Session 8: Plant Immunity: Evolutionary Arms Race between Host and Pathogen

Overview:
Similar to animal immune systems, a plant's immune system is in a constant battle against pathogens. This session covers the basics of plant immunology and reviews two major pathways that activate the plant immune response: Effector-triggered immunity and Pattern-triggered immunity. In addition, you'll learn the fundamental factors that lead to host-pathogen co-evolution.

First video:
Title: Introduction to Plant-Pathogen Interactions
Speaker: Sheng-Yang He
Time: 19:28

Concepts: Effector-Triggered immunity, Avr genes versus resistance genes, bacterial Type-II Secretion system, indirect recognition by R proteins, and Pattern-Triggered immunity

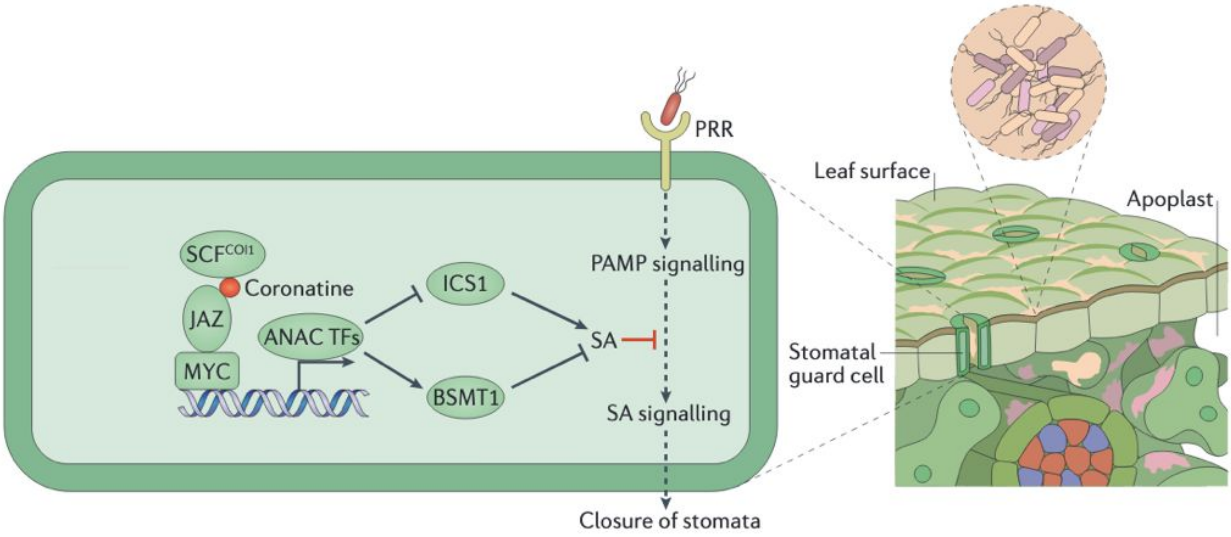
Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
    a. Plants have receptors that recognize molecular patterns found on pathogens.
    b. Resistance (R) proteins in plants can recognize multiple virulence factors.

2. Which of the following are common characteristics of resistance (R) proteins? (Select all that apply)
    a. Arise in the pathogen.
    b. Arise in the plant.
    c. Surface receptors.
    d. Homology to animal proteins.
    e. Homology to antibodies.

3. Briefly, compare and contrast pattern-triggered immunity (PRR/PAMP system) and effector-triggered immunity (R/Avr system) in plants. Give an example of each.

4. Shown below is the signaling pathway of a Pattern Recognition Receptor (PRR) upon recognition of a pathogen in the stomata of a plant.



Xin, XF et al. (2018) Nature Reviews Microbiology

a. What is the normal function of the stomata in the plant? Why would this organ represent a problem for the plant when it interacts with pathogens? Briefly explain.

b. Under normal conditions, what happens to the stomata when the plant recognizes that there is a pathogen in the vicinity?

c. High humidity inhibits the closure of the stomata. What would be the consequence for the plant? Briefly explain.

d. If you were a pathogen, how would you target this pathway to increase your infectivity rate? Briefly explain.

5. Plants have various defense mechanisms against bacterial infections. Researchers are studying how the infectivity of pathogens changes with different genetic mutations in plants. Shown below are the results of infecting mutant plants (fls2-17) and wild-type plants (Ler-0) with either bacteria (Pst DC3000) or vehicle (water).

Zipfel C, et al. (2004) Nature

   a. What is the consequence of the fls2-17 mutation in plants? Briefly explain.

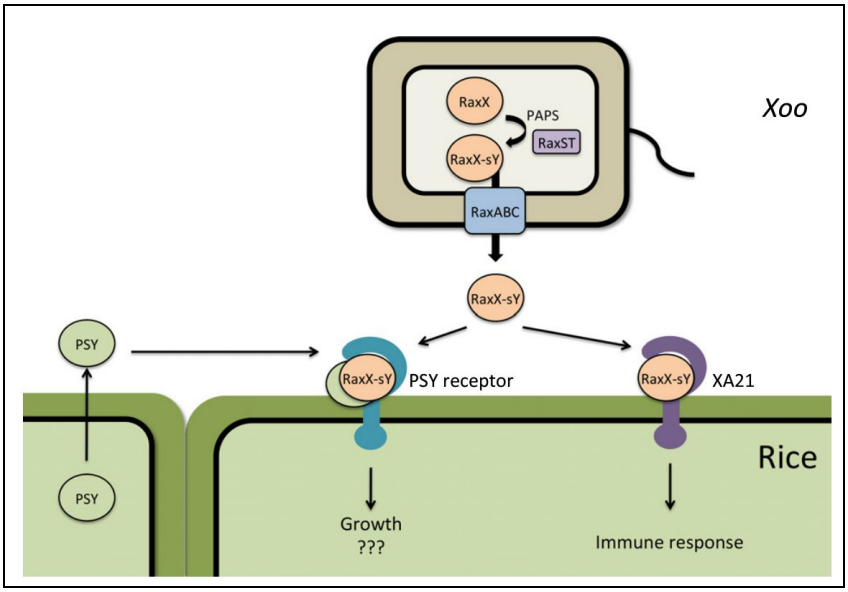
   b. Briefly describe the two major mechanisms that plants have for defense against pathogens.

   c. fls2-17 is a receptor that localizes close to the stomata. To which of the two mechanisms described in question (b) does this gene belong? Propose a mechanism of action.

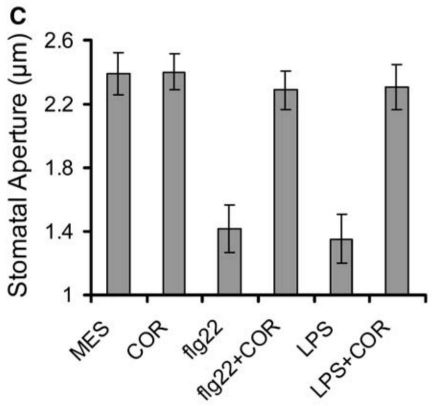
6. Explain how R proteins are able to recognize many types of pathogens, despite large variation in effector proteins.

7. Most R proteins detect pathogen effectors inside the plant cell. Predict a scenario in which pathogen effectors would function extracellularly.

8. In infection, there is a constant battle between host and pathogen. The host is winning the battle if it can mount an immune response and clear the pathogen, while the pathogen is winning if it can evade host detection systems. The diagram below depicts the current status of an immune battle between a bacterium, *Xoo*, and the rice cells it infects. Given the information that PSY is a growth factor, Xa21 is an immune receptor, and RaxX is a bacterial protein, arrange the components of this system (RaxX, Xa21, PSY, and PSY receptor) into their evolutionary history. (Reference: Pruitt et al., New Phytologist 2017).



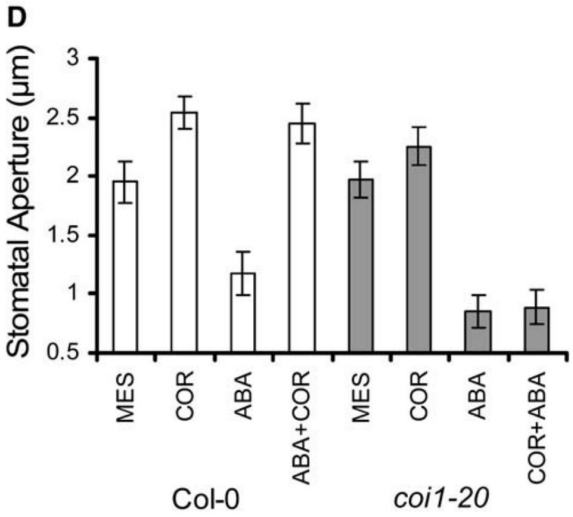
9. Plant stomata close in response to bacterial infection. (Reference: Melotto, Cell 2006)
    a. A group of researchers wanted to determine the effects of three different bacterial products - flagellin (flg22), lipopolysaccharide (LPS), and coronatine (COR) - on stomatal aperture. To address their question, they treated *Arabidopsis* leaves with each of the compounds. The data are shown below. MES = buffer used as negative control.



        i. Based on the data in the figure above, describe the effects of each compound on plant stomata.

ii. What type of immunity - pattern-triggered immunity or effector-triggered immunity - likely recognize each compound?

b. The researchers then compared the responses of two strains of *Arabidopsis* plants, Col-0 and coi1-20, to coronatine (COR). In the figure below, MES = buffer used as negative control; ABA = abscisic acid, a plant hormone that stimulates stomatal closing.

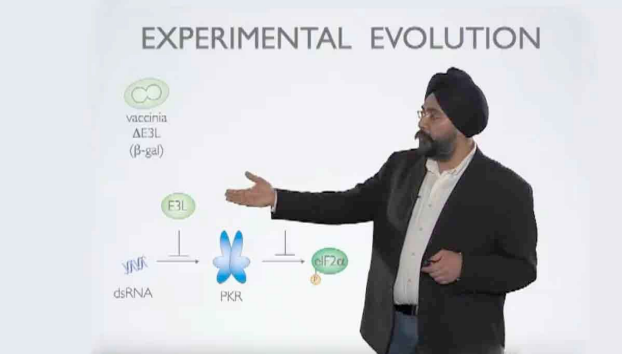


i. Based on the data above, what can you conclude about each strain's response to COR?

ii. Would you predict each strain to be resistant or susceptible to the bacteria that produces COR?

Second video:
Title: Host and Viral Evolution: Molecular Evolutionary Arms Race Between Primate and Viral Genomes
Speaker: Harmit Malik
Time: 32:50

Concepts: Molecular arms races between primate and viral genomes, host evolution, host-virus interactions, natural selection of mutations, and PKR signaling evolution across primates



Questions for Second Video:

1. Which of the following examples are ways by which a host can be "ahead" of a pathogen in an evolutionary arms race?
    a. A synonymous mutation in a protein involved in recognition of the pathogen.
    b. A synonymous mutation in a protein involved in the metabolism of the host.
    c. A single amino acid change that would interfere with the recognition of the pathogen by the host.
    d. A synonymous mutation in the pathogen in a protein involved in recognition of the pathogen.
    e. None of the above.

2. The polio virus gene X is involved in interaction with the host; therefore
    a. protein X is under positive selection.
    b. the host protein that interacts with protein X is under positive selection.
    c. protein X and the host protein that interacts with protein X are under positive selection.
    d. None of the above.

3. State whether the following statements are true or false. If false, explain why.
    a. eIF2a is a translational factor that has a high mutation rate due to co-evolution with viruses.
    b. Pathogen genes that code for proteins that bind to host immune proteins are prone to high selective pressures.
    c. Amino acid-altering mutations are more likely to occur than silent mutations by random chance.
    d. Genetic changes in pseudogenes are evolutionarily unfavorable.
    e. Diversifying selection results from an increased mutation rate.
    f. Single residue changes are sufficient to disable pathogen mimicry.
    g. Pathogen mimicry can result from copying a host gene into a viral genome.

4. You're designing a small molecule drug to treat patients who are infected with a pox-related virus. Janet discovers that the virus expresses a protein, K3L, that binds to the host kinase dimer PKR and reduces the translational inhibition induced by the host.
    a. Why is translational inhibition good for the host when it is infected by a virus? Briefly explain.

    b. Janet decides to use K3L as the target for her small molecule. Is this a good idea? Briefly explain your answer.

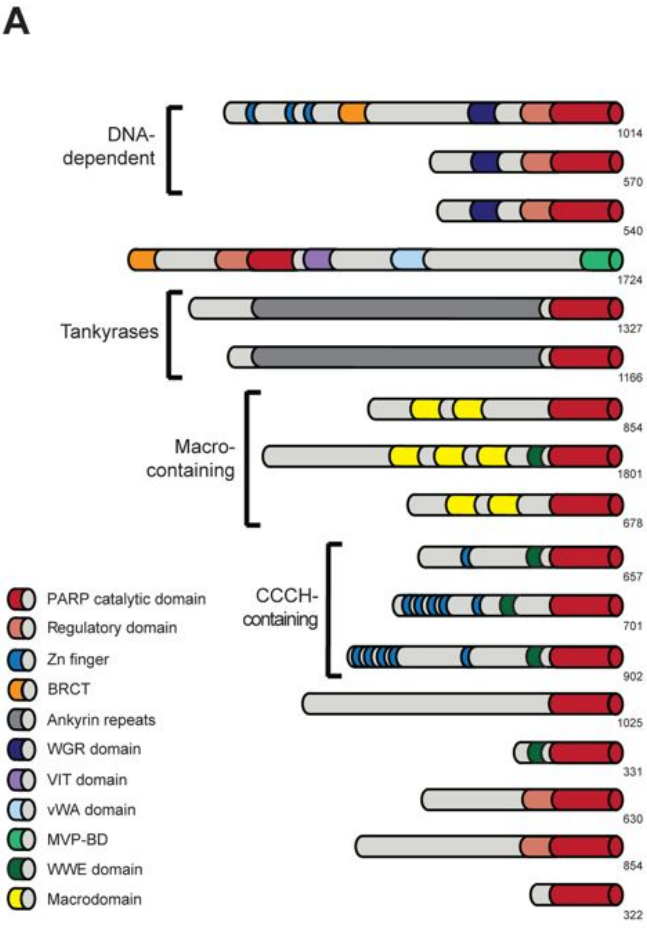
5. Compare and contrast purifying selection and diversifying selection. Give an example of each.

6. Explain why the mimicry of eIF2a by poxvirus K3L to inhibit PKR function is NOT analogous to a three-way rock-paper-scissors game.

7. Predict another way that the host can optimize its interaction between PKR and eIF2a to avoid mimicry by a viral protein.

8. Mutations allow organisms to adapt to their environment.
    a. How does co-evolution between host and pathogen drive "positive selection"? Briefly explain.

b. Shown below is the likelihood of positive selection (Ks/Ka) in the PARP family of genes. PARP is a family of host proteins involved in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. Note: A higher Ks/Ka number indicates increased mutability.



Gene name	Alternative name(s)	Enzymatic activity[1]	B PARRIS LRT	PARRIS p-value
PARP1	ARTD1	Poly-	<0.01	>0.99
PARP2	ARTD2	Poly-	<0.01	>0.99
PARP3	ARTD3	Mono-	<0.01	>0.99
PARP4	vPARP/ ARTD4	Poly-	**22**	**<0.01**
PARP5a	Tankyrase 1/ ARTD5	Poly-	<0.01	>0.99
PARP5b	Tankyrase 2/ ARTD6	Poly-	<0.01	>0.99
PARP9	ARTD9/ BAL1	Inactive[2]	**2.5**	**0.28**
PARP14	ARTD8/ BAL2	Mono-	**1.8**	**0.41**
PARP15	ARTD7/ BAL3	Mono-	**4.0**	**0.14**
PARP7	TiPARP/ ARTD14	Mono-	<0.01	>0.99
PARP12	ARTD12	Mono-	<0.01	>0.99
PARP13	ZAP/ ARTD13	Inactive[2]	**9.9**	**<0.01**
PARP10	ARTD10	Mono-	<0.01	>0.99
PARP11	ARTD11	Mono-	<0.01	>0.99
PARP6	ARTD17	Mono-	<0.01	>0.99
PARP8	ARTD16	Mono-	<0.01	>0.99
PARP16	ARTD15	Mono-	<0.01	>0.99

Daugherty MD, et al. (2014) PLoS Genet. 2014

i. PARP13/ZAP has broad antiviral activity. Does this information correspond with PARP13's Ks/Ka results? Briefly explain.

ii. If you're studying genes involved in host immunity, which other PARP gene would you choose to study? Briefly explain your answer.

iii. Although it is known that PARP proteins carry out ADP-ribosylation, a type of post-translational modification, most members of the PARP family are poorly characterized. What characteristics would you expect to find in pathogenic proteins that interact with PARP family members? Briefly explain.

9. Explain the selection pressures that would lead a residue to “toggle” between a few specific amino acids during its evolution.

10. Researchers discovered that a viral factor known as *Fv1* has persisted in the genomes of several species of mice for 7 million years. To determine the role of *Fv1*, they transduced two of its allelic variants*, Fv1n and Fv1m*, into the mouse cell line MDTF and then exposed the cells to three different murine viruses, AKV-N, AKV-B, and FBLV (collectively abbreviated as MLVs). Viral titers are reported in the table below. (Reference: Yan et al., PNAS 2009)

Table 2. Virus titers of MLVs on MDTF cells transduced with *Fv1^n* or *Fv1^m*

	Log_{10} Virus Titer*		
Cells	AKV-N	AKV-B	FBLV
MDTF	4.4	3.4	5.8
MDTF-*Fv1^n*	4.7	0.8	5.4
MDTF-*Fv1^m*	2.7	1.5	5.3

    a. How does expression of *Fv1* affect the cells’ susceptibility to virus infection?

    b. The researchers found evidence for strong positive selection of *Fv1* alleles in mouse genomes. Predict how *Fv1* might be involved in host-pathogen interactions.

    c. Do you predict that *Fv1* is derived from a virus that is more closely related to AKV-B or FBLV? Why?

11. Host-pathogen interactions lead to innovation of viral and cellular structures.
    a. Thinking about the eIF2a/PKR pathway, list two ways that viruses can evade host intracellular immunity.

    b. Predict how pathogen mimicry may be involved in a process other than PKR-dependent translational inhibition.

Optional video:
Title: Arabidopsis thaliana-Pseudomonas syringae interaction: The effect of climate in plant disease
Speaker: Sheng-Yang He
Time: 29:07

Concepts: *Arabidopsis - P. Syringae* model system of infection, role of environment (temperature & humidity) in plant disease, pattern-triggered immunity & effector inhibition, and water soaking as a symptom of bacterial infection in leaves

Questions for Optional Video:

1. Plants have two forms of defense: pattern-triggered immunity and effector-triggered immunity.
    a. How do effectors aid pathogens' infectivity?

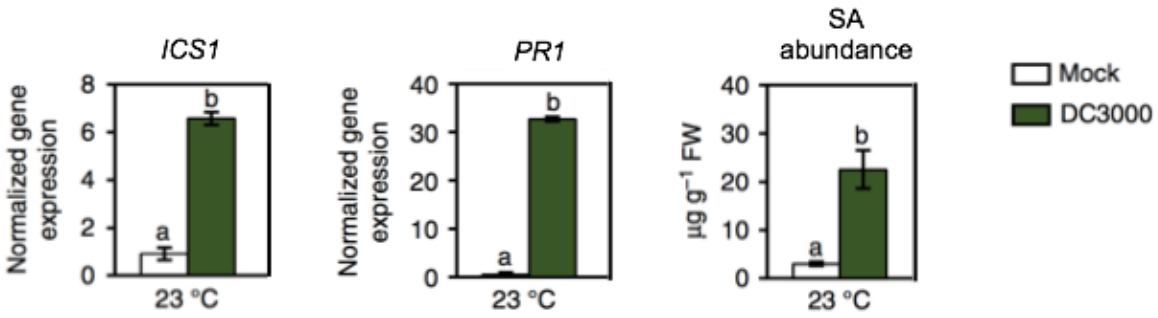
    b. Would a pathogen without effectors be able to infect a plant? Briefly explain.

2. The environment has a strong impact on plants' susceptibility to disease.
    a. Briefly explain how humidity affects plants' disease susceptibility.

    b. In low humidity, how can pathogens still use water to increase their infectivity? Briefly explain.

c. You know that pathogens can release molecules like HopM1 and AvrE to produce “water soaking” in plants. Can you engineer a plant that is less susceptible to the effect of humidity? Briefly explain.

3. A group of researchers sought to identify the molecular pathways involved in *Arabidopsis* response to *P. syringae* infection. (Reference: Huot et al., Nature Communications, 2017)

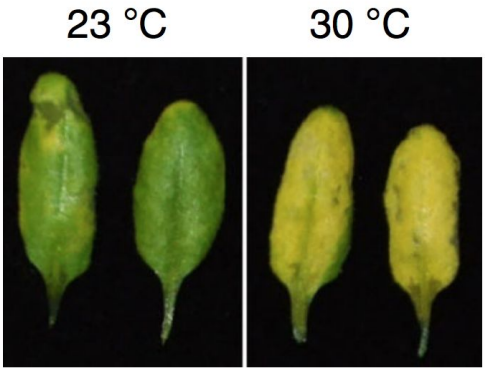


a. The researchers measured expression of two *Arabidopsis* genes (*ICS1* and *PR1*) that are involved in salicylic acid (SA) production, along with abundance of SA in *Arabidopsis* tissues, following infection with *P. syringae* strain DC3000. At 23°C, *P. syringae* does not infect *Arabidopsis.*

   i. Based on the information above, what do you predict is the role of SA in the host:pathogen interaction?

   ii. How could you test this hypothesis?

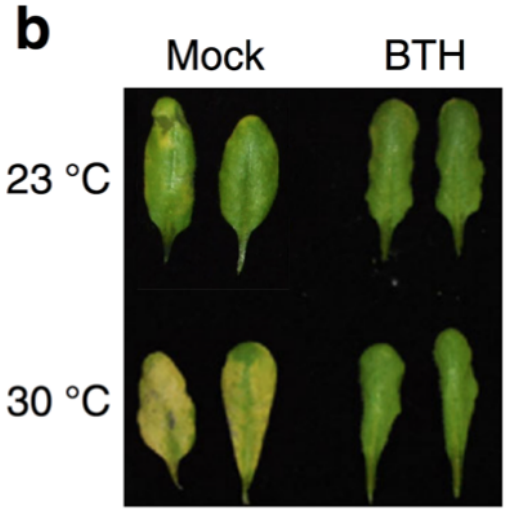
b. The researchers discovered that at 30°C, *Arabidopsis* becomes susceptible to *P. syringae* infection (see figure below).



i. Based on your prediction of the role of SA in infection, do you expect to see elevated or decreased levels of SA in *P. syringae*-inoculated *Arabidopsis* tissues at 30°C relative to tissues at 23°C?

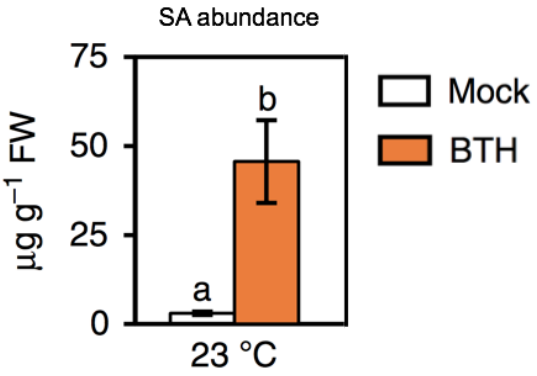
ii. Explain your prediction.

c. The researchers pre-treated *Arabidopsis* leaves with a synthetic compound, BTH, prior to *P. syringae* inoculation at two temperatures and observed the following results.

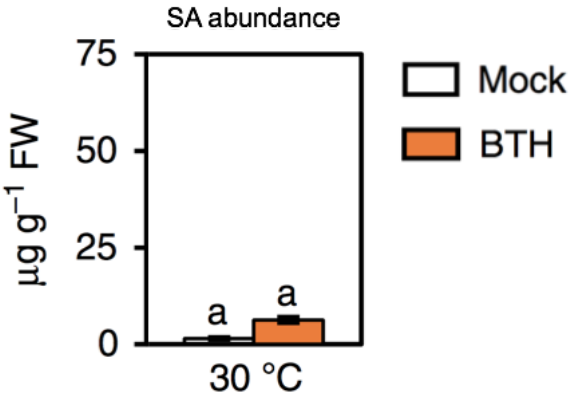


i. Do you predict that BTH is an agonist or antagonist of SA? Why?

ii. Imagine you wanted to test your prediction. To do so, you measure SA abundance in *Arabidopsis* tissues following BTH treatment at 23°C (in the absence of *P. syringae)* and obtain the following results. Do these results support your prediction?



iii. You then measure SA abundance in *Arabidopsis* tissues following BTH treatment at 30°C (in the absence of *P. syringae)* and obtain the following results. Do these results support your prediction?



iv. What can you conclude about BTH from the three pieces of data in part c?

Answers for Session 8:

Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
    a. Plants have receptors that recognize molecular patterns found on pathogens. **(True)**
    b. Resistance (R) proteins in plants can recognize multiple virulence factors. **(True)**

2. Which of the following are common characteristics of resistance (R) proteins? (Select all that apply)
    a. Arise in the pathogen.
    b. Arise in the plant.
    c. Surface receptors.
    d. Homology to animal proteins.
    e. Homology to antibodies.

3. Briefly, compare and contrast pattern-triggered immunity (PRR/PAMP system) and effector-triggered immunity (R/Avr system) in plants. Give an example of each.
    Pattern-triggered immunity detects conserved structures of pathogens, such as flagellin. Effector-triggered immunity detects intracellular changes caused by pathogen virulence effector proteins. An example of effector-triggered immunity is detection of post-translational modifications made on plant proteins by effectors. PRR are surface receptors while the R proteins are cytosolic, and each pathway activates different signal cascades.

    Both types of immunity use highly conserved proteins and activate an immune response. They also don't bind or recognize a specific pathogen, but can bind to antigens found in different pathogens.

4. Shown below is the signaling pathway of a Pattern Recognition Receptor (PRR) upon recognition of a pathogen in the stomata of a plant.
    a. What is the normal function of the stomata in the plant? Why would this organ represent a problem for the plant when it interacts with pathogens? Briefly explain.
    The stomata are the place where gases are exchanged in the plant. The stomata are big enough to allow pathogens to enter the leaf tissue.

    b. Under normal conditions, what happens to the stomata when the plant recognizes that there is a pathogen in the vicinity?
    The stomata close.

    c. High humidity inhibits the closure of the stomata. What would be the consequence for the plant? Briefly explain.
    The plant will be more susceptible to pathogens as they can more easily enter through the stomata.

d. If you were a pathogen, how would you target this pathway to increase your infectivity rate? Briefly explain.
Any correct prediction by the student should lead to an inhibition of SA. For example, you could have a protein that would bind to the ANAC binding site, and therefore inhibit the transcription of genes that enhances SA.

5. Plants have various defense mechanisms against bacterial infections. Researchers are studying how the infectivity of pathogens changes with different genetic mutations in plants. Shown below are the results of infecting mutant plants (fls2-17) and wild-type plants (Ler-0) with either bacteria (Pst DC3000) or vehicle (water).
   a. What is the consequence of the fls2-17 mutation in plants? Briefly explain.
   This mutation seems to reduce the plant's resistance to infection. The pathogen has higher infectivity in plants with this mutation.

   b. Briefly describe the two major mechanisms that plants have for defense against pathogens.
   Pathogen-triggered immunity – Recognition of a pathogen-derived molecule will induce an immune response in the plant.

   Effector-triggered immunity – Resistance (R) proteins in the plant will detect virulent (Avr) factors and elicit an immune response.

   c. fls2-17 is a receptor that localizes close to the stomata. To which of the two mechanisms described in question (b) does this gene belong? Propose a mechanism of action.
   Because it is a receptor, this gene belongs to the pathogen-triggered immunity pathway. Because it localizes close to the stomata, it's potentially detecting pathogens as they approach the stomata, and its activation is likely involved in stomata closure upon detection of pathogen.

6. Explain how R proteins are able to recognize many types of pathogens, despite large variation in effector proteins.
Through a process of indirect recognition: effector proteins can induce common modifications in plant proteins that are then recognized by the R proteins.

7. Most R proteins detect pathogen effectors inside the plant cell. Predict a scenario in which pathogen effectors would function extracellularly.
One example would be to bind and inhibit pattern-recognition receptors. Another would be to bind and sequester signaling molecules that are secreted between plant cells.

8. In infection, there is a constant battle between host and pathogen. The host is winning the battle if it can mount an immune response and clear the pathogen, while the pathogen is winning if it can evade host detection systems. The diagram below depicts the current status of an immune battle between a bacterium, *Xoo*, and the rice cells it infects. Given the information that PSY is a growth factor, Xa21 is an immune receptor, and RaxX is a bacterial protein, arrange the components of this system (RaxX, Xa21, PSY, and PSY receptor) into their evolutionary history. (Reference: Pruitt et al., New Phytologist 2017).
   PSY is a growth factor that binds and activates the PSY receptor. *Xoo* evolved to co-opt this interaction by secreting a peptide, RaxX, that activates PSY-R and encourages cell growth (which creates a comfy environment for the microbe to flourish). The rice host then evolved to detect RaxX via Xa21 so that it can mount an immune response.

9. Plant stomata close in response to bacterial infection. (Reference: Melotto, Cell 2006)
   a. A group of researchers wanted to determine the effects of three different bacterial products - flagellin (flg22), lipopolysaccharide (LPS), and coronatine (COR) - on stomatal aperture. To address their question, they treated *Arabidopsis* leaves with each of the compounds. The data are shown below. MES = buffer used as negative control.
      i. Based on the data in the figure above, describe the effects of each compound on plant stomata.
      Flg22 and LPS induce stomatal closing. COR blocks the stomatal closing induced by Flg22 and LPS.

      ii. What type of immunity - pattern-triggered immunity or effector-triggered immunity - likely recognize each compound?
      Flg22 and LPS are recognized by pattern-triggered immunity. COR is likely recognized by effector-triggered immunity.

   b. The researchers then compared the responses of two strains of *Arabidopsis* plants, Col-0 and coi1-20, to coronatine (COR). In the figure below, MES = buffer used as negative control; ABA = abscisic acid, a plant hormone that stimulates stomatal closing.
      i. Based on the data above, what can you conclude about each strain's response to COR?
      Strain Col-0 is susceptible to COR - its stomata stay open in combined ABA + COR treatment. Strain coi1-20 is resistant to COR - its stomata stay closed in combined ABA + COR treatment.

      ii. Would you predict each strain to be resistant or susceptible to the bacteria that produces COR?
      Col-0 is susceptible; coi1-20 is resistant.

Questions for Second Video:

1. Which of the following examples are ways by which a host can be "ahead" of a pathogen in an evolutionary arms race?
    a. A synonymous mutation in a protein involved in recognition of the pathogen.
    b. A synonymous mutation in a protein involved in the metabolism of the host.
    c. A single amino acid change that would interfere with the recognition of the pathogen by the host.
    d. A synonymous mutation in the pathogen in a protein involved in recognition of the pathogen.
    e. None of the above.

2. The polio virus gene X is involved in interaction with the host; therefore
    a. protein X is under positive selection.
    b. the host protein that interacts with protein X is under positive selection.
    c. protein X and the host protein that interacts with protein X are under positive selection.
    d. None of the above.

3. State whether the following statements are true or false. If false, explain why.
    a. eIF2a is a translational factor that has a high mutation rate due to co-evolution with viruses. **(False - its mutation rate is low because integrity of eIF2a is important for translation)**
    b. Pathogen genes that code for proteins that bind to host immune proteins are prone to high selective pressures. **(True)**
    c. Amino acid-altering mutations are more likely to occur than silent mutations by random chance. (**True**)
    d. Genetic changes in pseudogenes are evolutionarily unfavorable. (**False - because pseudogenes don't code for proteins, there is no advantage/disadvantage to changes in the code**)
    e. Diversifying selection results from an increased mutation rate. (**False - it results from a change in selection pressures**)
    f. Single residue changes are sufficient to disable pathogen mimicry. (**True**)
    g. Pathogen mimicry can result from copying a host gene into a viral genome. (**True**)

4. You're designing a small molecule drug to treat patients who are infected with a pox-related virus. Janet discovers that the virus expresses a protein, K3L, that binds to the host kinase dimer PKR and reduces the translational inhibition induced by the host.
    a. Why is translational inhibition good for the host when it is infected by a virus? Briefly explain.
    Translational inhibition will prevent virus-infected cells from becoming factories for virus production.

b. Janet decides to use K3L as the target for her small molecule. Is this a good idea? Briefly explain your answer.
Maybe. Reducing the effect of K3L is a good idea and it will help the host. But the mechanism of action of K3L is by mimicking eIF2a, an important translational factor. A small molecule that inhibits K3L would only be good if it cannot inhibit eIF2a. If it inhibits eIF2a it will presumably have bad side effects for the patient.

5. Compare and contrast purifying selection and diversifying selection. Give an example of each.
Purifying selection maintains an amino acid sequence; example is eIF2a, which is functionally equivalent in distantly related species. Diversifying selection prioritizes changes to an amino acid sequence. An example is the K3L : PKR interaction site. The mutation rate is presumed to be equal in both.

6. Explain why the mimicry of eIF2a by poxvirus K3L to inhibit PKR function is NOT analogous to a three-way rock-paper-scissors game.
eIF2a is under strong purifying selection pressure because of its essential role in translation in the cell, so its sequence doesn't change. Only K3L and PKR do.

7. Predict another way that the host can optimize its interaction between PKR and eIF2a to avoid mimicry by a viral protein.
The interaction between PKR and eIF2a could require scaffolding proteins, which would add another layer of complexity to the attempt at mimicry. Additional response: PKR could evolve to bind eIF2a at two motifs.

8. Mutations allow organisms to adapt to their environment.
    a. How does co-evolution between host and pathogen drive "positive selection"? Briefly explain.
    Genes involved in the interactions between host and pathogens experience a strong evolutionary pressure that increases the likelihood for these proteins to change within a population. These forces increase the positive selection (or diversifying selection) in a species.

    b. Shown below is the likelihood of positive selection (Ks/Ka) in the PARP family of genes. PARP is a family of host proteins involved in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. Note: A higher Ks/Ka number indicates increased mutability.
        i. PARP13/ZAP has broad antiviral activity. Does this information correspond with PARP13's Ks/Ka results? Briefly explain.
        Yes. It appears that a strong positive selection has taken place in the PARP13 gene. This correlates with genes involved in host-pathogen interactions.

        ii. If you're studying genes involved in host immunity, which other PARP gene would you choose to study? Briefly explain your answer.
        I would choose genes that seem to have strong positive selection (high Ks/Ka score). The genes marked in red (PARP4, PARP9, PARP14, PARP15, and PARP13) have high mutability.

iii. Although it is known that PARP proteins carry out ADP-ribosylation, a type of post-translational modification, most members of the PARP family are poorly characterized. What characteristics would you expect to find in pathogenic proteins that interact with PARP family members? Briefly explain.
Pathogenic proteins that interact with PARP family members probably mimic host proteins that need to be ribosylated.

9. Explain the selection pressures that would lead a residue to "toggle" between a few specific amino acids during its evolution.
There could be both purifying and diversifying selection acting on a residue. This could lead to a narrow window of options for the residue to change to and still allow the protein to function properly.

10. Researchers discovered that a viral factor known as *Fv1* has persisted in the genomes of several species of mice for 7 million years. To determine the role of *Fv1*, they transduced two of its allelic variants*, Fv1n and Fv1m*, into the mouse cell line MDTF and then exposed the cells to three different murine viruses, AKV-N, AKV-B, and FBLV (collectively abbreviated as MLVs). Viral titers are reported in the table below. (Reference: Yan et al., PNAS 2009)
    a. How does expression of *Fv1* affect the cells' susceptibility to virus infection?
    $Fv1^m$ decreases AKV-N and AKV-B infection, $Fv1^n$ decreases AKV-B infection. They do not appear to have an effect on susceptibility to FBLV.

    b. The researchers found evidence for strong positive selection of *Fv1* alleles in mouse genomes. Predict how *Fv1* might be involved in host-pathogen interactions.
    ***Fv1* appears to be co-opted by the mouse genome to somehow inhibit viral infection or replication. This represents an example of reverse mimicry.**

    c. Do you predict that *Fv1* is derived from a virus that is more closely related to AKV-B or FBLV? Why?
    ***Fv1* is most likely from a virus that is more closely related to AKV-B, because it is effective at inhibiting infection of this virus. This indicates that *Fv1* mimics a particle that is essential to AKV-B.**

11. Host-pathogen interactions lead to innovation of viral and cellular structures.
    a. Thinking about the eIF2a/PKR pathway, list two ways that viruses can evade host intracellular immunity.
    eIF2a-independent translation; inhibit dimerization of PKR; dephosphorylate eIF2a; hide its dsRNA from the cell; inhibit interaction between PKR and eIF2a.

    b. Predict how pathogen mimicry may be involved in a process other than PKR-dependent translational inhibition.
    Mimicry of eIF2a can activate translation. (Possible to have a range of accurate responses; i.e., mimicry of cytokine receptors can sequester cytokines away from cells)

Questions for Optional Video:

1. Plants have two forms of defense: pattern-triggered immunity and effector-triggered immunity.
    a. How do effectors aid pathogens' infectivity?
    Pathogens release effectors to counteract the pattern-triggered immune response. Inhibition of pattern-triggered immunity increases the chances for pathogens to successfully infect the plant.

    b. Would a pathogen without effectors be able to infect a plant? Briefly explain.
    Technically no. Without the effectors, the plant should be able to mount an immune response against the pathogens.

2. The environment has a strong impact on plants' susceptibility to disease.
    a. Briefly explain how humidity affects plants' disease susceptibility.
    Increased humidity increases the amount of water inside the leaf tissue, which creates an environment where bacteria multiply.

    b. In low humidity, how can pathogens still use water to increase their infectivity? Briefly explain.
    Pathogens can secrete chemicals that will produce "water soaking": accumulation of water inside the leaf tissue (apoplast).

    c. You know that pathogens can release molecules like HopM1 and AvrE to produce "water soaking" in plants. Can you engineer a plant that is less susceptible to the effect of humidity? Briefly explain.
    Possibly. If these are the only two factors that pathogens release to produce "water soaking" in plants, first you would have to know what are the target of these molecules. If the target can be deleted or modified so that it no longer interacts with HopM1 and/or AvrE without causing off-target damage to the plant, you could potentially create a plant that is less susceptible to high levels of humidity.

3. A group of researchers sought to identify the molecular pathways involved in *Arabidopsis* response to *P. syringae* infection. (Reference: Huot et al., Nature Communications, 2017)
    a. The researchers measured expression of two *Arabidopsis* genes (*ICS1* and *PR1*) that are involved in salicylic acid (SA) production, along with abundance of SA in *Arabidopsis* tissues, following infection with *P. syringae* strain DC3000. At 23°C, *P. syringae* does not infect *Arabidopsis.*
        i. Based on the information above, what do you predict is the role of SA in the host:pathogen interaction?
        Salicylic acid appears to be part of the response to *P. syringae* infection. It may be necessary for pathogen clearance.

        ii. How could you test this hypothesis?
        Inhibit SA production (for example, by knockdown of ICS1 or PR1). Loss of SA should decrease *Arabidopsis* resistance to *P. syringae* at 23°C.

b. The researchers discovered that at 30°C, *Arabidopsis* becomes susceptible to *P. syringae* infection (see figure below).
    i. Based on your prediction of the role of SA in infection, do you expect to see elevated or decreased levels of SA in *P. syringae*-inoculated *Arabidopsis* tissues at 30°C relative to tissues at 23°C?
    Decreased. (Responses may be different based on previous responses)

    ii. Explain your prediction.
    We predicted that SA is protective against *P. syringae* infection at 23°C. Since infection can take hold at 30°C, SA levels are predicted to be decreased.

c. The researchers pre-treated *Arabidopsis* leaves with a synthetic compound, BTH, prior to *P. syringae* inoculation at two temperatures and observed the following results.
    i. Do you predict that BTH is an agonist or antagonist of SA? Why?
    BTH is likely an agonist of SA signaling. It prevents infection at high temperatures.

    ii. Imagine you wanted to test your prediction. To do so, you measure SA abundance in *Arabidopsis* tissues following BTH treatment at 23°C (in the absence of *P. syringae)* and obtain the following results. Do these results support your prediction?
    Yes. BTH treatment increases SA abundance.

    iii. You then measure SA abundance in *Arabidopsis* tissues following BTH treatment at 30°C (in the absence of *P. syringae)* and obtain the following results. Do these results support your prediction?
    No. At 30°C, BTH treatment doesn't increase SA abundance.

    iv. What can you conclude about BTH from the three pieces of data in part c?
    BTH increases *Arabidopsis* resistance to *P. syringae*. At 30°C, it appears to do it through an SA-independent mechanism.