



# Redesigning Living Organisms to Survive on Mars

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# The Big Picture Issue:

Significant challenges are posed by the Martian Environment for Plant Growth.

Lack of Water

Lack of O<sub>2</sub>

Low pressure (about 6 mbars)

Extremely Cold Temperatures (-125 to -5°C)

UV and cosmic Radiation

Limited mineral nutrients

Image credits: NASA and  
The Hubble Heritage Team

## Project Concept:

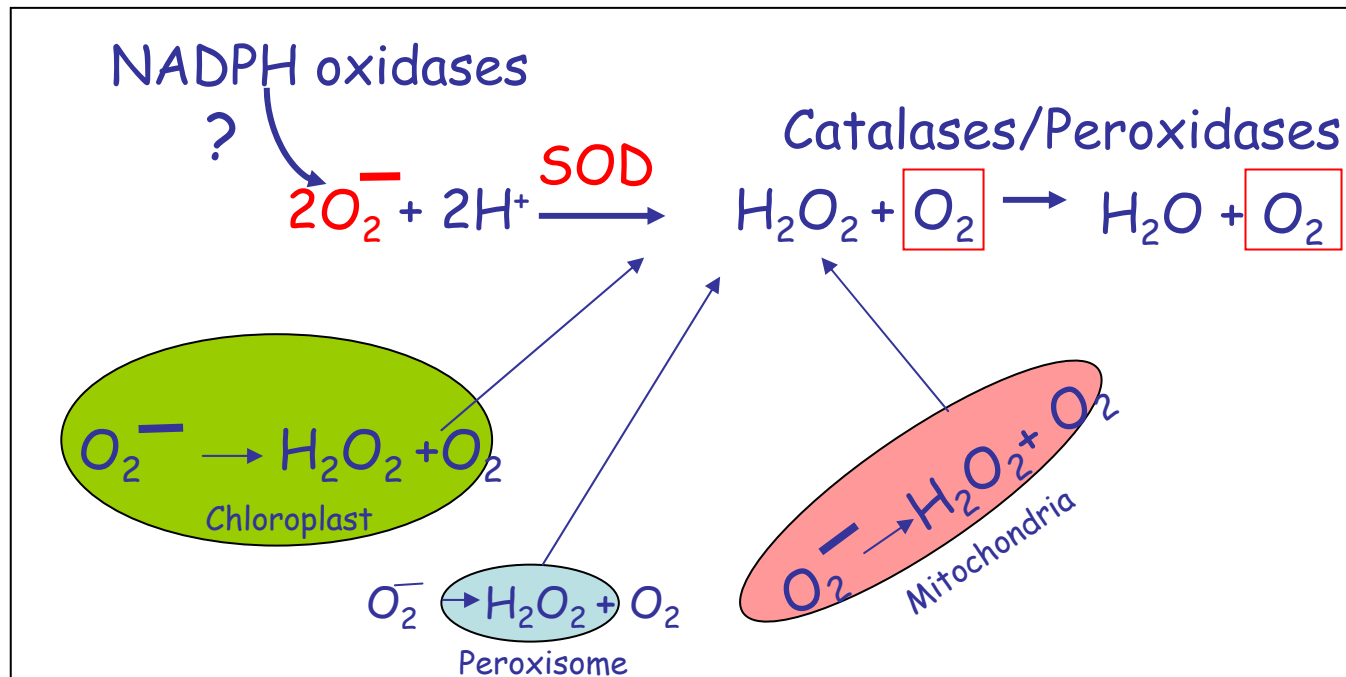
One can revolutionize life forms by selectively expressing extremophile genes that will collectively enable functional plant life in inhospitable environments.

## Project Rationale:

In response to environmental stresses such as radiation, cold, heat, anoxia and drought, plants produce reactive oxygen species (ROS) such as **superoxide** and **hydrogen peroxide**.

While the initial increase in ROS can serve as a stress signal, a sustained increase in ROS will kill the plant.

To prevent the build up of excess ROS, plants, like all aerobic organisms, have ROS detoxifying enzymes such as Superoxide Dismutases (**SOD**), Catalases and Peroxidases.



**SOD** = superoxide dismutase

We proposed to introduce a more efficient system to reduce ROS.

The initial ROS signal will occur but will not be sustained.

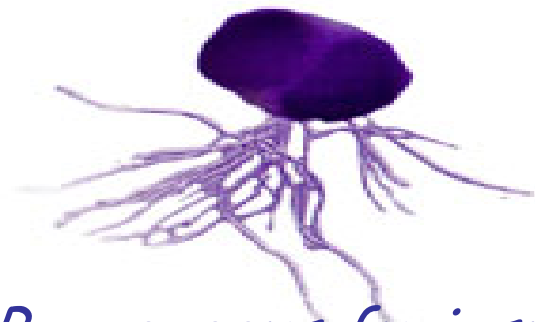
The byproduct, oxygen, will not be produced.

Our hypothesis is that this will decrease free radical propagation and ROS toxicity.

# The extremophile *Pyrococcus furiosus*

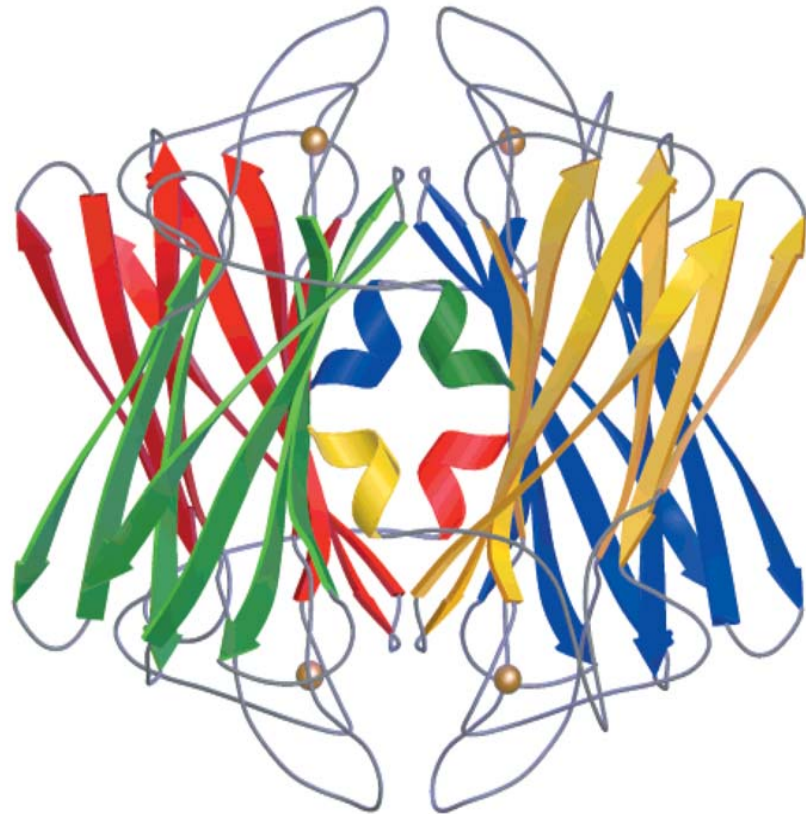


Jaun de Fuca smoker chimney



*Pyrococcus furiosus*

# Why *Pyrococcus furiosus* Superoxide Reductase (SOR)?



*P. Furiosus* SOR  
crystal structure

SOR is part of an oxygen detoxification system that could be beneficial to plants under stress conditions.

The pathway can function under both **aerobic** and **anaerobic** conditions.

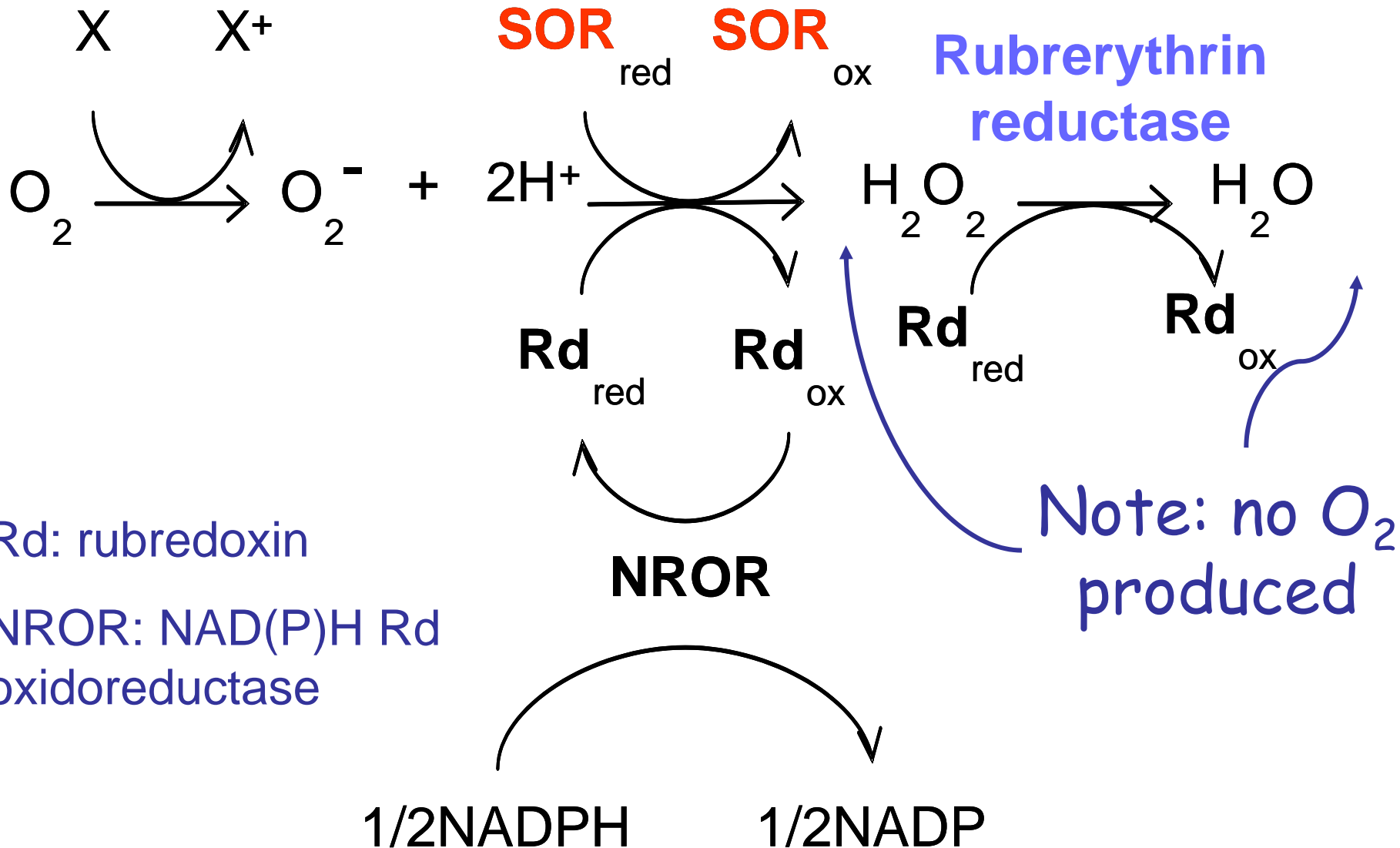
The pathway is not found in plants.

SOR is heat stable so that it can be assayed after heat denaturing the plant enzymes.

SOR is active from temperatures ranging from 0° to 100 °C.



# Superoxide Detoxification System in *Pyrococcus furiosus*



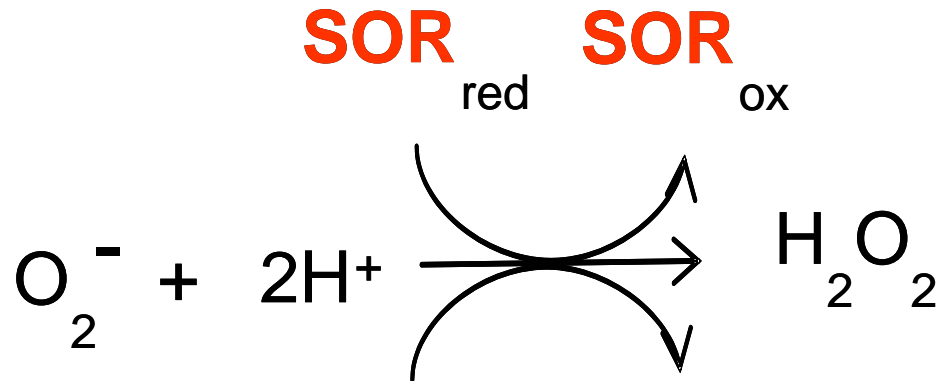
Rd: rubredoxin

NROR: NAD(P)H Rd  
oxidoreductase

# Project Goals

- A. To use genes from the extremophile *Pyrococcus furiosus* to introduce new mechanisms to reduce toxic free radicals in plants
- B. To use genes from the psychrophile, *Colwellia psychrerythraea* to develop new approaches to increase cold and dehydration tolerance
- C. Engage undergraduates in developing a preliminary design for virtual plants that would survive on Mars and evaluate possible mechanisms for providing increased resistance to radiation exposure

# Proof of concept: Expression of *P. furiosus* superoxide reductase (**SOR**) in plant systems



We anticipated that under normal conditions, the transgenic cells would have sufficient catalase activity to eliminate any excess hydrogen peroxide and that they would survive.

Im YJ, Ji ML, Lee AM, Boss WF, Grunden, AM. 2005. Production of thermostable archaeal superoxide reductase in plant cells. *FEBS Lett.* 579:5521-6.

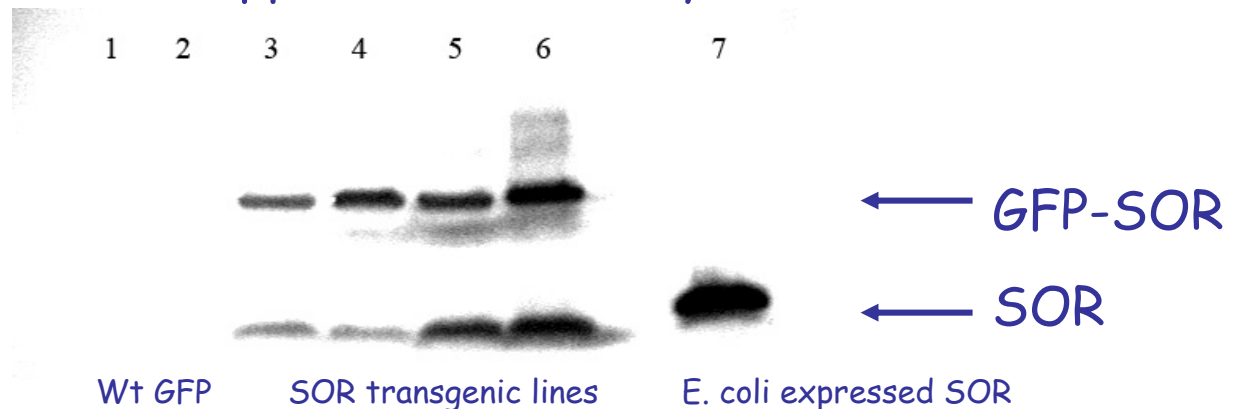
# Arabidopsis plants produce *P. furiosus* Superoxide Reductase

- We have screened and selected 4 homozygous lines. We raised antibodies against the recombinant *P. furiosus* SOR protein and confirmed that each line produce *P. furiosus* SOR.
- T4 generation seed is being used to characterize the SOR lines.

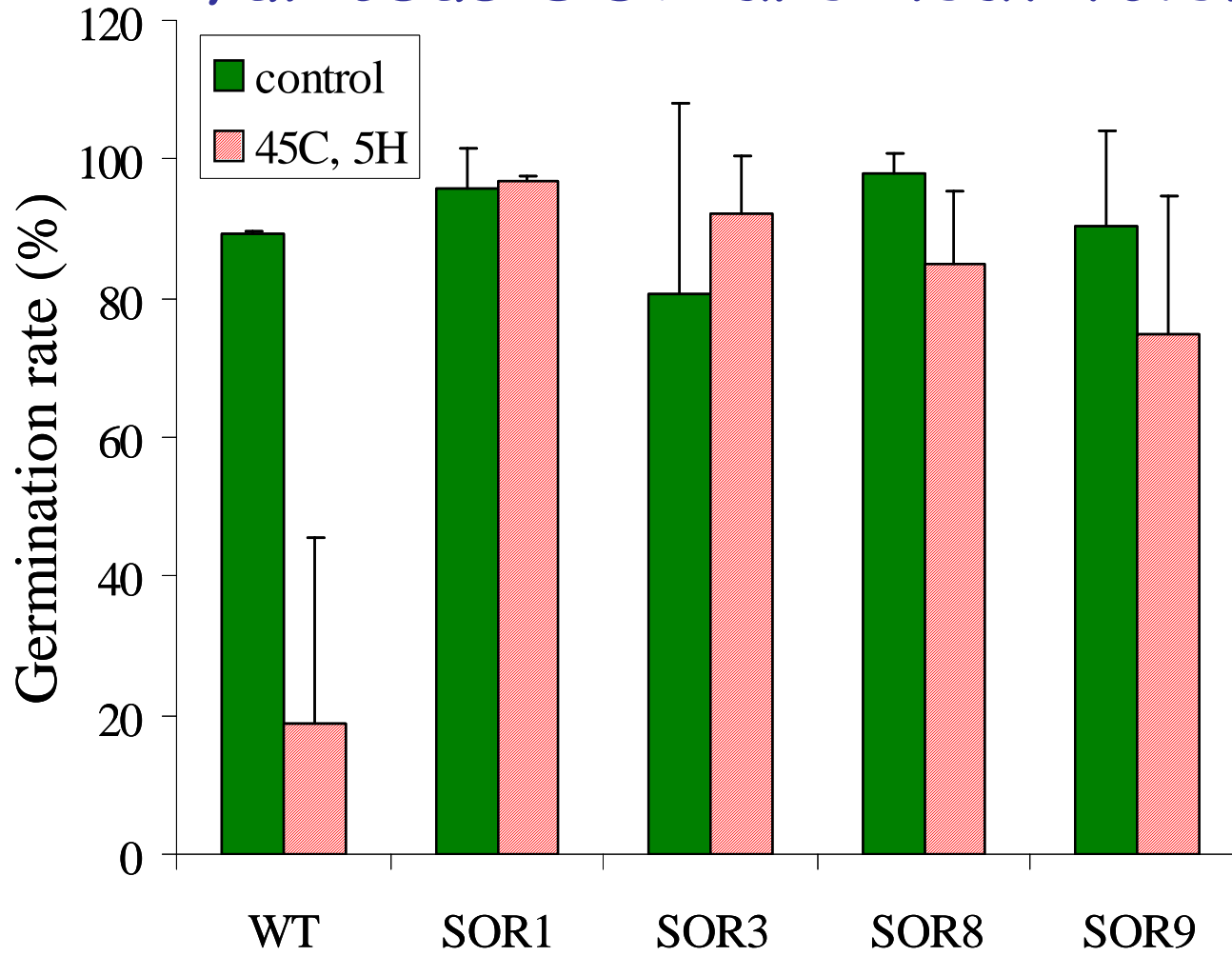
# The *P. furiosus* Superoxide Reductase is functional when produced in Arabidopsis

Sample	Specific Activity (U/mg)	
	Untreated Cell extract	Heat-treated cell extract <sup>a</sup>
GFP control	20.6	10.5
SOR-8	20.3	37.8
SOR-9	21.8	36.8

<sup>a</sup>Heat-treated samples were incubated at 85°C for 15 min. Anaerobic, reduced *E. coli* NC906 extract (reduced with DT + NADPH) was used to provide reductant to support SOR activity



# Seeds from *Arabidopsis* plants producing *P. furiosus* SOR are heat tolerant



Vernalized seeds were either incubated at 22C (solid green bars) or heat treated for 5 h at 45C (red stripes) and then incubated at 22C for 2 days and seed germination was monitored. Data are the average of 50 seeds from each line.

# Are the **SOR** Transgenic Seedlings Also Heat Tolerant?

Seedlings were grown in the dark



Exposed to heat stress



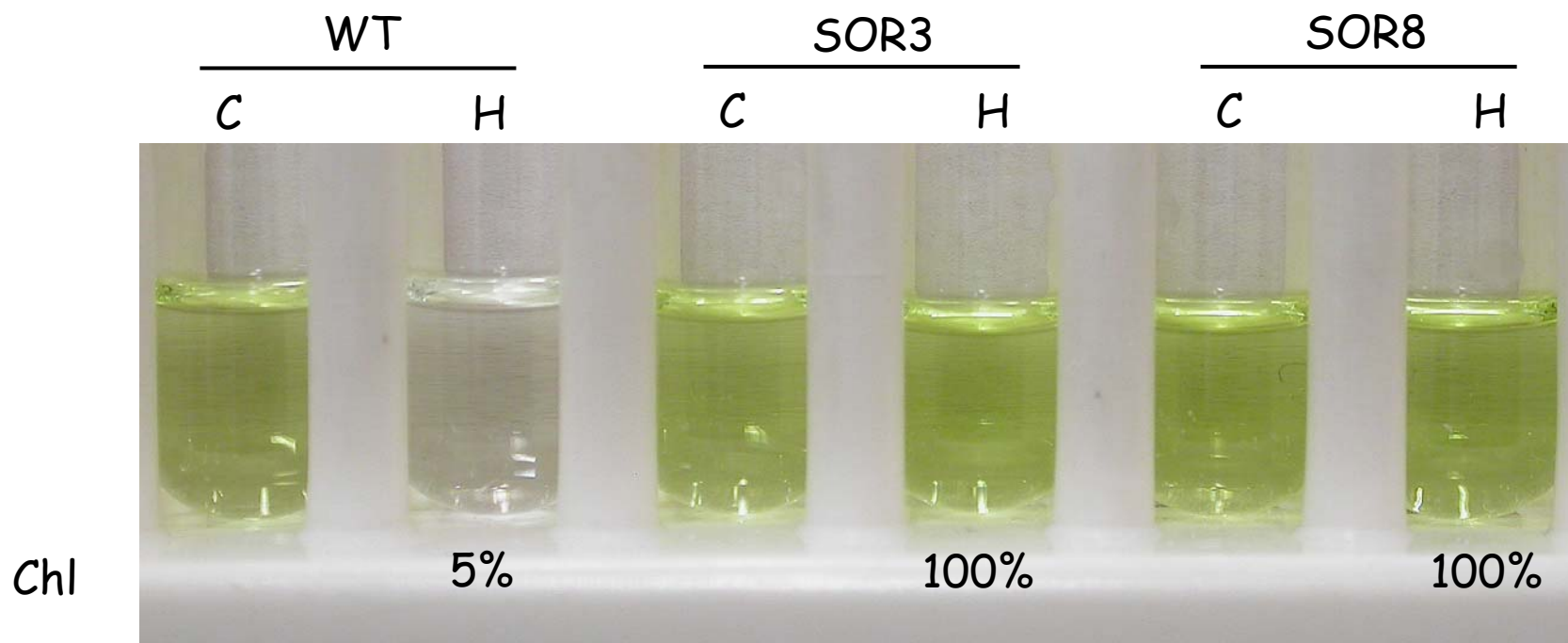
(48 C for 30 min)



Grown under continuous light for 24 h



**Chlorophyll** was extracted





The **SOR** transgenic lines should be making more  $H_2O_2$  and yet the seedlings are more heat tolerant.

Compensatory mechanisms including catalases and/or peroxidases must have been induced so that  $H_2O_2$  was removed and the **chloroplasts** developed normally and produced **chlorophyll**.

If the seedlings are heat tolerant as a result of a reduction of the ROS, what would be the phenotype of the plants? Would they also be resistant to stress?

To test this hypothesis, we monitored drought tolerance.

Plants produce ROS in response to drought.

Arabidopsis plants producing *P. furiosus* **SOR** are drought tolerant and continue to grow after 11 days without water.



Plants were not watered  
for 11 days



The Archaeon *Pyrococcus furiosus* superoxide reductase (**SOR**) gene produced a functional enzyme in plants.

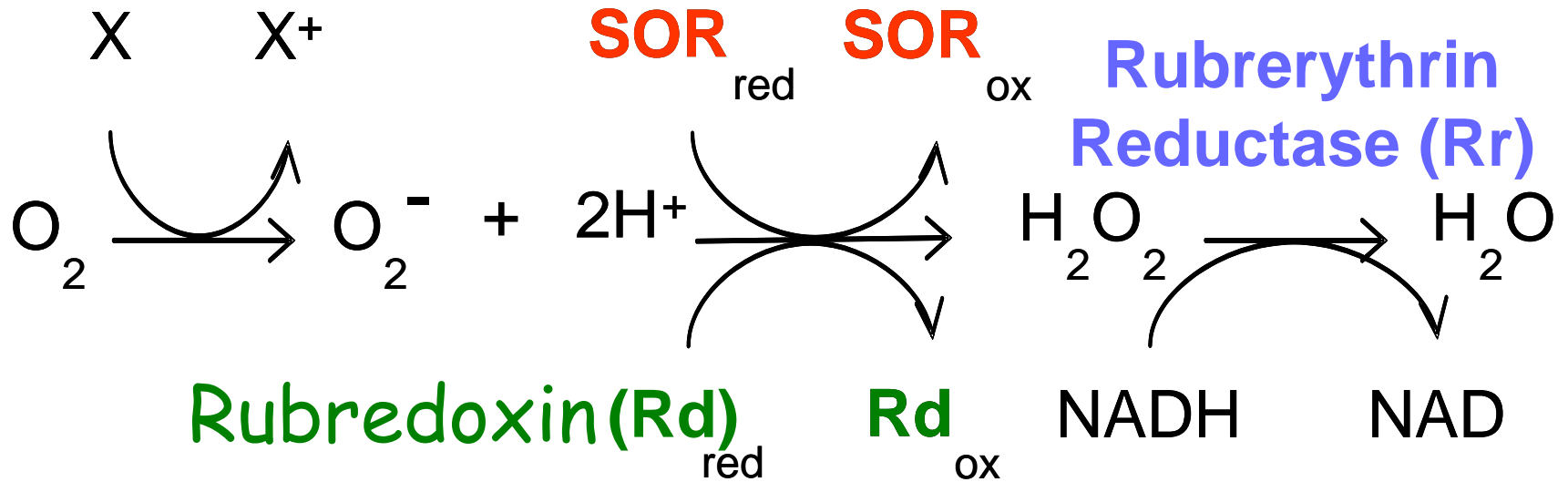
The **SOR** transgenic plants are surprisingly tolerant to heat, drought and high light suggesting compensatory pathways that reduce  $H_2O_2$  are functioning well in these plants.

Our working hypothesis is that by dampening the superoxide signal we have increased the flux through the ROS signaling pathway and induced compensatory pathways that decrease ROS-induced cell death.

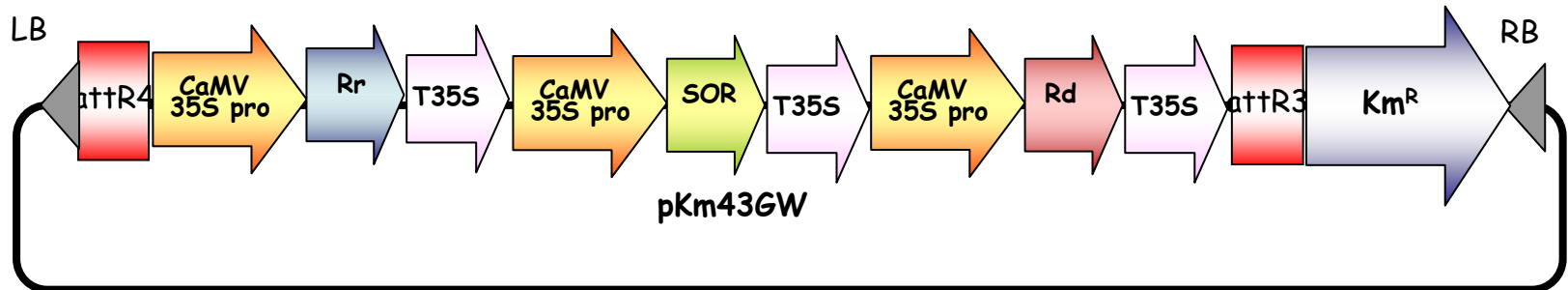
Can we improve and extend the stress tolerance of the **SOR** plants?

Can we produce more of the *P. furiosus* SOR pathway enzymes in plants?

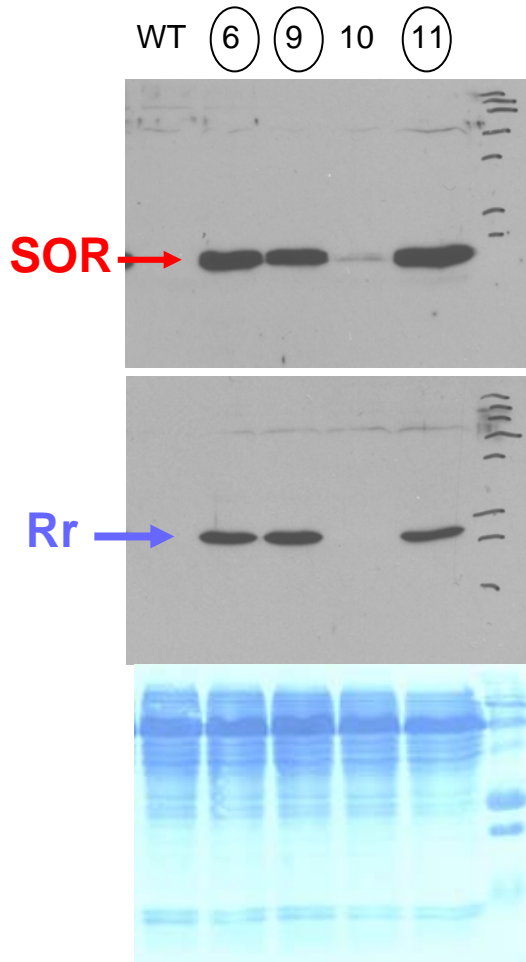
# Strategy for expressing more of the SOR pathway genes



The Gateway vector system was used to express **SOR** and **Rr** or **SOR, Rr** and **Rd** in tandem.



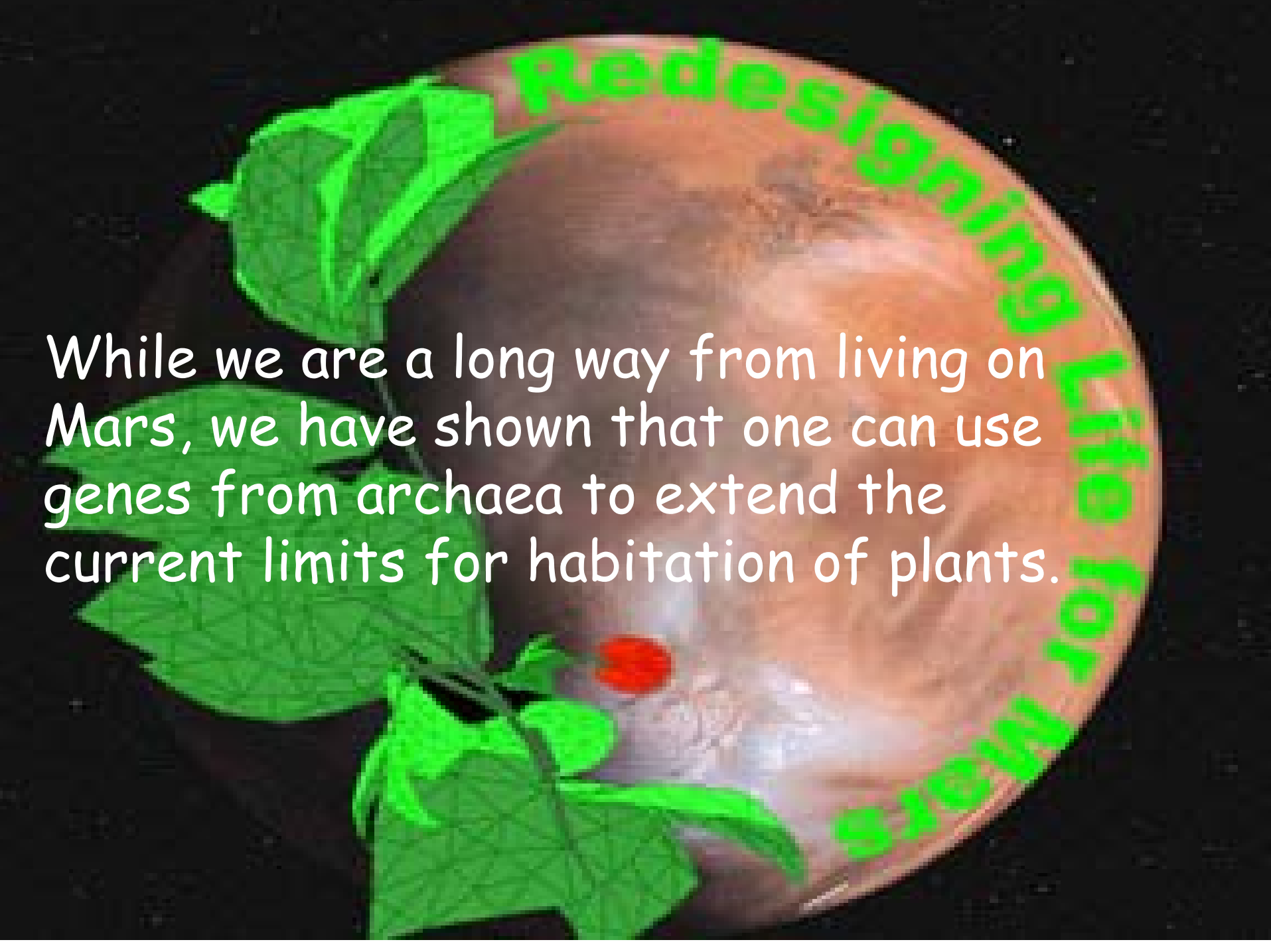
# Arabidopsis plants will produce both the **SOR** and **Rr** proteins



Three Arabidopsis plants were selected that produced both **SOR** and **Rr** based immunoblots with antibodies raised against these *P. furiosus* proteins.

These plants were self-pollinated and the seeds are currently being screened on kanamycin selection medium for homozygous lines.

Top 2 panels: immunoblots  
Lower panel: stained blot



While we are a long way from living on Mars, we have shown that one can use genes from archaea to extend the current limits for habitation of plants.



Mars is cold and if the greenhouses fail, the plants will be subjected to cold, low pressure and low humidity.

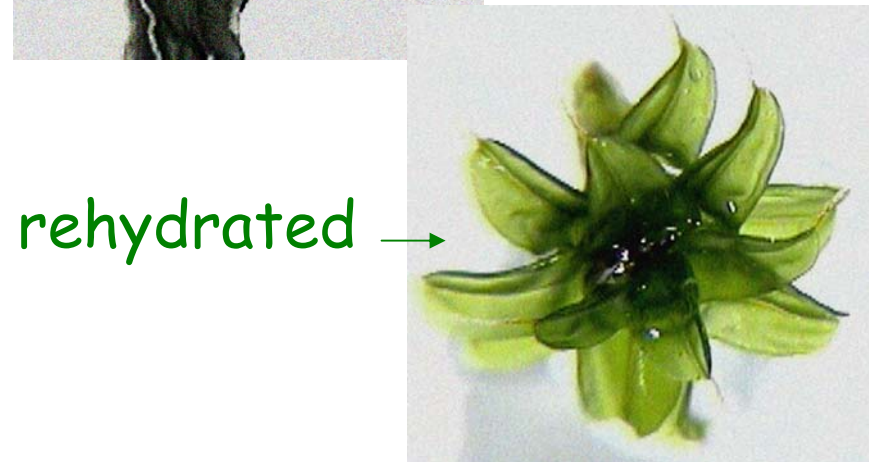
Can we generate a "fail-safe" system so the plants would survive until the greenhouse is fixed?

*Tortula ruralis* is a moss, which can dehydrate until it is brittle and breaks when touched, and yet when water is added, it will rehydrate and resume normal growth within 30 min.

*Tortula ruralis* has high levels of reduced glutathione (GSH), a small peptide, that will reversibly complex with cellular proteins and protect their structure as the plant dehydrates.



← dehydrated



rehydrated →

To produce more reduced glutathione in plants, we propose to express glutathione reductase from the psychrophile *Colwellia psychrerythraea*.

Glutathione  
Reductase



Reduced glutathione (GSH) will reversibly complex with proteins, stabilize their structure and prevent denaturing.



Glutathione reductase from *Colwellia psychrerythraea* will function at the growth temperatures of the organism (0° to 20 °C).

Our hypothesis is that increasing glutathione reductase with the psychrophilic enzyme will increase the pool of reduced glutathione available to complex with and stabilize proteins during rapid temperature fluctuations.

# Glutathione Reductase from *Colwellia psychrerythraea* was cloned and overexpressed in *E. coli* and is cold active



*C. Psychrerythraea*

Sample	Specific activity (units/mg)
GOR (25°C)	142.6
GOR (12°C)	100.8
GOR (5°C)	59.5

- We have cloned and expressed a functional psychrophilic glutathione reductase (GOR).
- We are currently transforming plants with GOR and will be screening and selecting the transformed plants over the next 10 months.

# Future Research Goals

- Complete the biochemical and physiological characterization of the SOR transgenic lines.
- Select homozygous lines expressing more of the *P. furiosus* pathway genes (SOR/Rr and SOR/Rr and Rd)

# Future Research Goals

- Complete the biochemical characterization of the recombinant *Colwellia psychrerythraea* Glutathione Reductase (GOR).
- Screen and select transgenic plants expressing GOR and test them for cold and drought tolerance.
- Assess the efficacy of expressing the SOR pathway genes in other biological systems



# Honors class of spring 2005 discussing their plans for redesigned plants



Four of these students are currently working on research projects in our labs.

# Spring of 2007 Honors Class

"Can genes from extremophilic microorganisms be used to generate radiation resistant eukaryotes?"

The goals for the class will be to learn about mechanisms of radiation resistance found in radiation tolerant microbes and to select potential mechanisms to test in eukaryotes.

# Phase II Redesigning Life for Survival on Mars Road-map



- Produce transgenic plants with improved growth and productivity at low temperature and water availability (5 years)

- Enhance plants' capacities to resist radiation damage by expressing DNA repair genes from radiation resistant microbes (10 years)

- Enhance plants capacity to resist ROS stress under extreme environmental conditions (Phase I and phase II, 3 years)

- Collaborate with scientists to use this technology in non-model systems. (10-15 years)



## The NC State NIAC Team

### Microbiologists



Alice Lee



Mikyung Ji

### Plant Biologists



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### Undergraduates:

Callie Barnwell  
Casey Lowder  
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