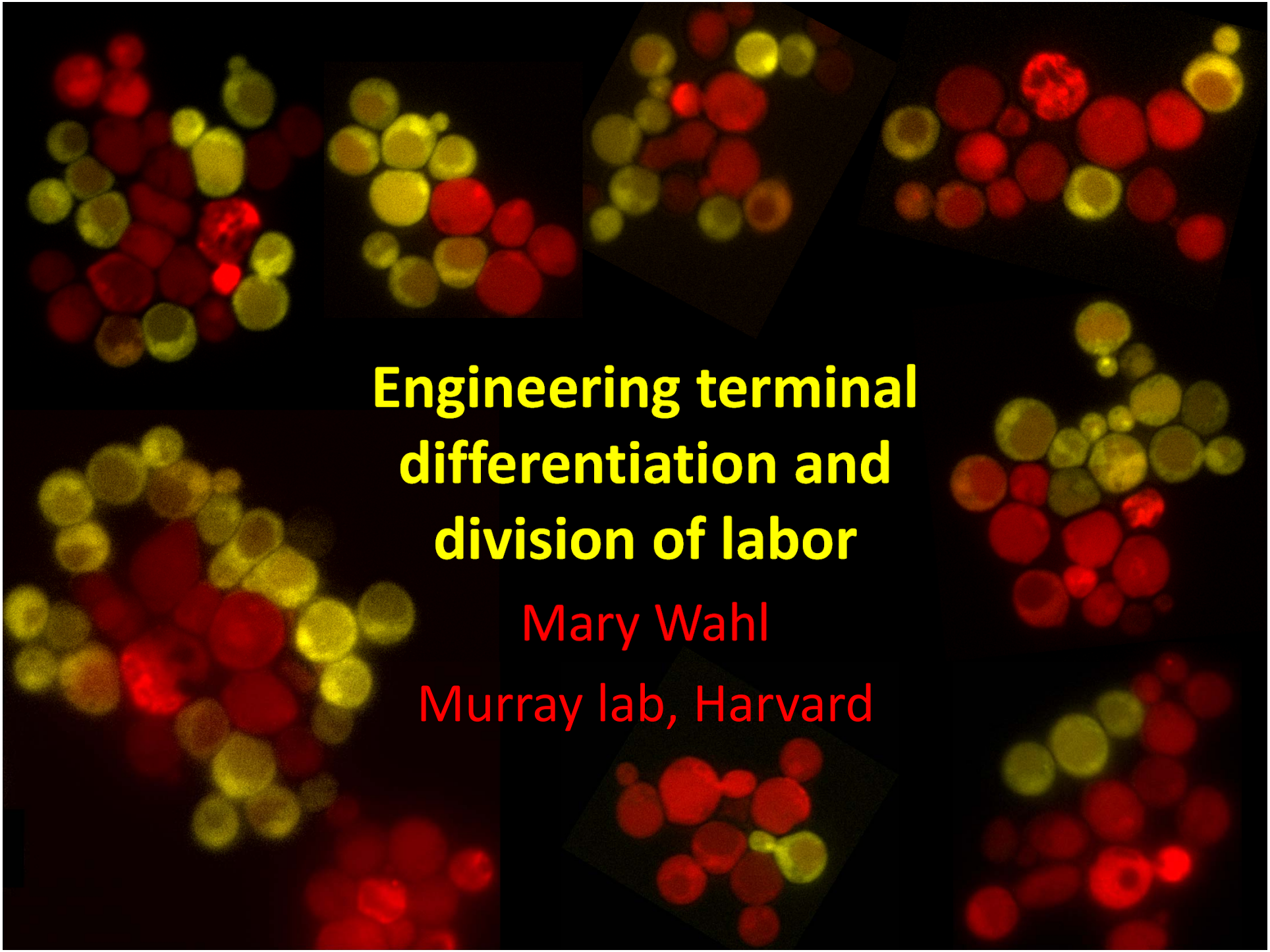
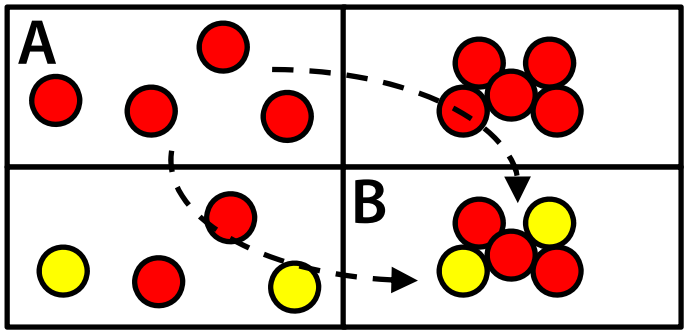
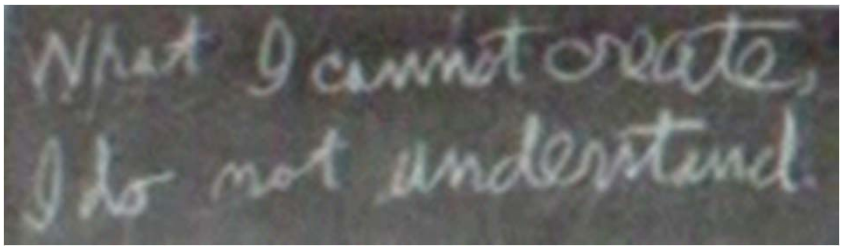

Engineering terminal differentiation and division of labor
Mary Wahl
Murray lab, Harvard

Why engineer differentiation/division of labor?

Model system for hypothesis testing

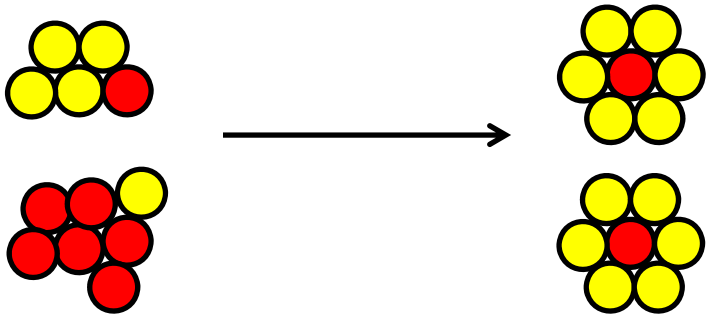


Bioengineering proof-of-principle



- Feynman (attrib.)

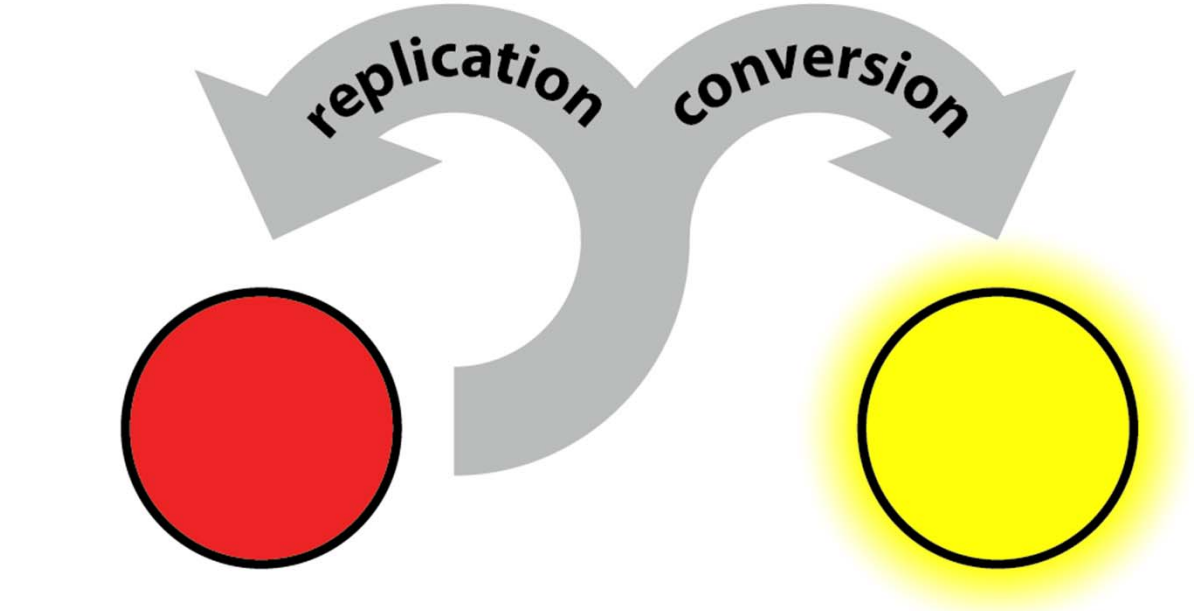
Starting point for experimental evolution



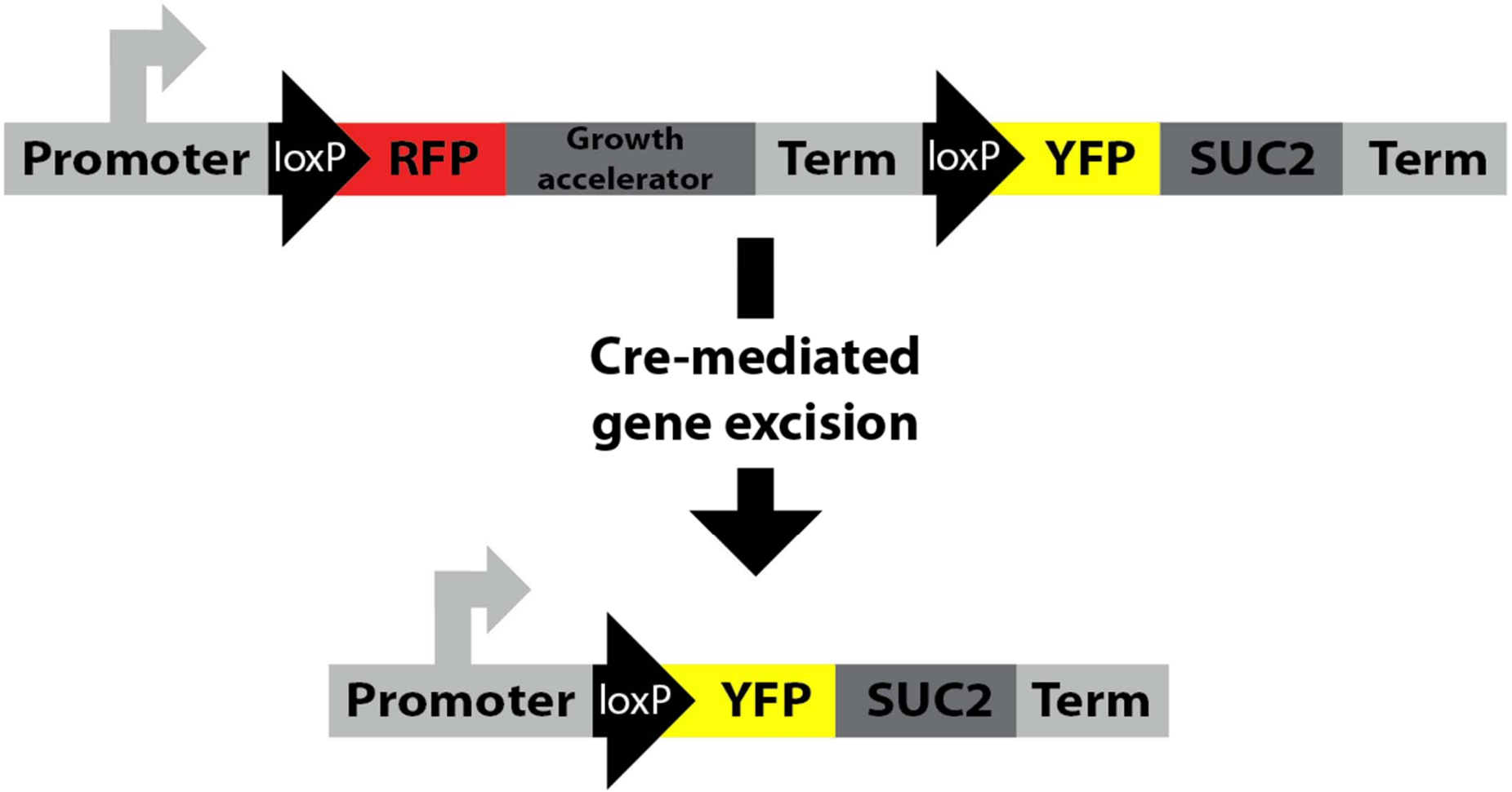
Terminal differentiation model: "germ-soma" division of labor

Necessary features:

- Irreversible differentiation
- Limited somatic cell growth
- Somatic cells help germ cells grow



A gene excision-based system for differentiation



Invertase secretion by somatic cells helps germ cells grow in sucrose media

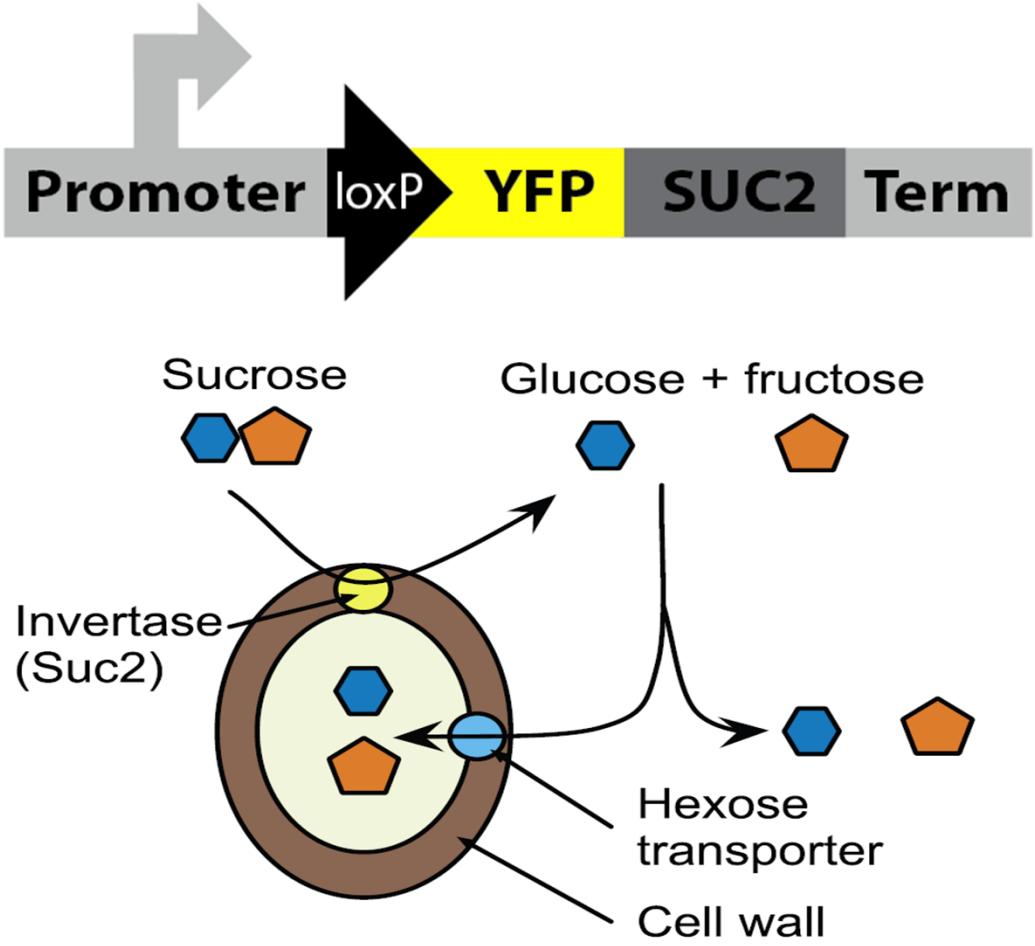
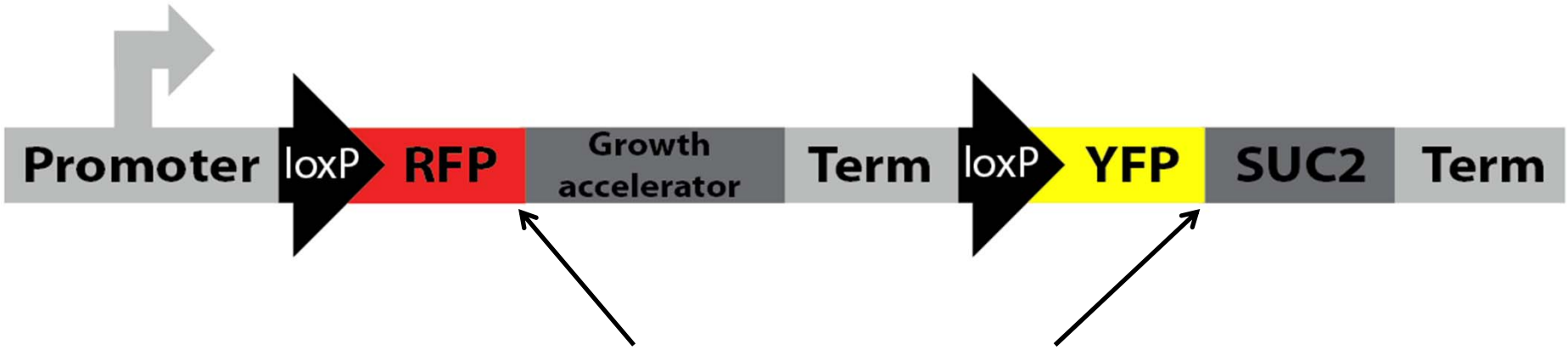


Figure from Koschwanez JH et al., 2011

Ubiquitin monomer linkers reduce troubleshooting of fusion proteins



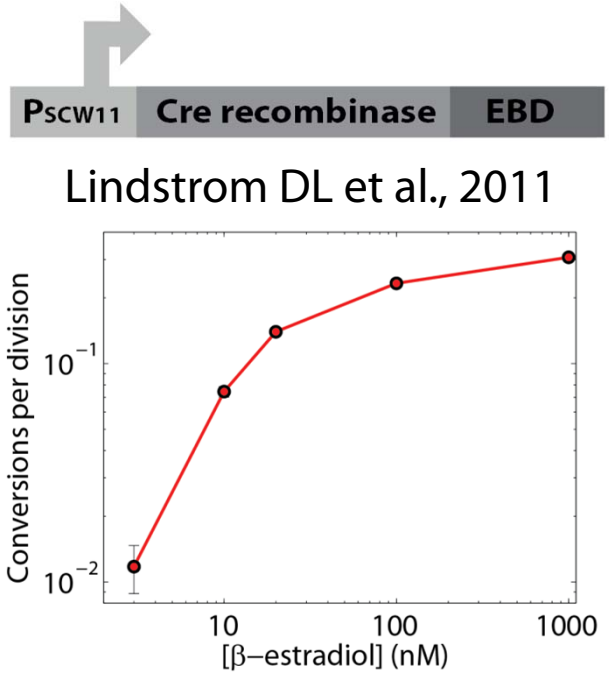
After cleavage by native yeast proteases:

- the C-terminal peptide has no tag and can still enter the secretory pathway
- the fluorescent protein retains the Ubq monomer and is active/stable

Other features of our system

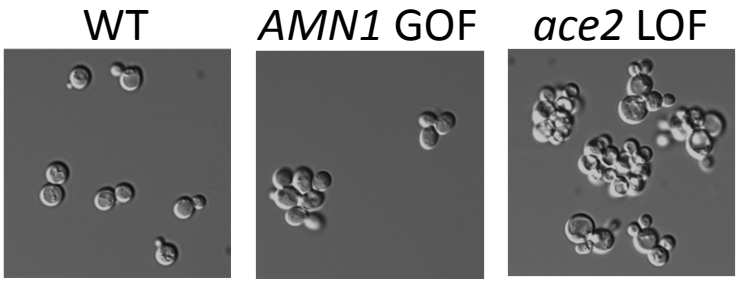
Conversion rate control:

- Only newborn daughters express Cre → "per division" conversion rates
- Conversion rate depends on inducer (β-estradiol) concentration

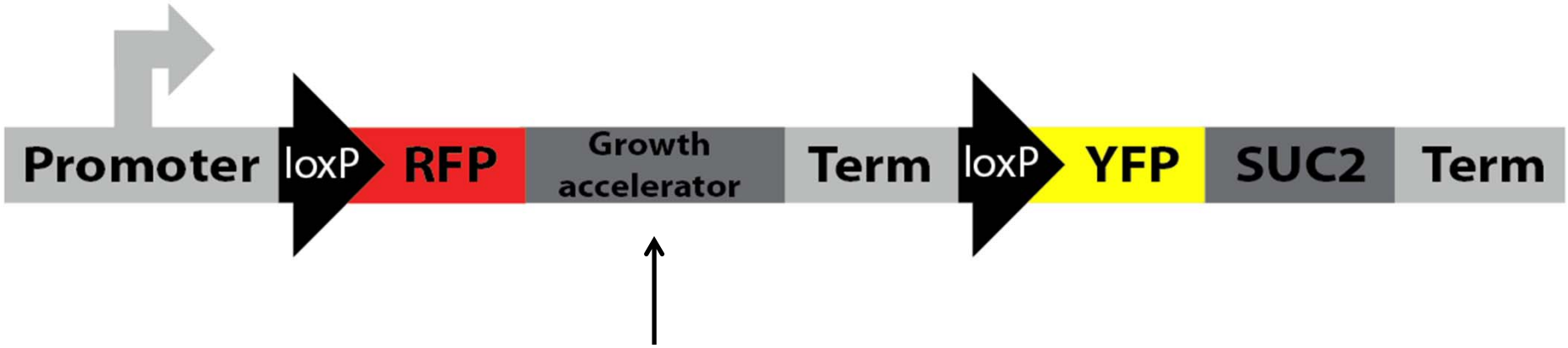


Variety of lifestyles:

- Unicellular
- Clonal groups
- Colonial growth
- Flocculation



Choosing the "growth accelerator"



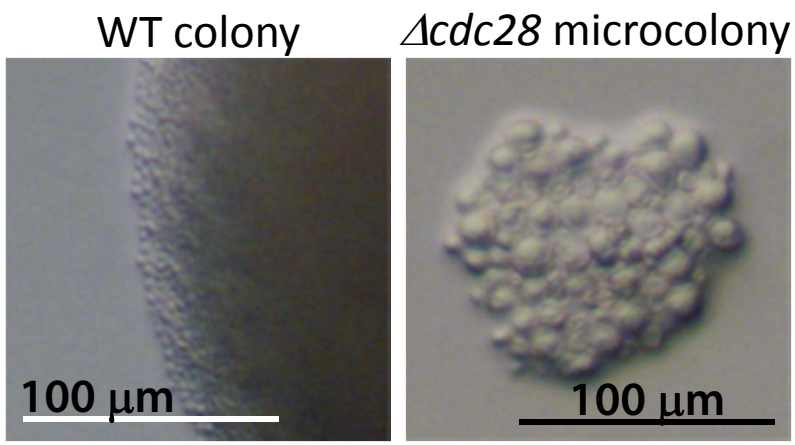
The "growth accelerator" gene can be:

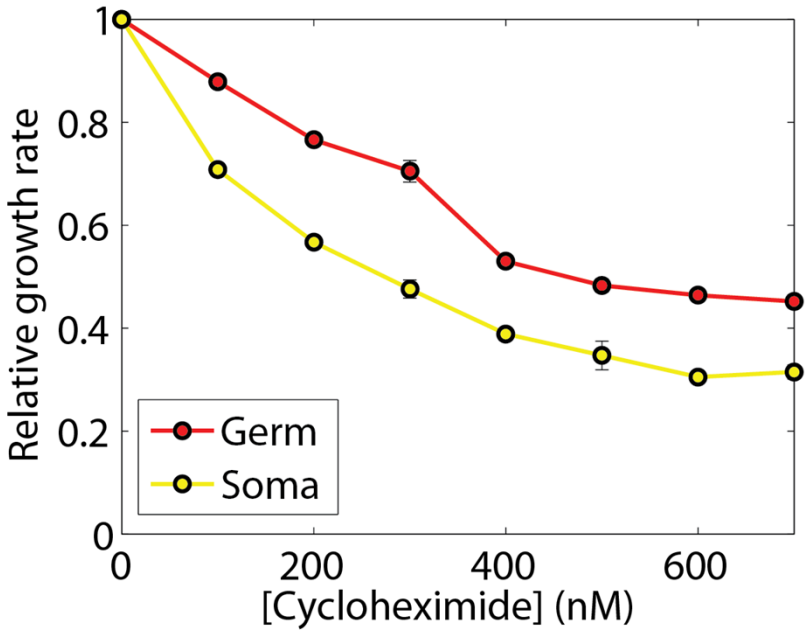
a) CDC28 – essential cell cycle gene; excision halts growth (if native CDC28 locus is deleted)

a) $cyh2^r$ – cycloheximide-resistant allele of ribosomal protein; excision slows growth in cycloheximide (if native copy of CYH2 is cycloheximide-sensitive)

Absence of the "growth accelerator" impedes somatic cell division

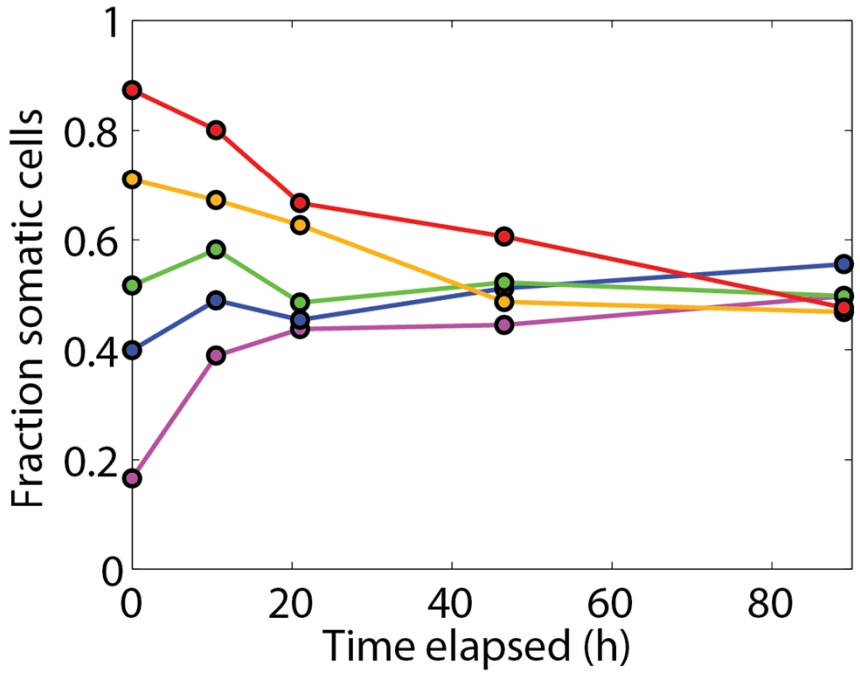
CDC28 *cyh2^{r}/cyh2^{s}*



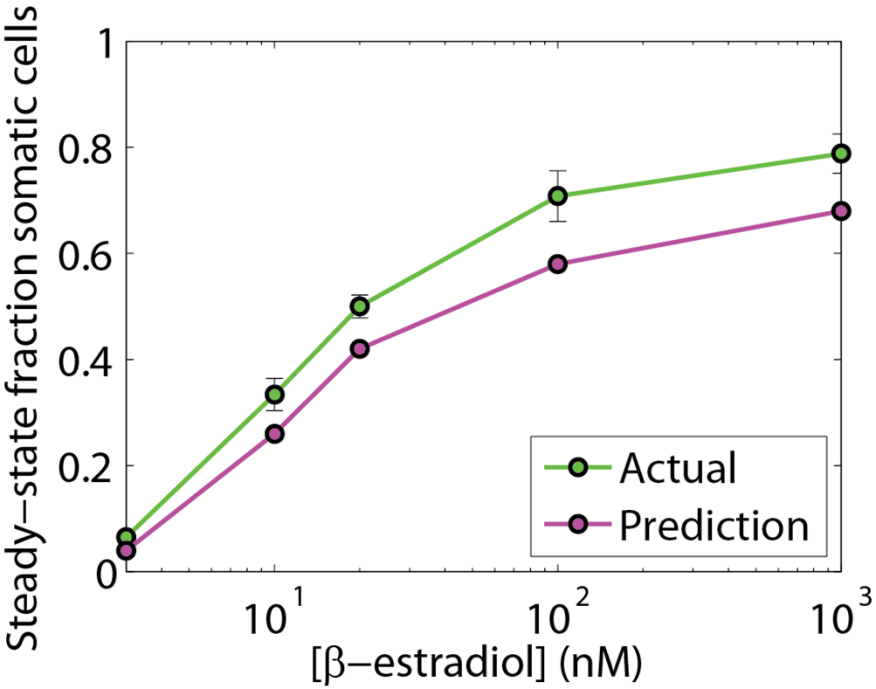


Cultures approach a steady-state ratio between cell types

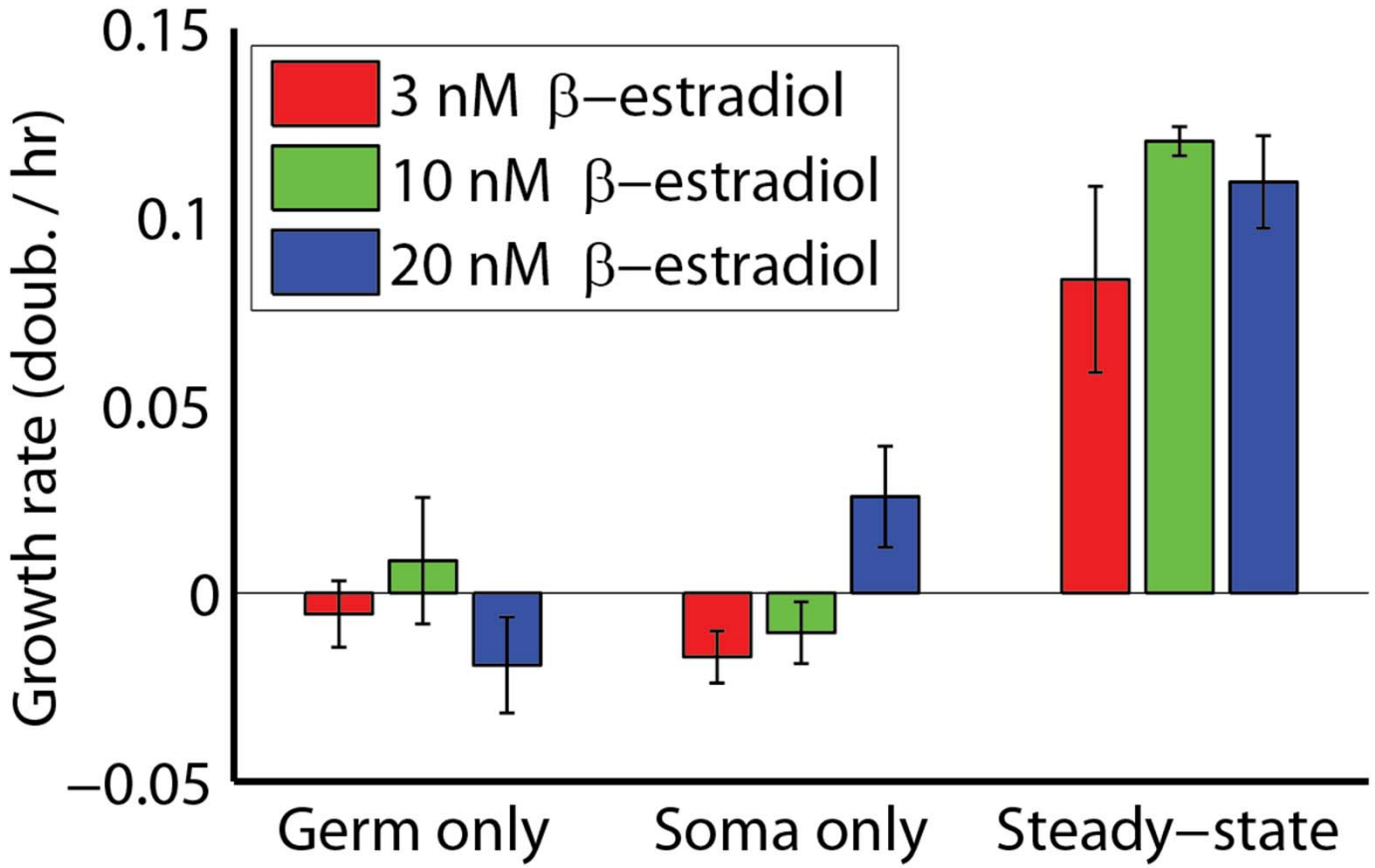
cyh2^{r}/cyh2^{s} in 500 nM cycloheximide



CDC28

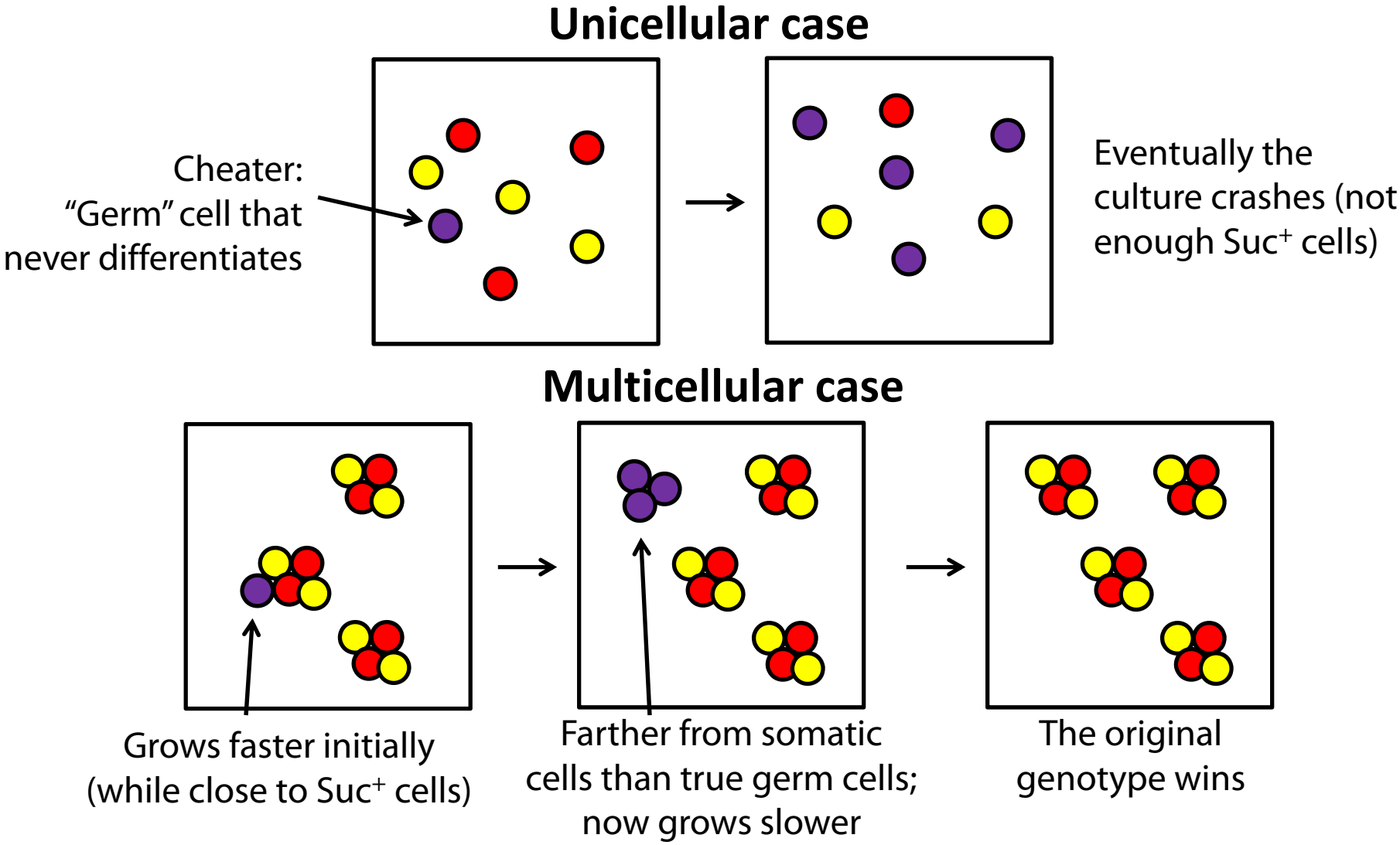


Cooperation between cell types

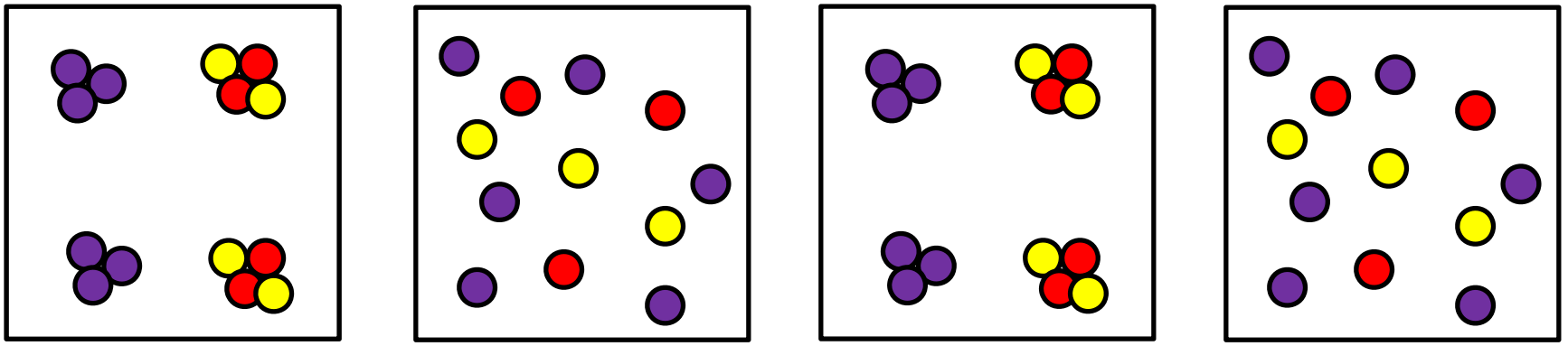


***cyh2*^{r}/*cyh2*^{s} in 0.5% sucrose with 500 nM cycloheximide**

Hypothesis testing: unicellular differentiation isn't an ESS



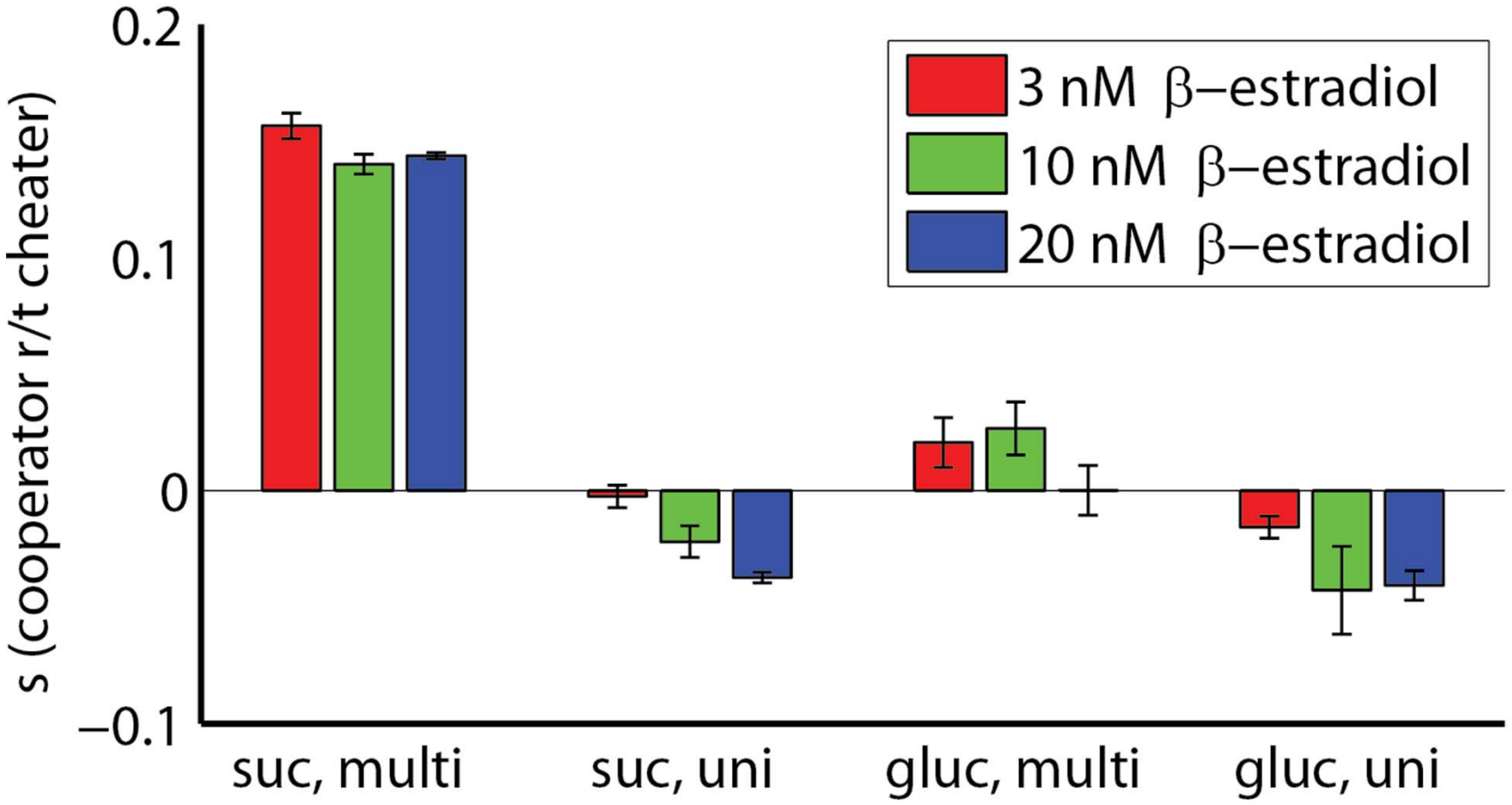
Competition assay design



We expect the cheater to increase in frequency:

- in the unicellular case, cheaters have the same access to monosaccharides as germ cells
- in glucose, conversions are always costly - somatic cells aren't useful

Competition assay results



Special thanks to:

Andrew Murray & co.
…esp. John Koschwanez
The Gottschling Lab
KITP Conference Organizers