

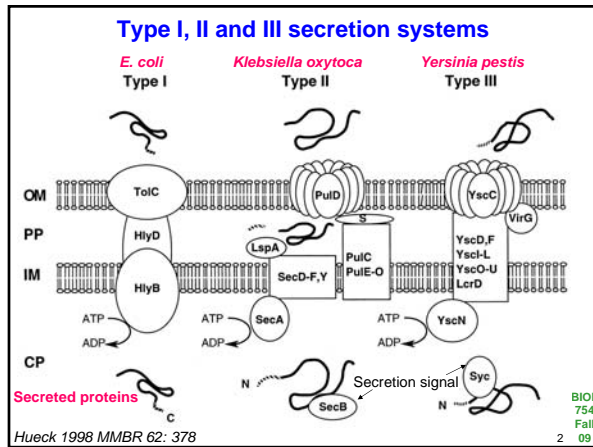
Lecture 19-2

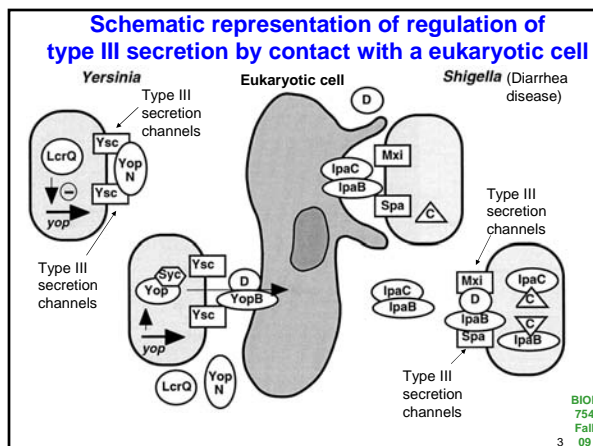
Biotic stress
Plant disease

A comparison between plant and animal disease (using a case study on the vitamin C-deficient Arabidopsis mutant *vtc1*)

The role of vitamin C (ascorbate) in plant defense against biotic and abiotic stress

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Plants have to cope with biotic and abiotic stress

Biotic stress: disease caused by **virulent** bacterial and fungal pathogens
Affected plants: crops in the *Brassicaceae* (cabbage) family (Crucifers)

Leaf Spot disease
 Pathogen: *Pseudomonas syringae*

Downey mildew disease
 Pathogen: *Hyaloperonospora parasitica*

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Plants have to cope with biotic and abiotic stress

Abiotic stress: air pollutants, nutrients, temperature, heavy metals, drought, salinity, excessive light

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Plant defense mechanisms

Biotic and abiotic stress → generation and accumulation of reactive oxygen species ($\cdot\text{O}_2$, H_2O_2 , $\cdot\text{O}_2^-$, $\cdot\text{OH}$); oxidative stress

↓

Oxidize proteins, nucleic acids, pigments, lipids...

Defense against oxidative stress

Antioxidants

Vitamin C (L-ascorbic acid, **AsA**)
 Vitamin E (α -tocopherol)
 Glutathione (**GSH**)

Antioxidant enzymes

Ascorbate peroxidase (**APX**)
 Ascorbate regenerating enzymes (**GR**,
MDAR, **DHAR**)

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**Vitamin C – Ascorbic acid:
A multi-faceted molecule**



Functions in humans

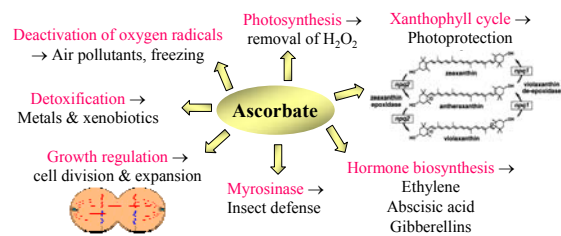
- Collagen biosynthesis (collagen deficiency → scurvy)
- Key antioxidant that is associated with resistance to oxidative stress and longevity
- Health-promoting effects: protection against diseases such as heart disease, obesity, hypertension, diabetes, some types of cancer

What is the function of ascorbate in plants?

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Vitamin C – Ascorbic acid: A multi-faceted molecule

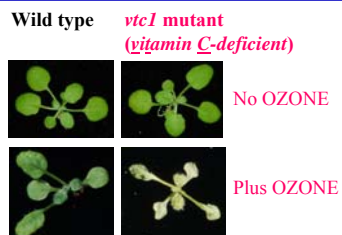
Functions in plants



Does ascorbate play a role in protection against disease in plants?

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The role of ascorbate in response to pathogens



- vtc1*:
- sensitive to O₃, SO₂, freezing (abiotic stress)
 - 30% of wild-type ascorbate content
 - defect in ascorbate biosynthesis

Responses of *vtc1* to pathogens (biotic stress)?

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Responses of *vtc1* to biotic stress

Infection of wild type and *vtc1* plants with *Pseudomonas syringae*

1. Grow up bacteria and dilute to desired OD

2. Infiltrate bacteria into leaves

3. Punch out leaf discs, macerate

4. Grow bacteria on plate

5. Count bacteria

Disease symptoms

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Bacterial growth of *Pseudomonas syringae* is lower in *vtc1*

Growth curve of *P. syringae* in leaves of wild type and *vtc1* (means \pm SD, n = 4).
Inoculation titer: 10^6 CFU/ml
CFU = Colony Forming Units

\Rightarrow *vtc1* is more resistant to *P. syringae* infection.

What are the molecular mechanisms of this disease tolerance?

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Mammals	Plants
<div style="border: 1px solid black; padding: 2px; display: inline-block;">Pathogen</div> Viruses, Bacteria, Fungi	<div style="border: 1px solid black; padding: 2px; display: inline-block;">Pathogen</div>
↓ Recognition	↓ Recognition
↓ Immune system	↓ "Immune system"
<div style="border: 1px solid black; padding: 2px; display: inline-block; color: red;">Defense gene induction</div>	<div style="border: 1px solid black; padding: 2px; display: inline-block; color: red;">Defense gene induction</div>
Antibodies	Pathogenesis-related (PR) proteins
↓	↓
<div style="border: 1px solid black; padding: 2px; display: inline-block; color: red;">Defense response</div>	<div style="border: 1px solid black; padding: 2px; display: inline-block; color: red;">Defense response</div>
Lysis of infected cells Fever (Innate immune response)	Pathogen resistance

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Innate immune response in animals

Innate immunity refers to antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to an antigen. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

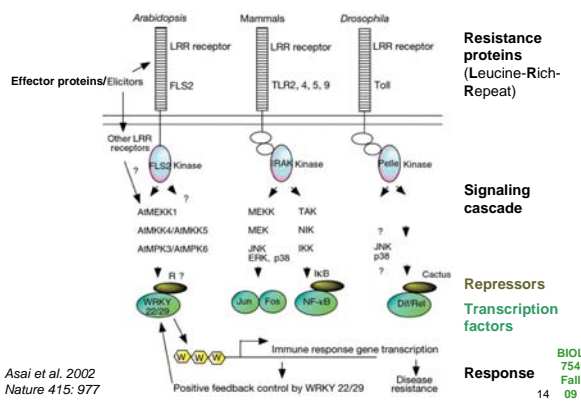
Innate immune responses involve:

- phagocytic cells (neutrophils, monocytes, and macrophages);
- cells that release inflammatory mediators (basophils, mast cells, and eosinophils);
- natural killer cells (NK cells)
- molecules such as complement proteins, acute phase proteins, and cytokines.

Examples of innate immunity include anatomical barriers, mechanical removal, bacterial antagonism, pattern-recognition receptors, antigen-nonspecific defense chemicals, the complement pathways, phagocytosis, inflammation, and fever.

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Model of innate immune signaling



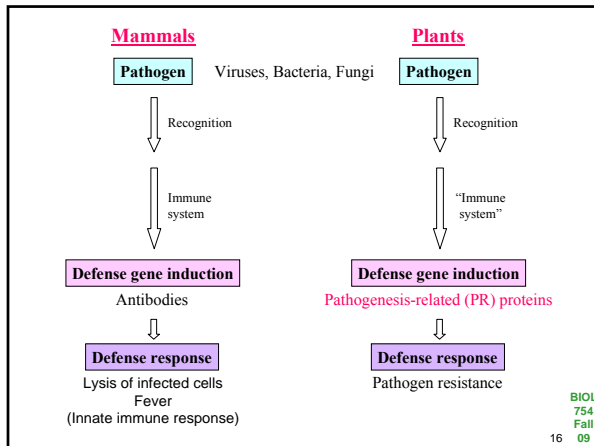
Example of innate immune response - Fever

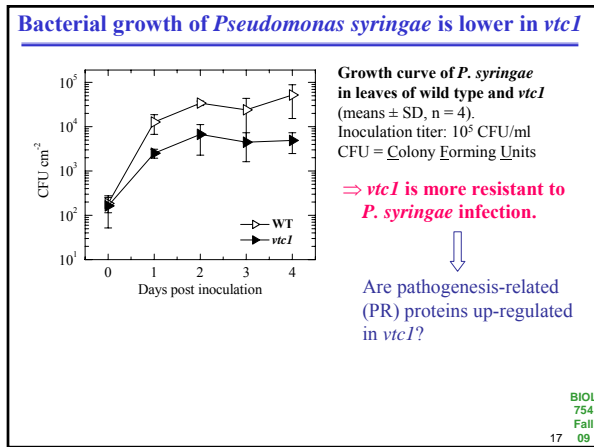
Activated macrophages and other leukocytes release proinflammatory cytokines (such as TNF-alpha and IL-1) when their toll-like receptors bind pathogen associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipopolysaccharide, mannans, flagellin, pilin, and bacterial DNA. There are also pattern-recognition molecules for viral double-stranded RNA (dsRNA) and fungal cell wall components such as lipoteichoic acids, glycolipids, and mannans.

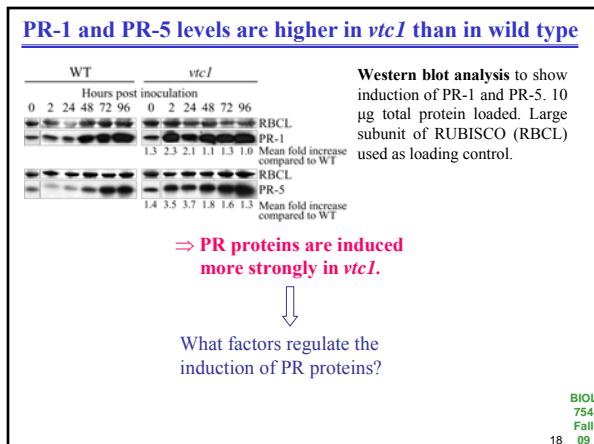
These cytokines **stimulate the anterior hypothalamus of the brain**, the part of the brain that regulates body temperature, **to produce prostaglandins that lead to an increase in body temperature**. Up to a certain point, fever is beneficial:

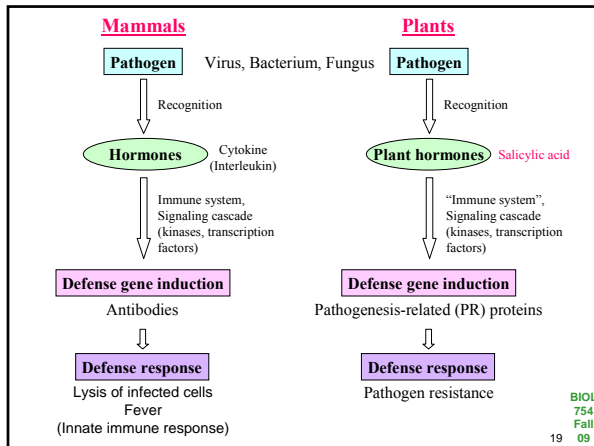
1. Fever **increases the environmental temperature above the optimum growth temperature for many microorganisms**. If the microorganisms are growing more slowly, the body's defenses have a better chance of removing them all.
2. Fever leads to **the production of heat shock proteins** that are recognized by some intraepithelial T-lymphocytes, resulting in the **production of inflammation-promoting cytokines**.
3. Fever **elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body**. An elevation in the rate of metabolism can increase the production and activity of phagocytes, speed up the multiplication of lymphocytes, increase the rate of antibody and cytokine production, increase the rate at which leukocytes are released from the bone marrow into the bloodstream, and speed up tissue repair. Too high of a body temperature, however, may cause damage by denaturing the body's enzymes.

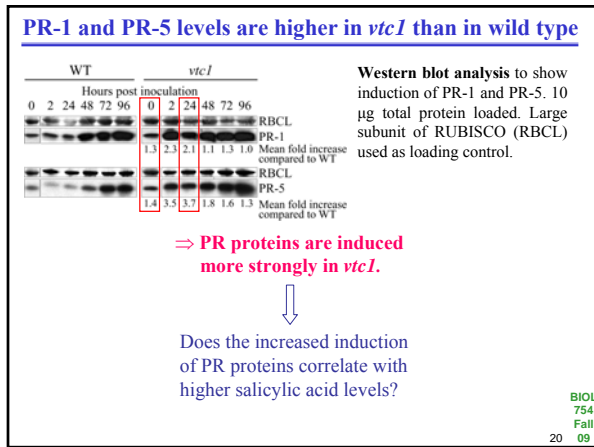
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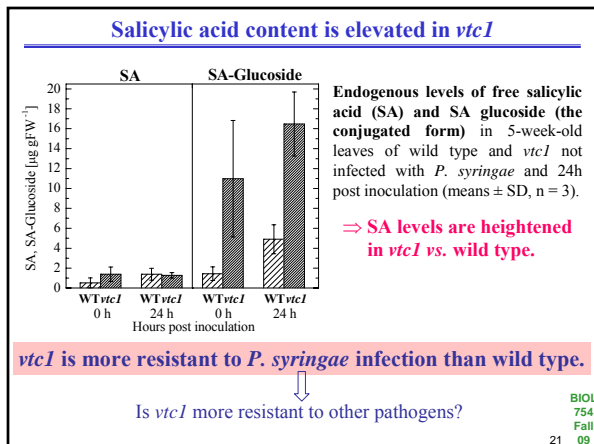













Responses of *vtc1* to biotic stress

Infection of wild type and *vtc1* plants with *Hyaloperonospora parasitica*

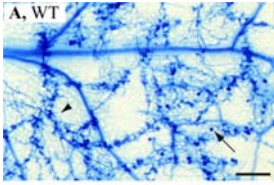


Conidiophores suspension of
H. parasitica

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Growth of *H. parasitica* is reduced in *vtc1*

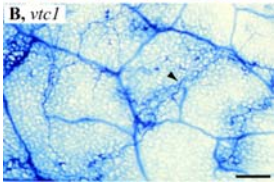
A, WT



Conidiophores production in wild-type and *vtc1* plants inoculated with *H. parasitica*. Infected leaves were analyzed seven days after inoculation. Scale bar, 100 μm.

← conidiophores
▲ hyphae

B, *vtc1*



⇒ *vtc1* is more resistant to *H. parasitica*.

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