Real time analysis of gene expression in living yeast cells: Novel approach for accurate cell damage detection

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Saccharomyces cerevisiae as a *Model Organism*

STRESS RESPONSE

BIOMEDICAL RESEARCH
TOXICOLOGY
DRUG DISCOVERY

BASIC RESEARCH

Yeast: A model for understanding cellular stress responses

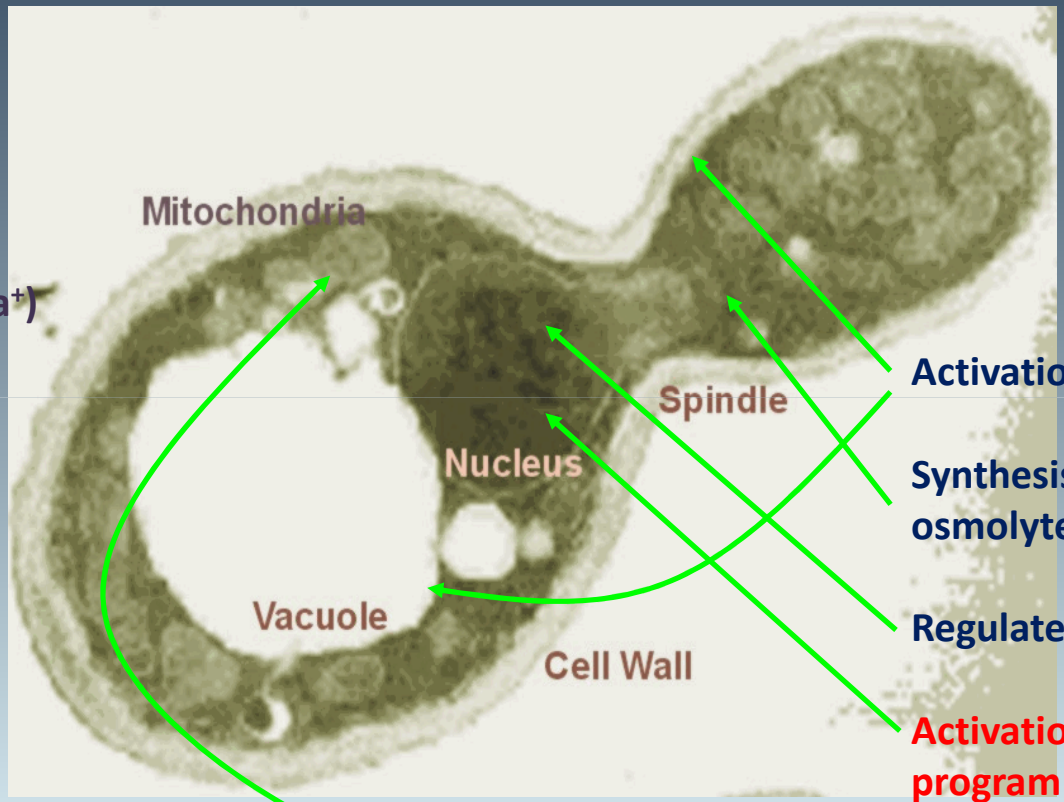
Hyperosmotic Stress

-Water efflux

-Cell shrinking

-Ionic imbalance (Na^+)

-Oxidative damage



Defense Mechanisms

Activation of ion transport

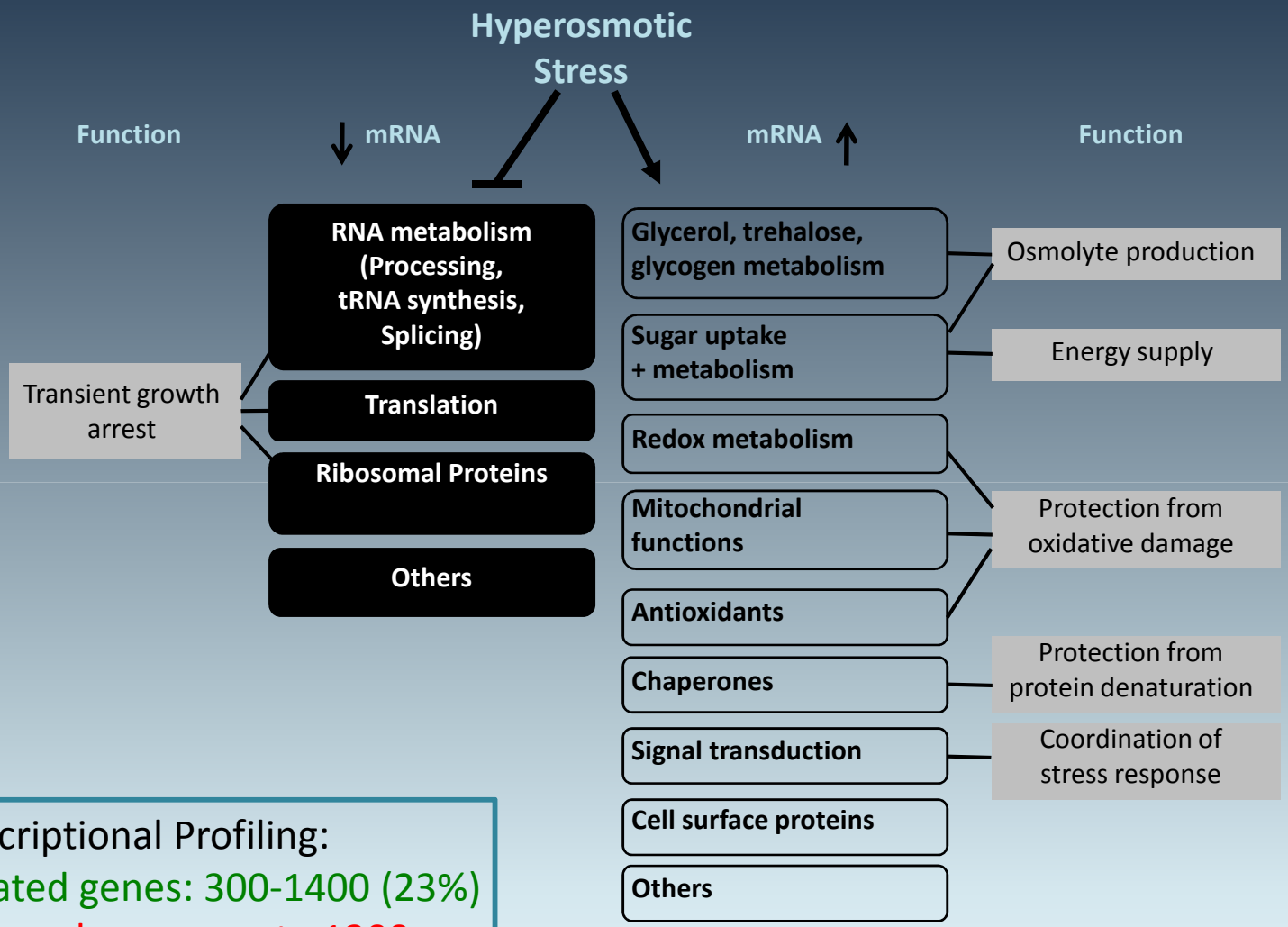
Synthesis/accumulation of osmolytes (glycerol)

Regulated cell cycle arrest

Activation of transcriptional program

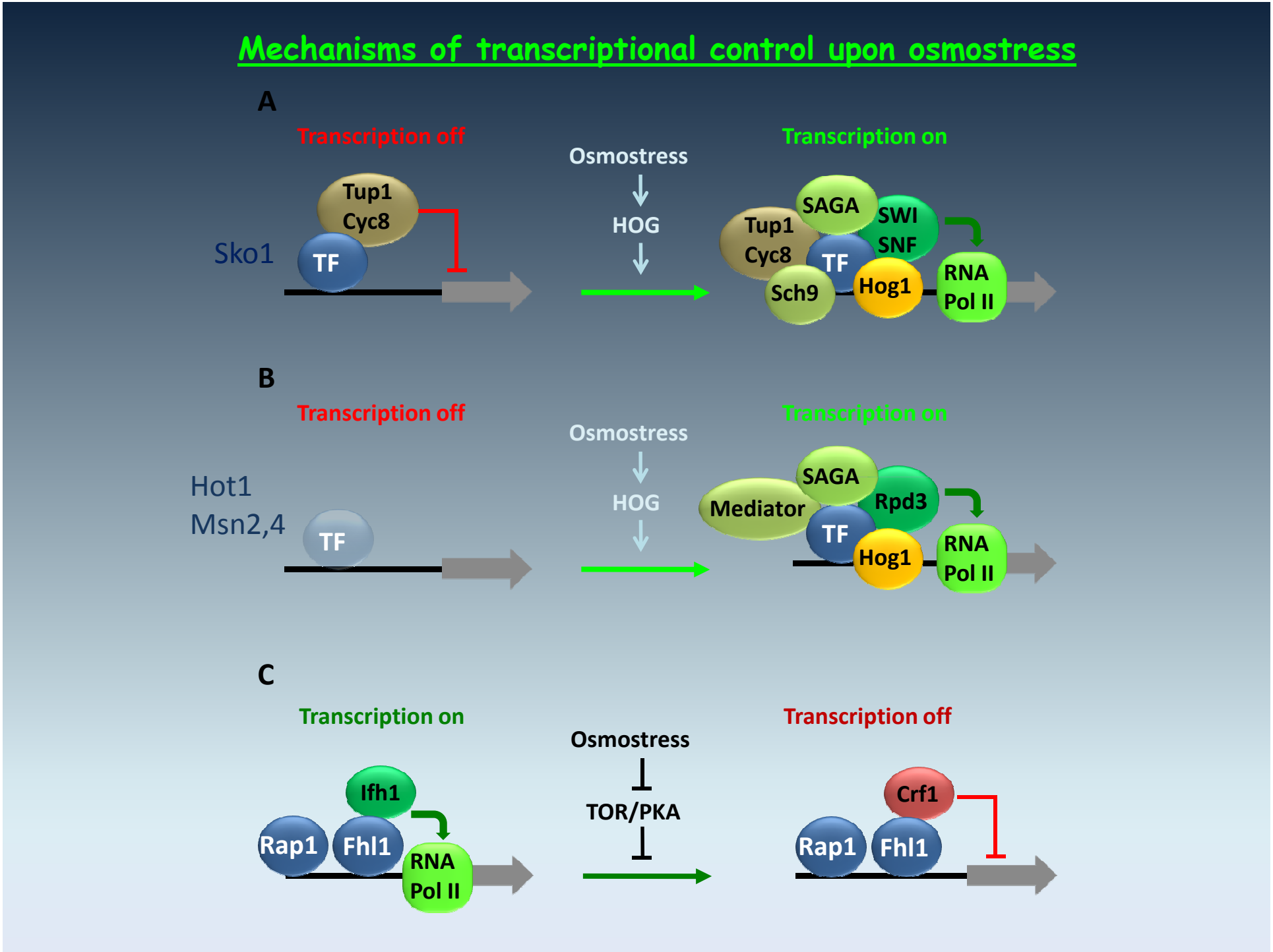
Modulation of mitochondrial activity

The transcriptional program upon osmostress in yeast



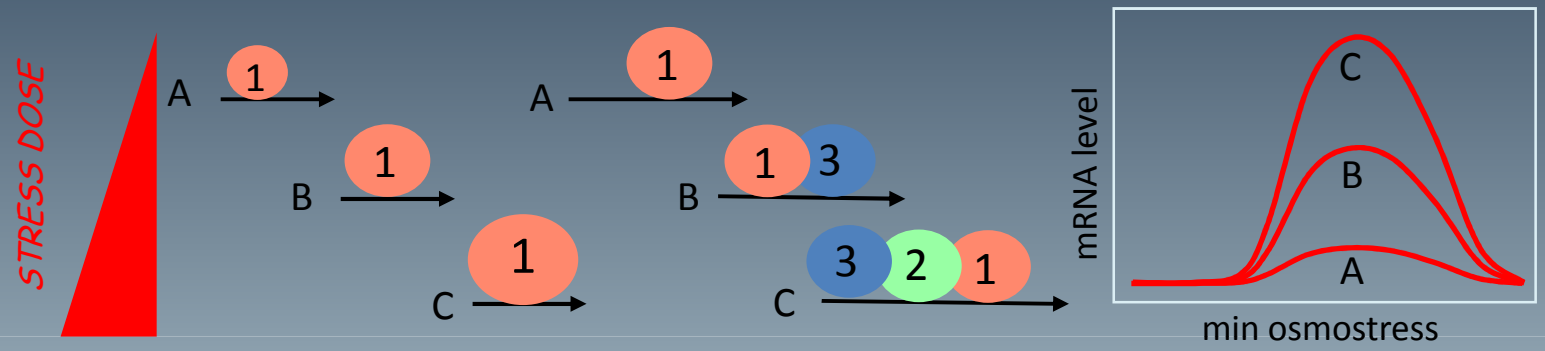
Transcriptional Profiling:
Activated genes: 300-1400 (23%)
Repressed genes: up to 1300

Mechanisms of transcriptional control upon osmostress



How is gene expression fine tuned during environmental changes?

Modes of dynamically modulate transcription at different stress doses



-How is promoter activity gradually regulated over a range of stress doses?

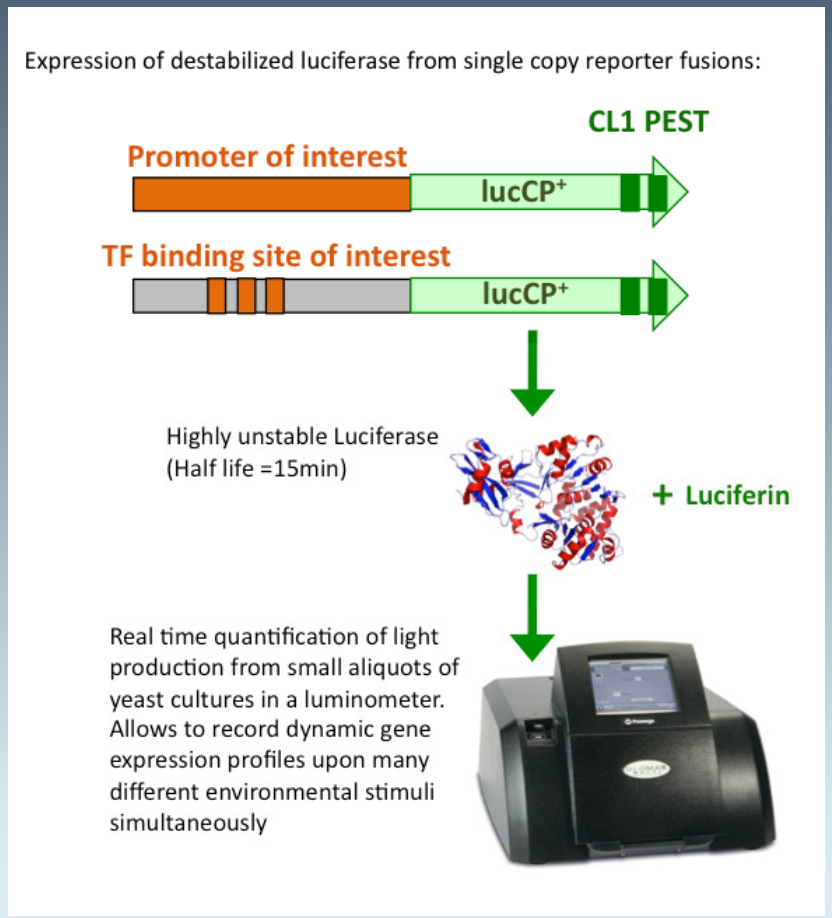
-What are the molecular mechanisms which confer dose dependent promoter activity?

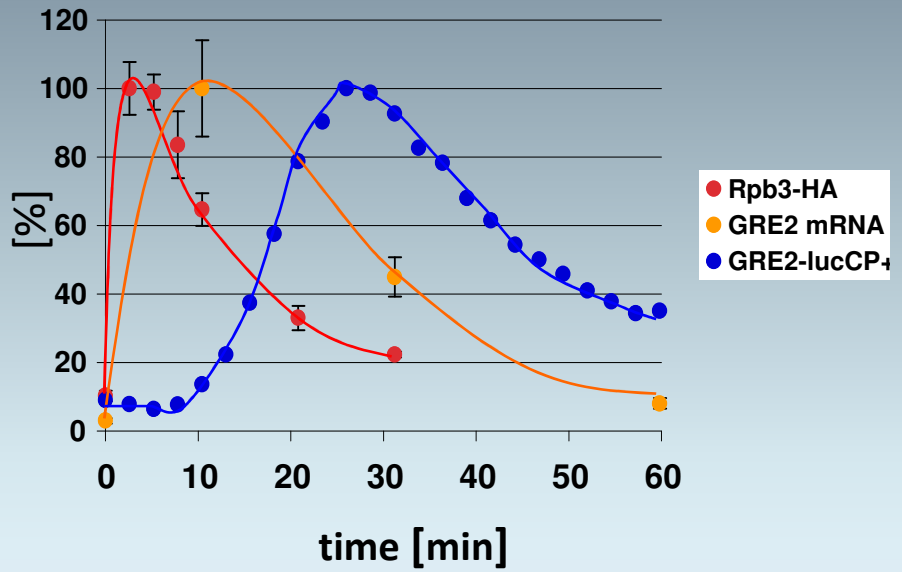
-How does cell physiology change the dose sensitive gene expression?

We need: *A continuous, real time transcription assay in the living cell adaptable to multiple stress doses*

The use of a real time luciferase assay to monitor promoter dynamics in the living yeast cell

Destabilized firefly luciferase (<15min half life in S. cerevisiae)
Measurements in small culture aliquots in multiwell setup allow parallel analyses upon many environmental conditions - DOSE-RESPONSE for a given promoter
We can obtain a quantitative value the EC50.

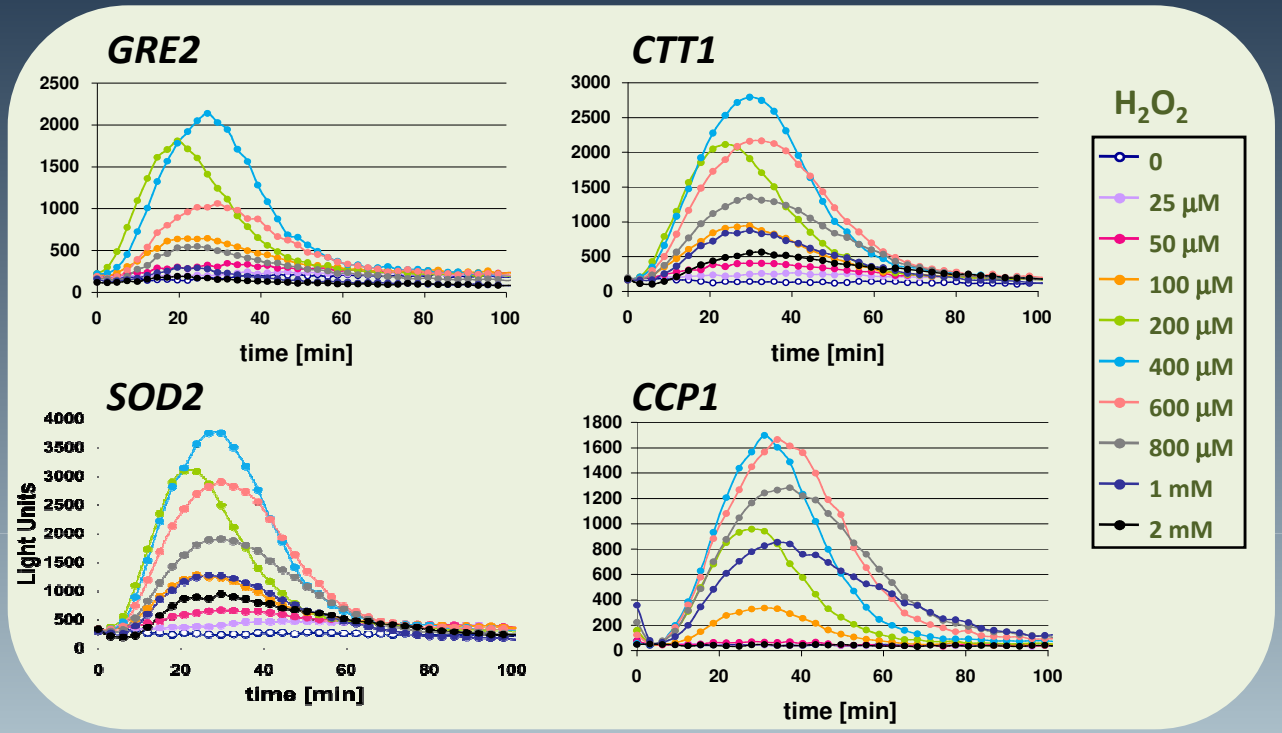


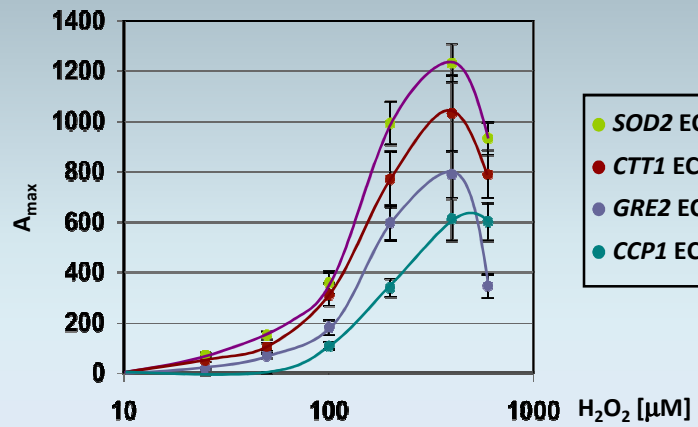


GRE2 induction by 0.3M NaCl

Rienzo et al., Yeast 2012

Quantitative Analysis of Natural Promoters upon Oxidative Stress





Dose-Response behavior of the GRE2, CTT1, SOD2 and CCP1 promoters

SOD2 and CTT1 activities are especially sensitive to hydrogen peroxide stress

Dolz-Edo et al., Mol Cell Biol 2013

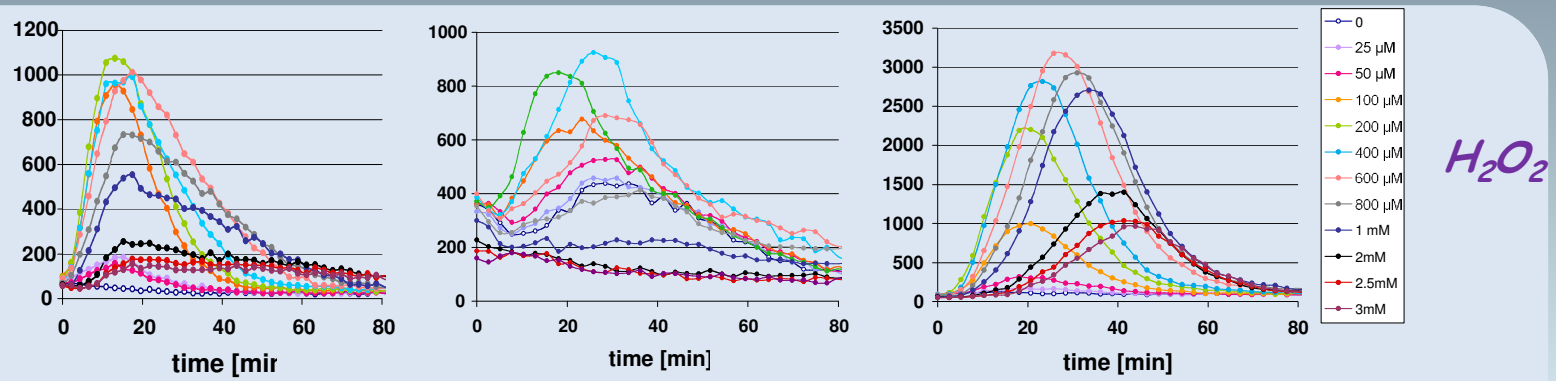
Quantitative Analysis of cis elements upon Oxidative Stress

Synthetic Reporter Genes: 3x[TF binding site]-lucCP+

Osmostress → HOG → Sko1 → CRE

Stress → Msn2,4 → STRE

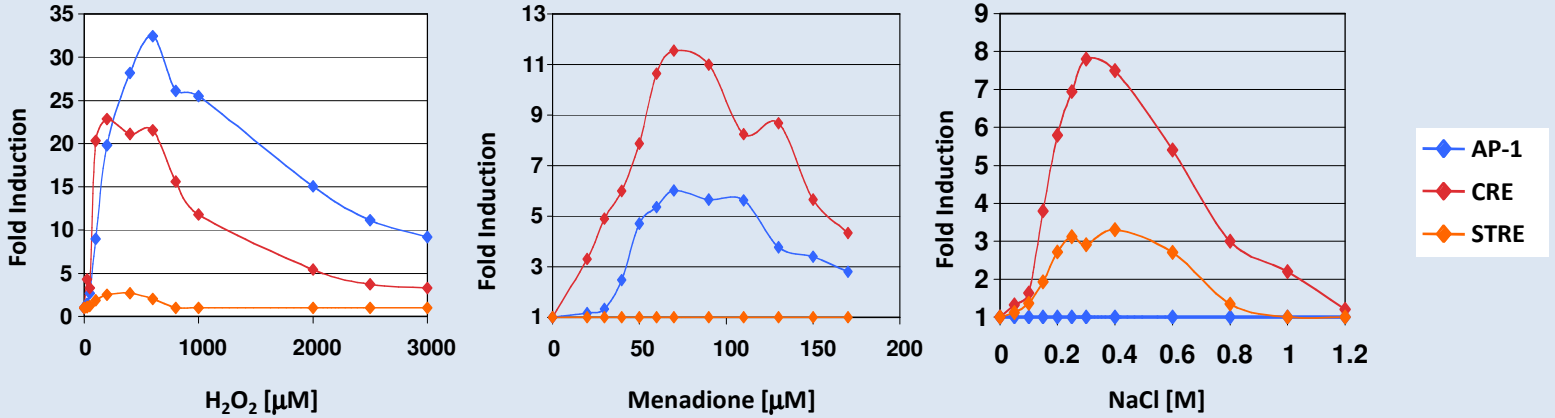
Yap1 ← Oxidative Stress → AP-1



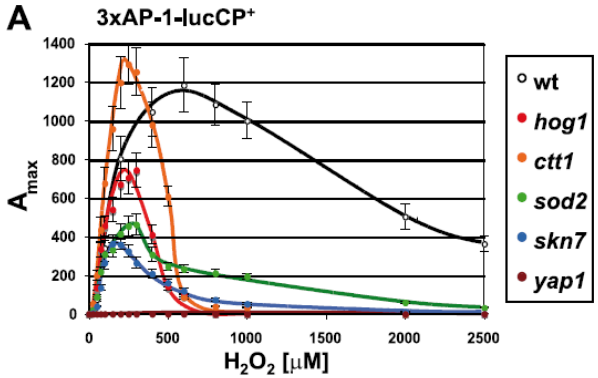
H_2O_2

Dolz-Edo et al., Mol Cell Biol 2013

Dose Response for each element under all stress conditions ($NaCl$, H_2O_2, Menadione)



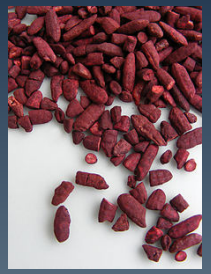
Cell physiology modulates the dose response of stress-activated gene expression

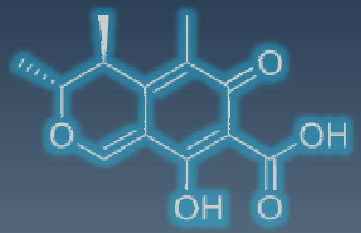


Dolz-Edo et al., Mol Cell Biol 2013

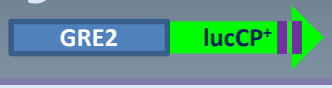
Application of quantitative yeast model to study mycotoxins

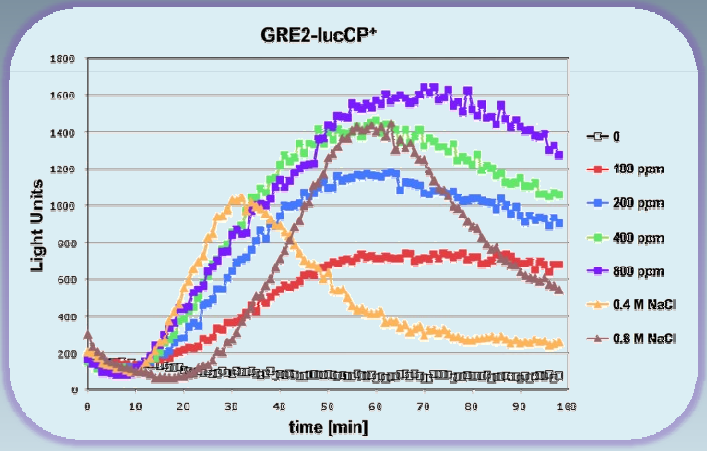
Toxicological study of citrinin.

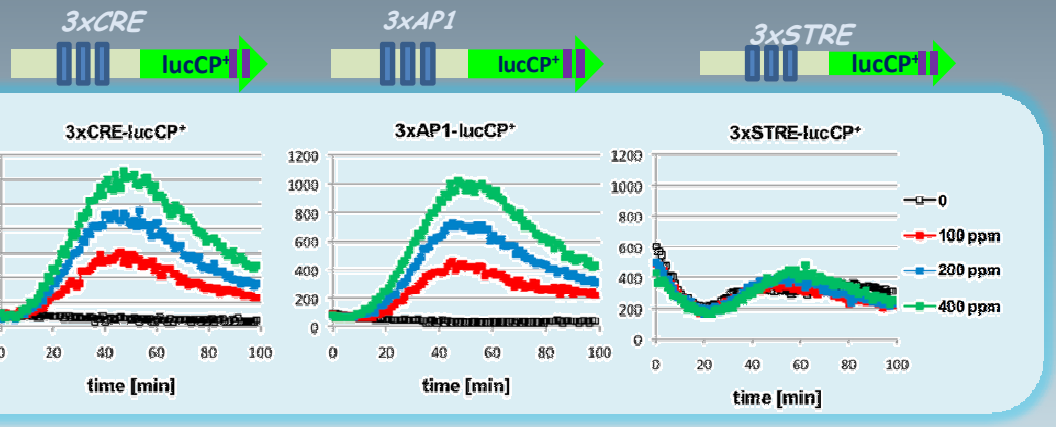




Citrinin triggers an immediate response to oxidative stress characterized by a strong and dose-dependent induction of natural genes.





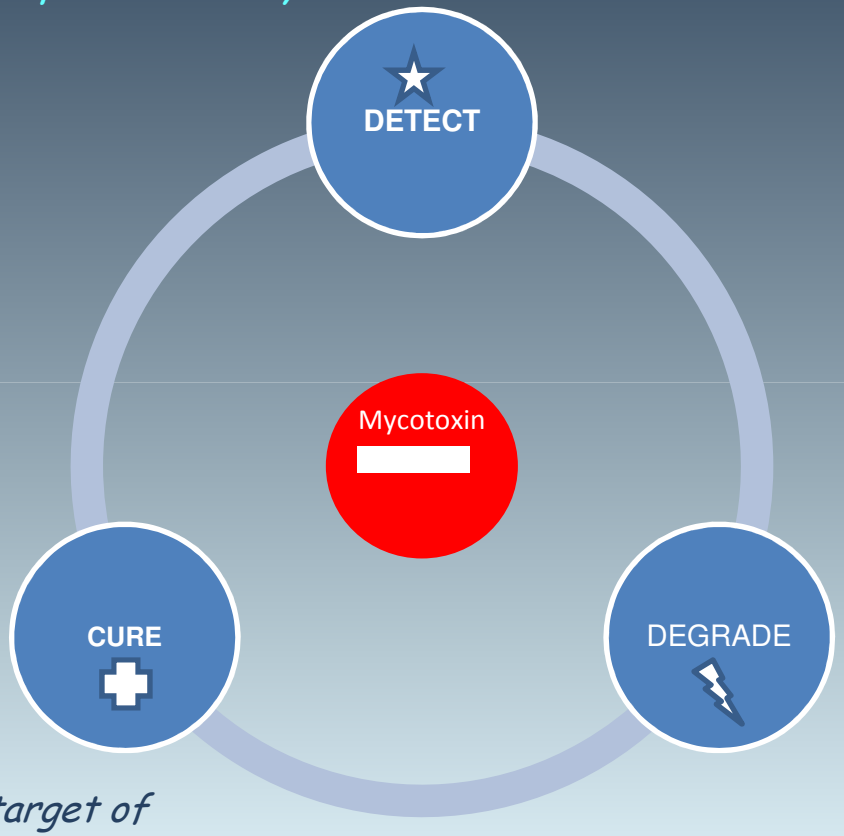


At present we are using the "dose-response assays" to study the transcriptional behavior of other stress response promoters (Oxidative , DNA Damage, Mitochondrial dysfunction, cell cycle)

Pascual-Ahuir et al., Nutrients 2014

Current projects:

-Generate artificial promoters, with optimized sensitivity and specificity to one particular mycotoxin .



-Identify the molecular target of toxicity of citrinin. Expand the dose-response assays to study the toxicological effect of Ochratoxin A.

-Identify new enzymes for biodegradation .

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