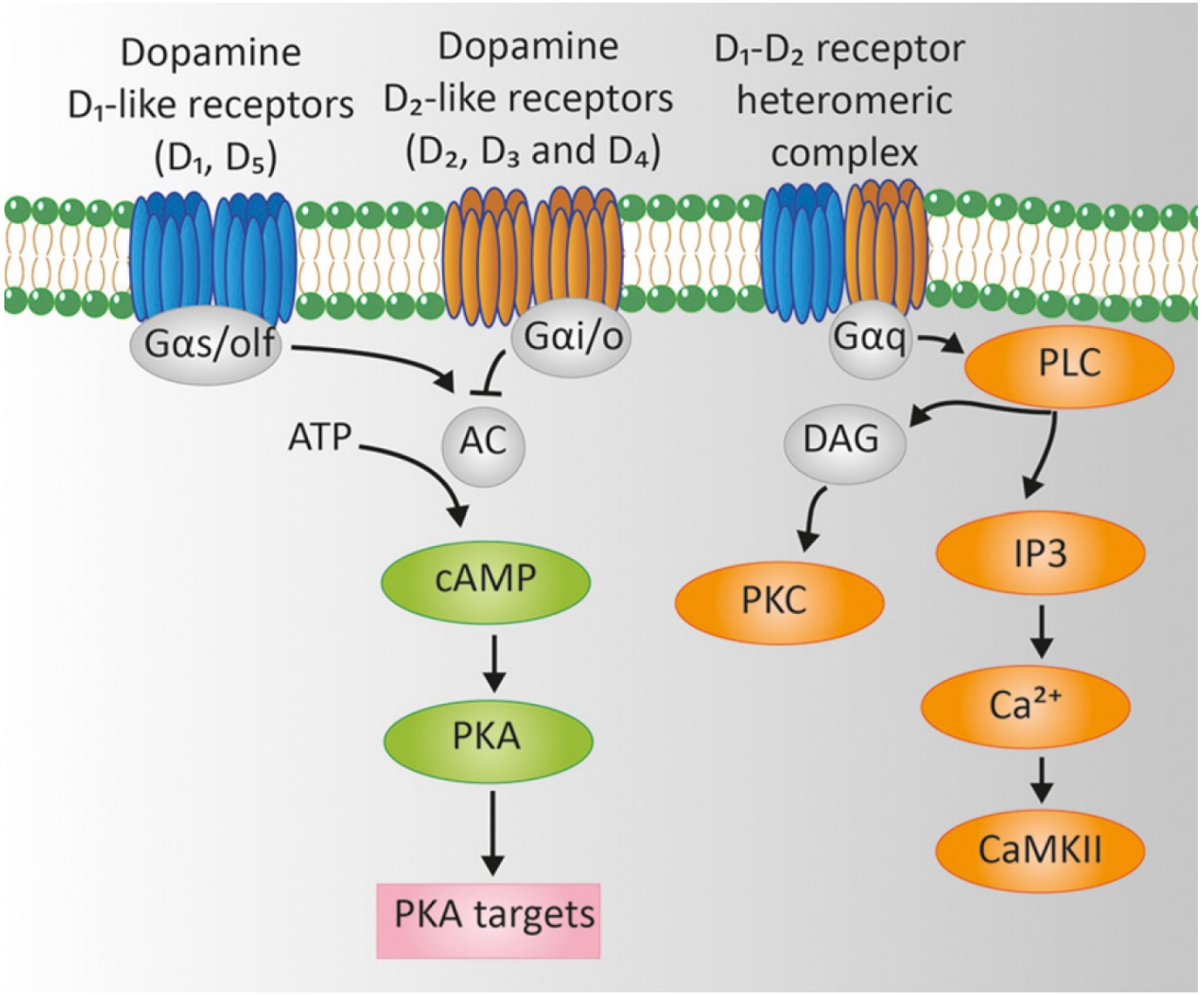
- Protein expression can be tightly regulated
  - Location specificity
  - Condition specificity
- Endogenous Fet4 expression is regulated by ROX1
- Rox1 is a transcriptional repressor of hypoxia induced genes





Does the target interact with other proteins?

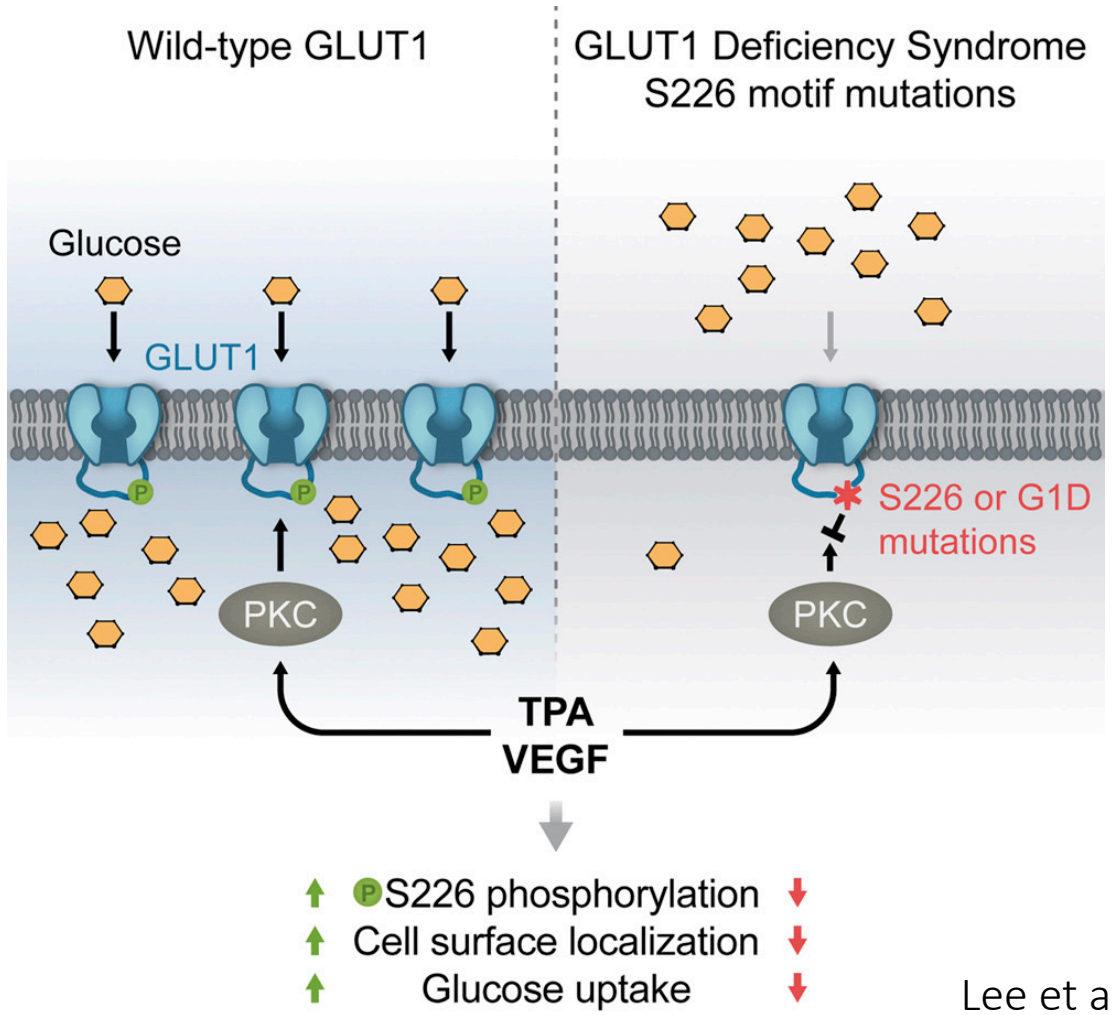
- Proteins form complex interactions with other proteins
- Homo / heterodimers /tetramers
- Obligate vs non-obligate
- Stable vs transient



Osinga 2017

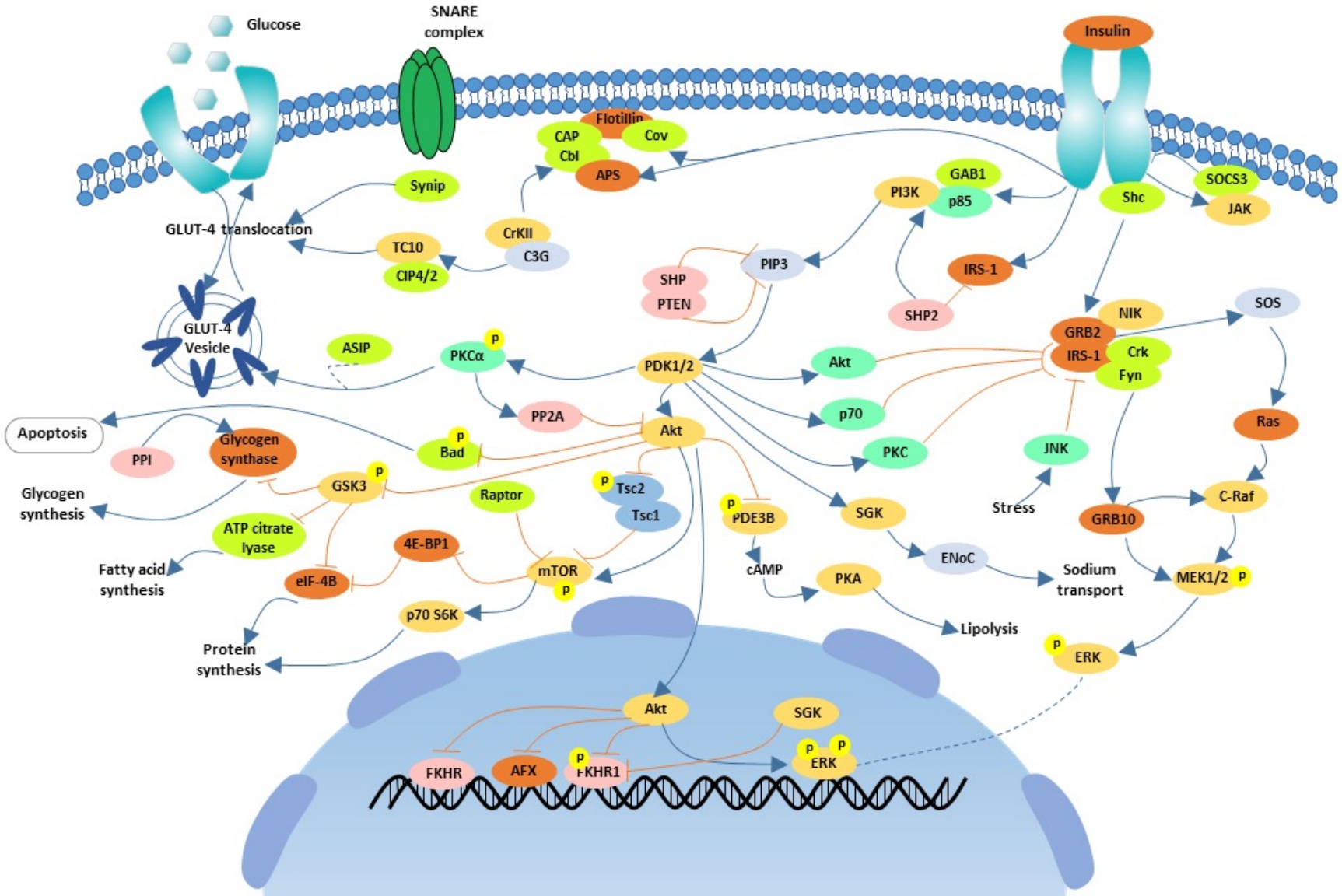
How would modulating this target affect its native function?

- Change binding affinity or specificity?
- Change access to or preference for binding partners?
- Modulate function vs destroy function



Lee et al. 2015

What are the downstream effects of modulating the target?



Cusabio

Why was Fet4 chosen as a target for this project?

1. Previous functional observations
   - Transporter does not show strong specificity for a particular metal
   - Wild type Fet4 can take up cadmium

2. Expressed at the cell surface

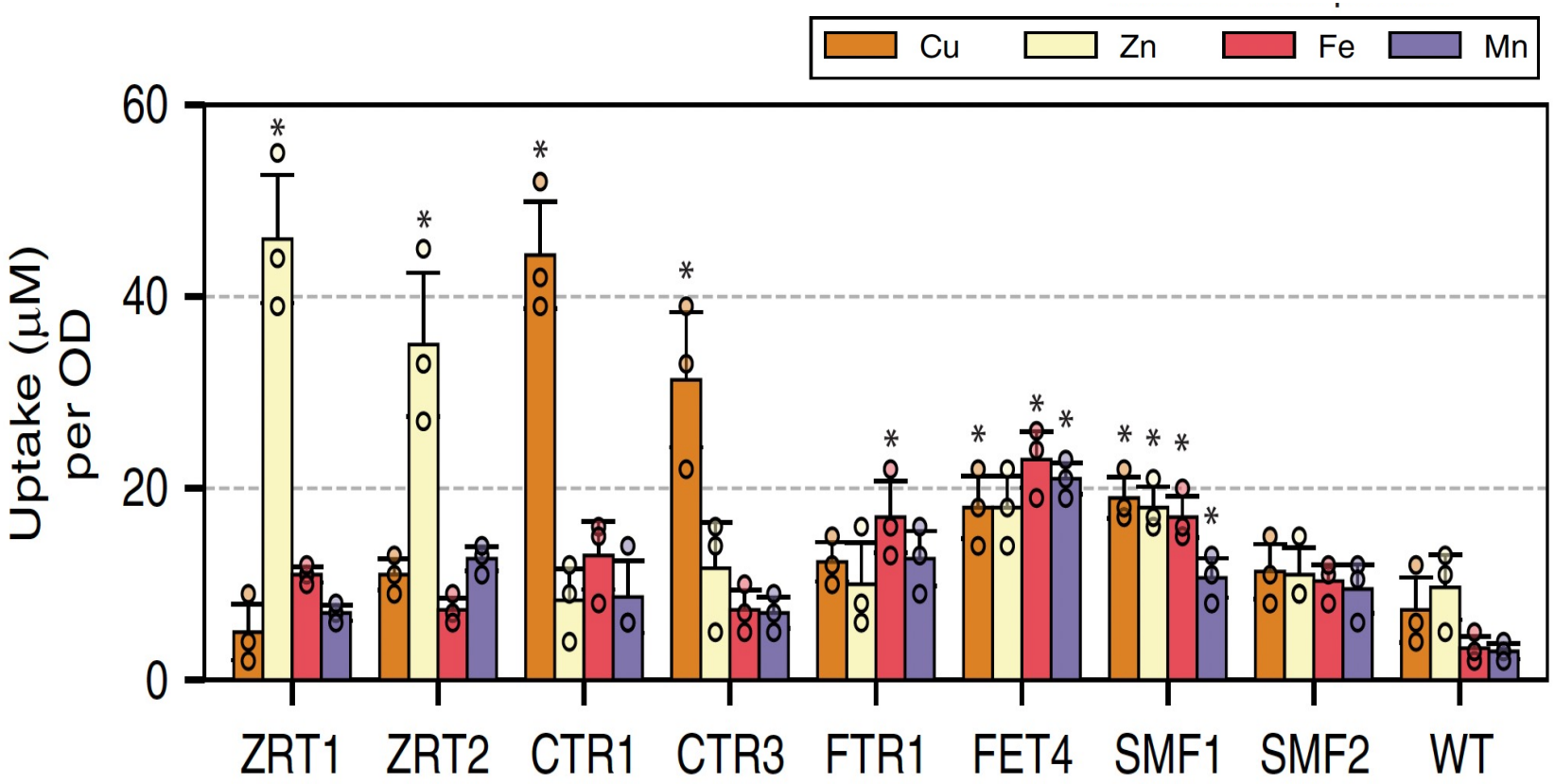
3. Low affinity iron transporter
   - Iron uptake is redundant under normal conditions

4. Low basal expression in aerobic cultures
   - Overexpress our mutant without as much wild type noise

5. Not a highly conserved, ubiquitous protein
   - Fewer considerations for off target effects

What are other potentially effective S. cerevisiae cell surface metal transporters to target?

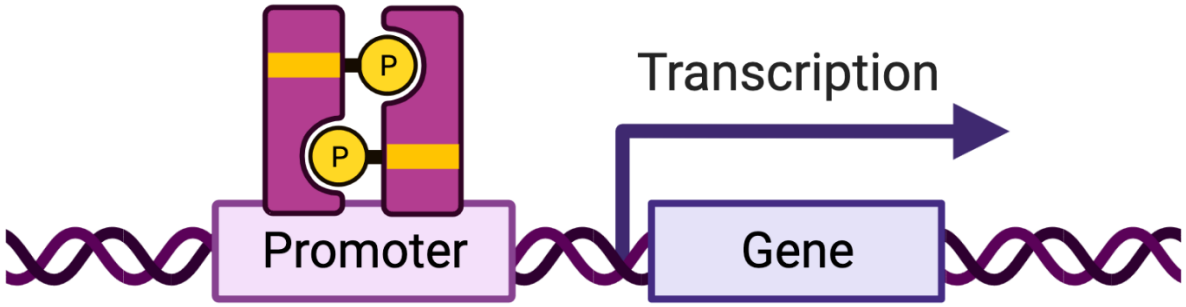
- SMF1:
  - high affinity manganese transporter

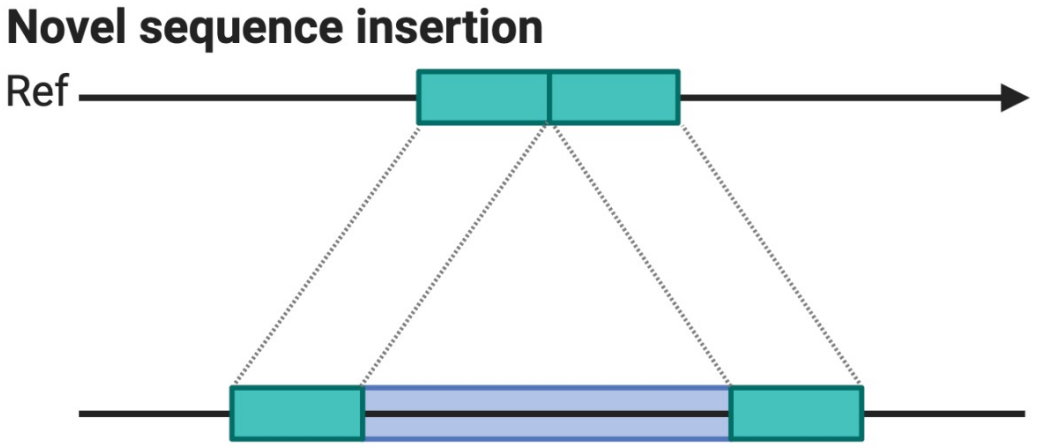


Genetic engineering to modify a target

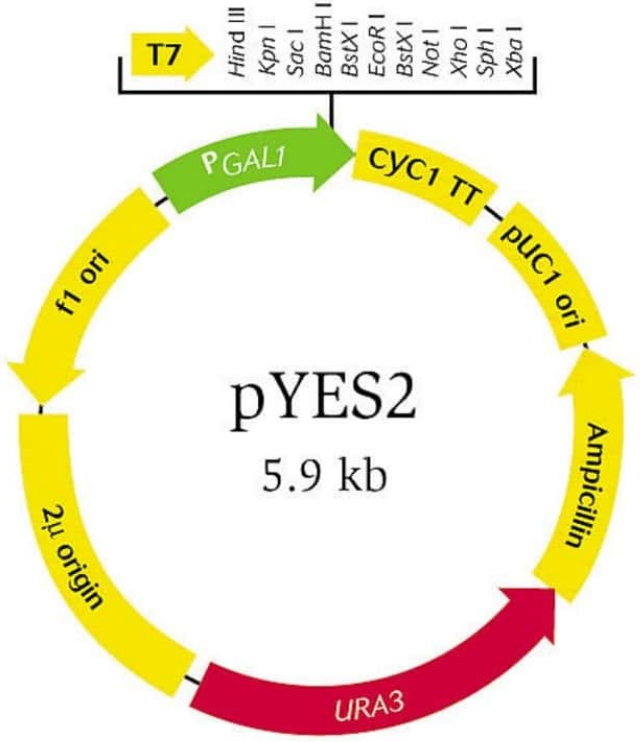
Genetic engineering encompasses a broad range of tools to manipulate targets

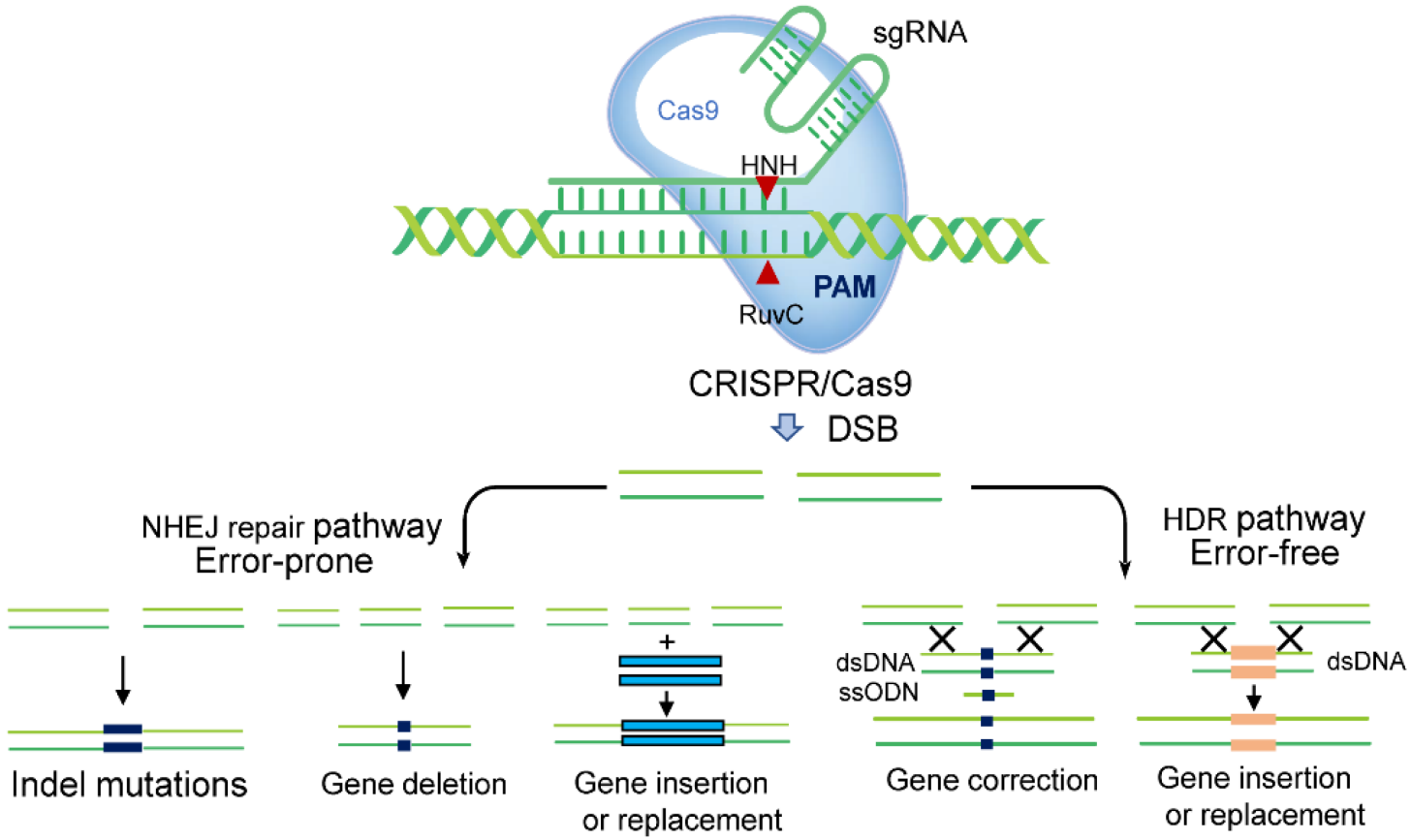
- Repression
  - Inhibit promoter
  - Inhibit RNA Polymerase
- Overexpression
  - Change endogenous promoter
  - Exogenous expression
- Addition
  - Integrate new gene into the genome
  - Exogenous expression of new gene
- Mutation
  - Alter gene sequence
  - Single or multiple changes





Exogenous expression and genomic integration can both be utilized for genetic engineering





Random mutagenesis approaches for library development

PCR

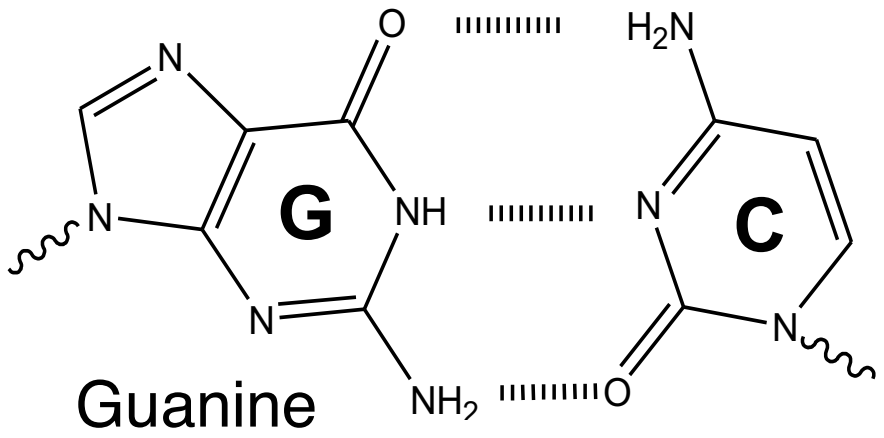
dCTP, dTTP
dGTP, dATP
Mg^{2+}

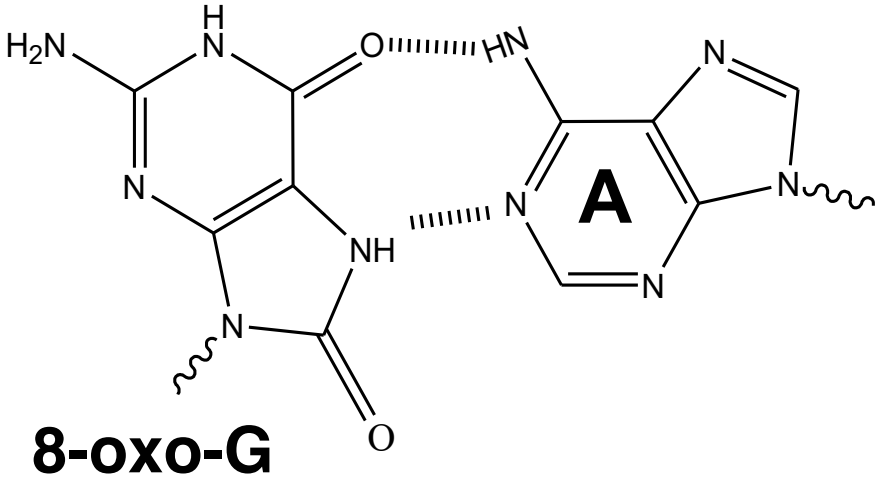
High fidelity
Taq

Error-Prone PCR

dCTP, dTTP ↑
dGTP, dATP ↓
Mg^{2+} ↑
Mn^{2+}

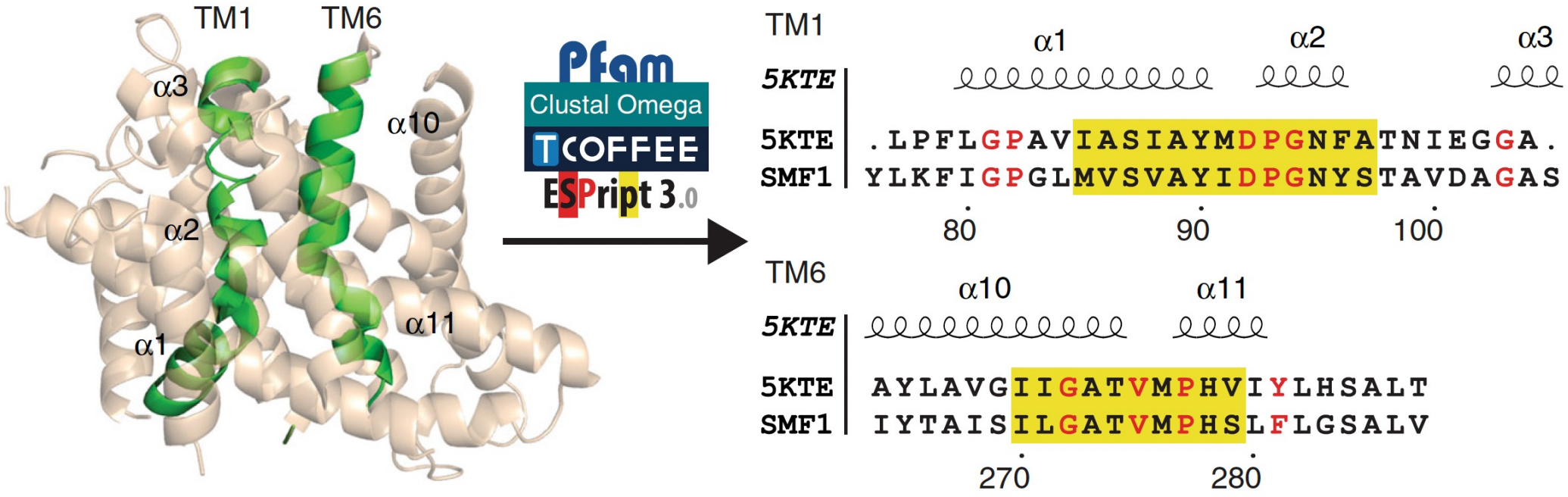
Low fidelity
Taq





Using rational design for selective mutations

- Examine what is known about target:
  - Function
  - Structure
  - Orthologs



Pros and Cons of mutagenesis strategies

Random mutagenesis

Pros

- Don’t need as much information about the gene
- Unbiased screen casts a wider net

Cons

- Lots of “junk” to sort through
  - truncations, silent mutations, etc…
- Multiple mutations in a gene

Site directed mutagenesis

Pros

- Able to selectively create single mutations
- Smaller population to screen for effects

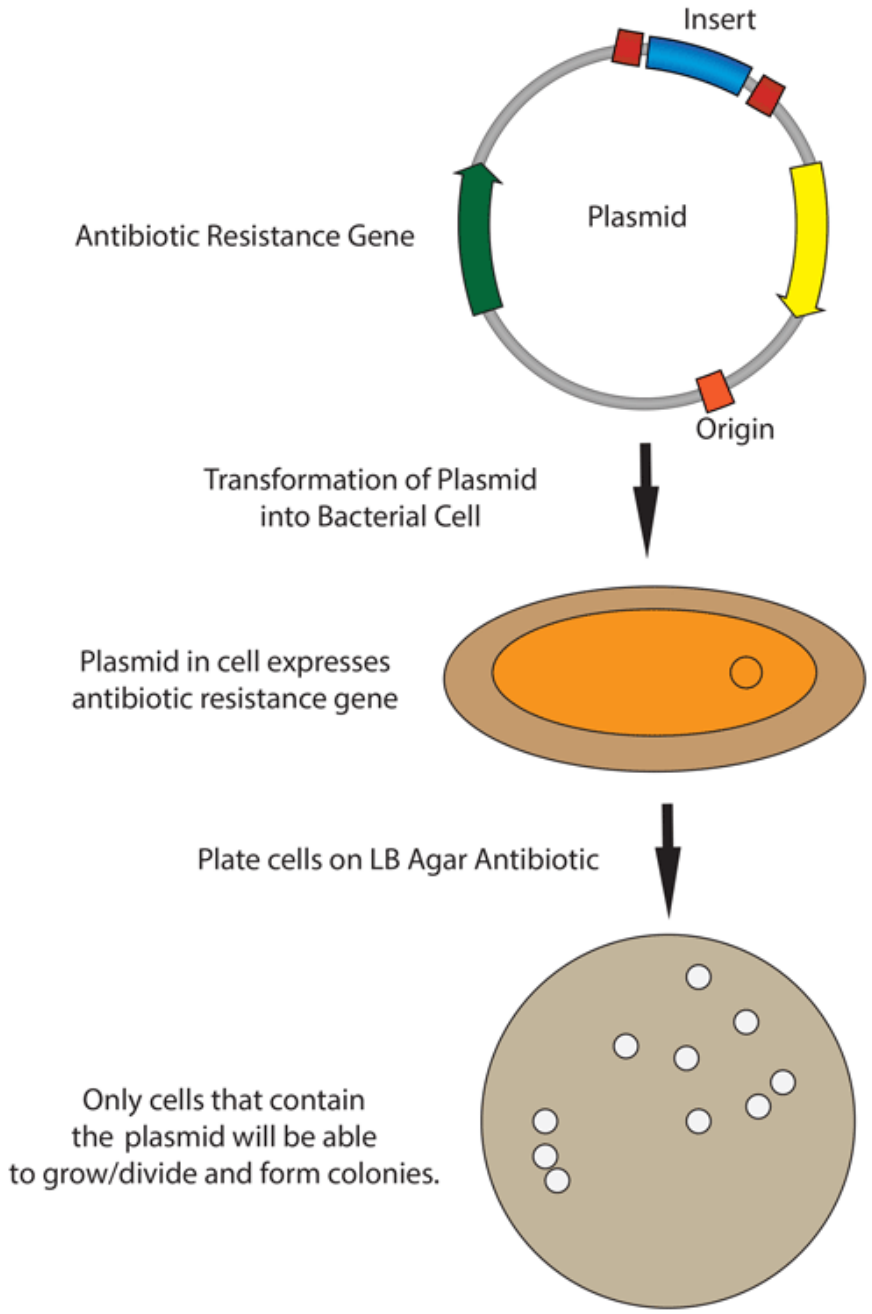
Cons

- Requires more background planning
- Easy to miss a potentially valuable mutant

What are you doing in lab?

- Follow through with the actual mutagenesis based on your rational design

- Transform your mutagenesis mixture into *E. coli*
  - Will purify colonies from this transformation to identify plasmid that has your mutation



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