Genome Editing and Engineering
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Module - 06
Transcription activator-like effector nuclease (TALEN) Technology
Lecture - 01
Basics of TALEN-Part A

Welcome to my course on Genome Editing and Engineering. Today we are going to discuss about TALEN, which is an editing technology little bit different from the last technology that we discussed the zinc finger nucleases. The word TALEN is also a hybrid between TAL effectors and the word nuclease. In this lecture, we are going to discuss about the basics of this technology platform.

So, we will start with a discussion with TAL effectors, their biology and how these TAL effectors are finally fused with nucleus proteins to produce artificially made TALENs.

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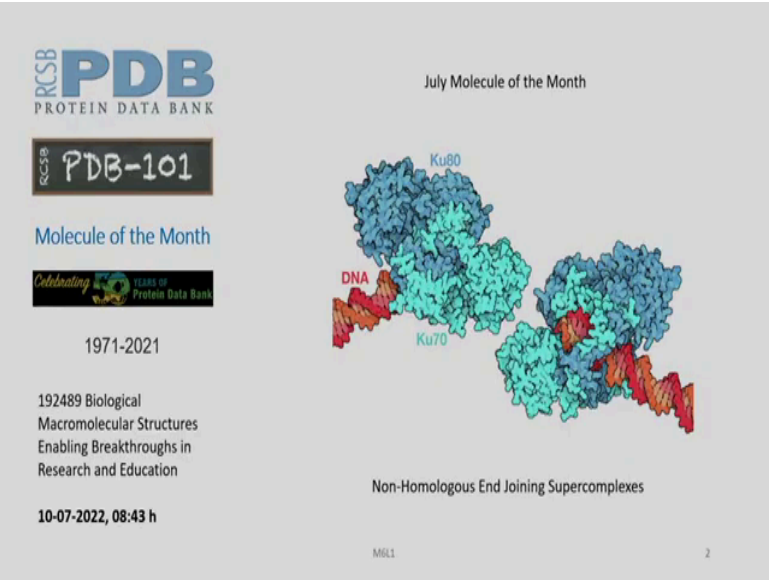

So, let us start with one interesting molecule, which has been listed as the "molecule of the month" at the time of recording this lecture. So, every month Protein Data Bank highlights a protein molecule or a complex as the molecule of the month. So, this month the non-homologous end joining super-complexes has been listed by PDB as such.

You know about the DNA double strand break mechanisms and how the DNA double strand breaks are repaired by the cellular mechanisms; amongst them the NHEJ is one of the major players. And this molecular complex is also important from the point of view of the TALEN technology, because once we use TALEN to induce, to define the double strand breaks on DNA sequences; it has to undergo repair either by the homology repair pathway or the NHEJ pathway.

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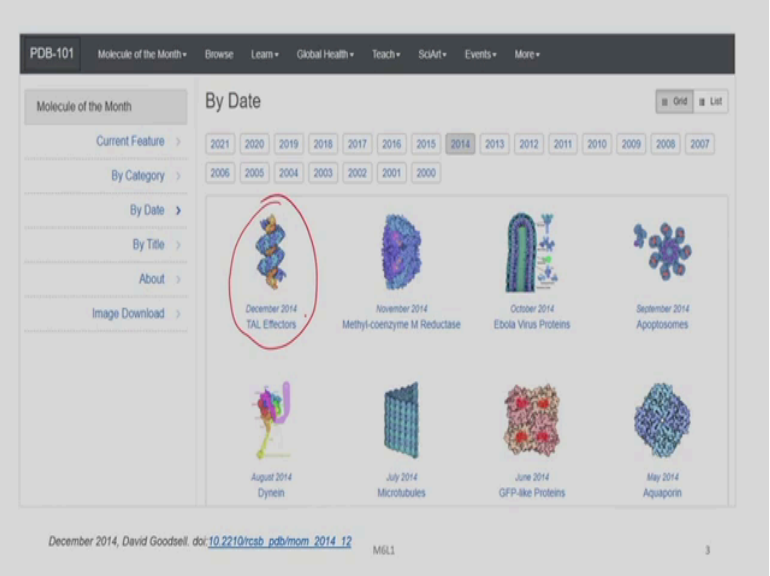

Similarly, a few years back in 2014, PDB listed these TAL effectors in December 2014 as the "molecule of the month". Today we are going to spend our time in discussing at large about these TAL effectors.

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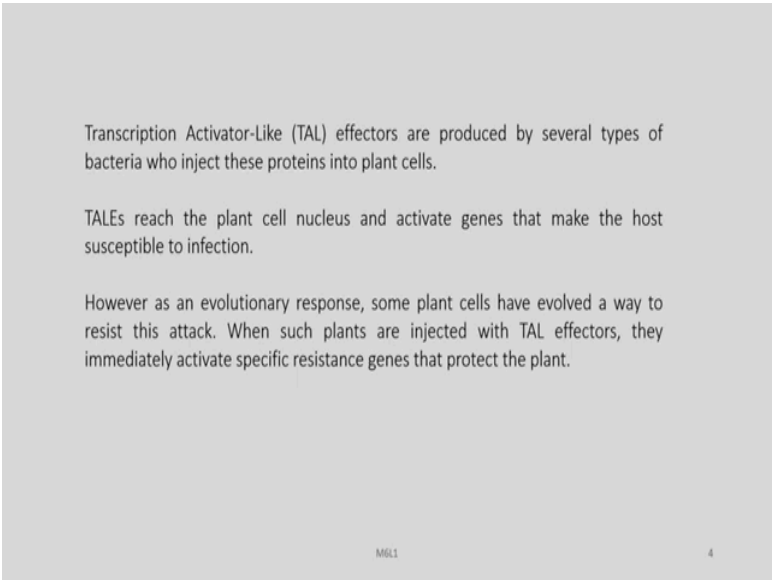

So, what are these TAL effectors? So, TAL stands for Transcription Activator-Like effectors and these are produced by several types of bacteria, who inject these proteins into plant cells. The TALEs reach the plant cell nucleus and activate genes that make the host susceptible to infection; so, they are infectious entities.

However, during the course of evolution as a response to infection by bacteria and the reparatory of TALEs they use in the pathogenic mechanism, plant cells have evolved a way to resist this attack. And when such plants are injected with TAL effectors, they immediately activate special resistance genes that protect the plant.

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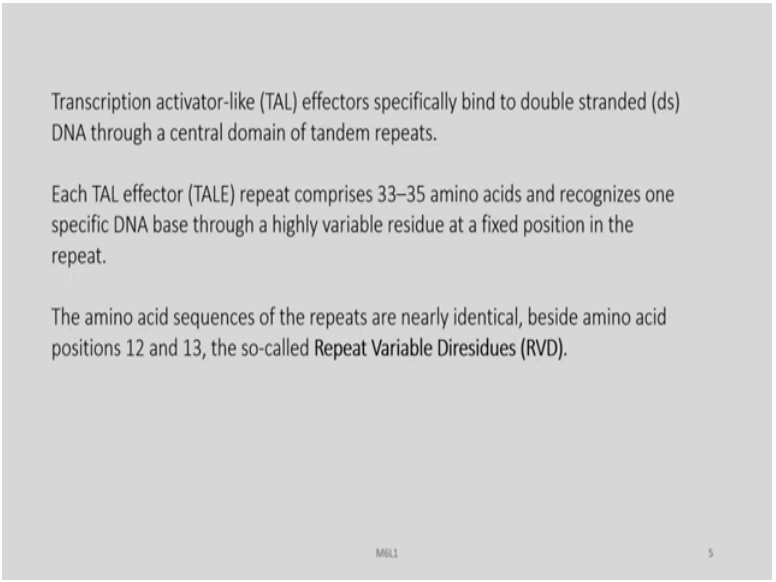

We will not be discussing about this defense mechanism; because our interest is just restricted to the use of TAL effectors in the development of the TALEN technology platform. But we will discuss slowly about the TALE biology and how they are involved in the pathogenicity.

These TAL effectors bind to double stranded DNA through a central domain of tandem repeats. So, we are going to repeat these tandem repeats again and again and there will be lot of redundancies in this discussion; but they are important from the conceptual point of view.

So, transcription activator like effectors specifically bind to double-stranded DNA through a central domain of tandem repeats. We have to remember the central domain of tandem repeats, which is very very important for understanding the function of TAL effectors. Each of these TAL effector repeats comprises around on average 34 amino acids and they recognize one specific DNA base through a highly variable residue at a fixed position in the repeat.

The amino acid sequences of the repeats are nearly identical, besides amino acid positions 12 and 13; these are called the repeat variable diresidues. So, out of this 34 except 12 and 13, everything else is identical.

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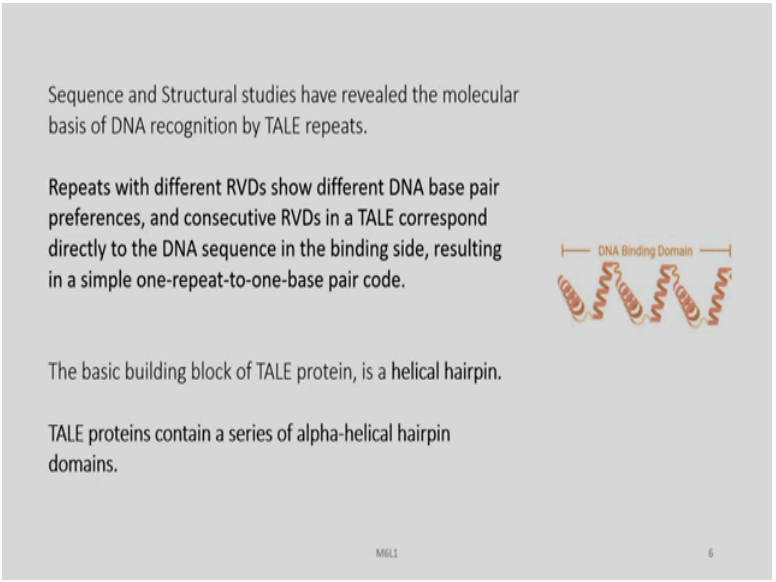

A sequence analysis and structural studies have revealed the molecular basis of DNA recognition by the TALE repeats.

So, repeats with different RVDs, with different amino acids at the 12 and 13 positions show different DNA base pair preferences and consecutive RVDs in a TALE correspond directly to the DNA sequence in the binding site, resulting in a simple one repeat to one base pair code. The basic building block of TALE protein, we have to remember, is a helical hairpin. TALE proteins contain a series of alpha-helical hairpins arranged in a continuity or a tandem.

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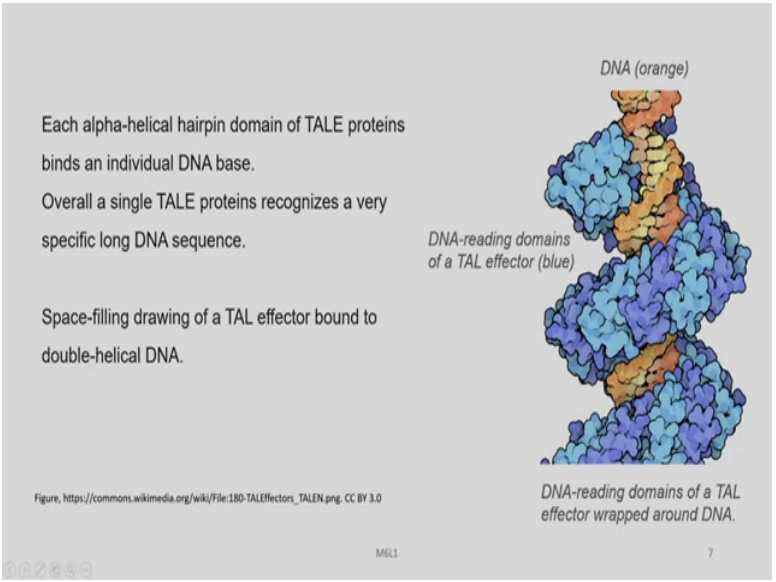

Each of these alpha-helical hairpin domain of TALE proteins binds an individual DNA base as we have already discussed. Overall, a single TALE protein would recognize a very long specific DNA sequence. On the right you can see a space-filling drawing of a TAL effector bound to double helical DNA, which is colored in orange and the DNA reading domains of the TAL effector is being shown in blue, which is wrapped around DNA.

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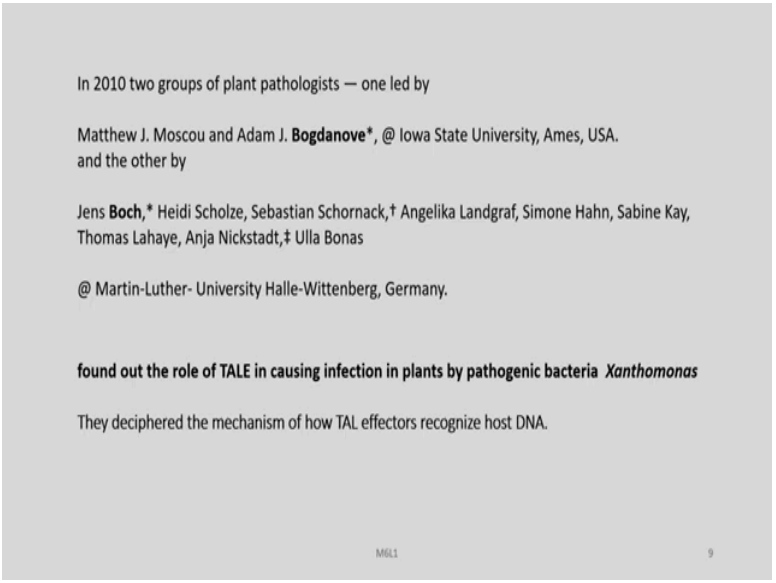

Let us learn a little bit about the discovery of TALE, who are the people who discovered TALE. In 2010, two groups who were working independently in plant pathology; one led by

Bogdanove at the Iowa State University and the other by Jens Boch at the Martin Luther University in Germany, found out the role of TALE in causing infection in plants by pathogenic bacteria *Xanthomonas*. And they also deciphered the mechanism of how these TAL effectors recognize host DNA.

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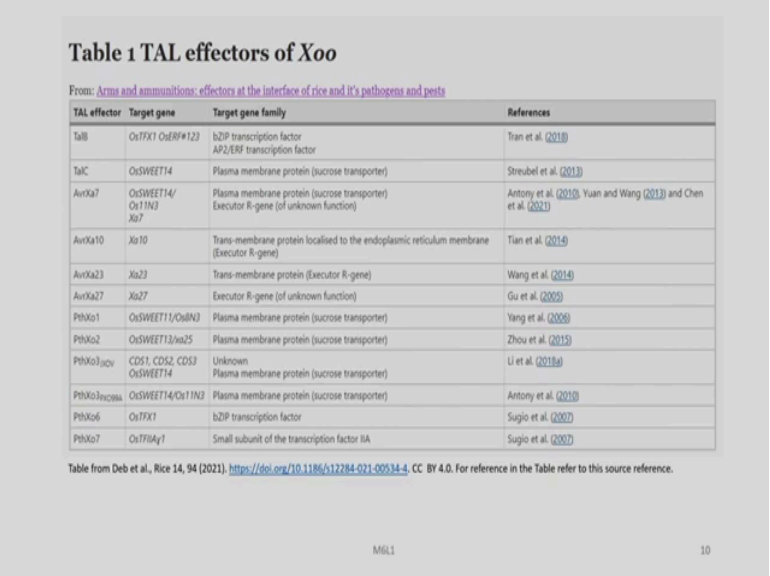

Table 1 TAL effectors of *Xoo*

From: Arms and ammunitions: effectors at the interface of rice and it's pathogens and pests

TAL effector	Target gene	Target gene family	References
TalB	*OsTFX1 OsERF#123*	bZIP transcription factor AP2/ERF transcription factor	Tran et al. (2018)
TalC	*OsSWEET14*	Plasma membrane protein (sucrose transporter)	Streubel et al. (2013)
AvrXa7	*OsSWEET14/* *Os11N3* *Xa7*	Plasma membrane protein (sucrose transporter) Executor R-gene (of unknown function)	Antony et al. (2010), Yuan and Wang (2013) and Chen et al. (2021)
AvrXa10	*Xa10*	Trans-membrane protein localised to the endoplasmic reticulum membrane (Executor R-gene)	Tian et al. (2014)
AvrXa23	*Xa23*	Trans-membrane protein (Executor R-gene)	Wang et al. (2014)
AvrXa27	*Xa27*	Executor R-gene (of unknown function)	Gu et al. (2005)
PthXo1	*OsSWEET11/Os8N3*	Plasma membrane protein (sucrose transporter)	Yang et al. (2006)
PthXo2	*OsSWEET13/xa25*	Plasma membrane protein (sucrose transporter)	Zhou et al. (2015)
$PthXo3_{MDV}$	*CDS1, CDS2, CDS3* *OsSWEET14*	Unknown Plasma membrane protein (sucrose transporter)	Li et al. (2018a)
$PthXo3_{PXO99A}$	*OsSWEET14/Os11N3*	Plasma membrane protein (sucrose transporter)	Antony et al. (2010)
PthXo6	*OsTFX1*	bZIP transcription factor	Sugio et al. (2007)
PthXo7	*OsTFIIAγ1*	Small subunit of the transcription factor IIA	Sugio et al. (2007)

Table from Deb et al., Rice 14, 94 (2021). https://doi.org/10.1186/s12284-021-00534-4. CC BY 4.0. For reference in the Table refer to this source reference.

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The list of TAL effector effectors is quite large and you can see here some of them listed in column one and each TAL effector has a particular target gene to which it would go and bind and they are part of a target gene family. And if you are interested to know about the details of each effector you can go to the papers published by the original authors and the full references are available in this publication by Deb et al.

Let us just focused on one or two of these; for example you have an effector called TalB and another called TalC; For TalC the target gene is OsSWEET14 one of the very famous genes. Then you have others like PthXo. So, this also binds to OsSWEET 11 and then others like AvrXa7, AvrXa23 and they target various members of different gene families.

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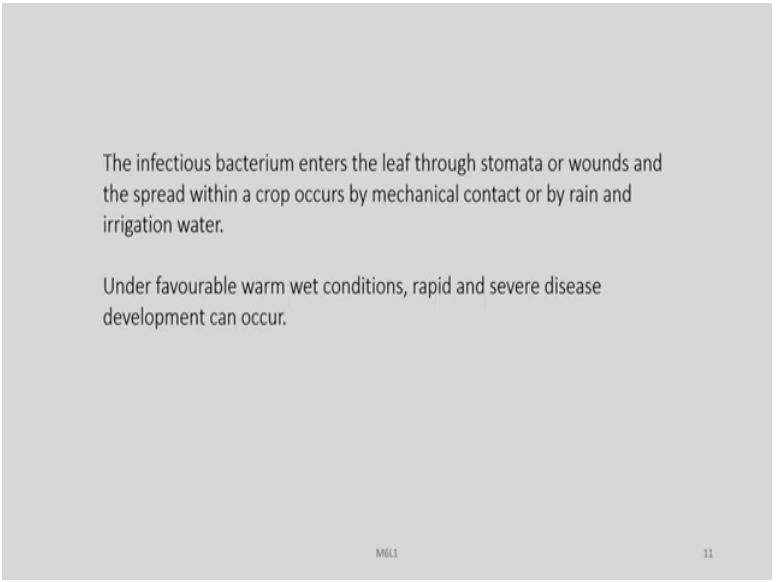

So, these particular bacteria which is an infectious agent, enter the plant leaf through the stomata or any mechanical wounds caused by maybe insect injury or other mechanical injury and then spread within the crop by mechanical contact or by rain and irrigation water. When it finds a favourable warm wet condition, the organism will cause rapid and severe disease in the plant.

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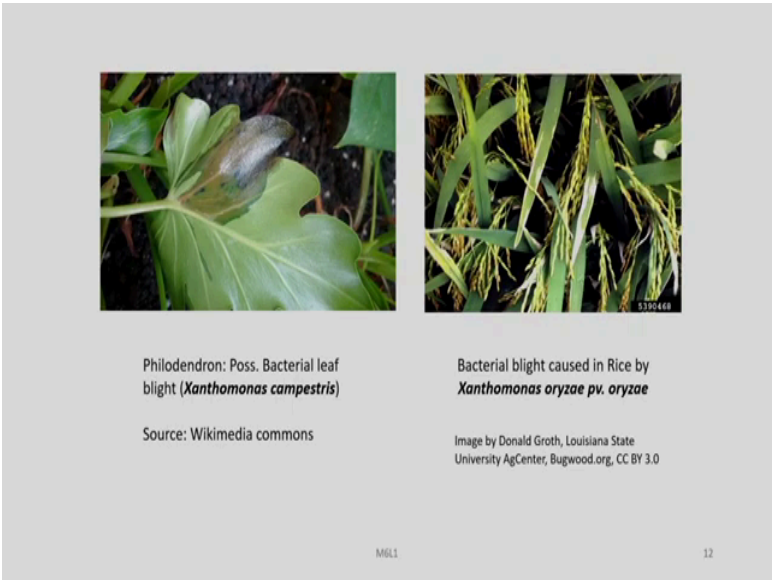

And these are some of the symptoms you can see in some of the host species; for example, in philodendron, you can see the bacterial leaf blight caused by Xanthomonas compestris and

you can see the clear symptom over here. And this is the cereal crop rice, in which the bacterial blight has happened due to infection by Xanthomonas oryzae pathovar oryzae; this organism is very important in the understanding of the TAL effectors.

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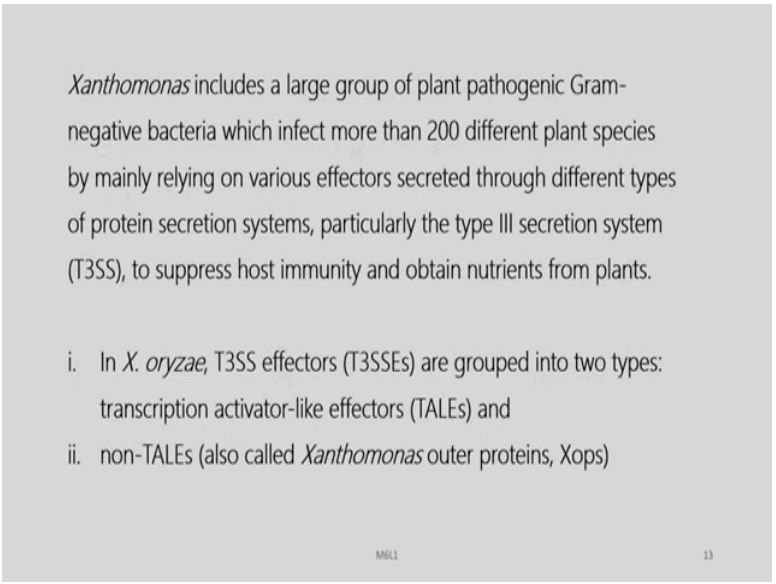

In general Xanthomonas includes a large group of plant pathogenic gram-negative bacteria, which infect more than 200 different plant species and we showed you only just two of them in the earlier slide. And they rely on various effectors secreted through different types of protein secreted systems, in particular the type III secretion system T3SS, to suppress host immunity and obtain nutrients from the plants.

In Xanthomonas oryzae, the T3SS effectors are grouped into two types: transcription activator like effectors and non TALEs, which are also called Xanthomonas outer proteins or Xops.

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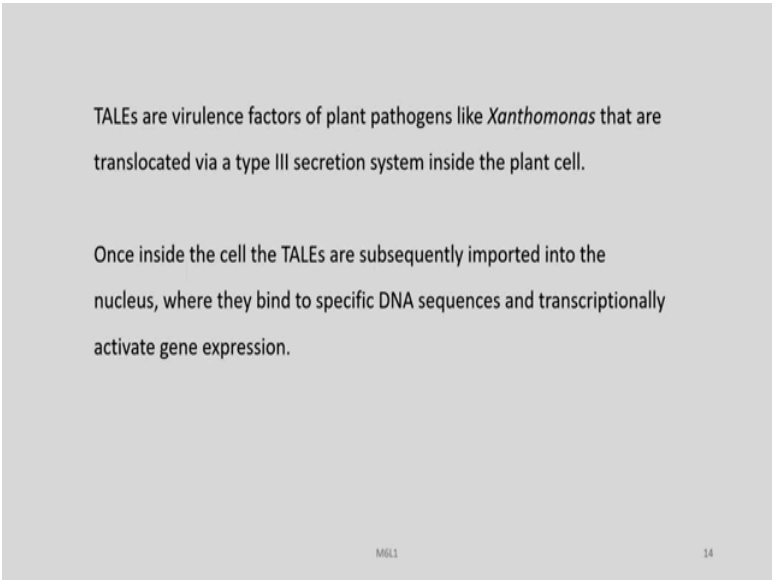

So, we know that TALEs are virulence factors of the plant pathogen; Xanthomonas and these are translocated via a type III secretion system inside the plant cell. And once inside the plant cell these TALEs are subsequently imported into the nucleus, where they bind to specific DNA sequences and transcriptionally activate a gene expression and most of the times these causes susceptibility in the host.

But sometimes the plant as a response to these types of infections will produce some kind of a defense system and these very TALEs may in fact bind to the immune response genes and help the plant in fighting off the infection. We are not going to discuss that part much in this particular lecture.

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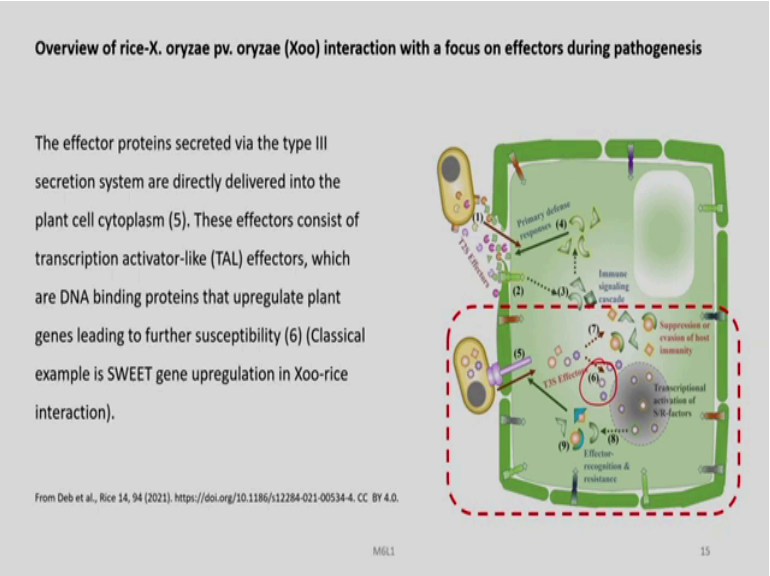

Let us have a little bit of detail view of the rice-Xanthomonas oryzae, pathovar oryzae or Xoo interaction with focus on effectors during pathogenesis. The effector proteins are secreted via the type III secretion system. This is the type II secretion system; we are not going to study about it, we are going to study only about the type III secretion system.

So, the T3 effectors which are secreted via the type III secreted system; you can see these T3 effectors and these are actually pumped inside the plant cell through these beautiful apparatus. So, these are directly delivered into the plant cell cytoplasm. These effectors consist of TAL effectors, which are DNA-binding proteins that up-regulate plant genes leading to further susceptibility, you can see over here. The classical example is SWEET gene up-regulation in Xoo-rice interaction.

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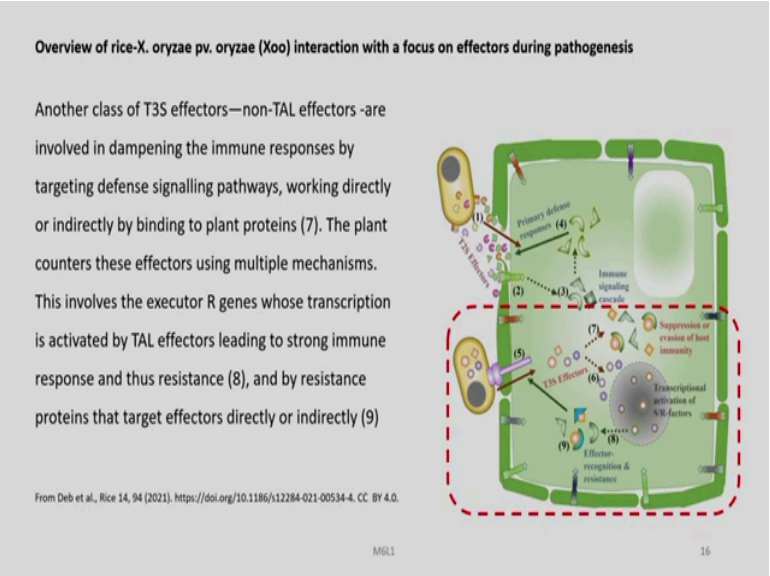

Another class of T3S effectors, the non-TAL effectors are involved in dampening the immune response by targeting defense signaling pathways, working directly or indirectly by binding to plant proteins. So, this is the pathway by which the immune response is triggered. The plant would counter these effectors using multiple mechanisms and it involves the executor R genes whose transcription is activated by TAL effectors leading to strong immune response and resistance. And by resistance proteins that target effectors directly or indirectly.

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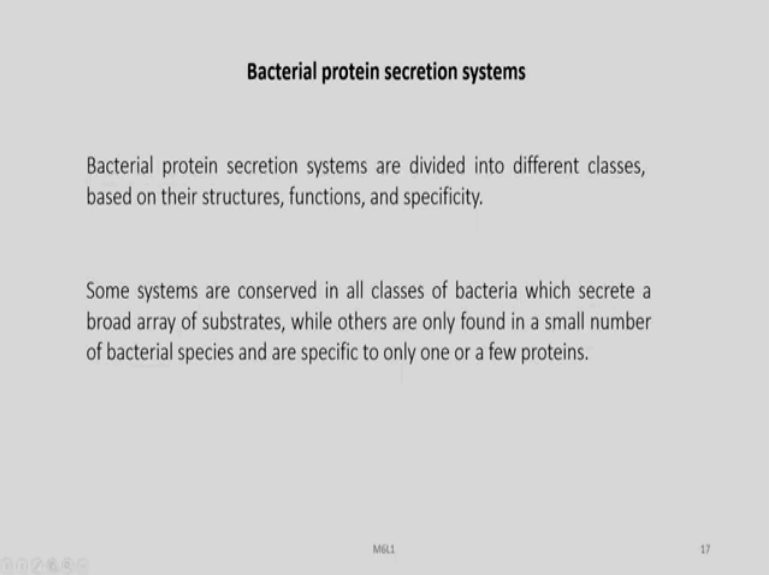

So, let us learn a little bit about the bacterial protein secretion system. We already know that there are at least two such systems; the T2SS and the T3SS. Bacteria deploy a multiple array of secretion systems and we can divide them into different classes based on their structure, function, and specificity.

So, some of these systems are conserved in all classes of bacteria which secrete a broad array of substrates, while others are only found in a small number of bacterial species and are specific to only one or a few proteins.

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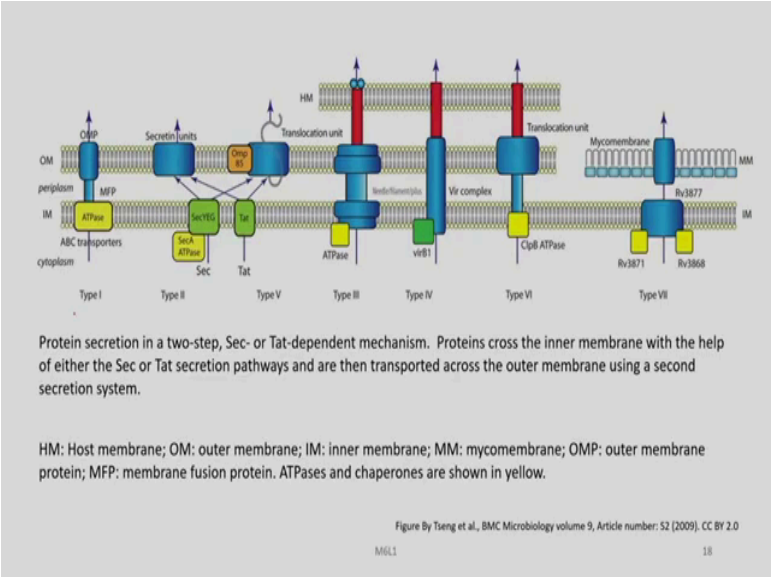

Protein secretion in a two-step, Sec- or Tat-dependent mechanism. Proteins cross the inner membrane with the help of either the Sec or Tat secretion pathways and are then transported across the outer membrane using a second secretion system.

HM: Host membrane; OM: outer membrane; IM: inner membrane; MM: mycomembrane; OMP: outer membrane protein; MFP: membrane fusion protein. ATPases and chaperones are shown in yellow.

So, you can see here. So, many different typeS: type I, II, III, IV, V, VI and VII; all of them are different and some of them can be grouped into larger groups. So, in general protein secretion from bacteria is a two-step method. So, here Sec or Tat is involved in the first transportation step and we call it a Sec or Tat dependent mechanism.

So, here the proteins will cross the inner membrane with the help of either the Sec or Tat secretion pathways. And then they will land up here in this periplasm. And from here they are transported across the outer membrane using a second secretion system. So, this is a two-step Sec or Tat dependent mechanism of a protein transport.

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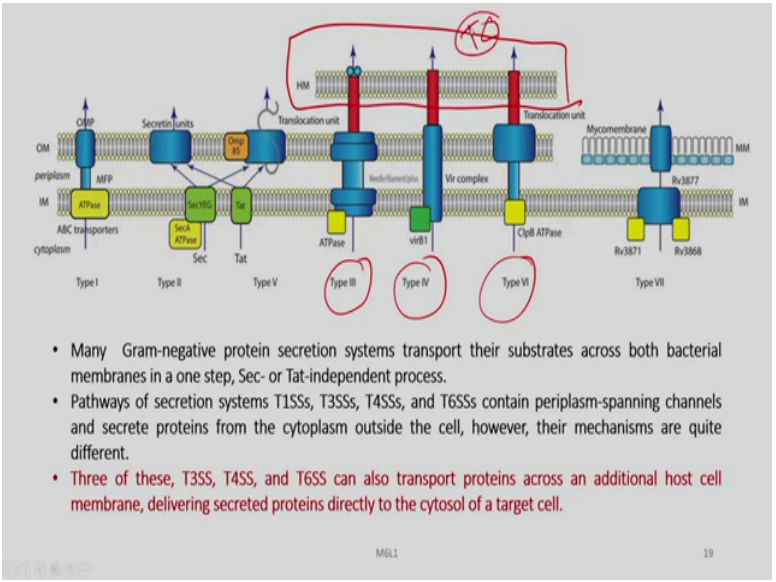

Many Gram negative protein secretion system transport their substrate across both bacterial membranes in one-step, Sec or Tat independent process. Sec or Tat is a two-step transportation system, but some of the bacteria do not use that particular system; it uses a one-step Sec or Tat independent. The pathways of the secretion system T1, T3, T4 and T6 contain the periplasm-spanning channels and secrete proteins from the cytoplasm outside the cell, however, their mechanisms are quite different.

Three of these, the T3, the T4 and the T6 can also transmit protein across an additional host membrane. So, this is this membrane belongs to the host and these three transportation systems can inject through the host cell wall or a membrane and deliver the secreted proteins directly into the cytosol of the target cell.

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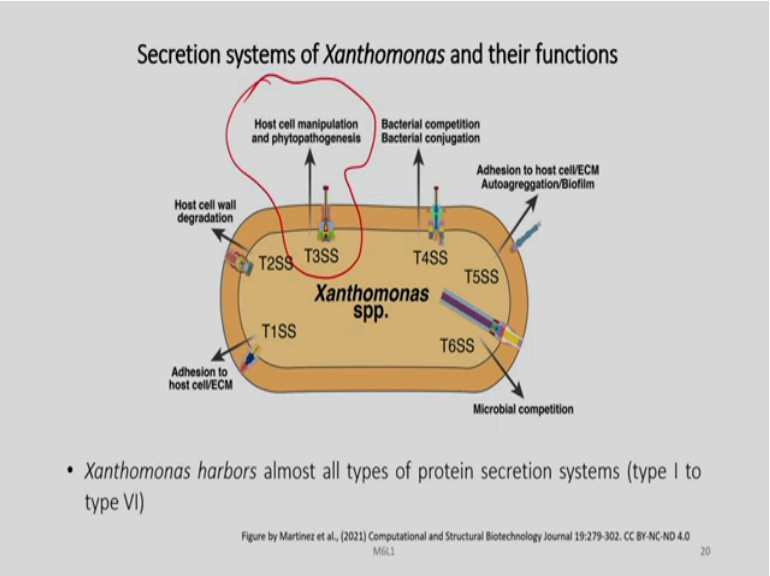

So, let us see a little bit in detail about the various secretion systems of Xanthomonas and their functions. So, the T2 system is responsible for host cell wall degradation; T1 is responsible for addition to the host cell and the extracellular matrix, T6 is involved with the microbial competition and T5, adhesion to host cell and autoaggregation and biofilm production.

And then the bacterial conjugation is carried out by the T4SS; amongst all these, the T3SS is involved with a host cell manipulation in phytopathogenesis and this is the focus of our study.

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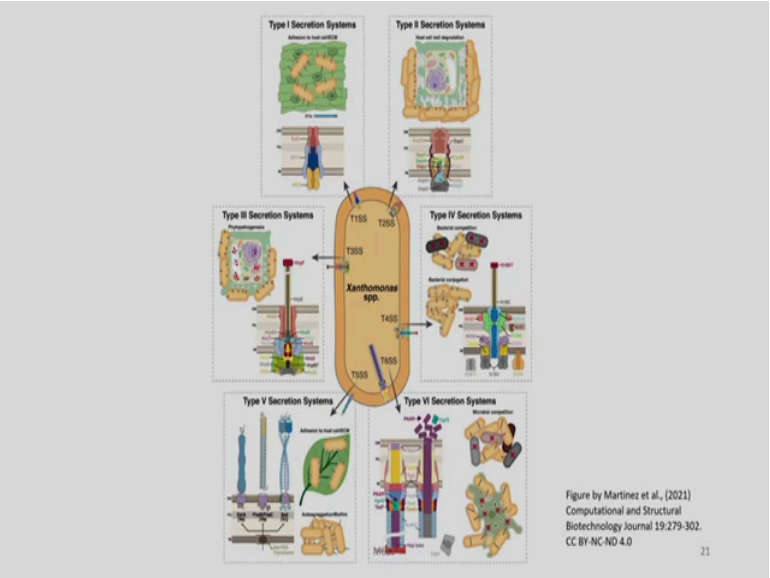

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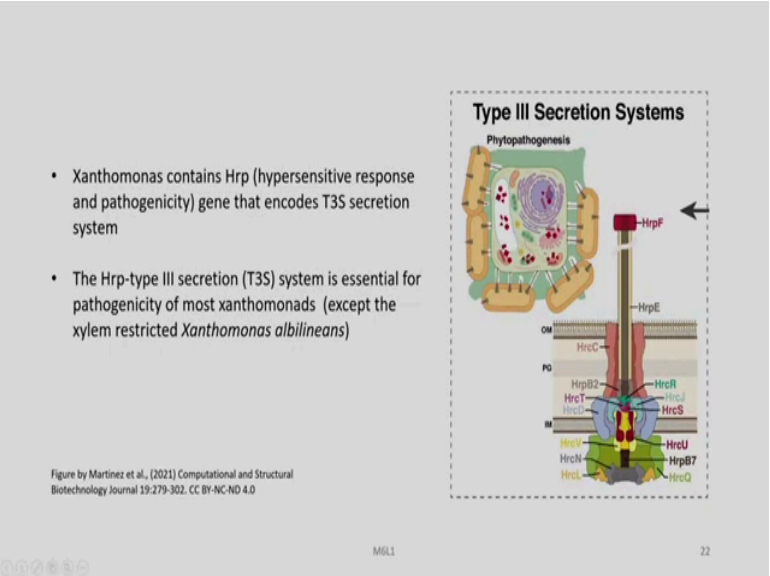

So, you can see here all these systems have a diverse components and mechanisms; we are focusing our discussion on the T3 secretion system. And the Xanthomonas T3 secretion system contains a Hrp hypersensitive response and pathogenicity gene that encodes T3 secretion system. The Hrp type III secretion system is essential for pathogenicity of most xanthomonads, except xylem restricted Xanthomonas albilineans.

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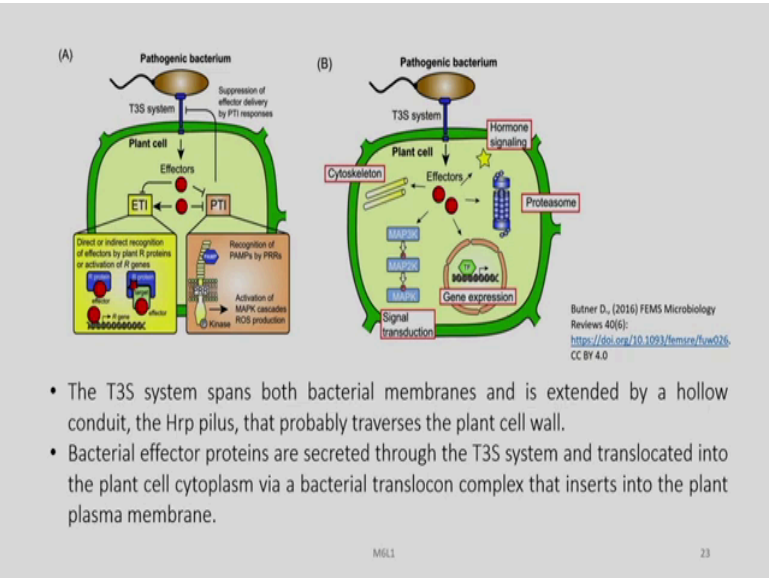

So, this T3 secretion system spans both bacterial membranes and is extended by a hollow conduit, the Hrp pilus, that traverses the plant cell wall. Bacterial effector proteins are

secreted through these T3 secretion system and translocated into the plant cell cytoplasm via bacterial translocation complex, that inserts into the plant plasma membrane.

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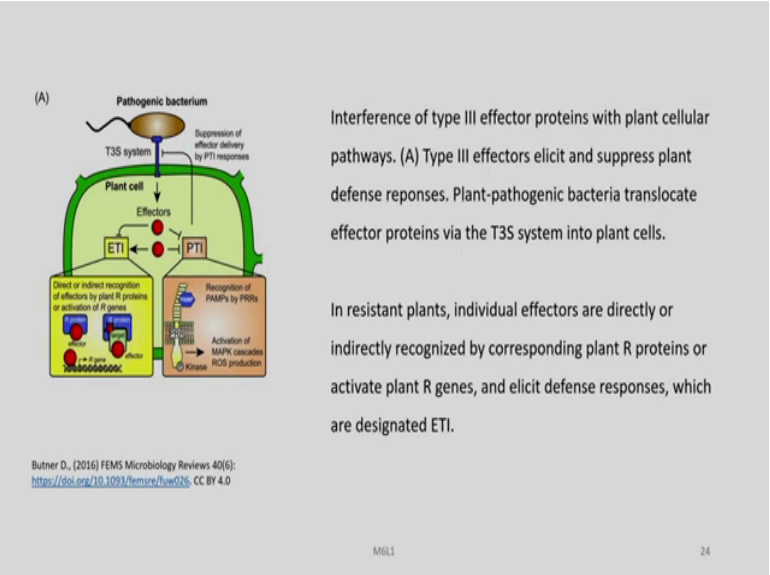

Interference of type III effector proteins with plant cellular pathways happens. So, in A you can see the type III effectors elicit and suppress plant defense responses. Plant pathogenic bacteria translocate effector proteins via the T3 secretion system into the plant cells through that pilus. In resistant plants, individual effectors are directly or indirectly recognized by corresponding plant R proteins or activate the plant R genes, which elicits a defense response and these are designated as the ETI.

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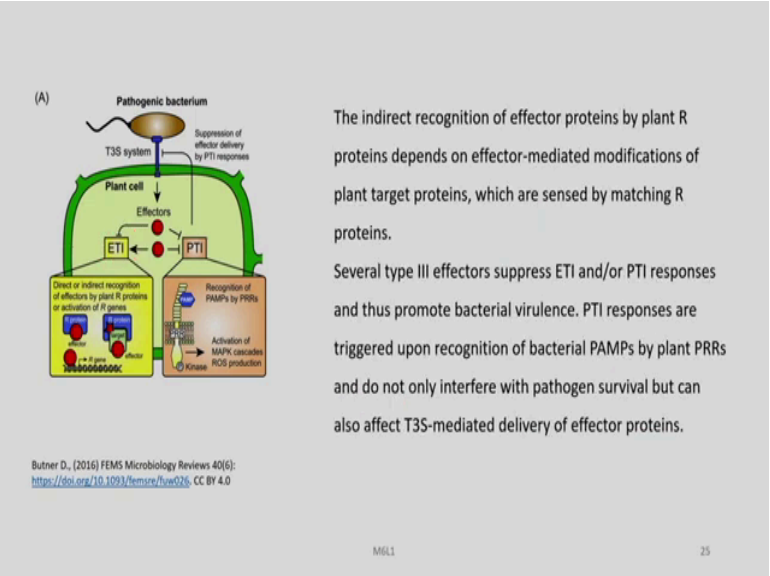

The indirect recognition of effector proteins by plant R proteins depends on effector-mediated modifications of the plant target proteins, which are sensed by matching R proteins. Several type III effectors suppress the ETI and/or PTI responses and thus promote bacterial virulence. PTI responses are triggered upon recognition of bacterial PAMPs by plant PRRs and do not only interfere with pathogen survival but can also affect T 3 secretion-mediated delivery of the effector proteins.

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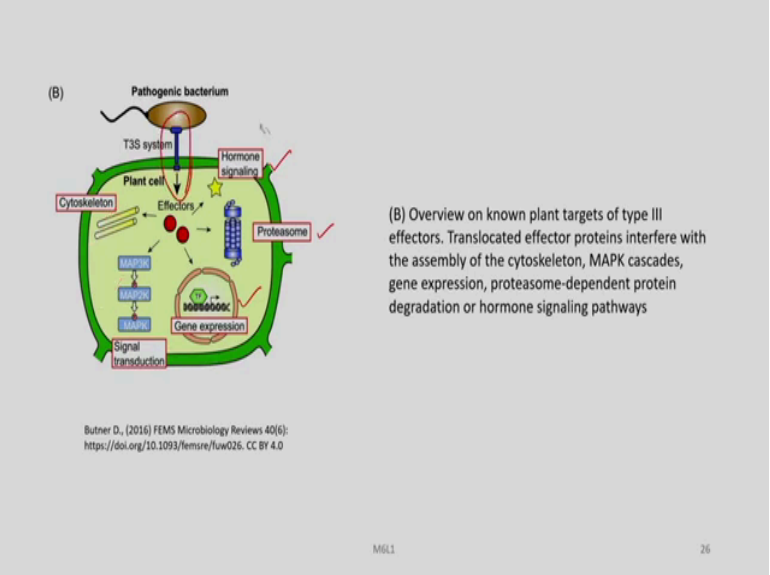

This is the overview on known plant targets of type III effectors. The translocated effector proteins interfere with the assembly of the cytoskeleton, then MAPK cascades, and then gene expression, proteasome-dependent protein degradation or hormone signaling pathway. So, this particular T3 system is a conduit for so many different functions happening inside the host plant.

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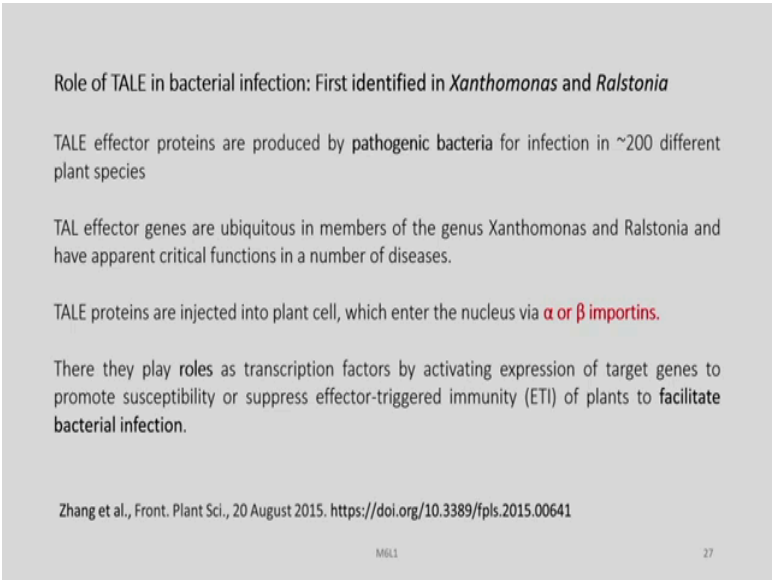

Let us now briefly look into the role the TAL effectors play in the bacterial infection, which was first identified in Xanthomonas and Ralstonia.

We already know that these TAL effectors are produced by pathogenic bacteria and they infect over around 200 different plant species. These TAL effector genes are ubiquitous in members of the genus Xanthomonas and Ralstonia and have apparent critical functions in a number of diseases.

The TALE proteins are injected into plant cell which entered the nucleus via alpha or beta importins. There they play roles as transcription factors by activating the expression of target genes to promote susceptibility or suppress effector-triggered immunity ETI of plants to facilitate bacterial infection.

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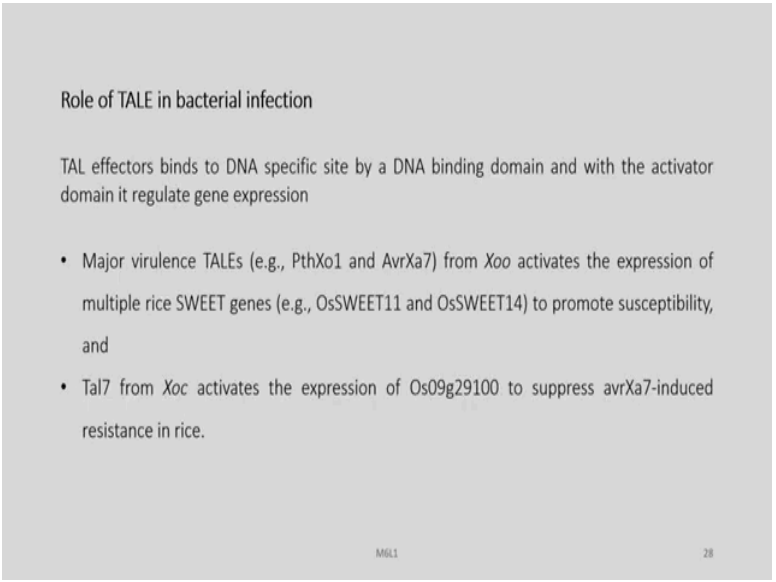

The TAL effectors binds to DNA specific site by DNA binding domain and with the activator domain it regulates gene expression. Major virulence TALEs from Xoo activates the expression of multiple rice SWEET genes to promote susceptibility. And Tal7 from Xoc activates the expression of Os09g29100 to suppress the avrXa7 induced resistance in rice.

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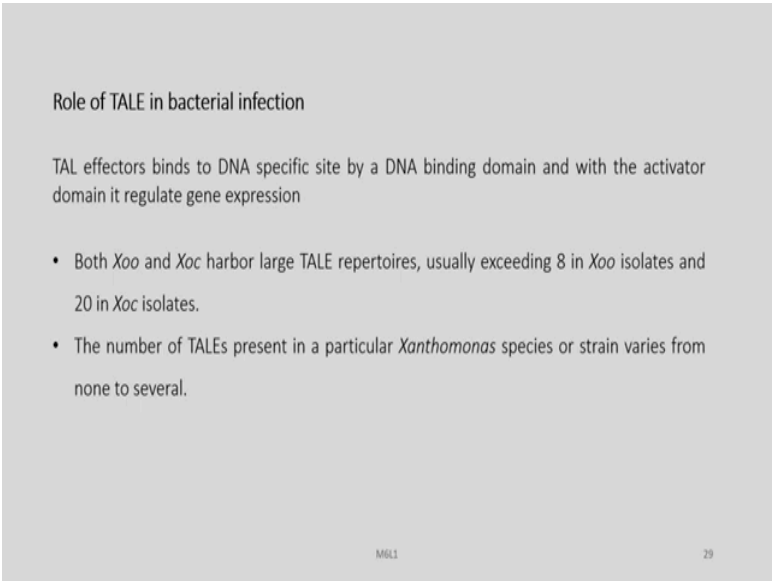

So, we know that the TAL effectors bind to DNA specific site by a DNA binding domain and with the activator domain it regulates gene expression. Both Xoo and Xoc harbor large TALE

repertories, usually exceeding 8 in Xoo isolates and around 20 in Xoc isolates. The number of the TALEs present in a particular Xanthomonas species or strain varies from none to several.

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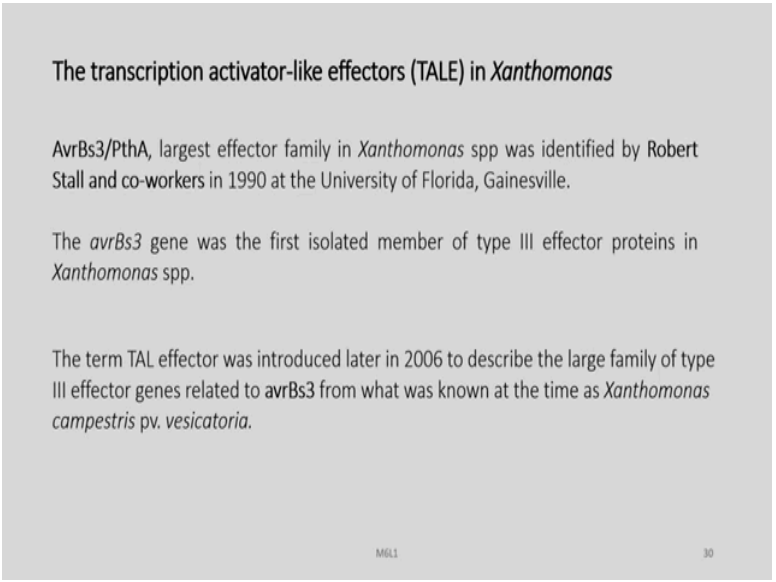

The transcription activator-like effectors in Xanthomonas avrBs3, PthA are the largest effector family in Xanthomonas species and was identified by Robert Stall and co-workers in 1990 at the University of Florida. The avrBs3 was the first isolated member of the type III effector proteins in Xanthomonas species.

The term TAL effector was introduced much later in 2006 to describe the large family of type III effector genes related to avrBs3 from what was known at the time as Xanthomonas compestris pathovar vesicatoria. So, with this we come to the end of our part A.

Thank you; in the next part, we will be discussing about the various domains in the TAL effector proteins.