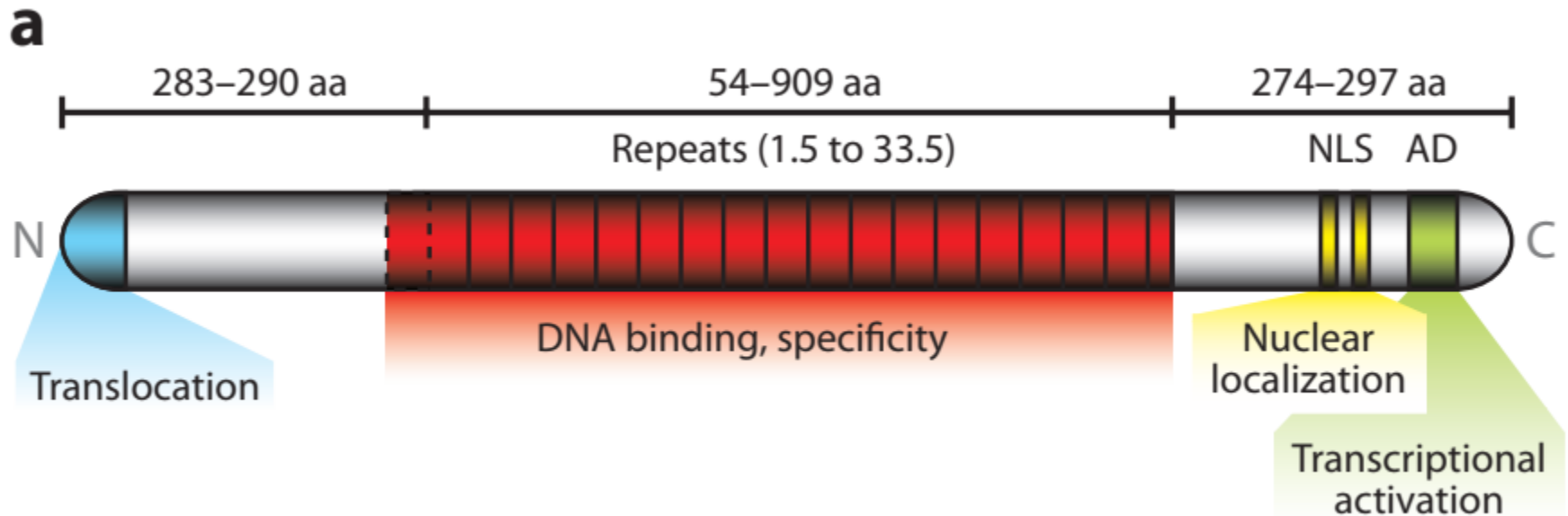# Genome Editing and Engineering
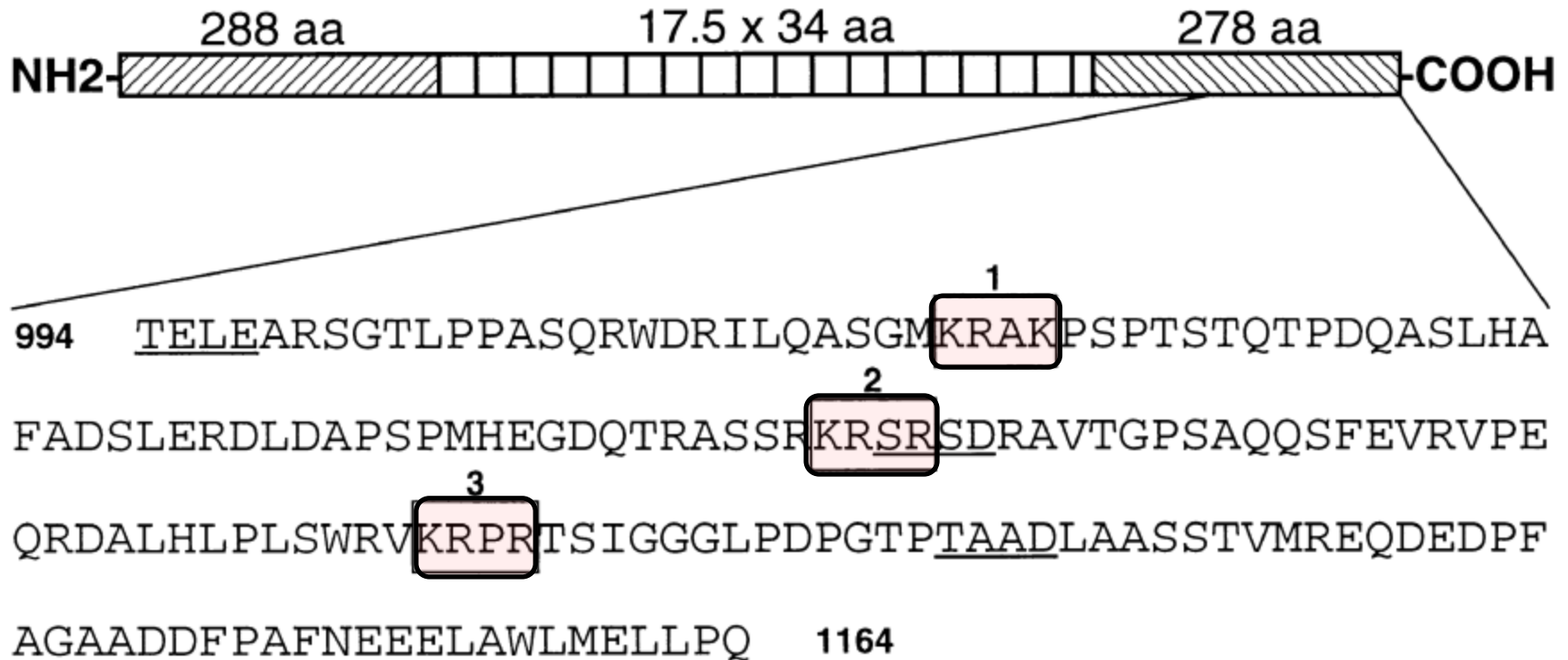
# Course No: BT-637

# LECTURE-14

Dr. Kusum K. Singh
Department of Biosciences and Bioengineering
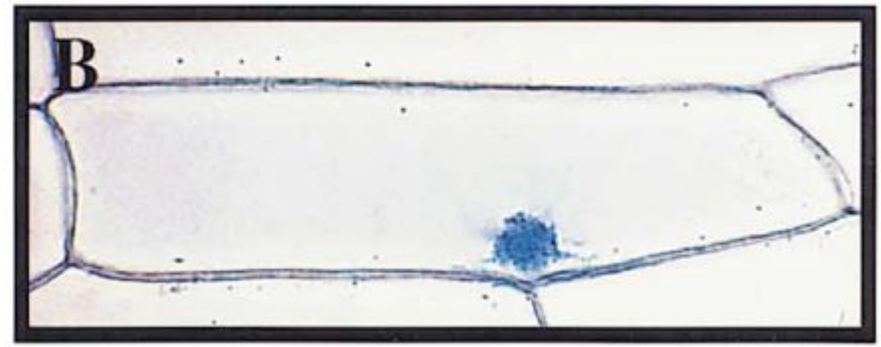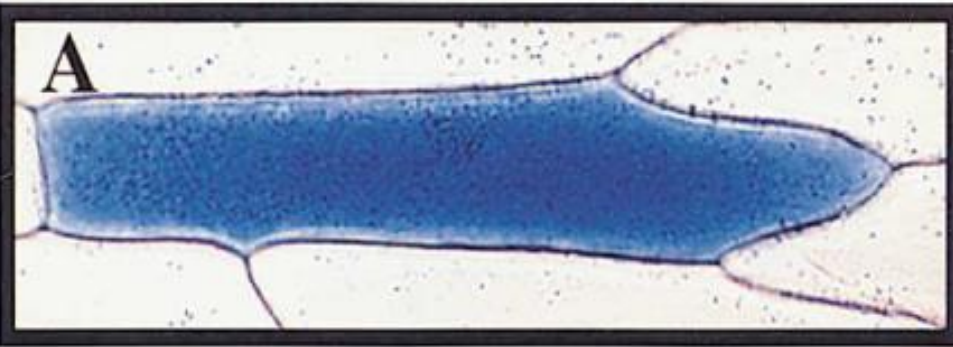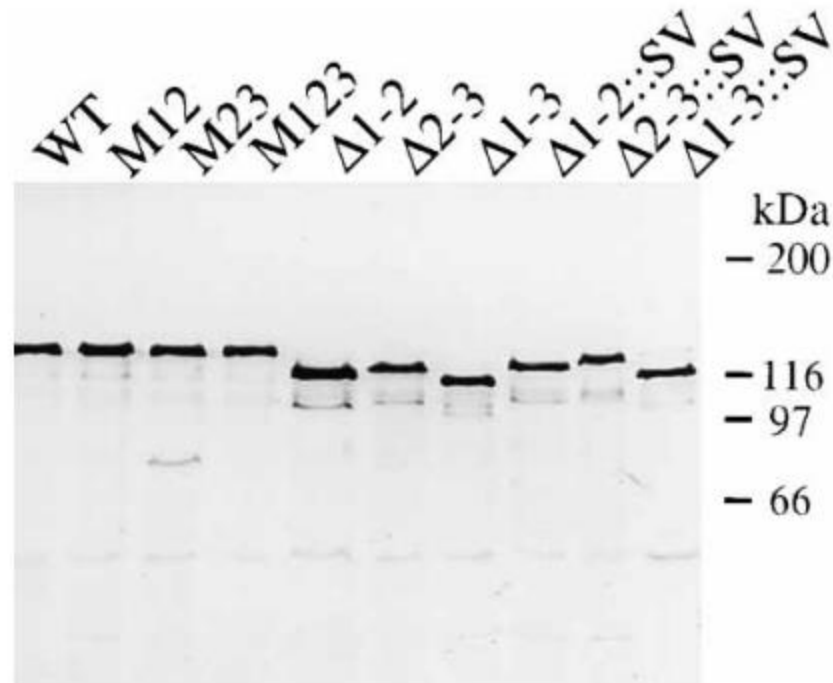Indian Institute of Technology Guwahati

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)



```
        NLS1                                              NLS2                                          NLS3
WT   MKRAKPSPTSTQTPDQASLHAFADSLERDLDAPSPMHEGDQTRASSFKRSRSDRAVTGPSAQQSFEVRVPEQRDALHLPLSWRVKRPRTSI
M1   .T...................................................................................................
M2   .........................................................T.A.........................................
M3   ...............................................................................................T.A....
```

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

Table 2. Nuclear Localization Signals in the AvrBs3 C-Terminal Region Are Required for HR Induction on Pepper Genotype *Bs3*

| *avrBs3* Construct | Intact NLS | HR on Pepper Genotype *Bs3*[a] | Localization of GUS Activity[b] |
|---|---|---|---|
| WT | 1, 2, 3 | +++ | N |
| M1 | 2, 3 | +++ | nd |
| M2 | 1, 3 | +++ | nd |
| M3 | 1, 2 | +++ | nd |

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

**Table 2.** Nuclear Localization Signals in the AvrBs3 C-Terminal Region Are Required for HR Induction on Pepper Genotype *Bs3*

| *avrBs3* Construct | Intact NLS | HR on Pepper Genotype *Bs3*[a] | Localization of GUS Activity[b] |
|---|---|---|---|
| WT | 1, 2, 3 | +++ | N |
| M1 | 2, 3 | +++ | nd |
| M2 | 1, 3 | +++ | nd |
| M3 | 1, 2 | +++ | nd |
| M12 | 3 | +++ | nd |
| M13 | 2 | +++ | N |
| M23 | 1 | + | nd |
| M123 | − | +/− | C>N |

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

**Table 2.** Nuclear Localization Signals in the AvrBs3 C-Terminal Region Are Required for HR Induction on Pepper Genotype *Bs3*

| avrBs3 Construct | Intact NLS | HR on Pepper Genotype Bs3[a] | Localization of GUS Activity[b] |
|---|---|---|---|
| WT | 1, 2, 3 | +++ | N |
| M1 | 2, 3 | +++ | nd |
| M2 | 1, 3 | +++ | nd |
| M3 | 1, 2 | +++ | nd |
| M12 | 3 | +++ | nd |
| M13 | 2 | +++ | N |
| M23 | 1 | + | nd |
| M123 | − | +/− | C>N |
| Δ1-2 | 3 | +++ | N>C |
| Δ2-3 | 1 | − | C |
| Δ1-3 | − | − | C |

57

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

**Table 2.** Nuclear Localization Signals in the AvrBs3 C-Terminal Region Are Required for HR Induction on Pepper Genotype *Bs3*

| *avrBs3* Construct | Intact NLS | HR on Pepper Genotype *Bs3*[a] | Localization of GUS Activity[b] |
|---|---|---|---|
| WT | 1, 2, 3 | +++ | N |
| M1 | 2, 3 | +++ | nd |
| M2 | 1, 3 | +++ | nd |
| M3 | 1, 2 | +++ | nd |
| M12 | 3 | +++ | nd |
| M13 | 2 | +++ | N |
| M23 | 1 | + | nd |
| M123 | − | +/− | C>N |
| Δ1-2 | 3 | +++ | N>C |
| Δ2-3 | 1 | − | C |
| Δ1-3 | − | − | C |
| Δ1-3::SV | c | ++ | N |
| Δ1-3::VS | − | − | nd |
| Δ1-2::SV | 3[c] | +++ | nd |
| Δ2-3::SV | 1[c] | ++ | nd |

# The AvrBs3: contains eukaryotic motifs (NLS and AD)



a

283–290 aa      54–909 aa      274–297 aa

Repeats (1.5 to 33.5)      NLS   AD

N      C

Translocation

DNA binding, specificity

Nuclear localization

Transcriptional activation
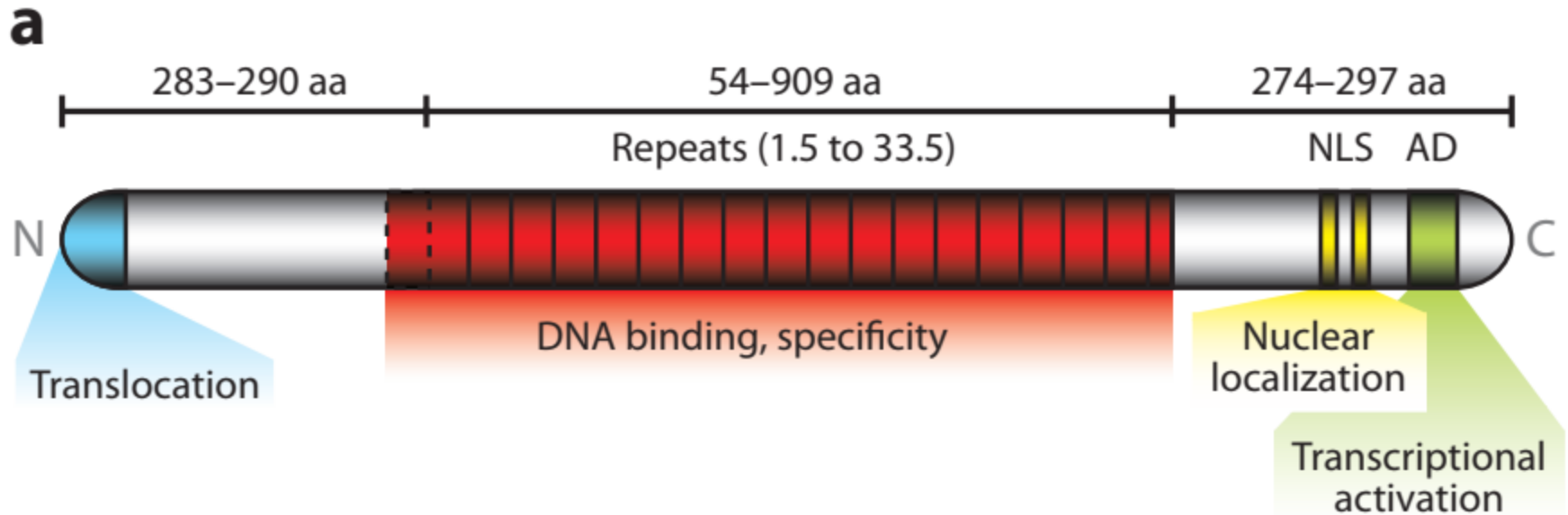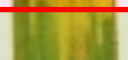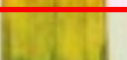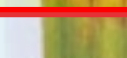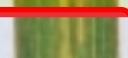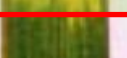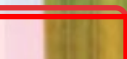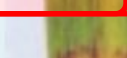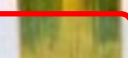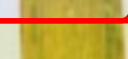
# The AvrBs3: contains eukaryotic motifs (NLS and AD)

```
              1060          1070          1080          1090          1100
              |.........|.........|.........|.........|.........|..
                  *       **                                  *******
AvrXa10  TVMWEQDAAPFAGAADDFPAFNEEELAWLMELLPQSGSVGGTI
AvrBs3   TVMREQDEDPFAGAADDFPAFNEEELAWLMELLPQ
Avrb6    TVMREQDEDPFAGAADDFPAFNEEELAWLMELLPQ
PthA     TVMREQDEDPFAGAADDFPAFNEEELAWLMELLPQ


OPAQUE-2  49  GDGDMMDQQHATEWTFERLLEEEALTTSTPPP      80
VP16(1)  422   GDELRLDGEEVDMTPADALDDFDLEMLGDVESPSP  456
VP16(2)  457    GMTHDPVSYGALDVDDFEFEQMFTDAMGIDDFGG  490
p53        1  MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLP  36
GAL4     850   GITTGMFNTTTMDDVYNYLFDDEDTPPNPKKE     881
```

# The AvrBs3: contains eukaryotic motifs (NLS and AD)



**Table 1.** Requirement of the C terminus

| Plasmid (avirulence gene) | Resi... Xa10 | 3 |
|---|---|---|
| pFWX10-F2 (*avrXa10*) | R | |
| pFWB3S-F2 (*avrBs3*) | S | |
| pFWB3SH-F2 (*avrBs3*) | S | |
| pFWX7S-F2 (*avrXa7*) | S | Γ |
| pZW17 (*avrXa10$^{TGA1064}$*) | S | Γ |
| pZW18 (*avrXa7$^{TGA1064}$*) | NT | Γ |
| pZW19 (*avrBs3$^{TGA1064}$*) | NT | |

R S S

1 2 3

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

**Table 1.** Requirement of the C terminus for avirulence activity

**Plasmid (avirulence g**

pFWX10-F2 (*avrXa10*)
pFWB3S-F2 (*avrBs3*)
pFWB3SH-F2 (*avrBs3*)
pFWX7S-F2 (*avrXa7*)
pZW17 (*avrXa10*$^{TGA100}$)
pZW18 (*avrXa7*$^{TGA1064}$)
pZW19 (*avrBs3*$^{TGA1064}$)

# The AvrBs3: contains eukaryotic motifs (NLS and AD)

**Table 1.** Requirement of the C terminus for avirulence activity

| Plasmid (avirulence gene) | Resistance gene and reaction[a] | | |
| --- | --- | --- | --- |
| | *Xa10* | *Xa7* | *Bs3* |
| pFWX10-F2 (*avrXa10*) | R | S | S |
| pFWB3S-F2 (*avrBs3*) | S | NT | R |
| pFWB3SH-F2 (*avrBs3*) | S | NT | R |
| pFWX7S-F2 (*avrXa7*) | S | R | NT |
| pZW17 (*avrXa10$^{TGA1064}$*) | S | NT | NT |
| pZW18 (*avrXa7$^{TGA1064}$*) | NT | S | NT |
| pZW19 (*avrBs3$^{TGA1064}$*) | NT | NT | S |

VP16(1)   422   GDELRLDGEEVDMTPADALDDFDLEMLGDVESPSP   456

VP16(2)   457   GMTHDPVSYGALDVDDFEFEQMFTDAMGIDDFGG   490

**Mutational & swapping studies showed both NLS and AAD are critical for AvrBs3 activity.**

# The AvrBs3: flanked by conserved Inverted Repeats

```
ATGCGGTTTTGCGCCGCCACCAGCACGGCGTTGCAGGCAACCAGCGTGTCATCACGCACA      60
                   IR-L
TAGTTCTGATCGAGGGTCGGCAGGGATTGGTGTAAAAAACAGCCAAAAGTGAGCTAACTC    120

GCTGTCAGCACAGAAATTTTTCACAACCTTCTGCCGATCCTCCATGCGGGTCCGTGATCG    180

CCTTCATGTCTGCGCCTCACCCTGGTCGTCGAGGGTTGCCAGGATCACCCGAAGTTGTGT    240

ACTGCCATGCGGCCTCGGAAGCTATGTAGGAACCACAGACCGCTAGTCTGGAGGCGACCA    300
                         BamHI
TGTAAAGAGGTATGCCTGATGGATCCCATTCGTTCGCGCACACCAAGTCCTGCCCGCGAG    360
      SD              M   D   P   I   R   S   R   T   P   S   P   A   R   E
                                            PstI
CTTCTGCCCGGACCCCAACCCGATGGGGTTCAGCCGACTGCAGATCGTGGGGTGTCTCCG    420
L   L   P   G   P   Q   P   D   G   V   Q   P   T   A   D   R   G   V   S   P

CCTGCCGGCGGCCCCCTGGATGGCTTGCCCGCTCGGCGGACGATGTCCCGGACCCGGCTG    480
P   A   G   G   P   L   D   G   L   P   A   R   R   T   M   S   R   T   R   L
```

# The AvrBs3: flanked by conserved Inverted Repeats

```
CTGCATTTGCCCCTCAGTTGGAGGGTAAAACGCCCGCGTACCAGTATCGGGGGCGGCGGT    3660
L   H   L   P   L   S   W   R   V   K   R   P   R   T   S   I   G   G   G   G

ACGCCCACGGCTGCCGACCTGGCAGCGTCCAGCACCGTGATGCGGGAACAAGATGAGGAC    3720
T   P   T   A   A   D   L   A   A   S   S   T   V   M   R   E   Q   D   E   D

CCCTTCGCAGGGGCAGCGGATGATTTCCCGGCATTCAACGAAGAGGAGCTCGCATGGTTG    3780
P   F   A   G   A   A   D   D   F   P   A   F   N   E   E   E   L   A   W   L

ATGGAGCTATTGCCTCAGTGAGGCTCAGTCGGTGACTACCTGAGCGTCGGCAGGGATTGG    3840
M   E   L   L   P   Q   *
                    IR-R

TGTAAGTAACCTTTACTGACAGCGAGTTAGCCCACTTTTGGCTGTTTTTTACACAAATCC    3900

CTGCCTCCCCTCTAGTTGCGCAAGATGCCCGCGCTTGGTACCAGAGGCATCGTAGGCCGT    3960
    211
GAGCTTGTTCAGTGGCGGCAACCGCTTGAAGCCTGCTGCC    4000
```

66

# The AvrBs3: Structural features

Central Repeats (1.5 to 33.5 times)
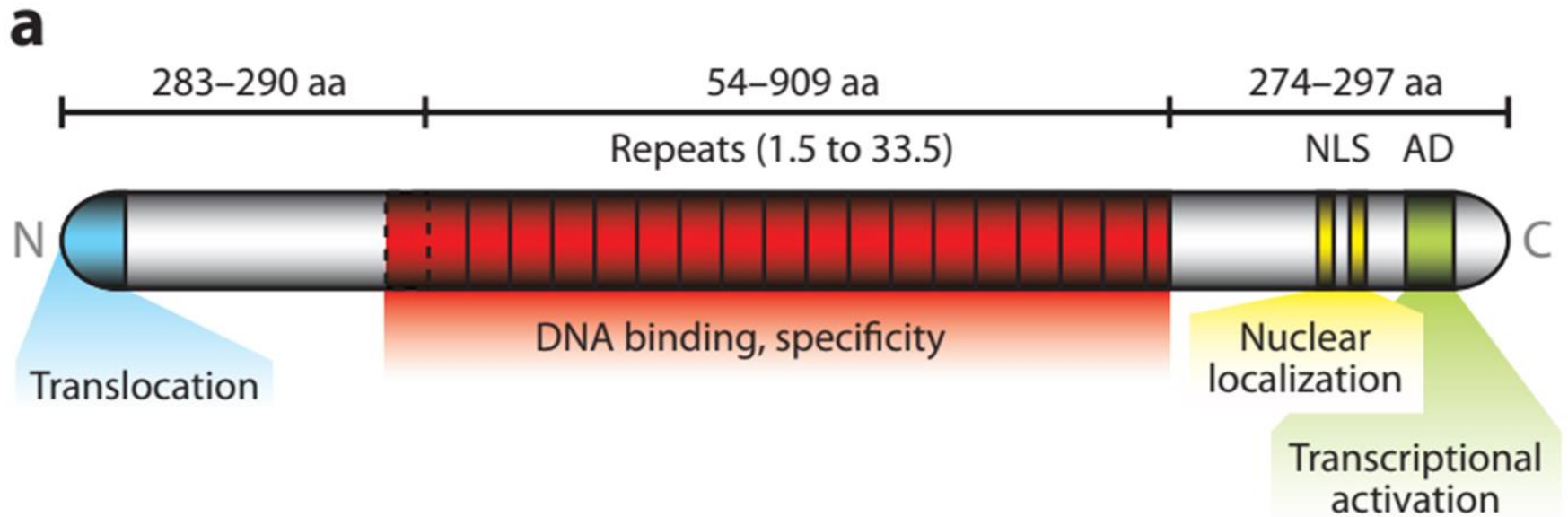102 bp (34 aa), $12^{th}$ and $13^{th}$ HVR

C-terminal:  3 Nuclear Localization Signal
of which $2^{nd}$ and $3^{rd}$ is critical

C-terminal: Acidic Activation domain,
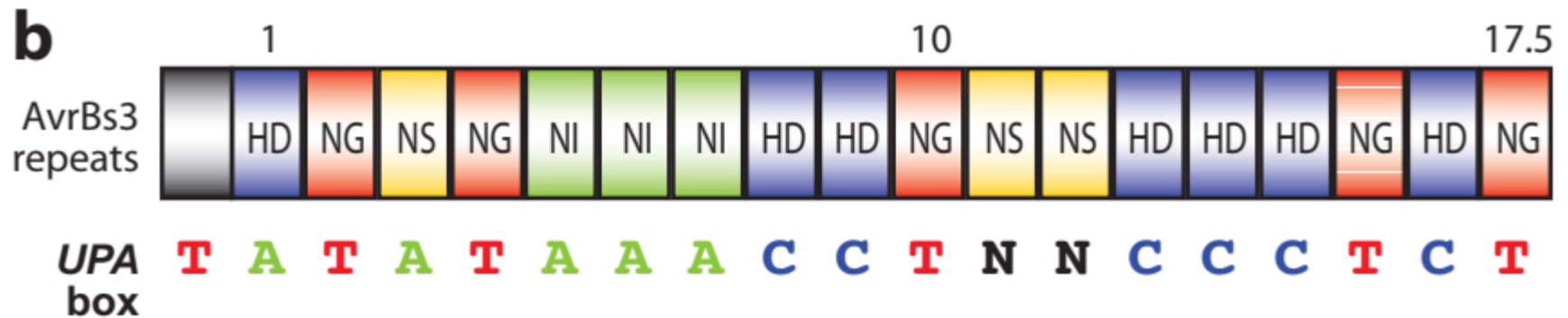important for AvrBs3 activity and

Left & right inverted repeats of 62 bp

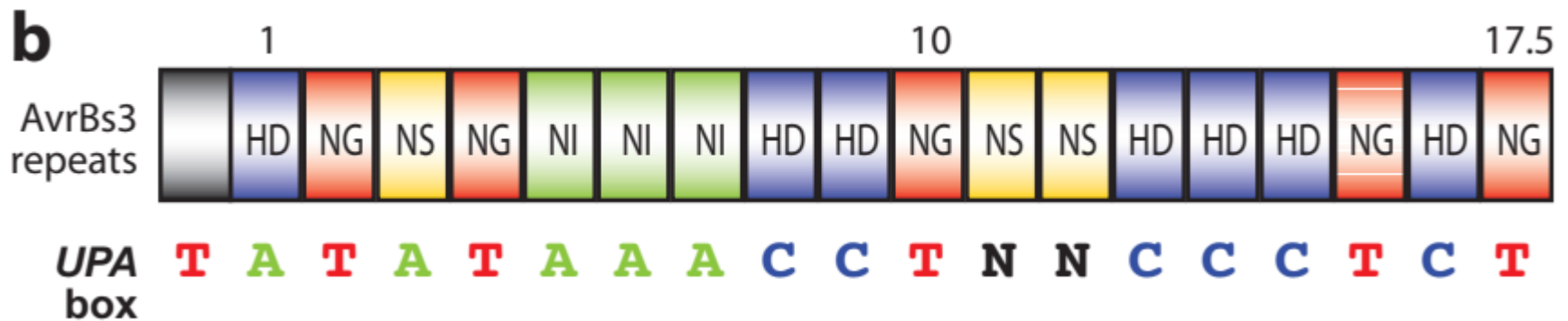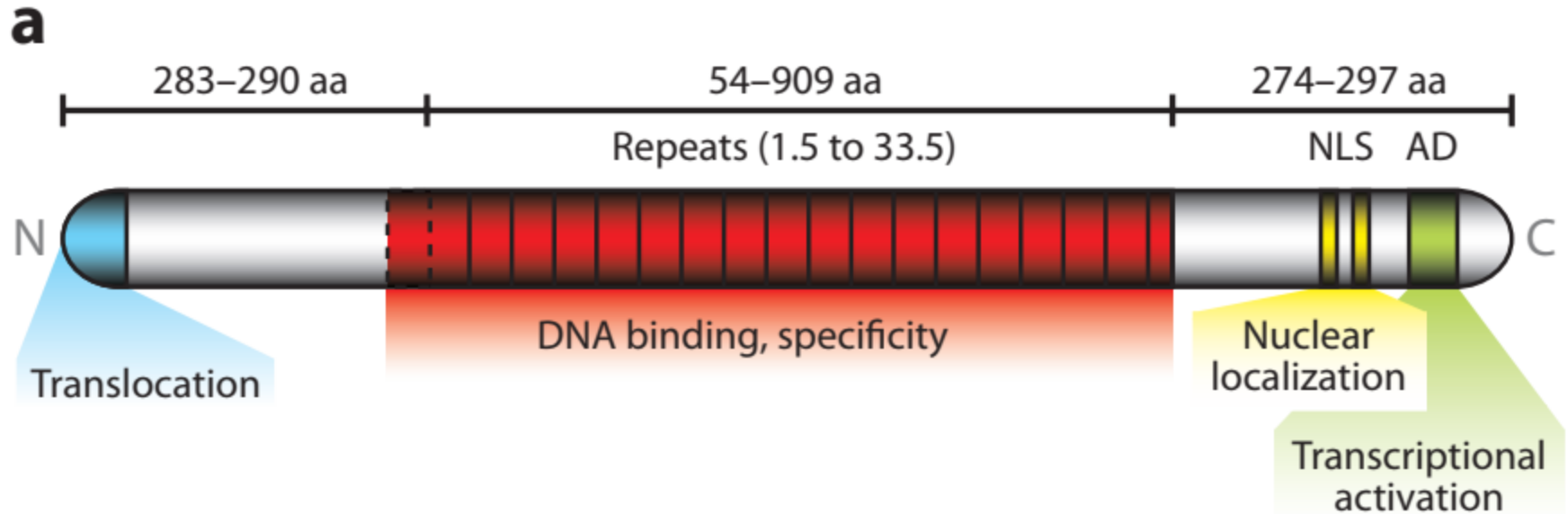N terminal has translocation signal

# The AvrBs3: Structural features

# Targets of AvrBs3: Upregulated by AvrBs3 (UPA)

# Isolations of avrBs3 homologs

# Conclusions of Lecture-14

- **TALE has NLS and AAD (eukaryotic motifs).**

- **It was found to function in plants.**

- **Mutational studies showed both NLS and AAD are critical for AvrBs3 activity.**

- **Transposons like gene transfer = short conserved regions = 62 bp.**

- **It targets UPA genes by binding to specific conserved UPA box.**

# Questions?