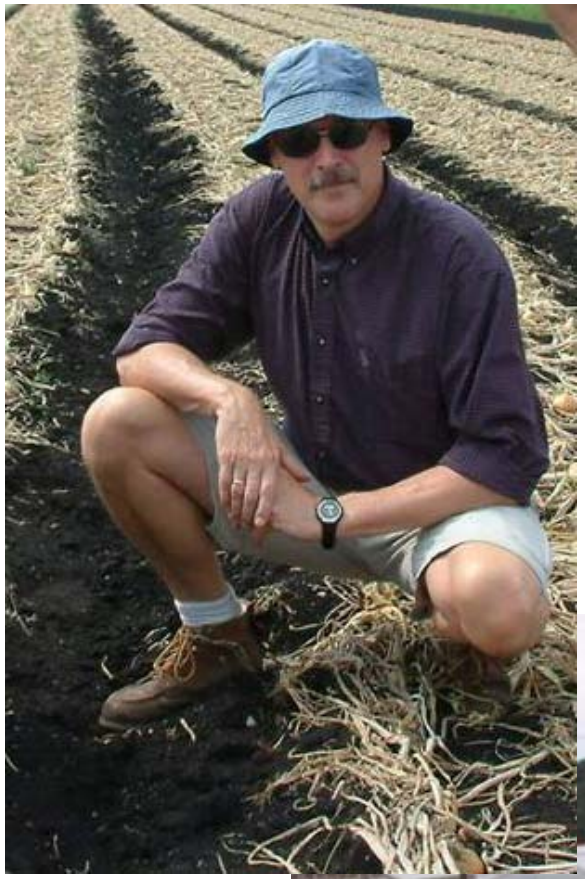
USDA-ARS Research Geneticist

and

Professor of Horticulture

University of Wisconsin-Madison

Onion Breeding and Genetics



Onion Breeding and Genetics

Biennial (2 years per generation)

Onion Breeding and Genetics

Year 1: Seed to Bulb in Field

Onion Breeding and Genetics

Year 1: Seed to Bulb in Field

Onion Breeding and Genetics

Bulb storage and vernalization

Onion Breeding and Genetics

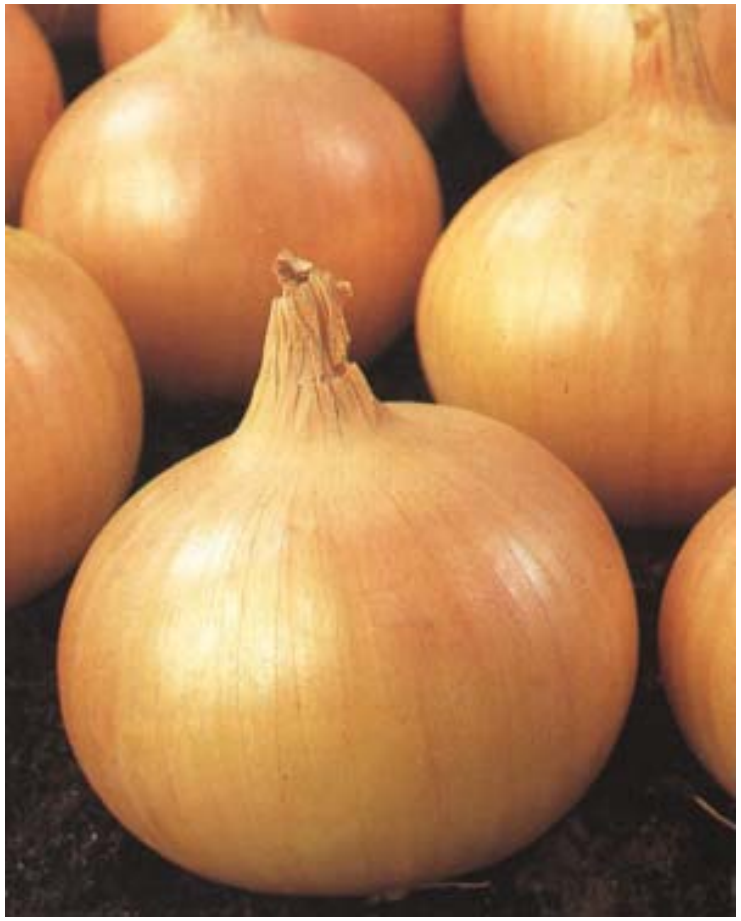
Year 2: Bulb selection for seed production

Onion Breeding and Genetics

Classical crossing and seed production

Onion Breeding Goals

- For Growers:
  - High quality seed
  - Multiple pest resistances
  - High yields
  - Low post-harvest losses
- For Consumers:
  - Attractive bulbs
  - Good flavor
  - Heath-enhancing attributes
- For Processors:
  - Uniformity
  - Single centers
  - High dry matter



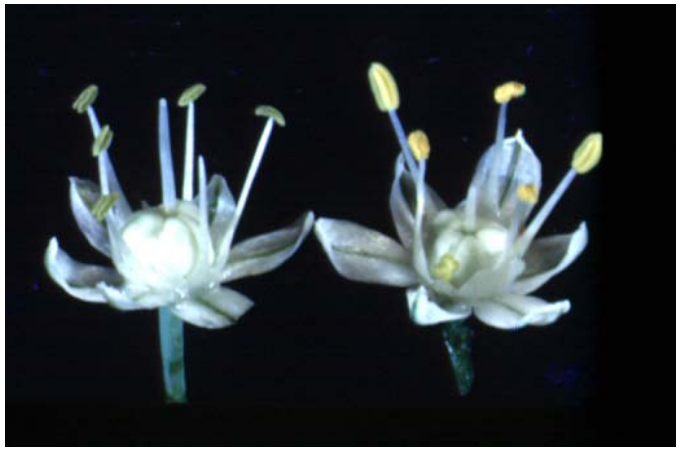
USDA Hybrids

- 'Granex' (1952)
  - YB986A x TEG951C
  - Short-day onion (Vidalia)
  - Flat shape with low pungency
  - Short storage ability
- 'Spartan Banner 80' (1979)
  - MSU(611-1Ax611B) x 2399B
  - Long-day pungent onion
  - Good storage and pink root resistance

http://southeastagnet.com

Inbred Releases

- B8667 A&B
  - $F_1MSM_2SM_2$
  - CMS Line is BC_6
  - Deep red color to internal rings
  - Medium pungency
- Ski-1 A&B
  - $F_1MSM_2SM_2$
  - CMS Line is BC_5
  - Early maturity, good storage



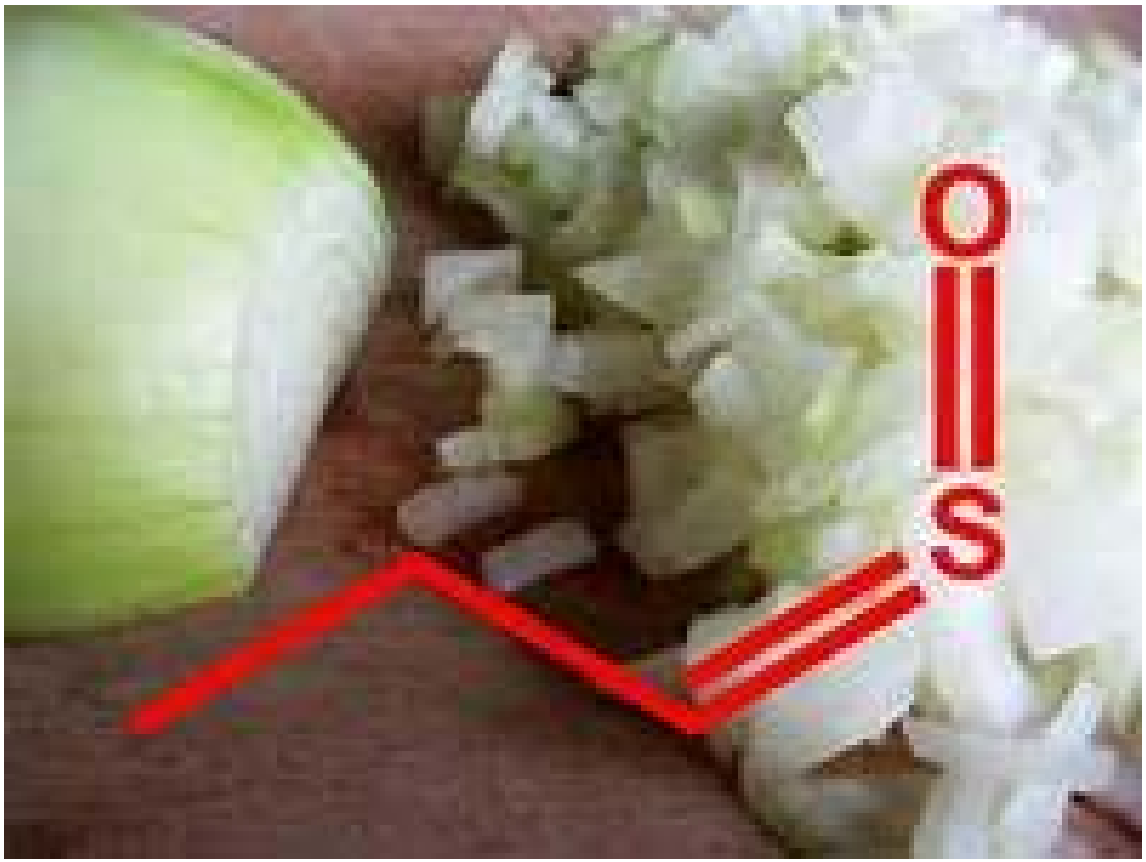
Development of Value-Added Onions

Traits recognized by the consumer

Development of Value-Added Onions

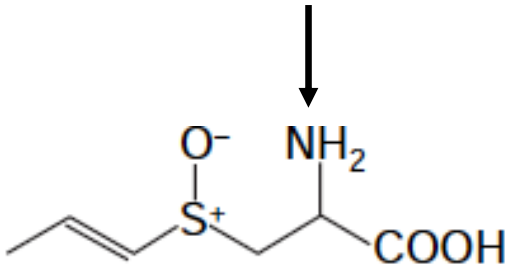
#1 Consumer Complaint: Onions Make You Cry

LF = Lachrymatory Factor

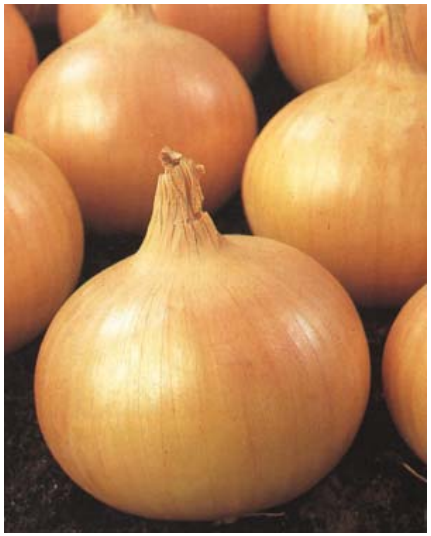


Onion Thiosulfinates

SO_4 → Cysteine → Glutathione



"Flavor Precursors"

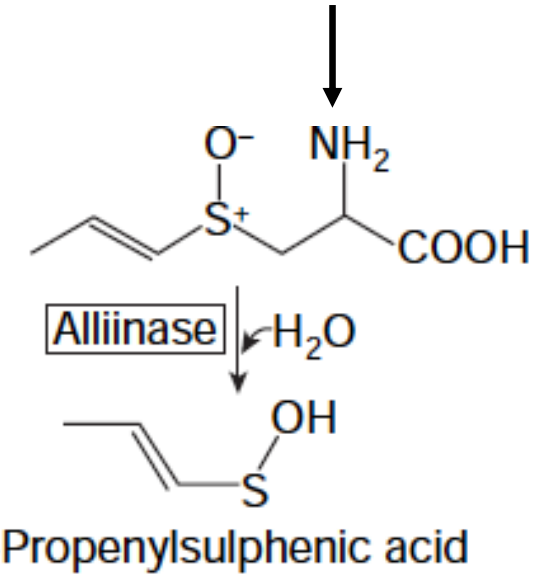


Nature 419:685

Onion Thiosulfinates

SO_4 → Cysteine → Glutathione

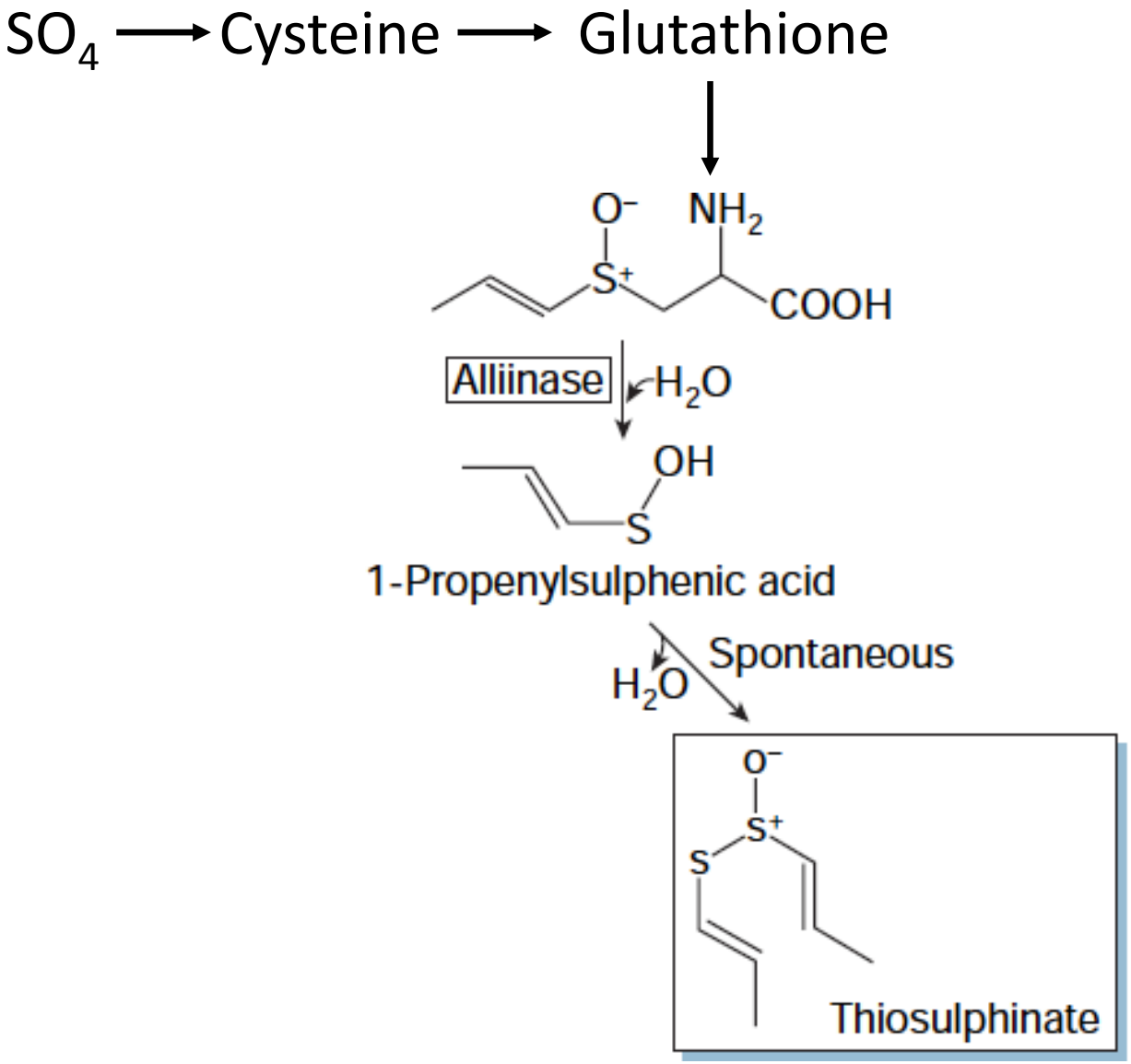
"Flavor Precursors"



1-Propenylsulphenic acid

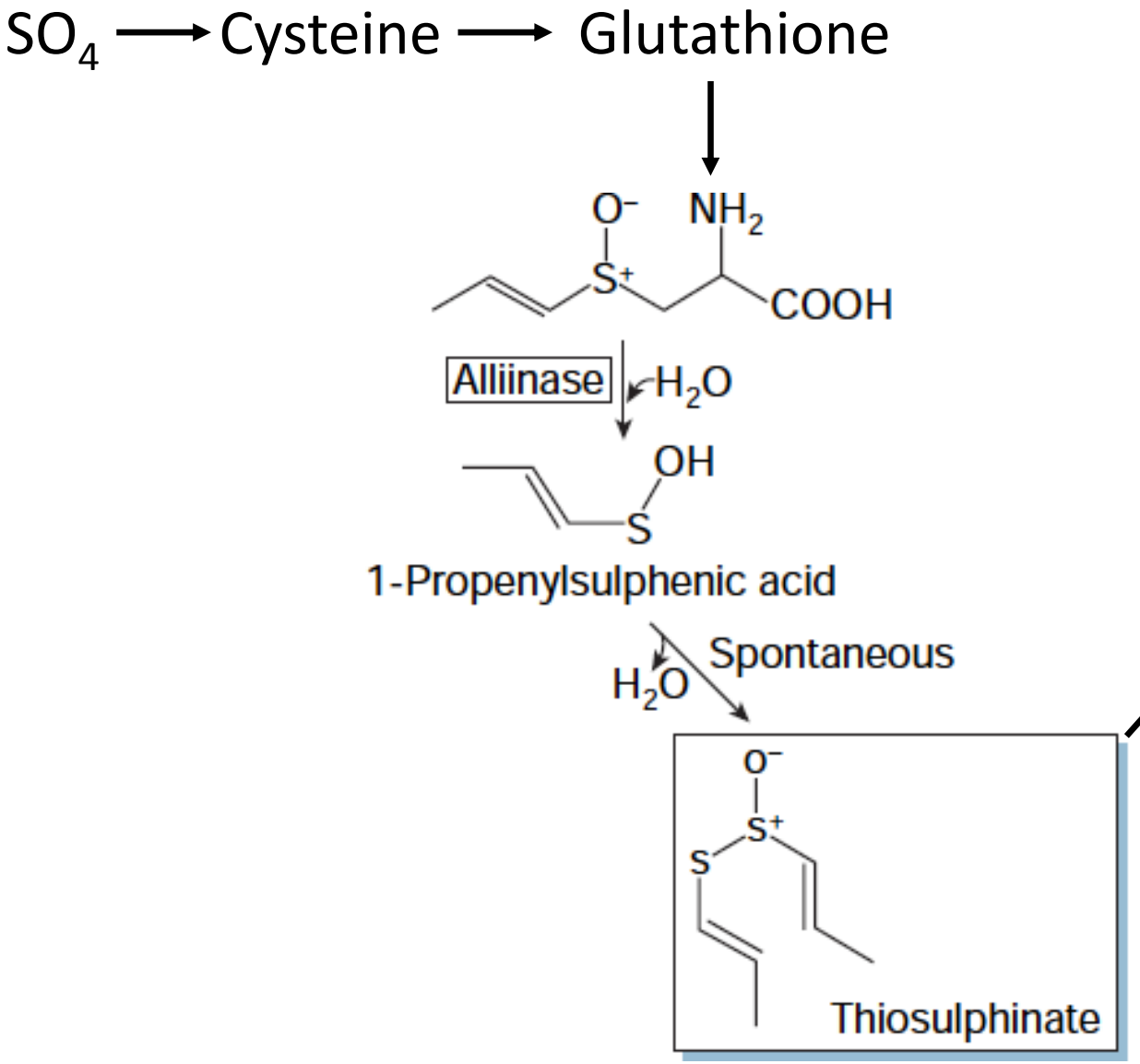
Nature 419:685

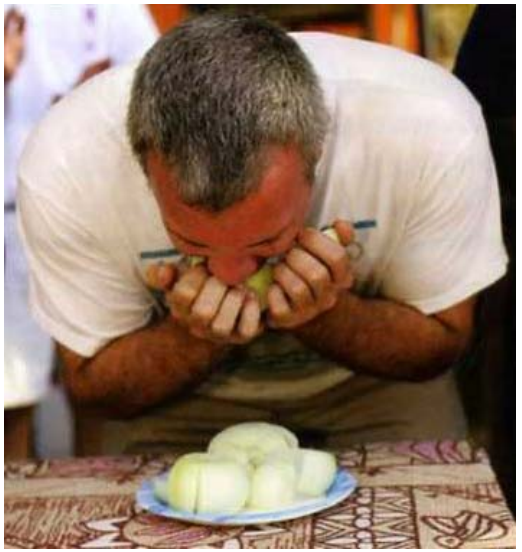
Onion Thiosulfinates



Nature 419:685

Onion Thiosulfinates

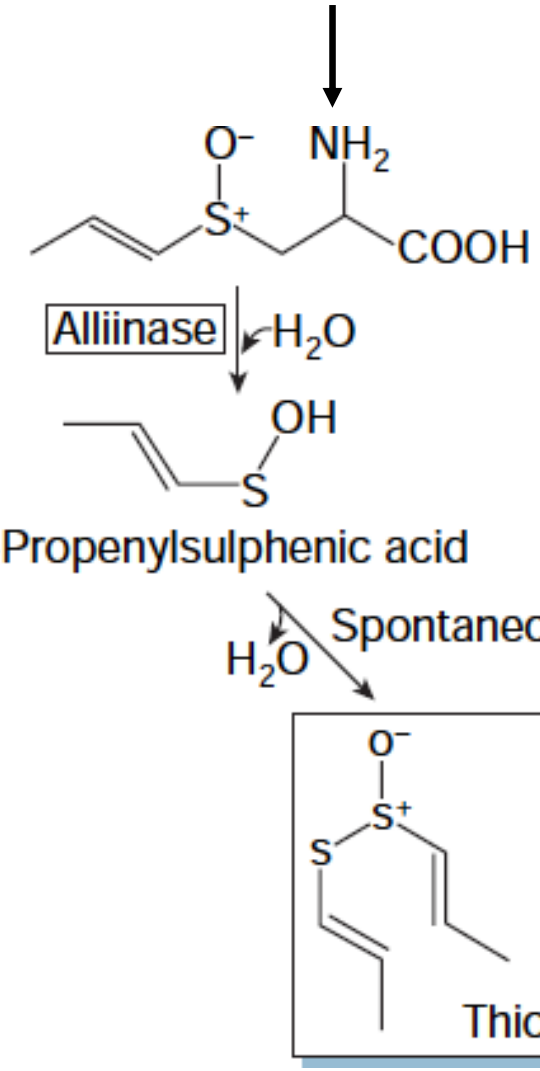


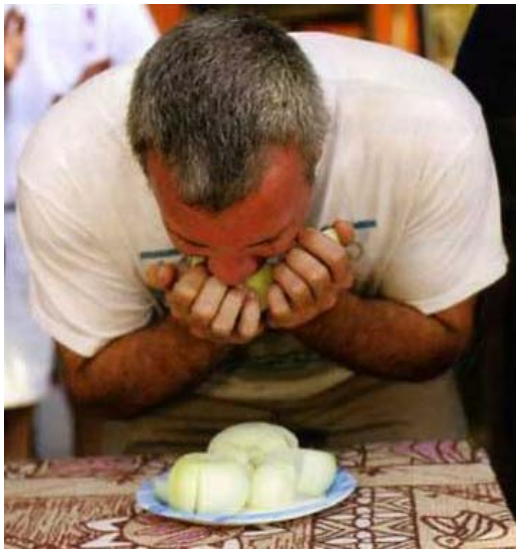


Pungent Flavor

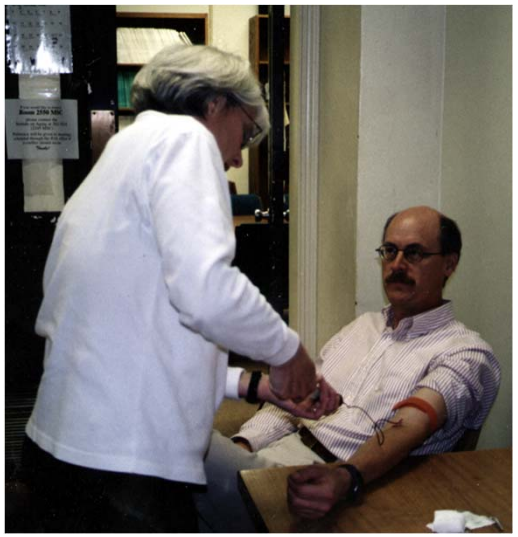
Onion Thiosulfinates

SO_4 → Cysteine → Glutathione





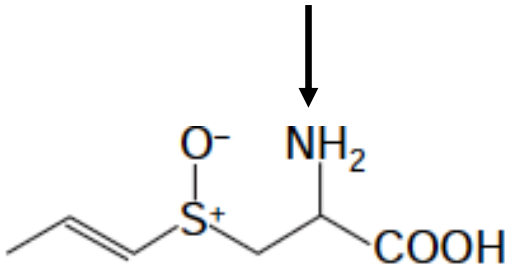
Pungent Flavor



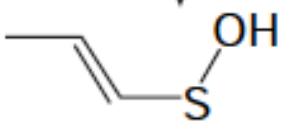
Reduce Aggregation of Blood Platelets

Onion Thiosulfinates

SO_4 → Cysteine → Glutathione

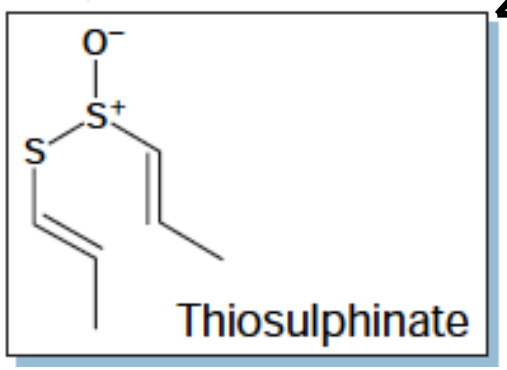


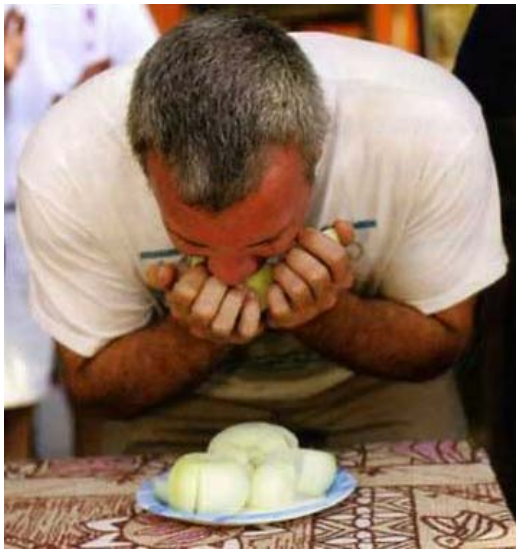
Alliinase, H_2O



1-Propenylsulphenic acid

H_2O, Spontaneous

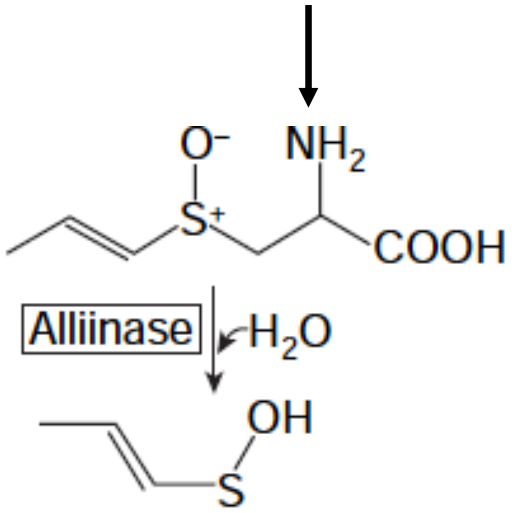




Pungent Flavor

Onion Thiosulfinates

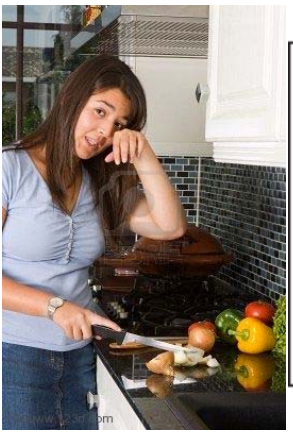
SO_4 → Cysteine → Glutathione

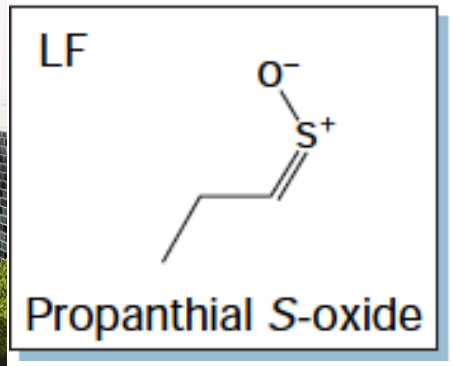


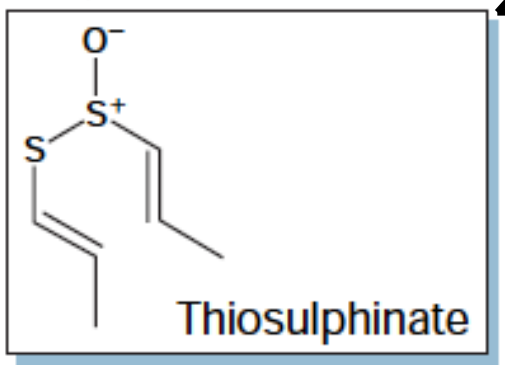
1-Propenylsulphenic acid

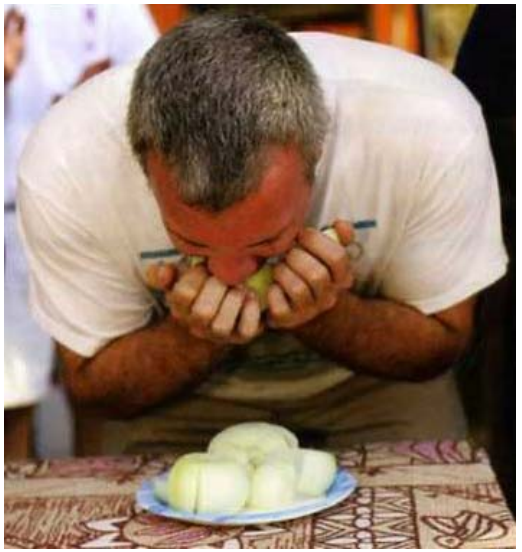
LF synthase

H_2O Spontaneous





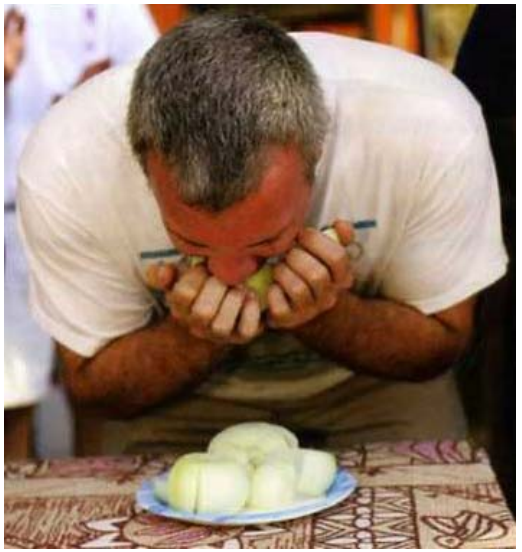




Pungent Flavor

Nature 419:685

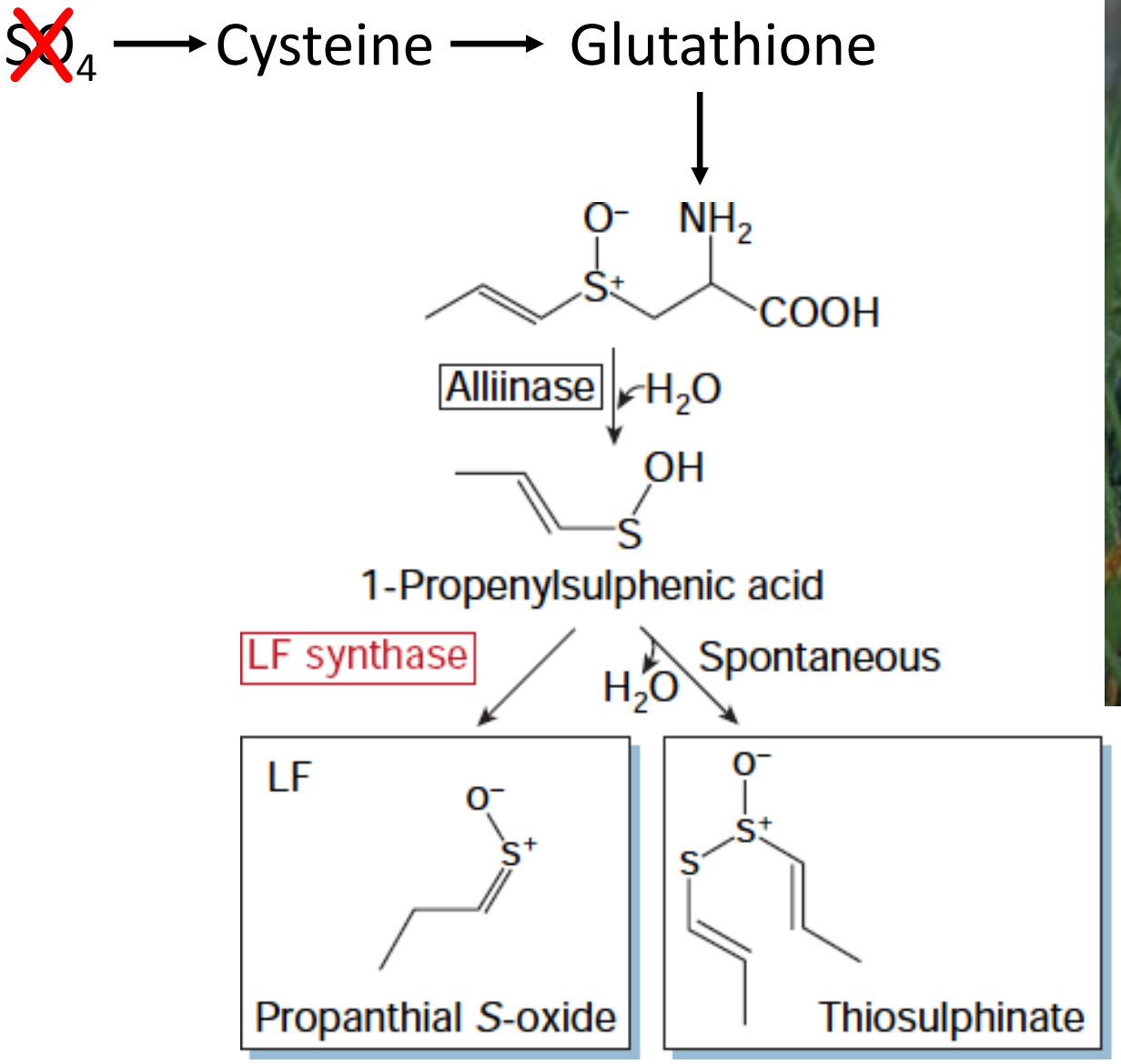
'Tearless Onion'



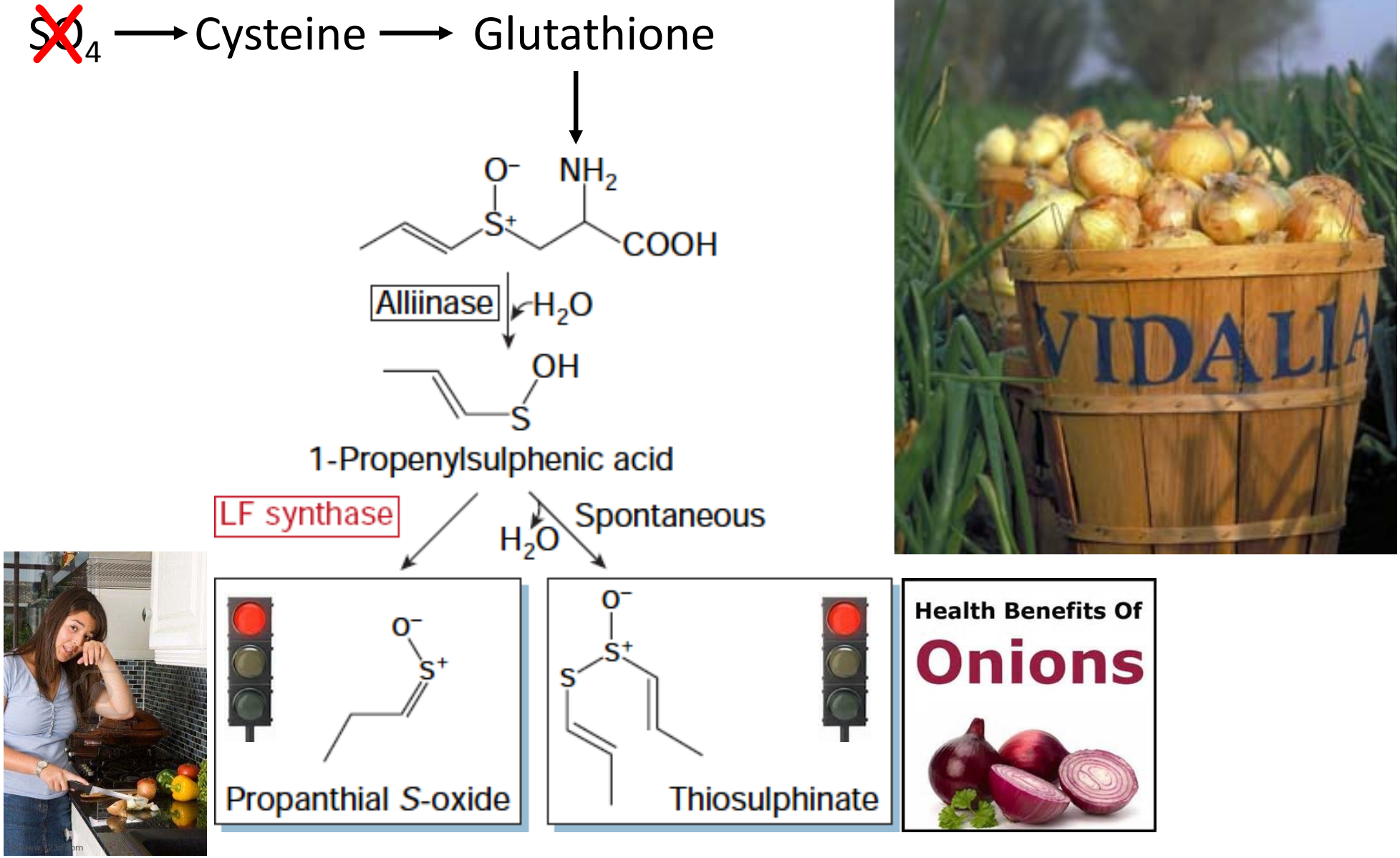
Pungent Flavor

Health Benefits

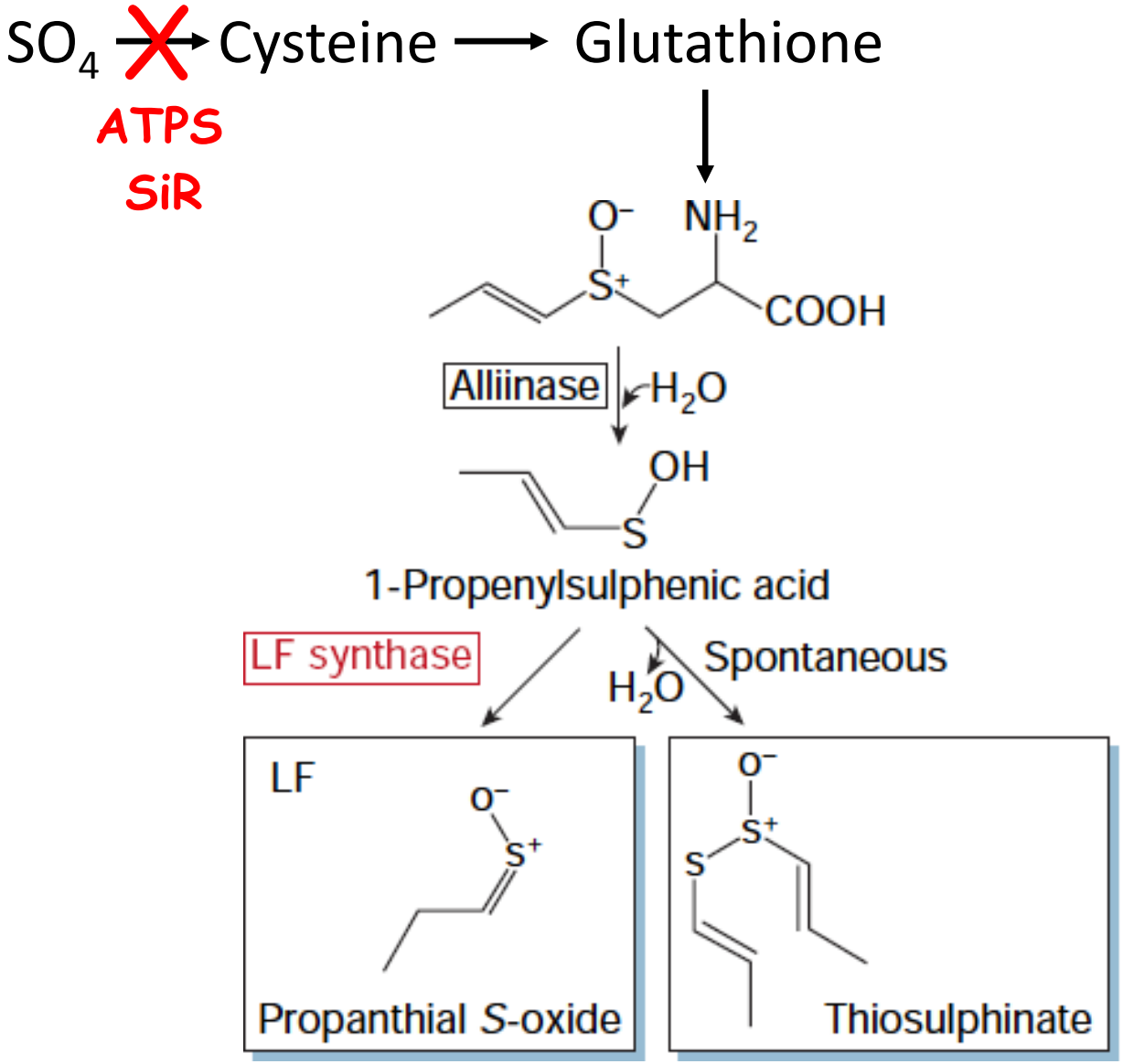
Onions Grown on Low Sulfur Soils

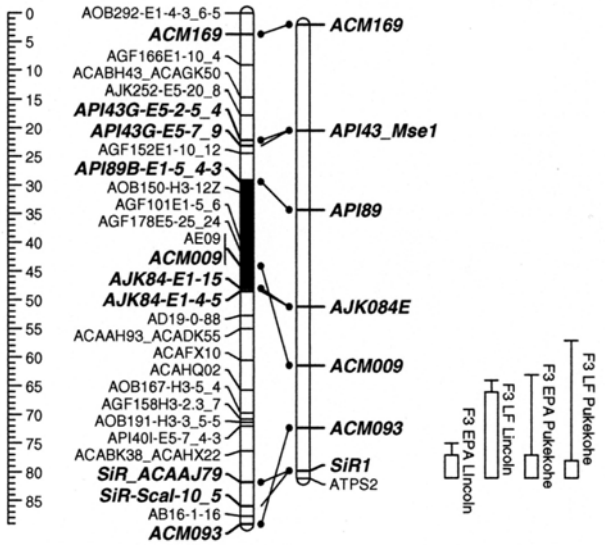


Onions Grown on Low Sulfur Soils



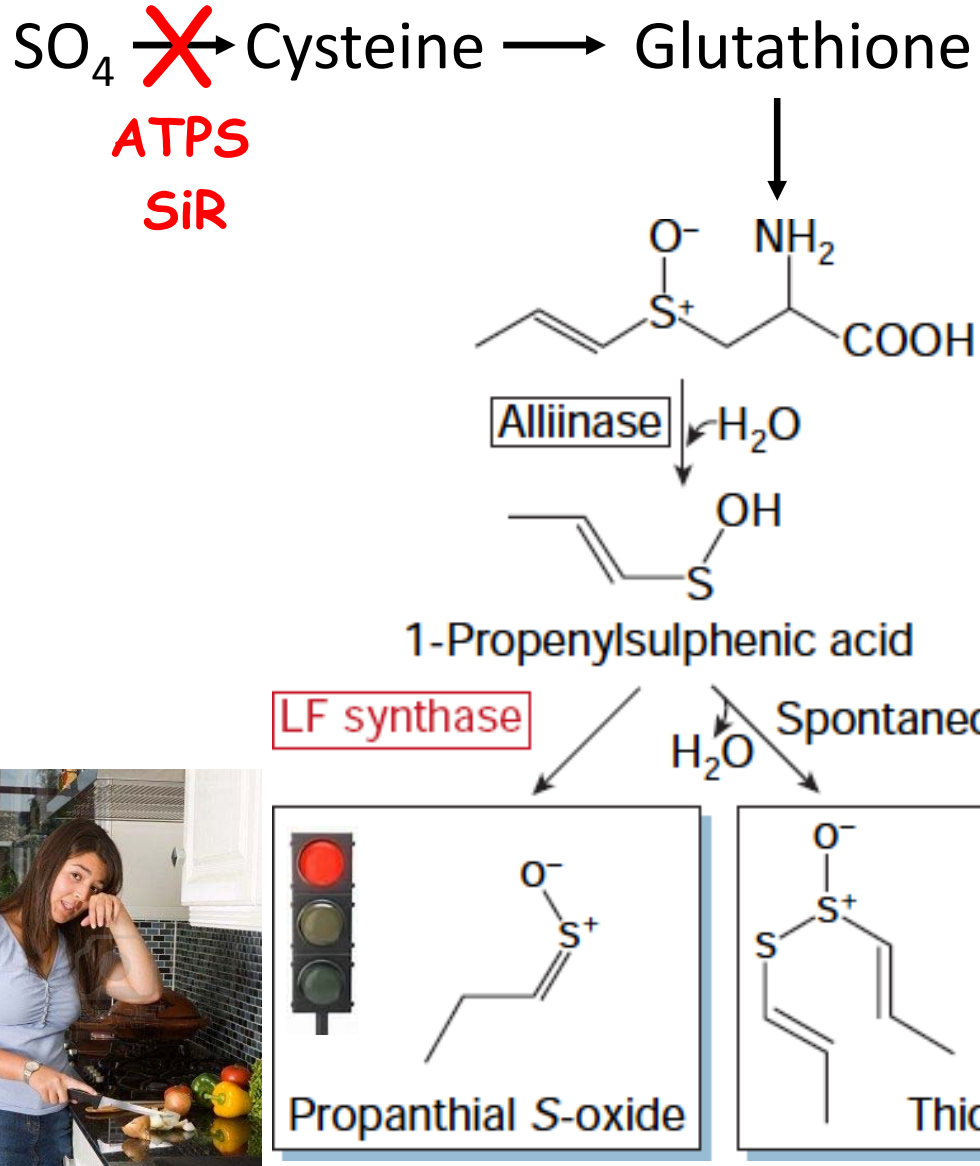
Genetic Variation for Low Pungency





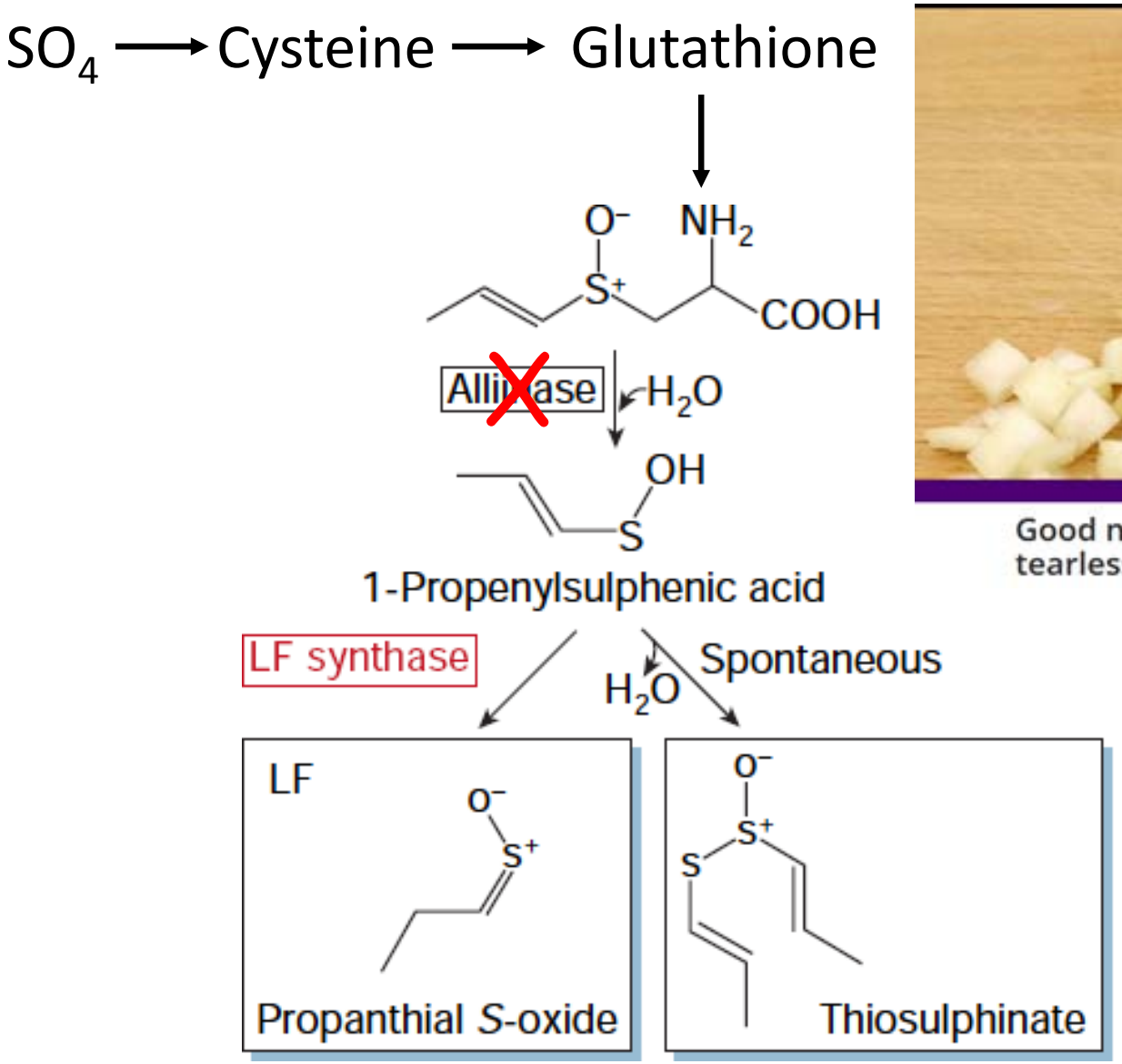
MGG 265:543-551
TAG 114:815-822

Genetic Variation for Low Pungency



MGG 265:543-551
TAG 114:815-822

Mutation Breeding for Low Tearing

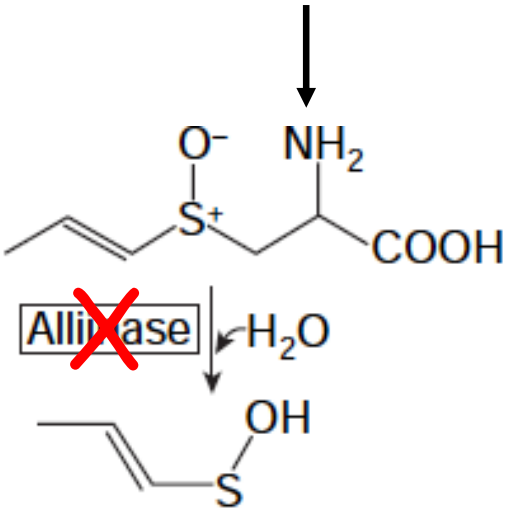


Good news! Japanese scientists have created a tearless onion

Sci. Rep. 6:23779

Mutation Breeding for Low Tearing

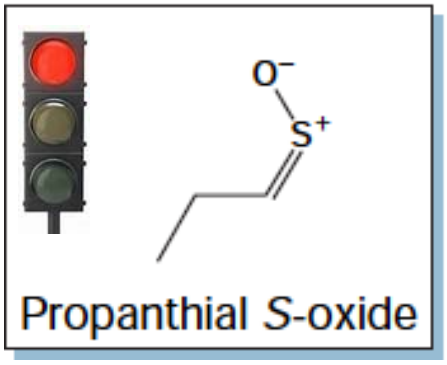
SO_4 → Cysteine → Glutathione

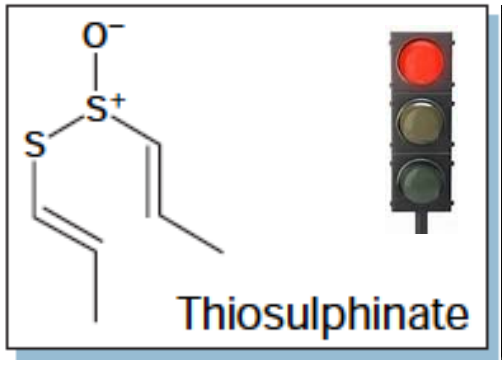


Good news! Japanese scientists have created a tearless onion

LF synthase

H_2O Spontaneous





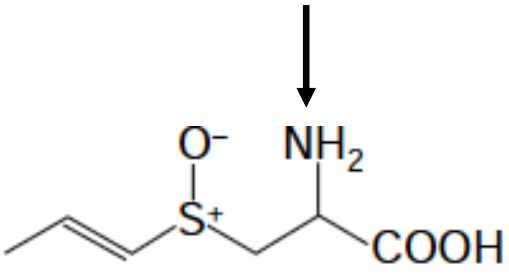
Sci. Rep. 6:23779

Reduced Tearing and Thiosulfinates

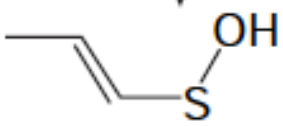
- Low sulfur soil
- Low sulfur uptake
- Alliinase mutation

Targeted Mutation of LFS

SO_4 → Cysteine → Glutathione



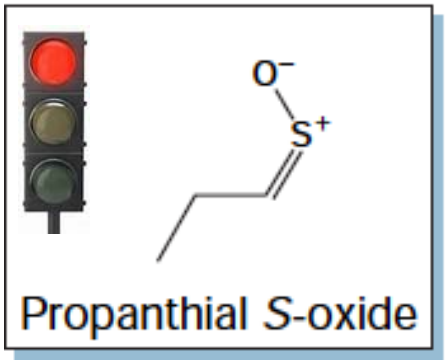
Alliinase H_2O

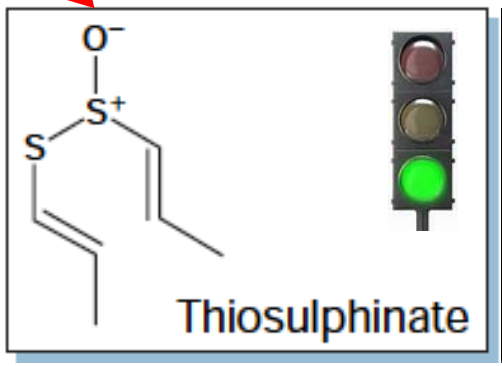


1-Propenylsulphenic acid

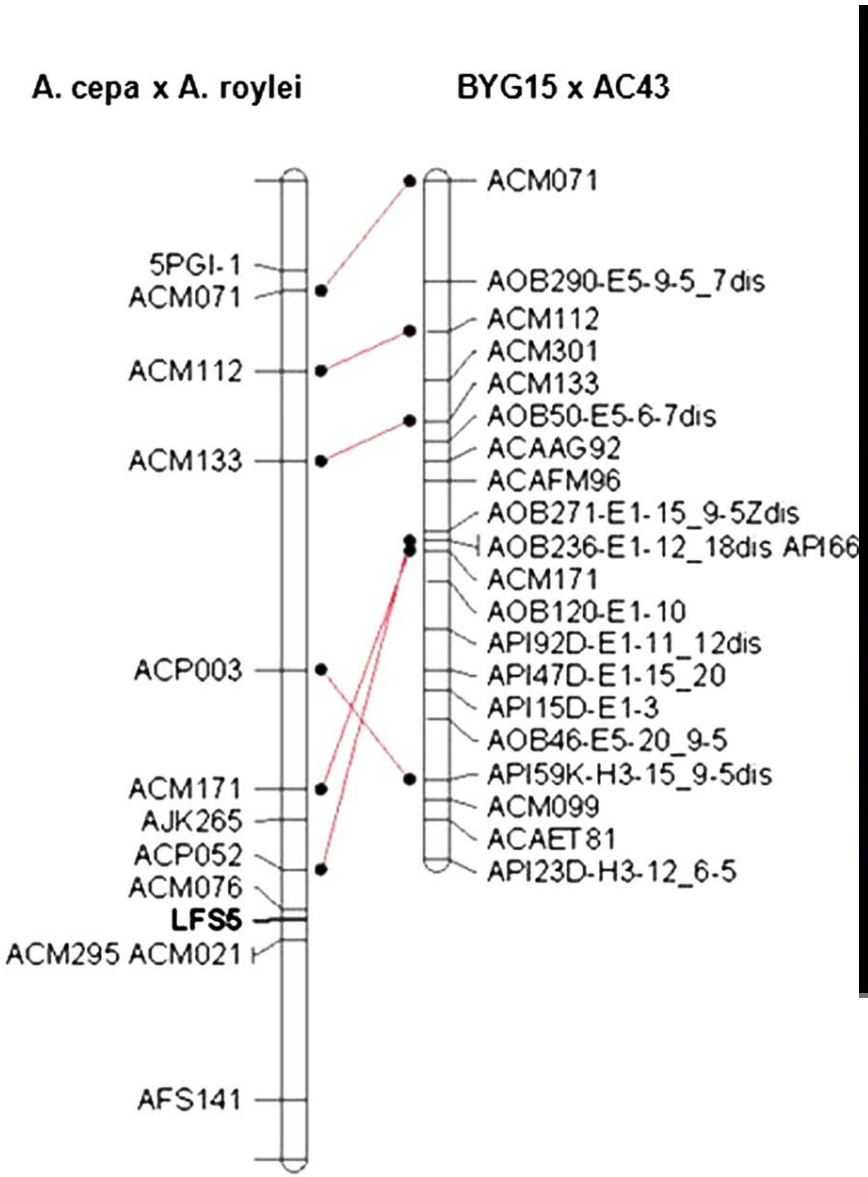
LF synthase

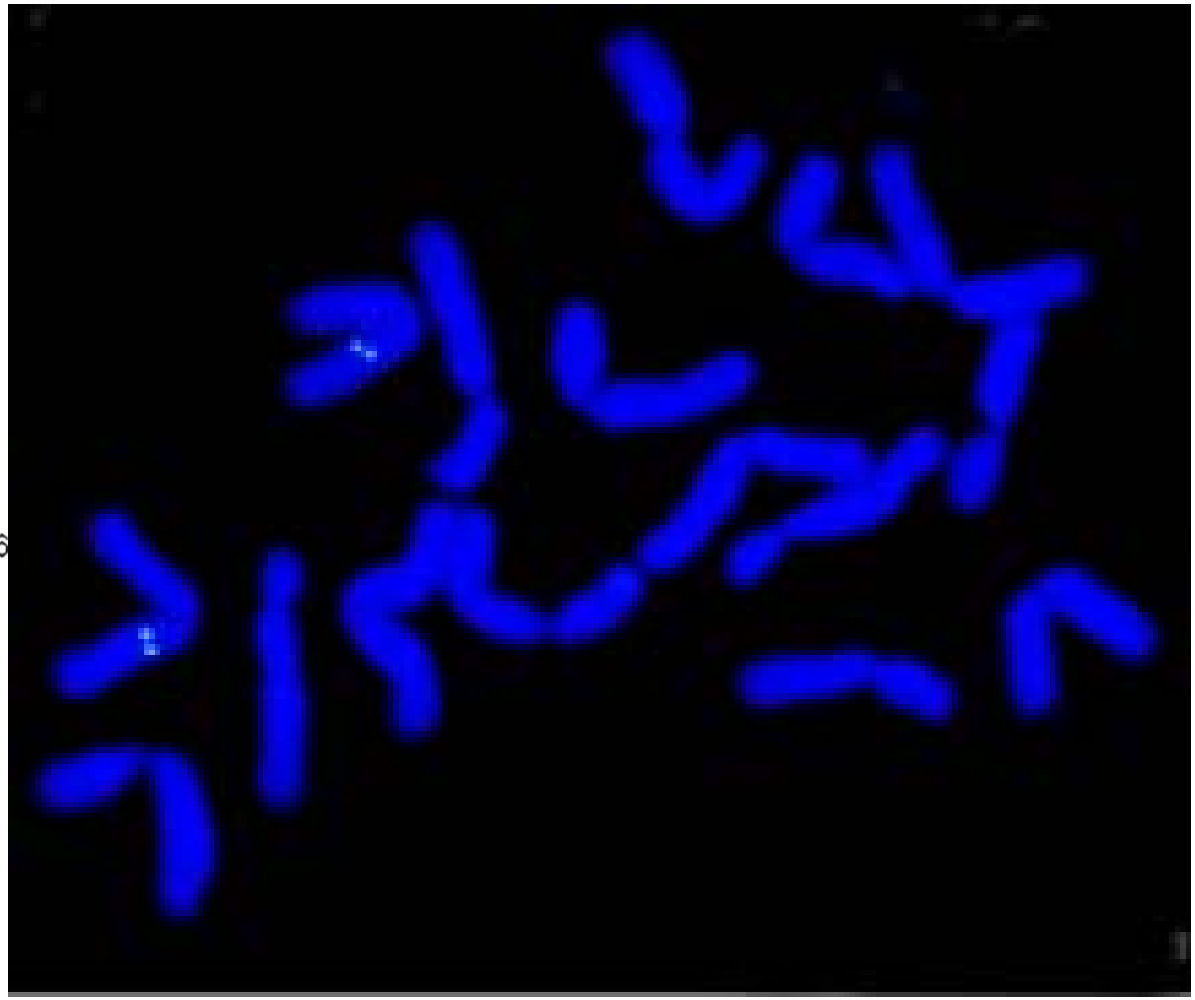
H_2O Spontaneous





LFS Maps to Chromosome 5

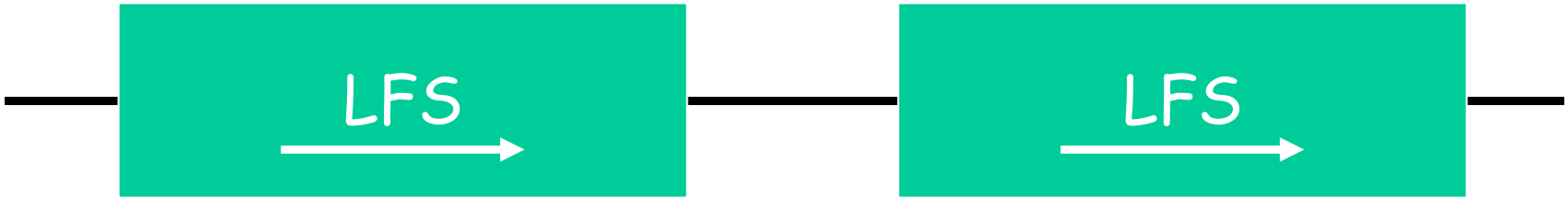




G3 2:643-651

Tandem Duplication of LFS

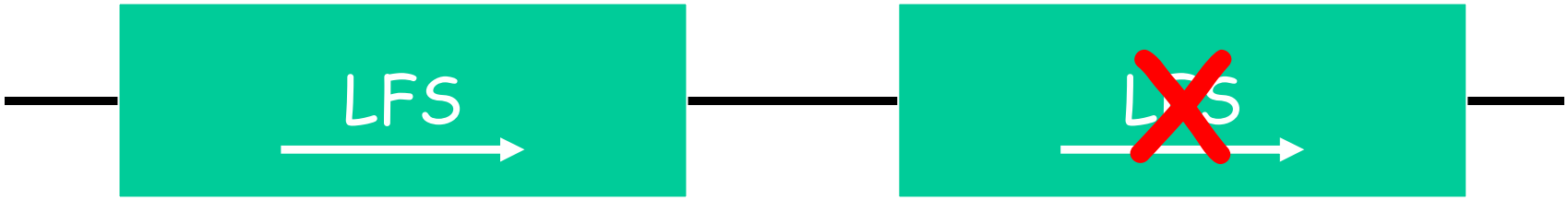
- Two copies of LFS are tandemly linked



G3 2:643-651

Tandem Duplication of LFS

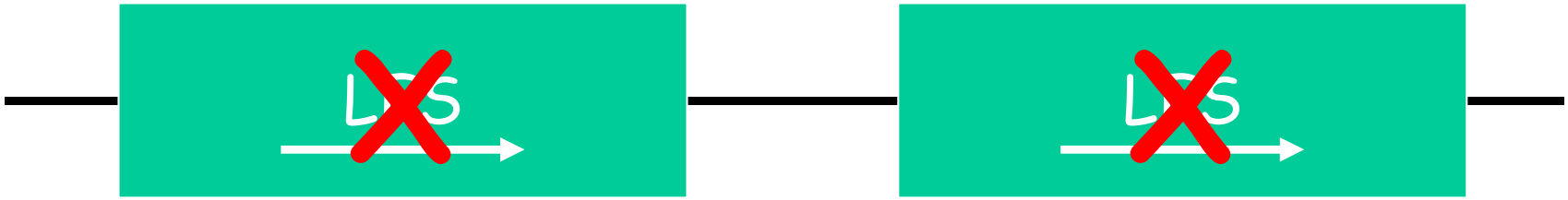
- Two copies of LFS are tandemly linked
- Low probability for mutating BOTH copies



G3 2:643-651

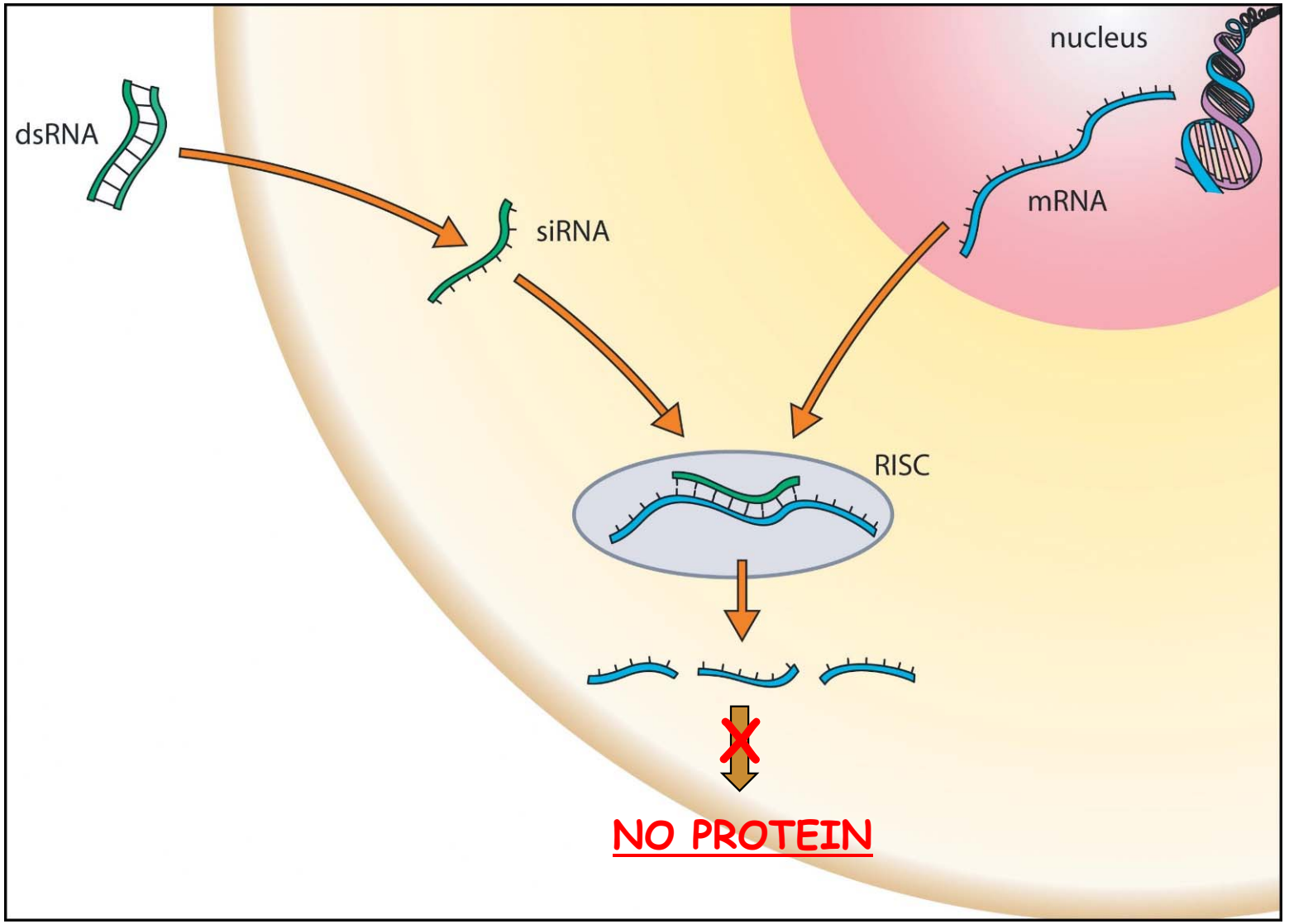
Tandem Duplication of LFS

- Two copies of LFS are tandemly linked
- Low probability for mutating BOTH copies



- Targeted knock-out of **both** copies of LFS with interfering RNA or gene editing

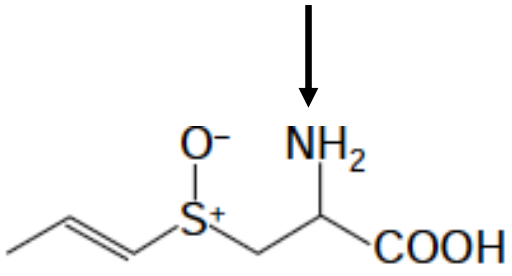
Interfering (i) RNA



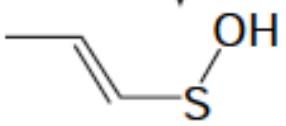
http://upload.wikimedia.org/wikipedia/commons/6/6c/RNAi.jpg

RNAi for LFS Produced First 'Tearless' Onion

SO_4 → Cysteine → Glutathione



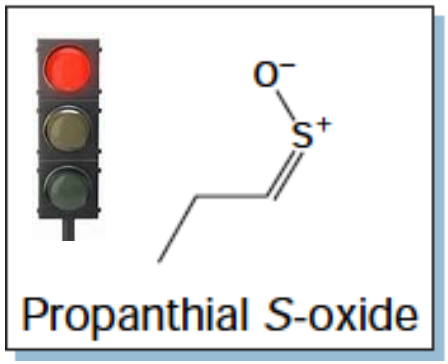
Alliinase ← H_2O



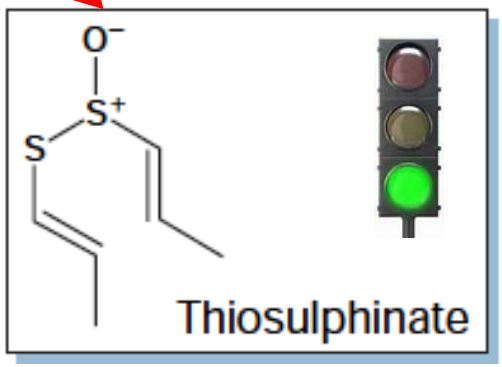
1-Propenylsulphenic acid

LF synthase

H_2O Spontaneous



Propanthial *S*-oxide

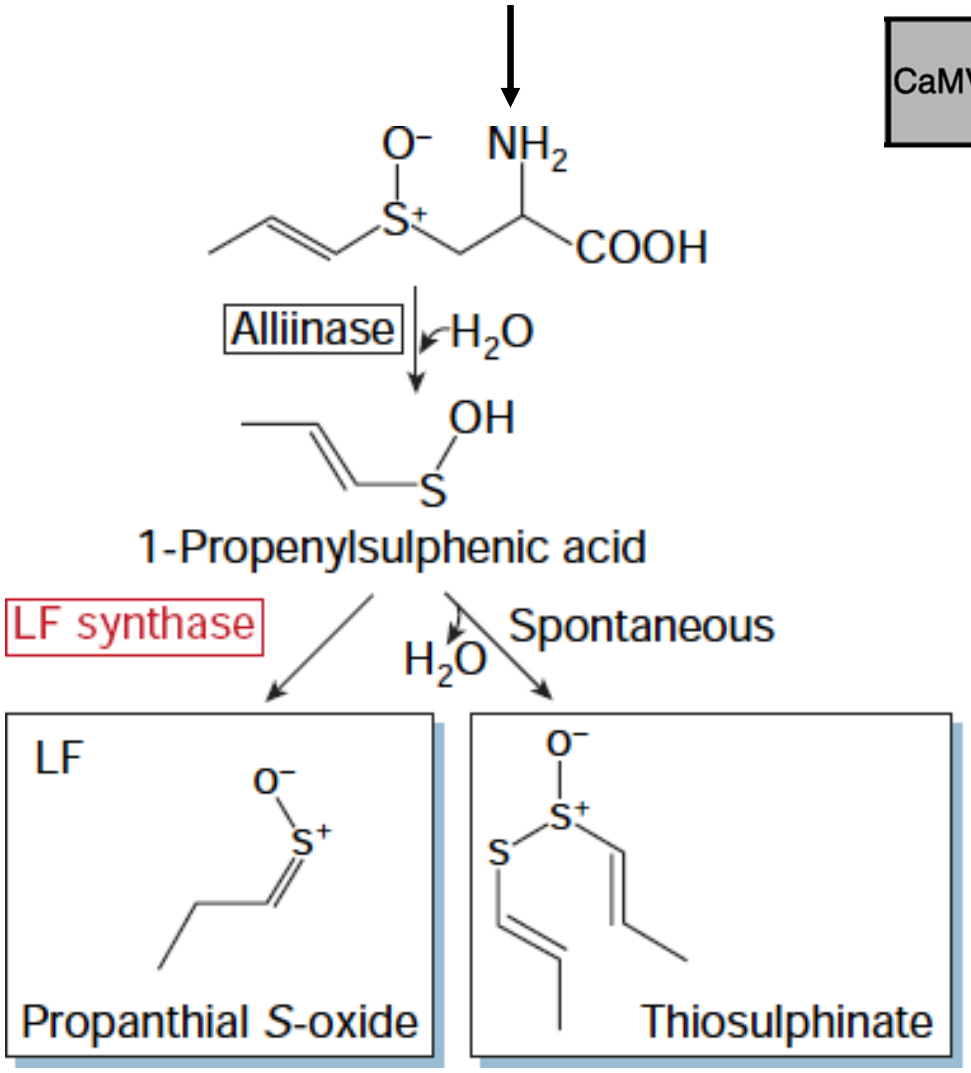


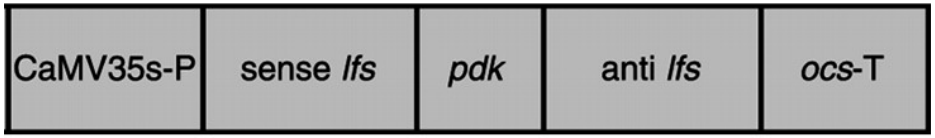
Thiosulphinate

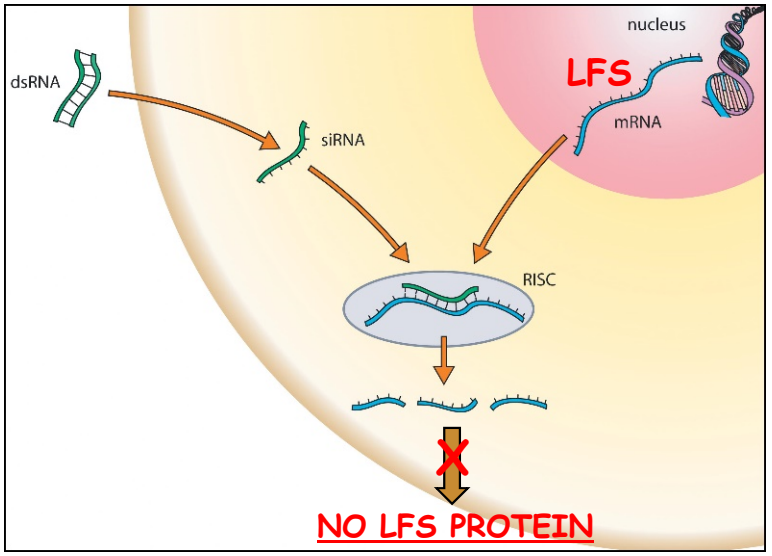
Plant Physiol 147:2096

RNAi for LFS Produced First 'Tearless' Onion

SO_4 ⟶ Cysteine ⟶ Glutathione

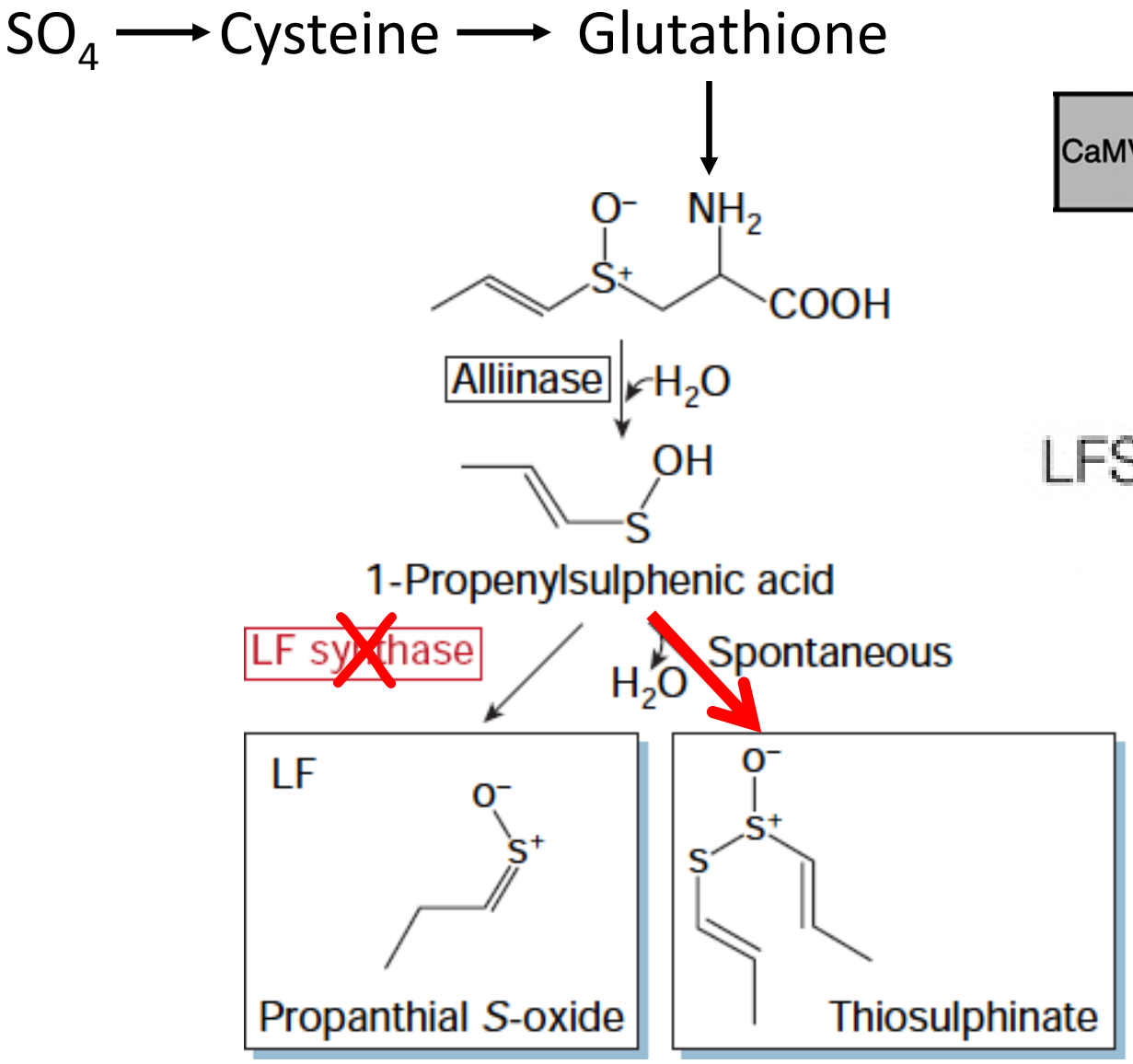


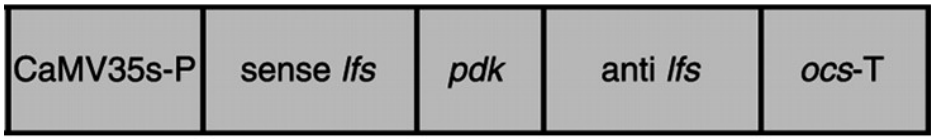


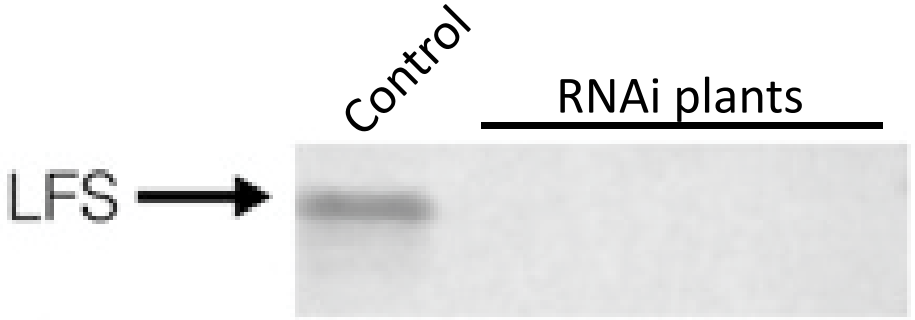


Plant Physiol 147:2096

RNAi for LFS Produced First 'Tearless' Onion





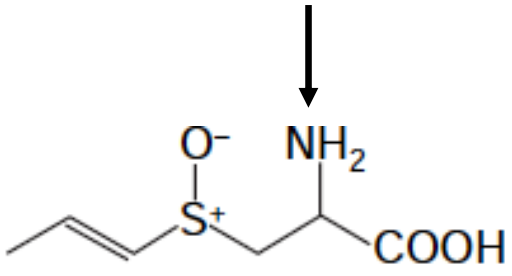


Protein Gel

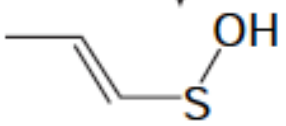
Plant Physiol 147:2096

RNAi for LFS Produced First 'Tearless' Onion

SO_4 → Cysteine → Glutathione

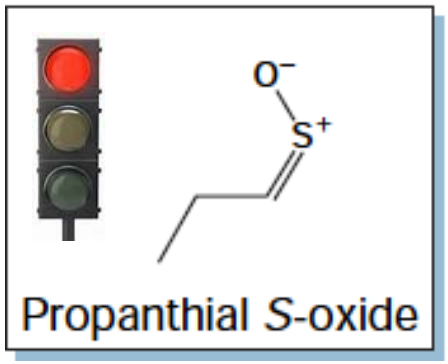


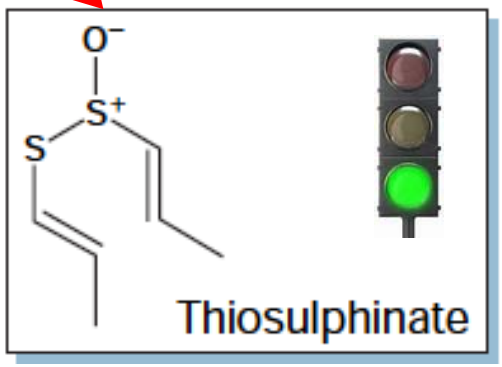
Alliinase + H_2O

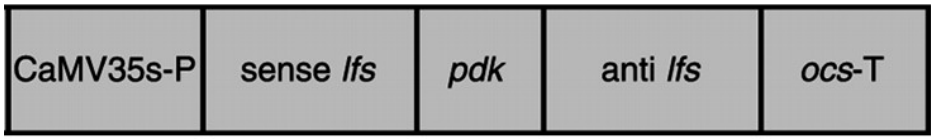


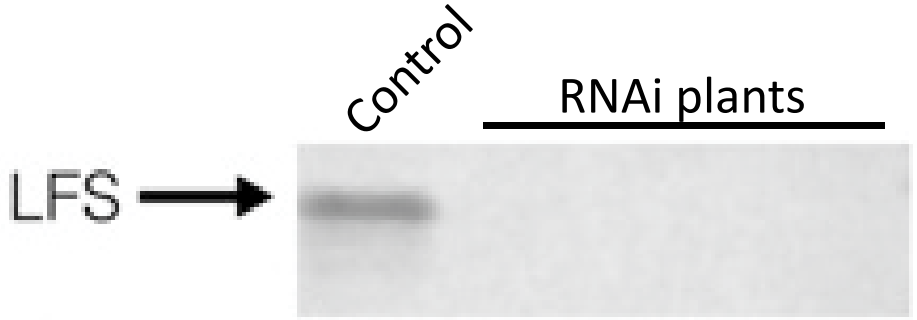
1-Propenylsulphenic acid

LF synthase (✗) + H_2O → Spontaneous







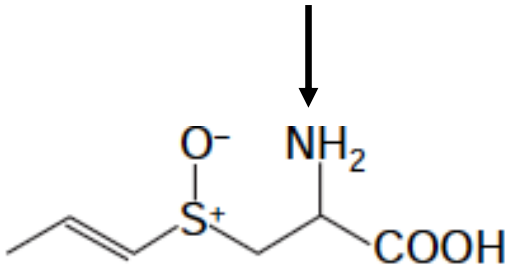


Protein Gel

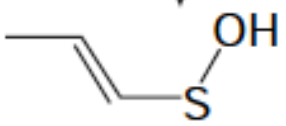
Plant Physiol 147:2096

RNAi for LFS Produced First 'Tearless' Onion

SO_4 → Cysteine → Glutathione



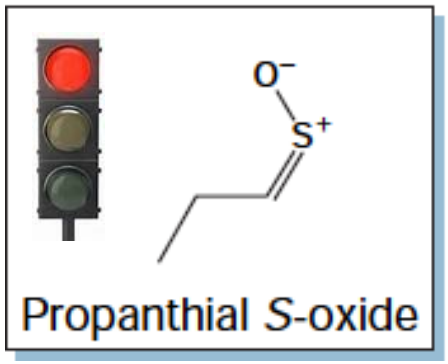
Alliinase H_2O



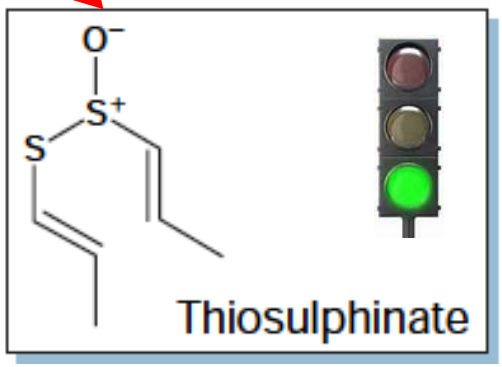
1-Propenylsulphenic acid

LF synthase

H_2O Spontaneous



Propanthial *S*-oxide



Thiosulphinate

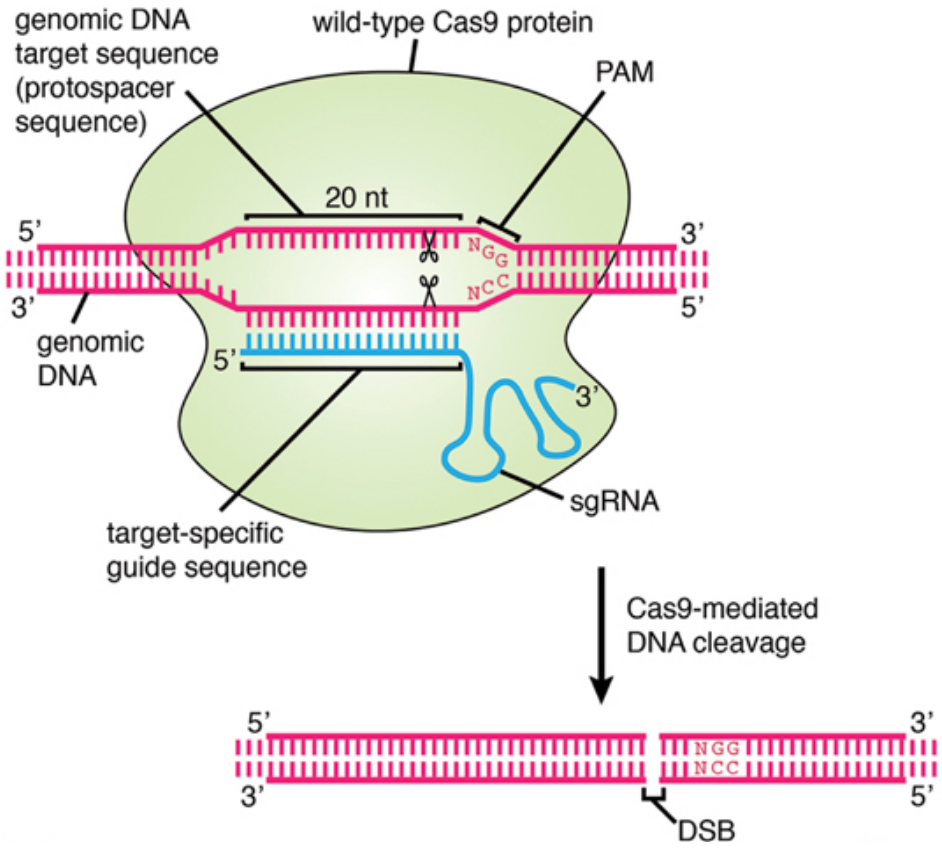
Plant Physiol 147:2096

'Tearless Onion'

RNAi knock-down does **NOT** cause tearing AND accumulates **MORE** healthy thiosulfinates!!

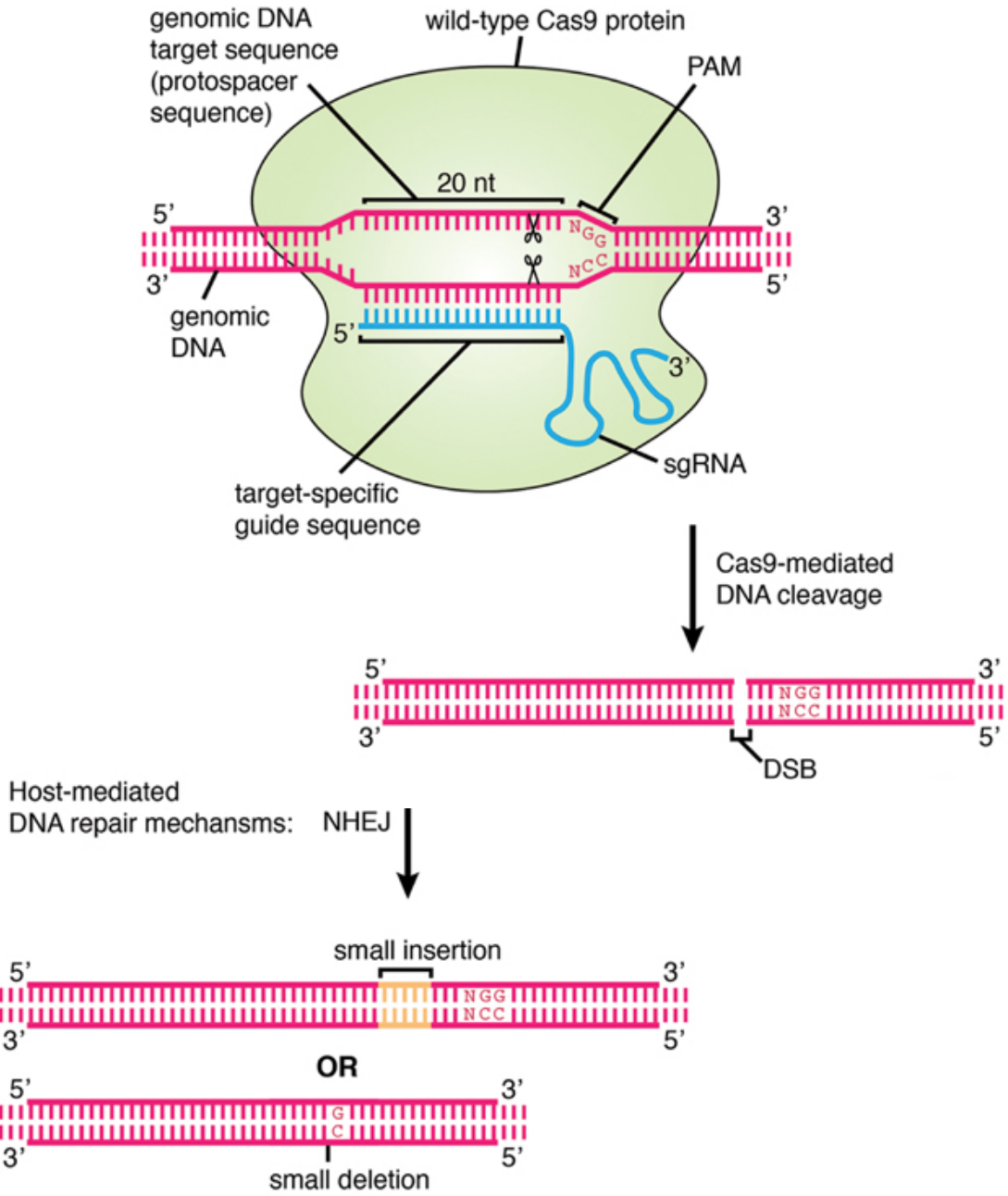
- Transgenic onion
- No commercialization
  - Consumer reluctance for GMOs
  - Regulatory and licensing costs
- *Great product; large market;*
  No go!

CRISPR/Cas9 Targeted Gene Mutation



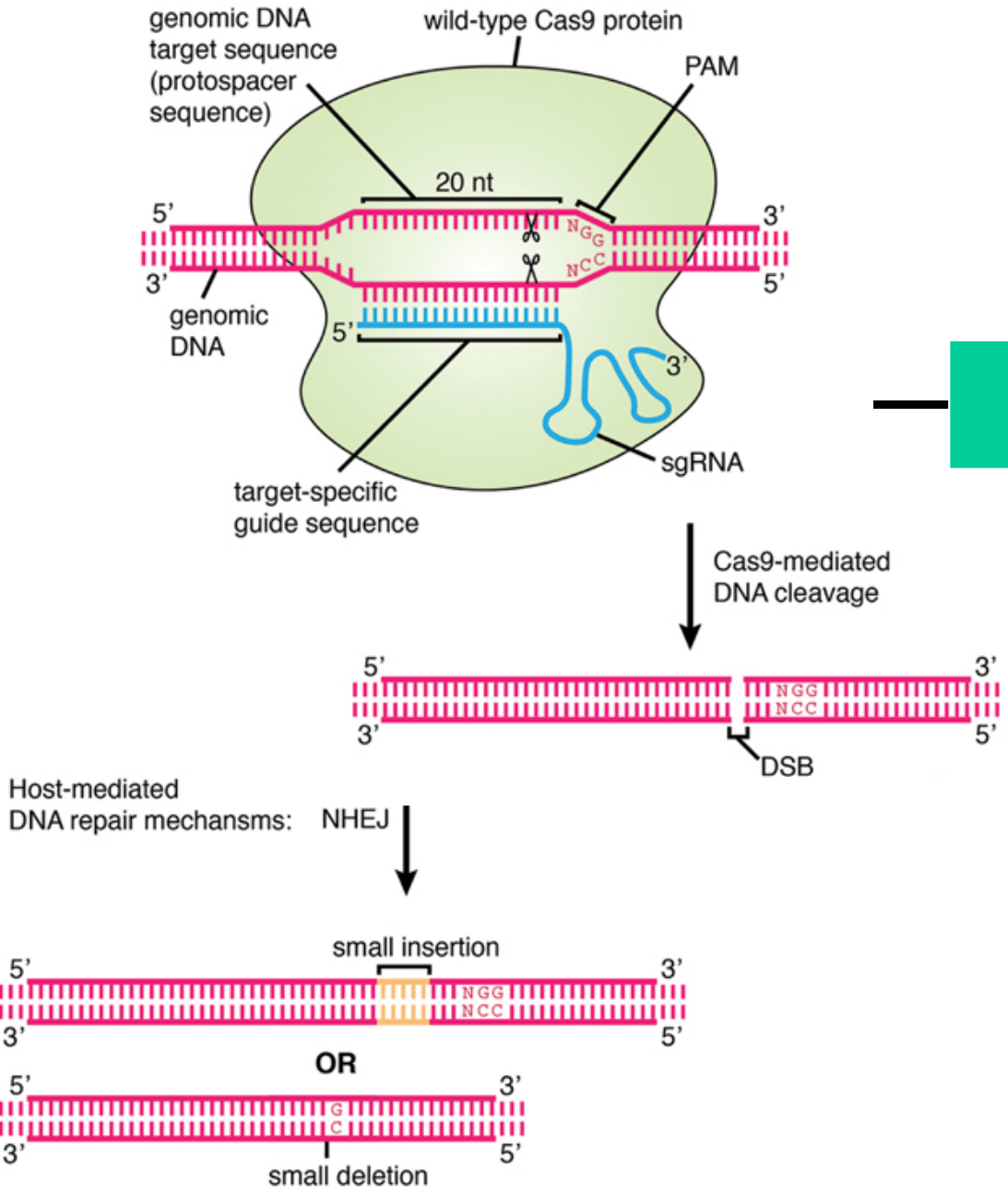
https://www.frontiersin.org/files/Articles/162559/fgene-06-00300-HTML/image_m/fgene-06-00300-g001.jpg

CRISPR/Cas9 Targeted Gene Mutation



https://www.frontiersin.org/files/Articles/162559/fgene-06-00300-HTML/image_m/fgene-06-00300-g001.jpg

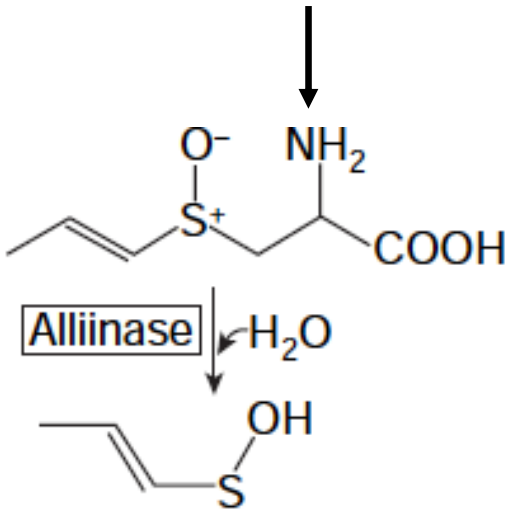
CRISPR/Cas9 Targeted Gene Mutation



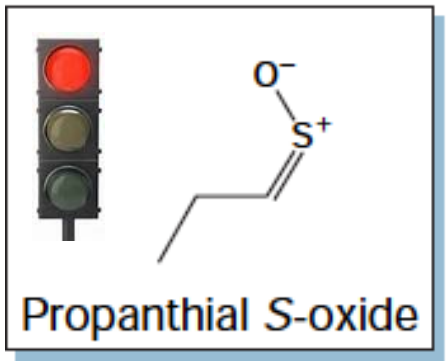
Knock-Out <u>BOTH</u> copies of LFS

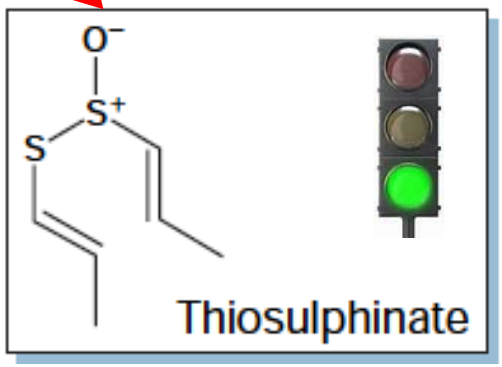
CRISPR/Cas9 Targeted Gene Mutation

SO_4 ⟶ Cysteine ⟶ Glutathione



Knock-out <u>BOTH</u> copies of LFS and NON-TRANSGENIC





Regulation of CRISPR/CAS

- Knock-out native gene
  - Targeted mutation
  - Presently not regulated
- Change protein
  - Same gene
  - May not exist naturally
  - Regulated?
- Build a new gene
  - Similar to transgenics
  - Regulated?

Polyphenol Oxidase

Onion Breeding Approaches

- Natural genetic variation
  - Basis of breeding for 1000s years
- Mutation breeding
  - Random approach
  - Few successes relative to effort
- Transgenics
  - Powerful approach and highly successful
  - Public opposition; high regulation costs
- Gene editing (CRISPR/Cas)
  - Non-random approach
  - Enormous potential for new variation
  - Regulation and public acceptance?

Excellent Research By

- Japan
  - House Foods
- New Zealand
  - Plant and Food Research
- USA
  - USDA and University of Wisconsin
  - J. Craig Venter Institute
  - Funding
    - USDA ARS, NRI, FRA, IFAFS, and SCRI programs
    - Seed companies