Harnessing RNA-sequencing to study pH responses in the fungal pathogen *Cryptococcus neoformans*

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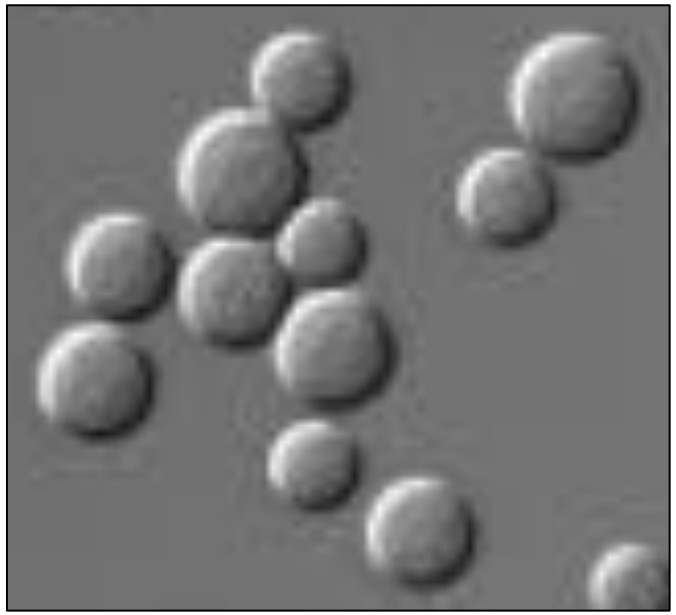
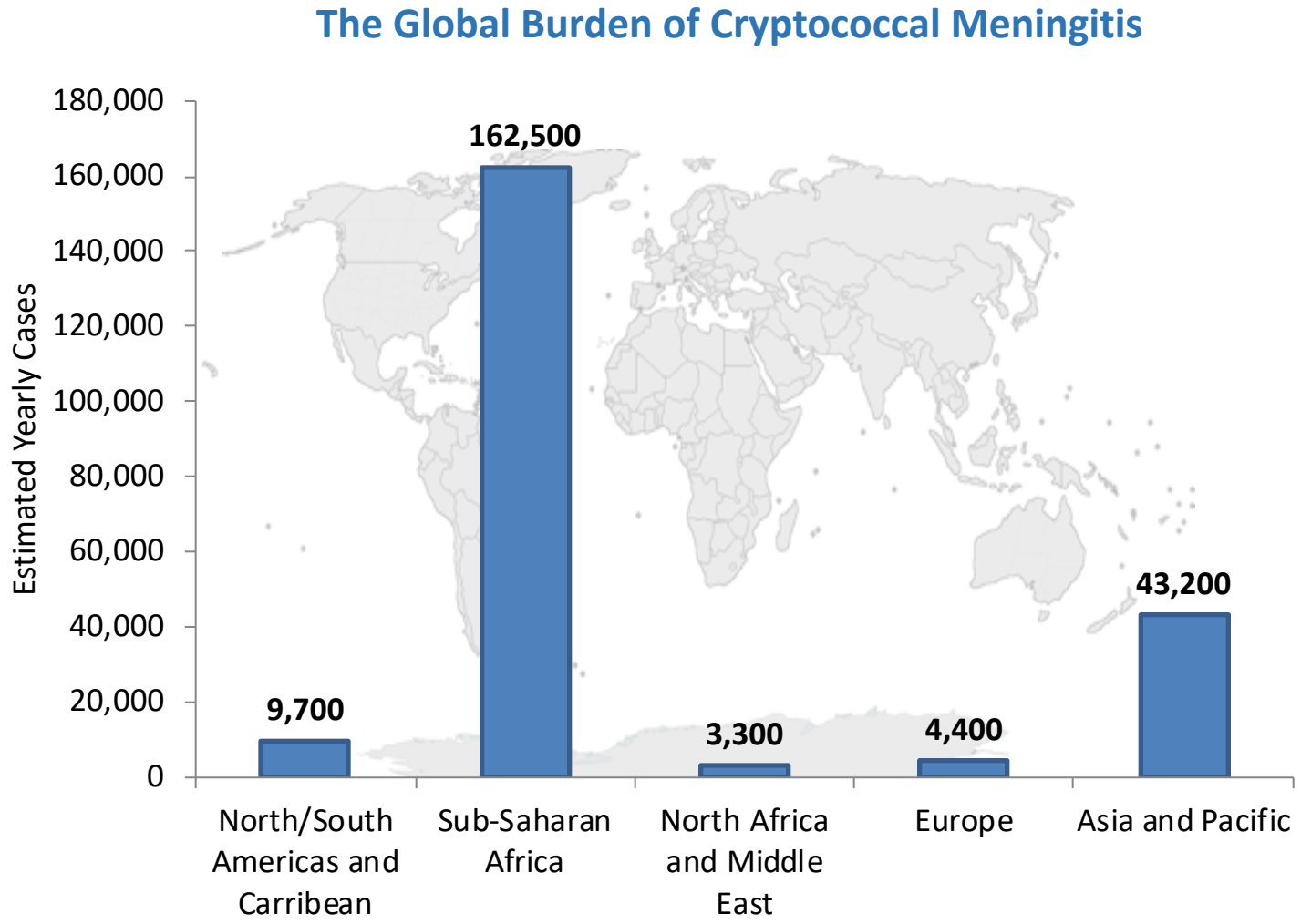
High-Throughput Sequencing Course

5/28/2019

Cryptococcosis remains a major problem in resource-limited regions

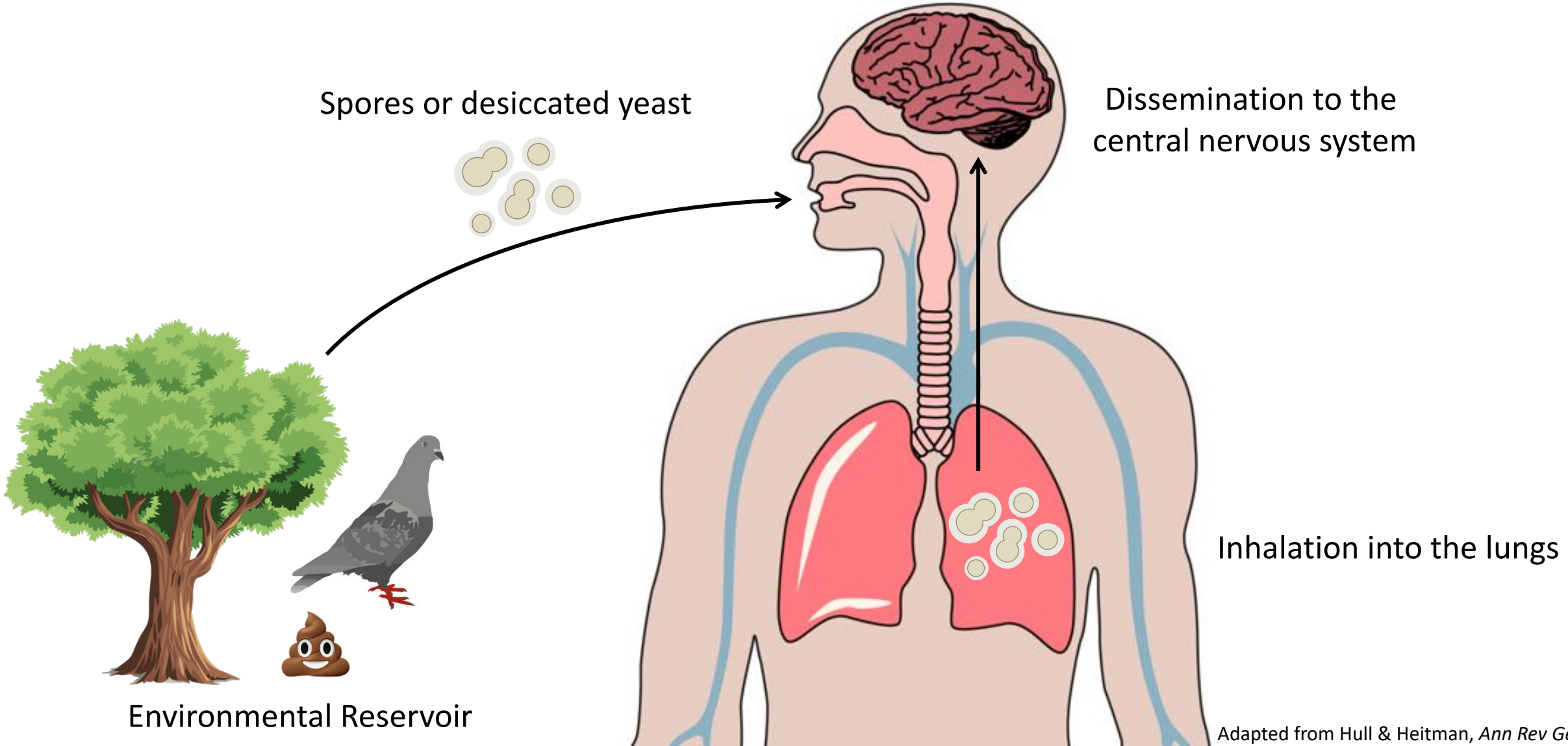


Cryptococcus neoformans



Adapted from Rajasingham *et al., Lancet Infect Dis*, 2017

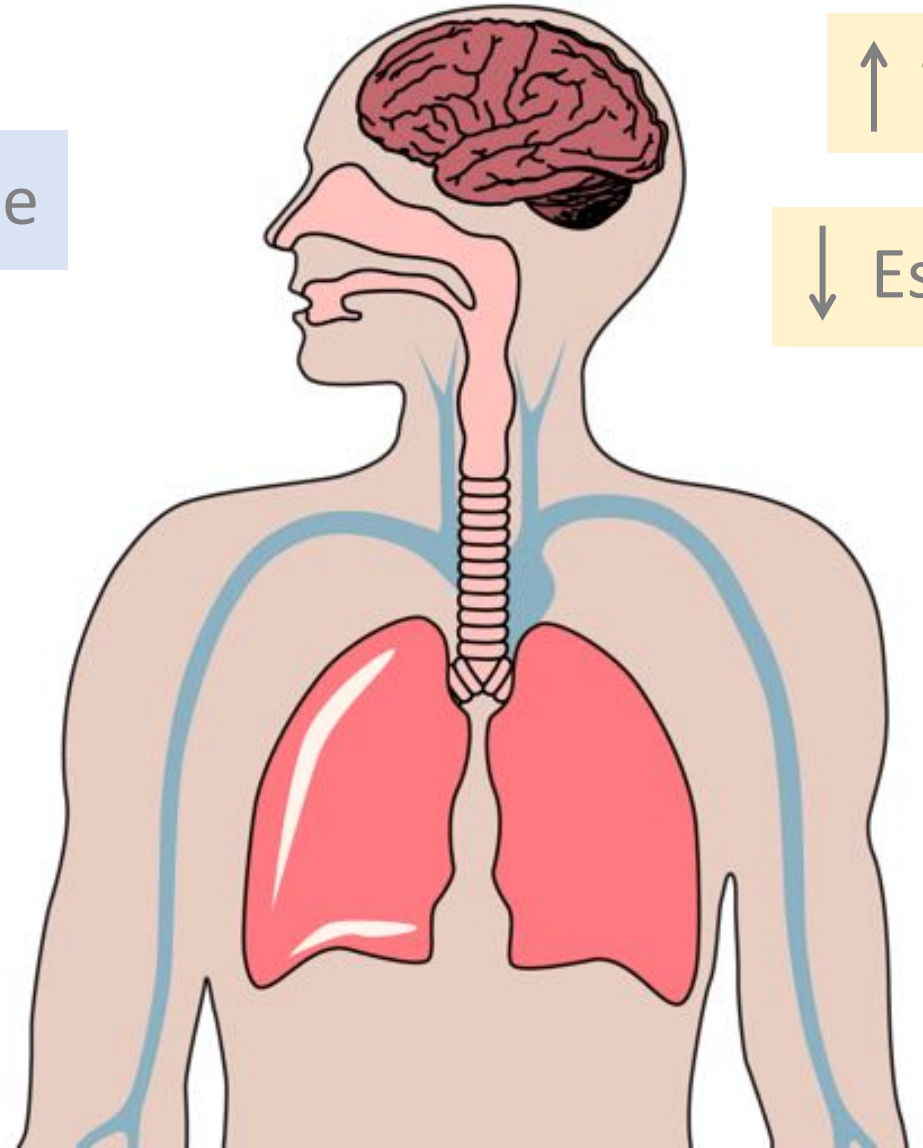
Cryptococcus neoformans is an environmental fungus



Adapted from Hull & Heitman, *Ann Rev Genet,* 2002

Fungal pathogens must adapt to their hosts

The Host Immune Response



↑ Temperature

↓ Essential nutrients

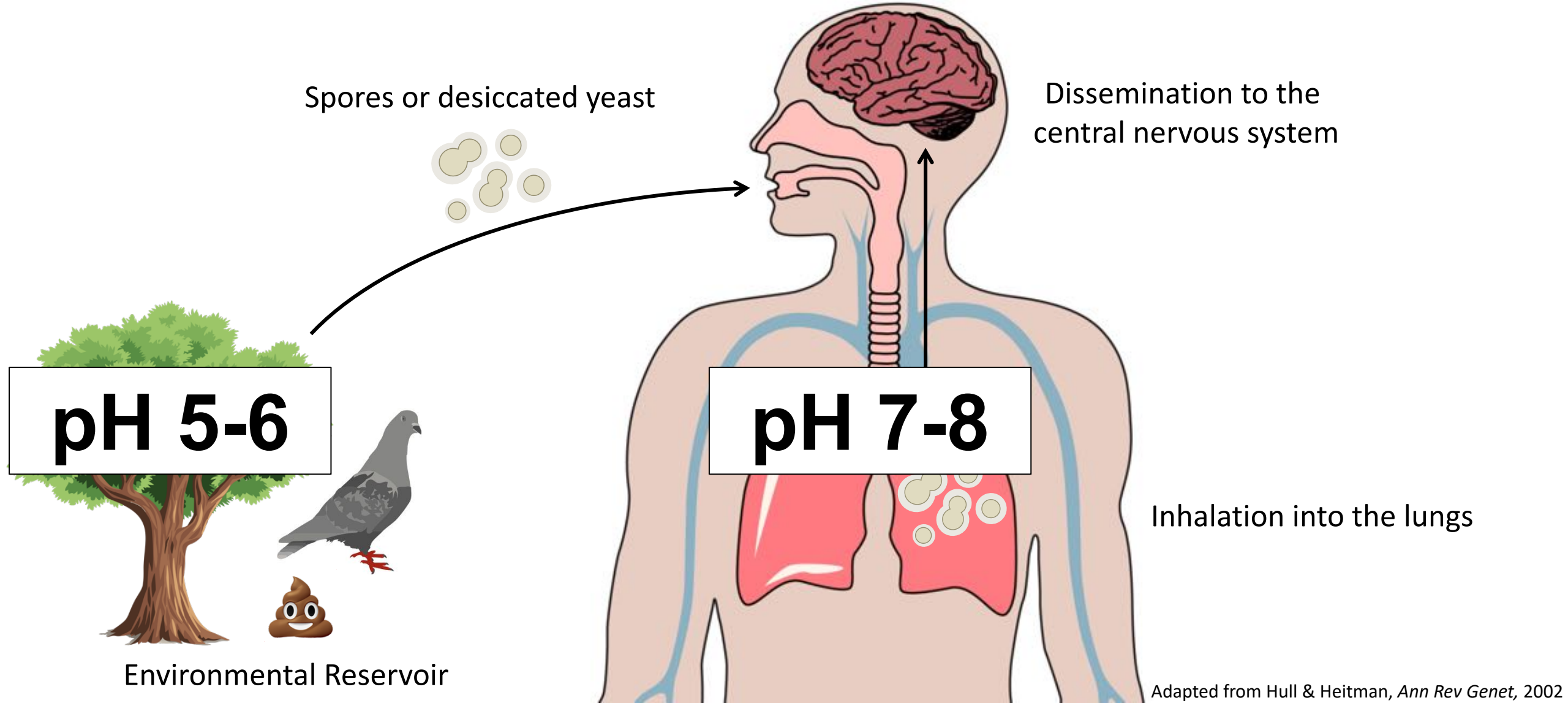
≷ Essential metals

↑ pH

↑ CO_2

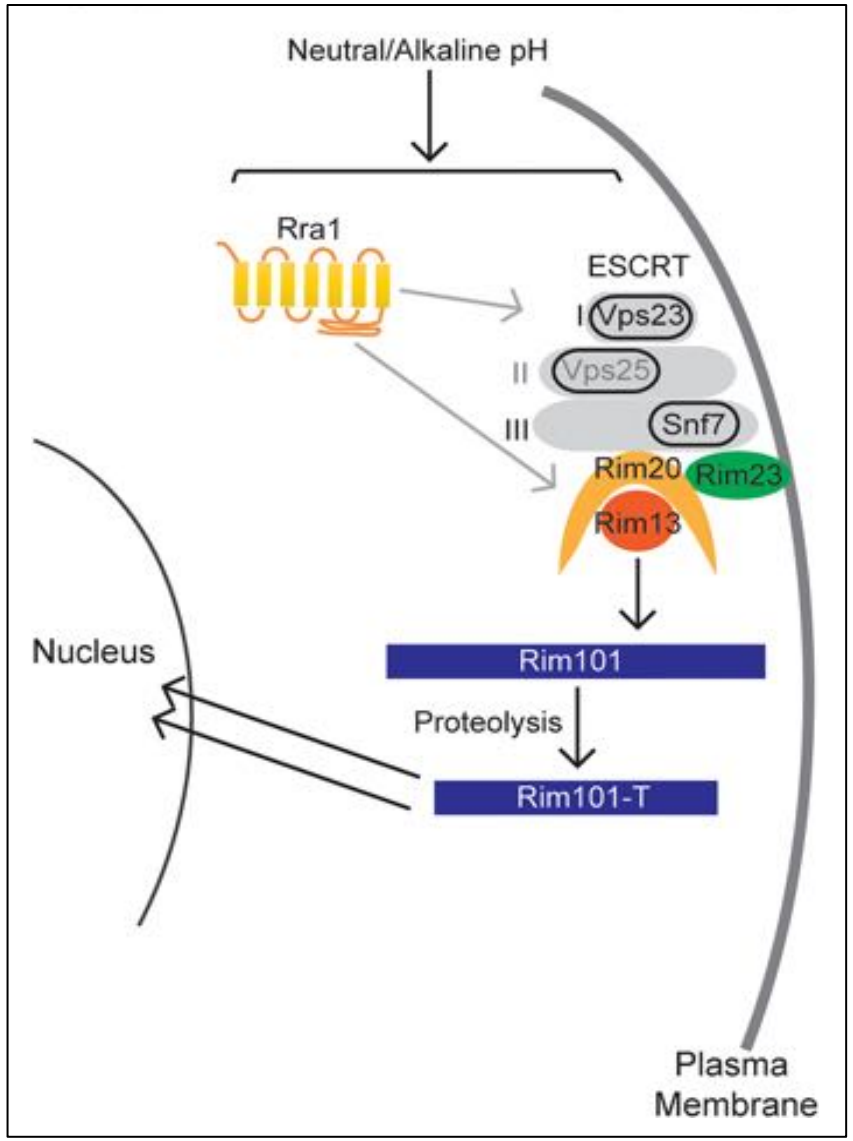
↓ O_2

Cryptococcus neoformans is an environmental fungus



Adapted from Hull & Heitman, *Ann Rev Genet,* 2002

The Rim pathway is the only characterized alkaline pH-sensing pathway

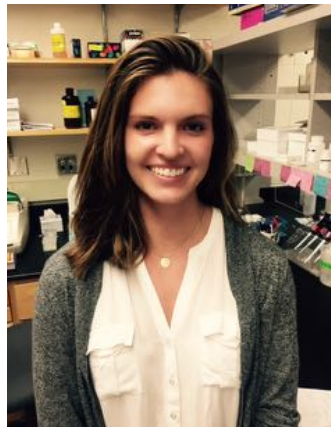


In alkaline pH, cells undergo changes that cannot be attributed to the Rim pathway

Stains with mutations in genes not involved in the Rim pathway show alkaline pH sensitivity

Ost et al., *PLoS Pathog.*, 2015

What Rim-independent pathways regulate the alkaline pH response?

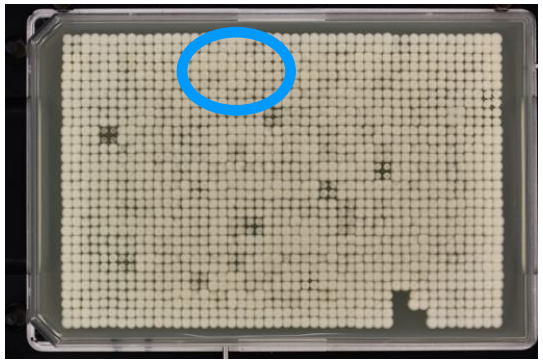


Hannah Brown

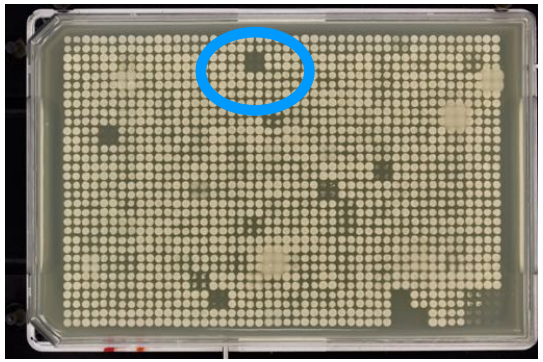
Random mutagenesis

Forward genetic screen to identify mutants with sensitivity to alkaline pH

SRE1

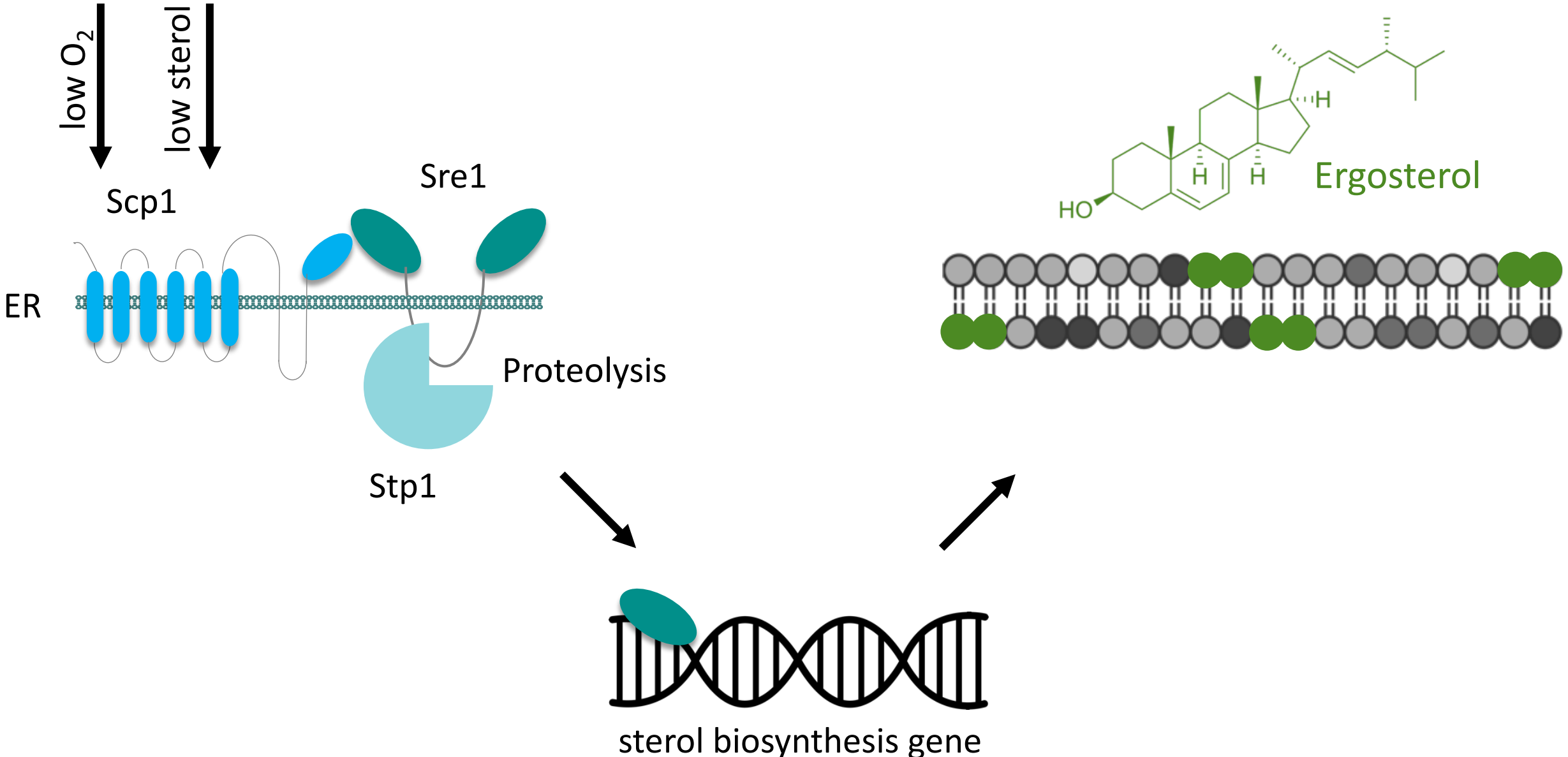


pH 5.5



pH 8

SRE1 is involved in sterol homeostasis in fungi



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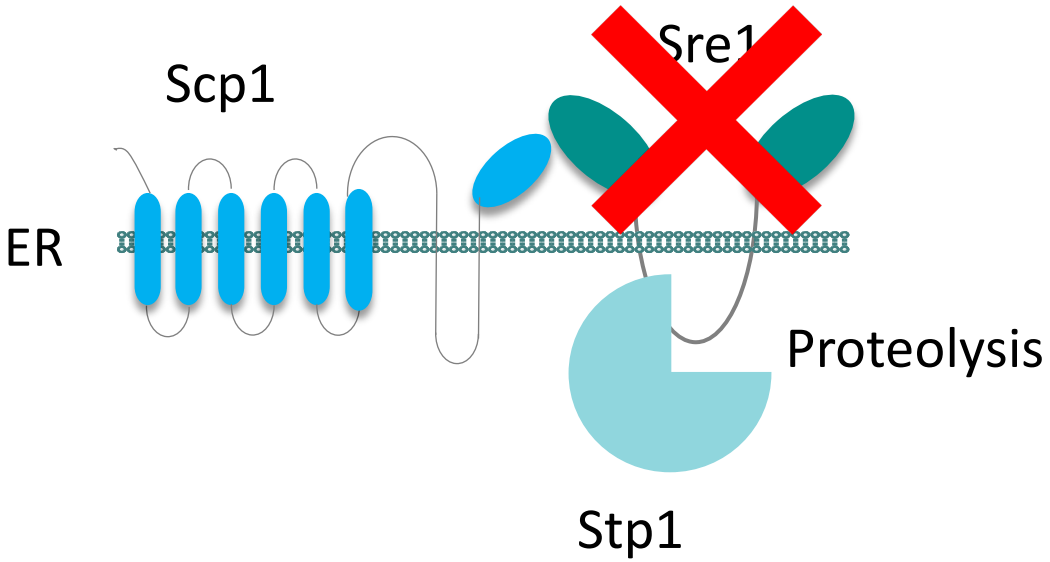
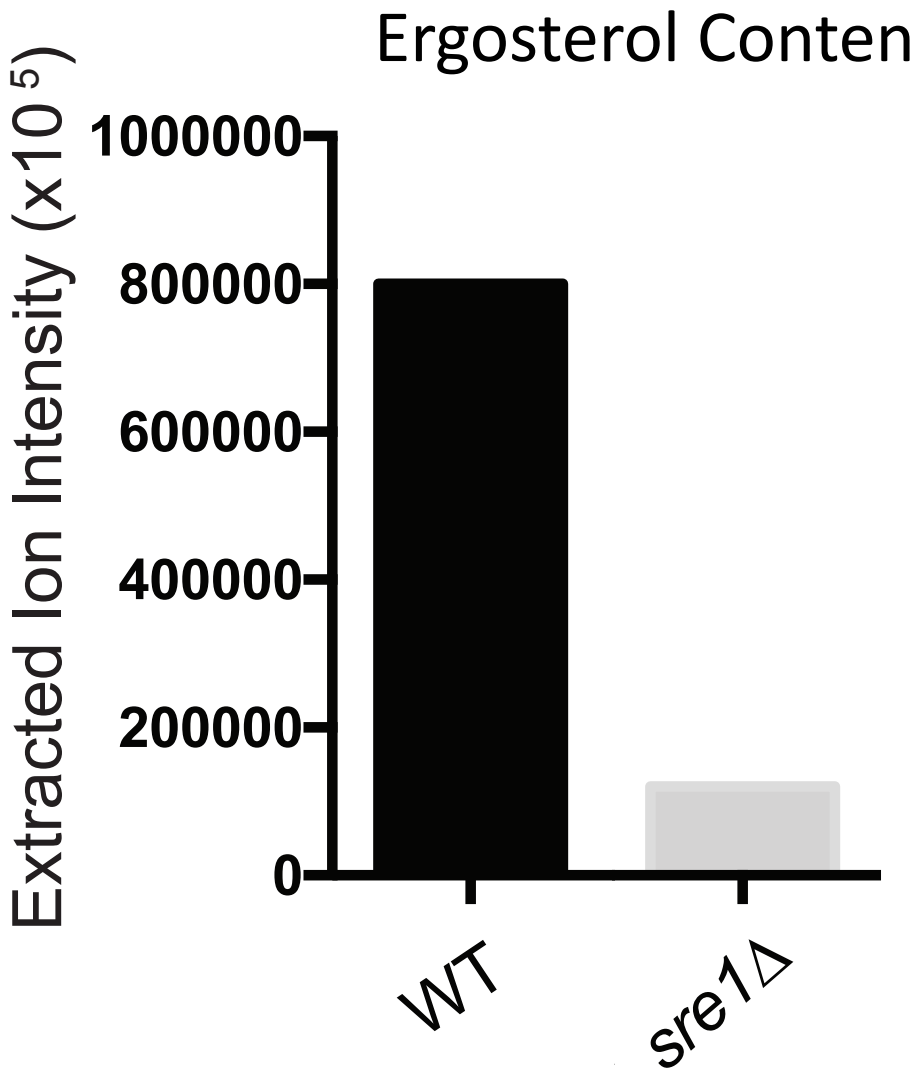
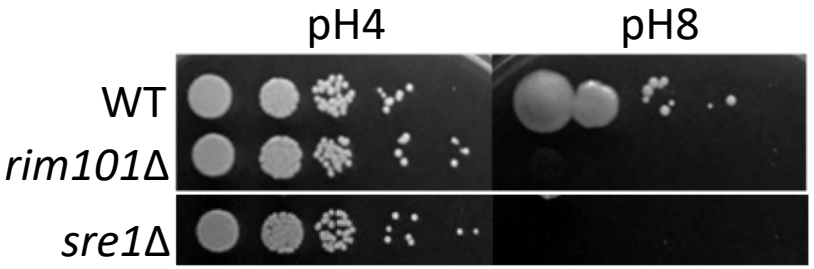
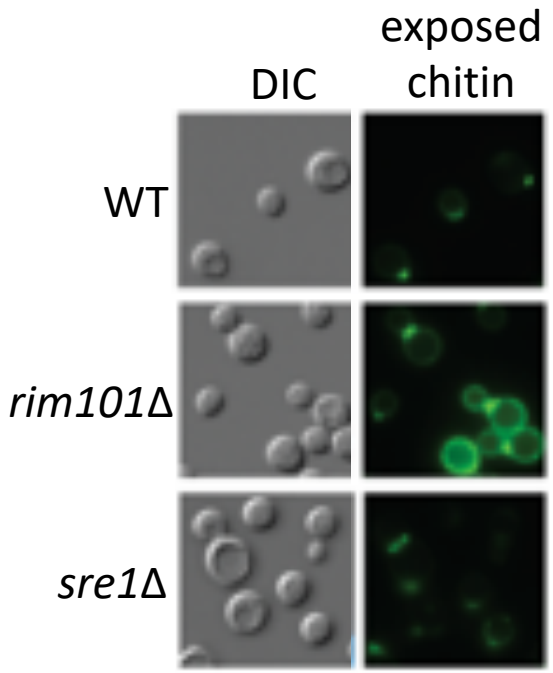


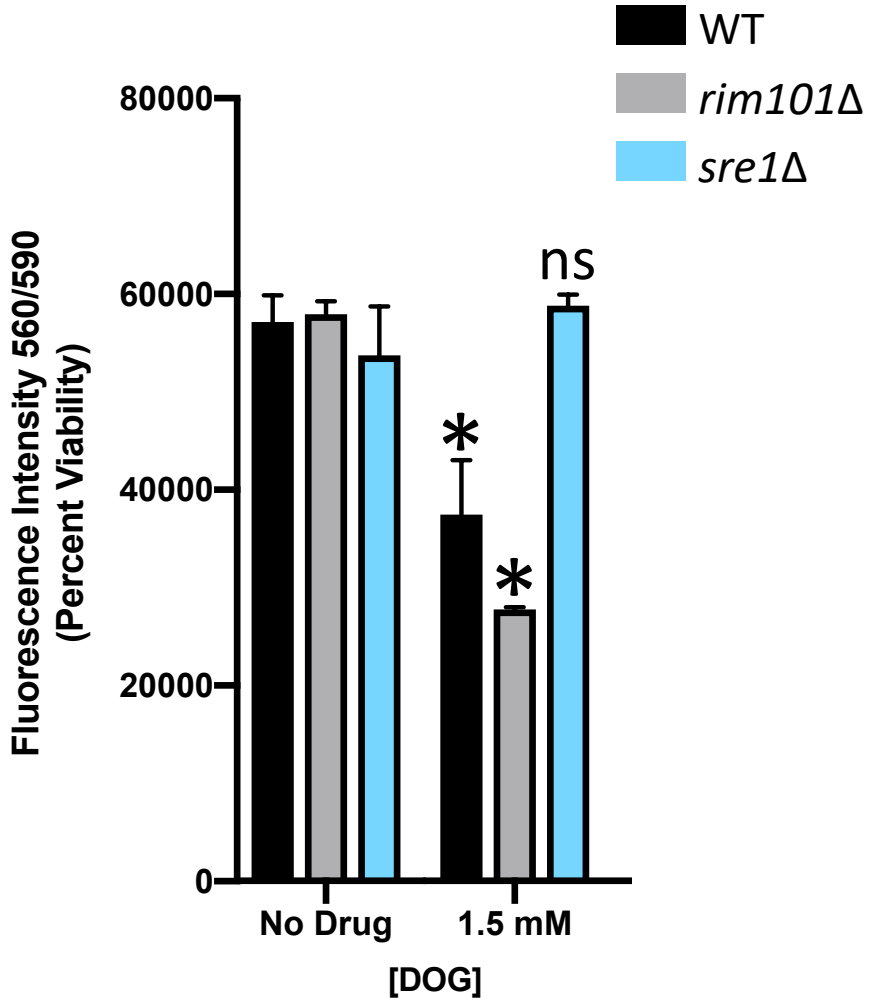


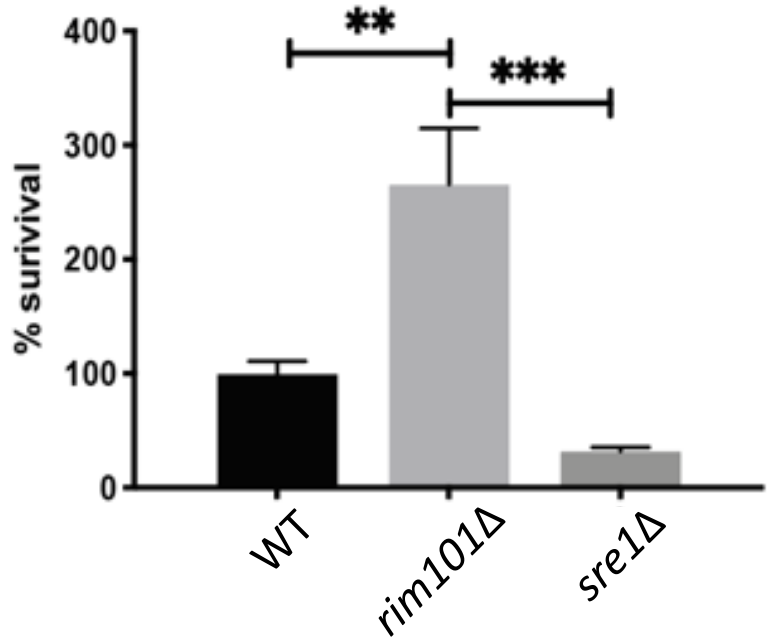
Hannah Brown, unpublished

SRE1 plays a Rim-independent role in alkaline pH-sensing in *C. neoformans*



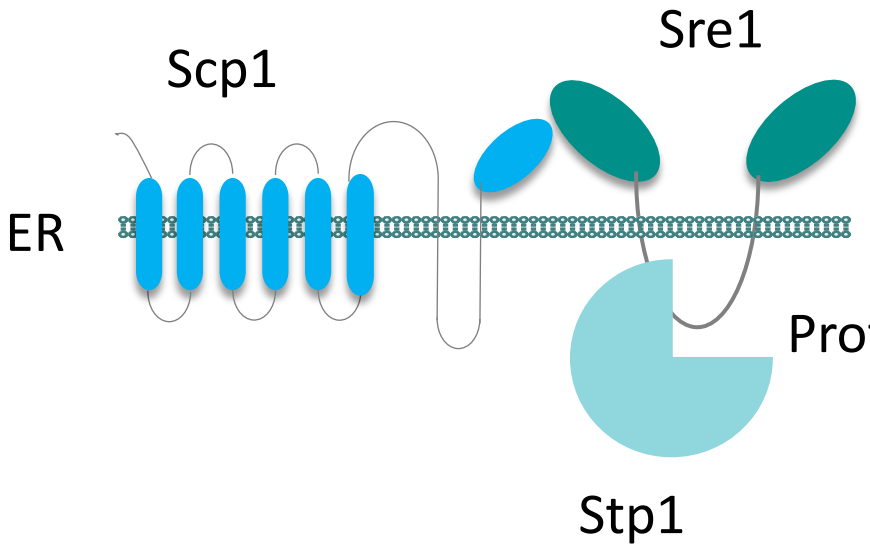


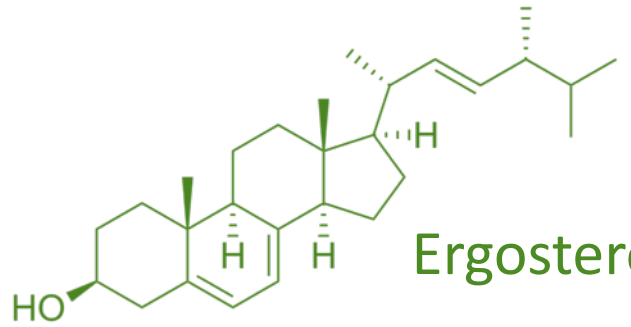




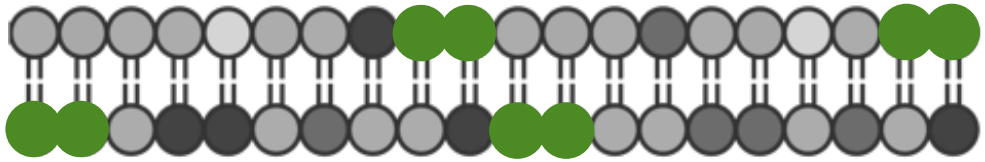
Hannah Brown, unpublished

How does sterol homeostasis contribute to alkaline pH-sensing?





Ergosterol



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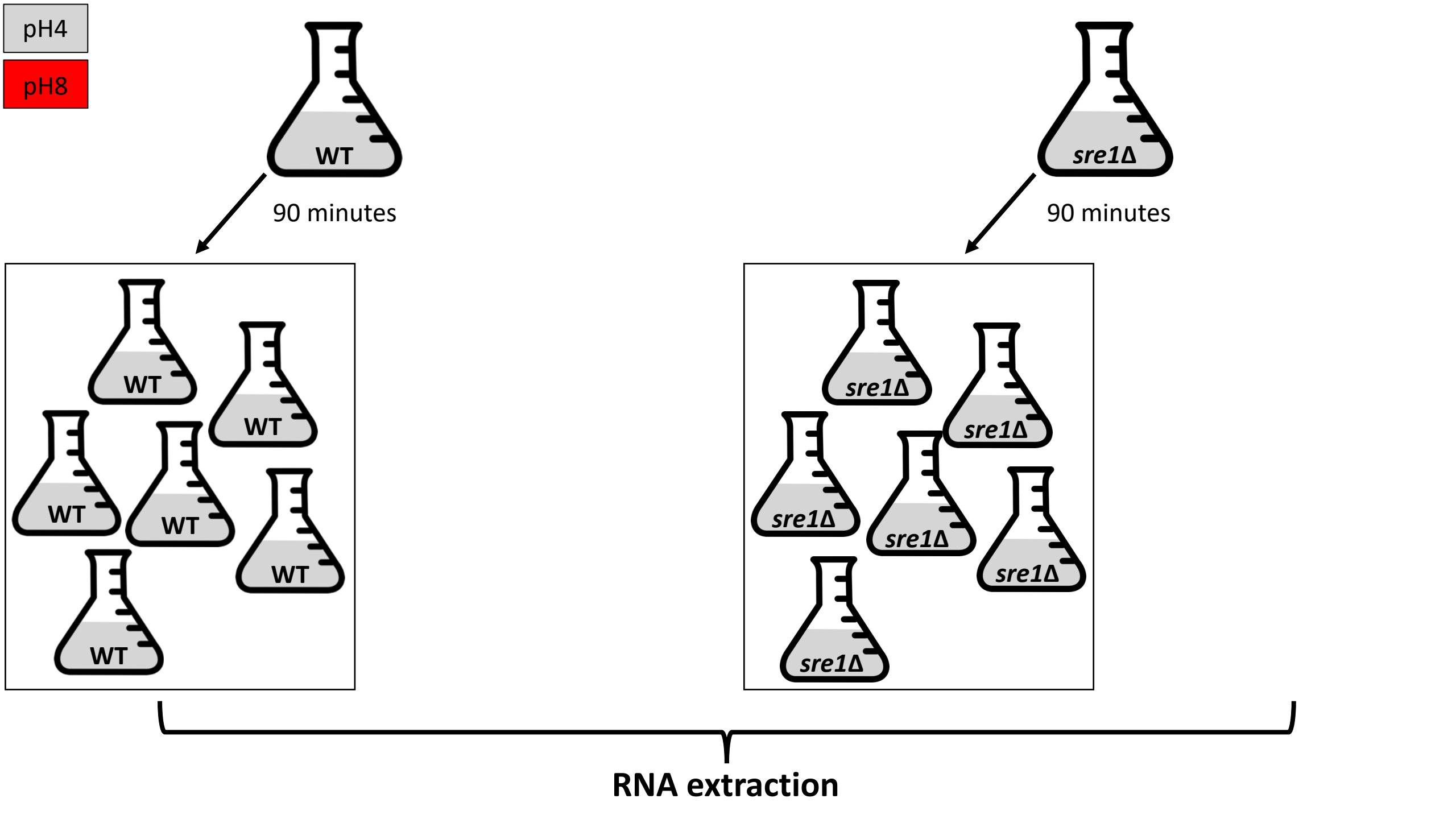
alkaline pH sensing/response


pH4
pH8
WT
90 minutes
sre1Δ
90 minutes
WT
WT
WT
WT
WT
WT
sre1Δ
sre1Δ
sre1Δ
sre1Δ
sre1Δ
sre1Δ
sre1Δ
RNA extraction

RNA-sequencing can be used to identify and understand novel pH-sensing mechanisms

- Sterol and cell membrane synthases
- Polysaccharide and lipid metabolism
- Rim pathway components
- Genes we have not yet investigated
  - other transcription factors
  - cell surface receptors

} Hypothesis generators!