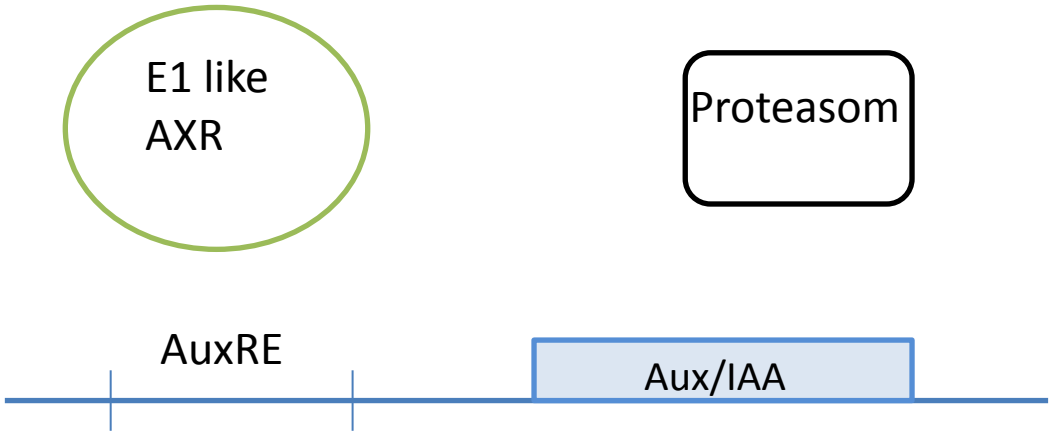
ARF1, a Transcription Factor That Binds to Auxin Response Elements

Tim Ulmasov, Gretchen Hagen, Tom J. Guilfovle (1997)

Introduction



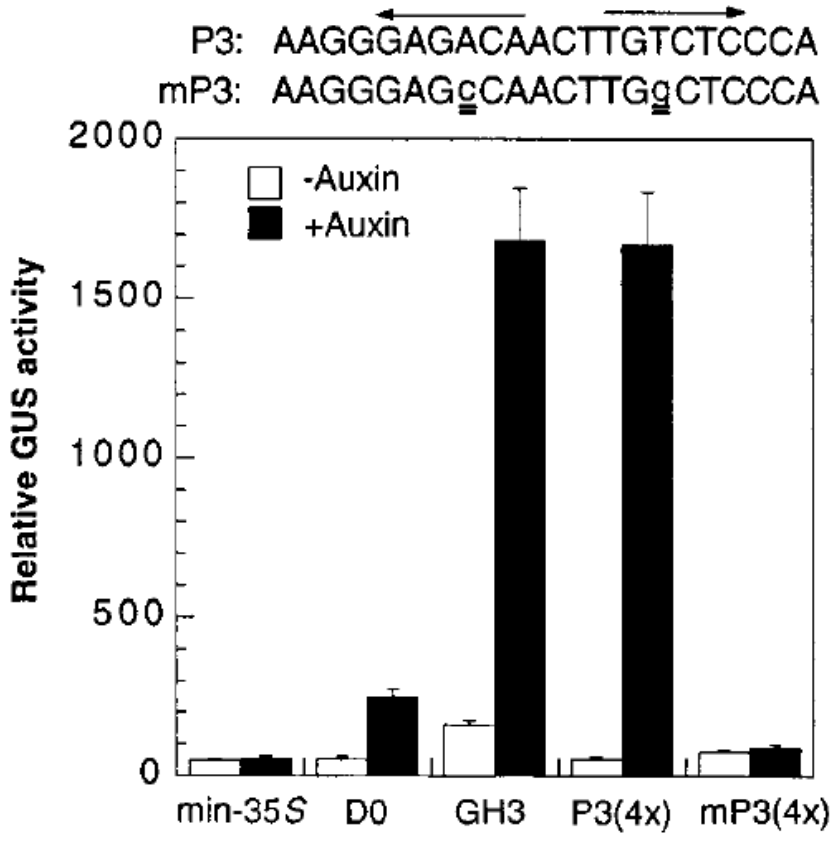
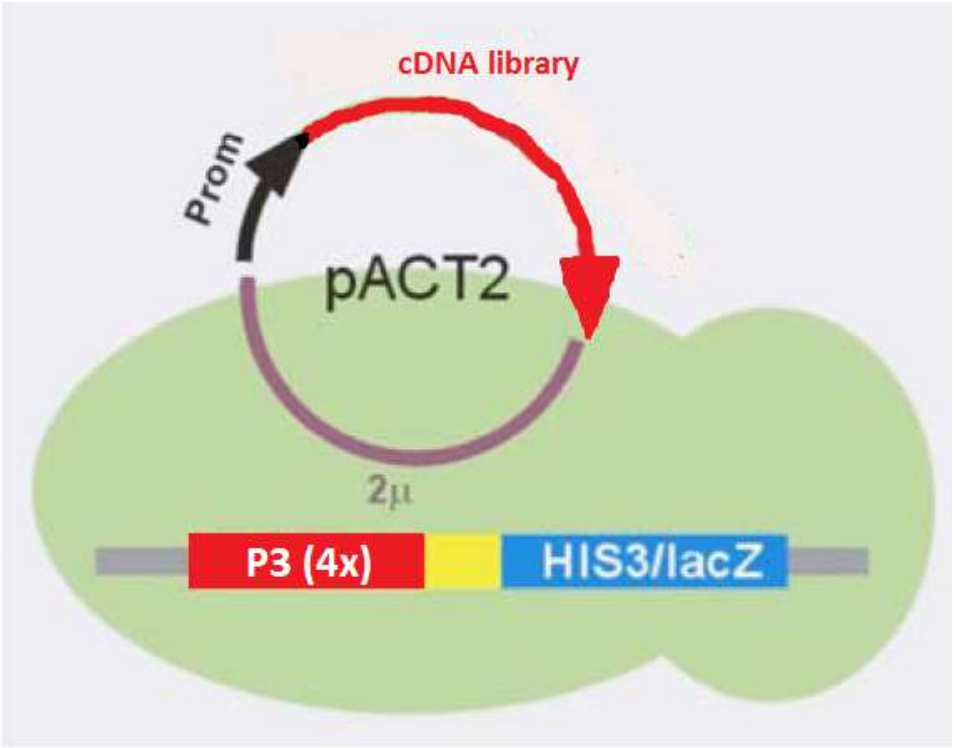


Fig. 1. Palindromic copies of the TGTCTC function as AuxREs. Constructs were tested in transfected carrot protoplasts with or without auxin (*3*). min-35*S*, −46 CaMV 35*S* promoter; D0, one copy of the 74–base pair (bp) D0 AuxRE from the GH3 promoter; GH3, 592-bp GH3 promoter; P3(4×), four tandem copies of the P3 element; mP3(4×), four tandem copies of a mutated P3 element.

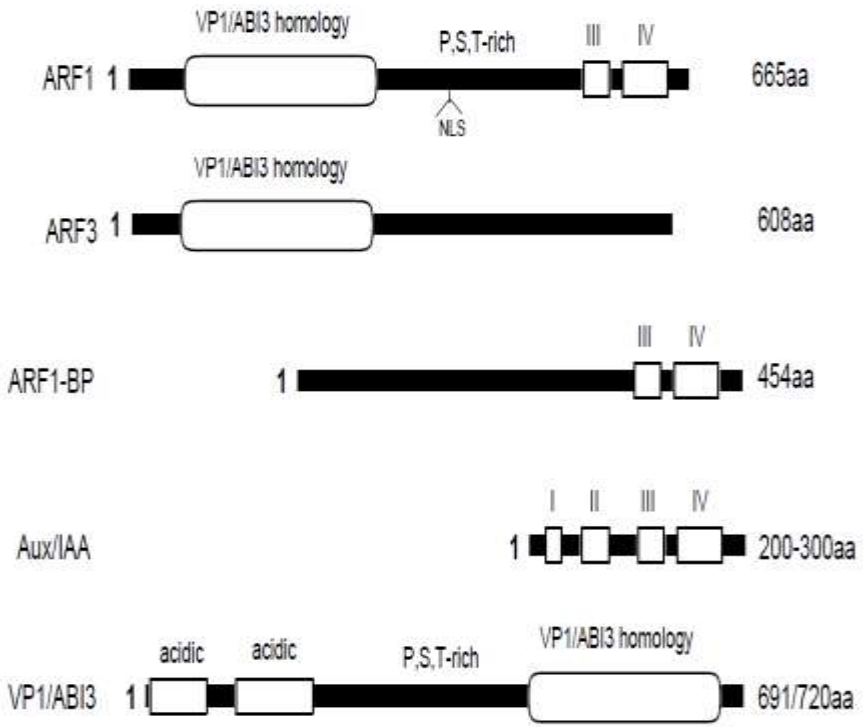
- Test for Identification of the consensus sequence
- Palindromic repeats of TGTCTC [P3(4x)] functioned as AuxREs
- Mutated P3(4x) were inaktive

Yeast-one-hybrid System



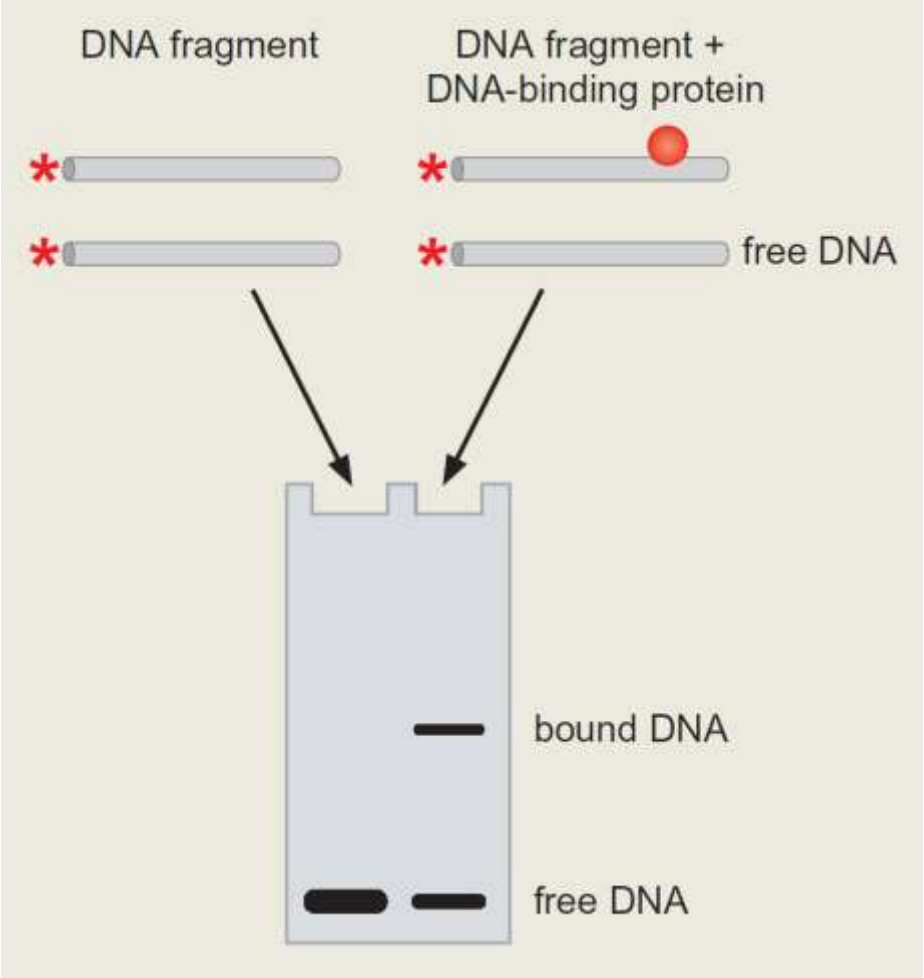
- Identification of cDNA encoding protein, that binds on target sequence
- Five cDNA clones were isolated that encoded same protein
- ARF 1 was found

Schematic diagrams of ARF1 and related proteins

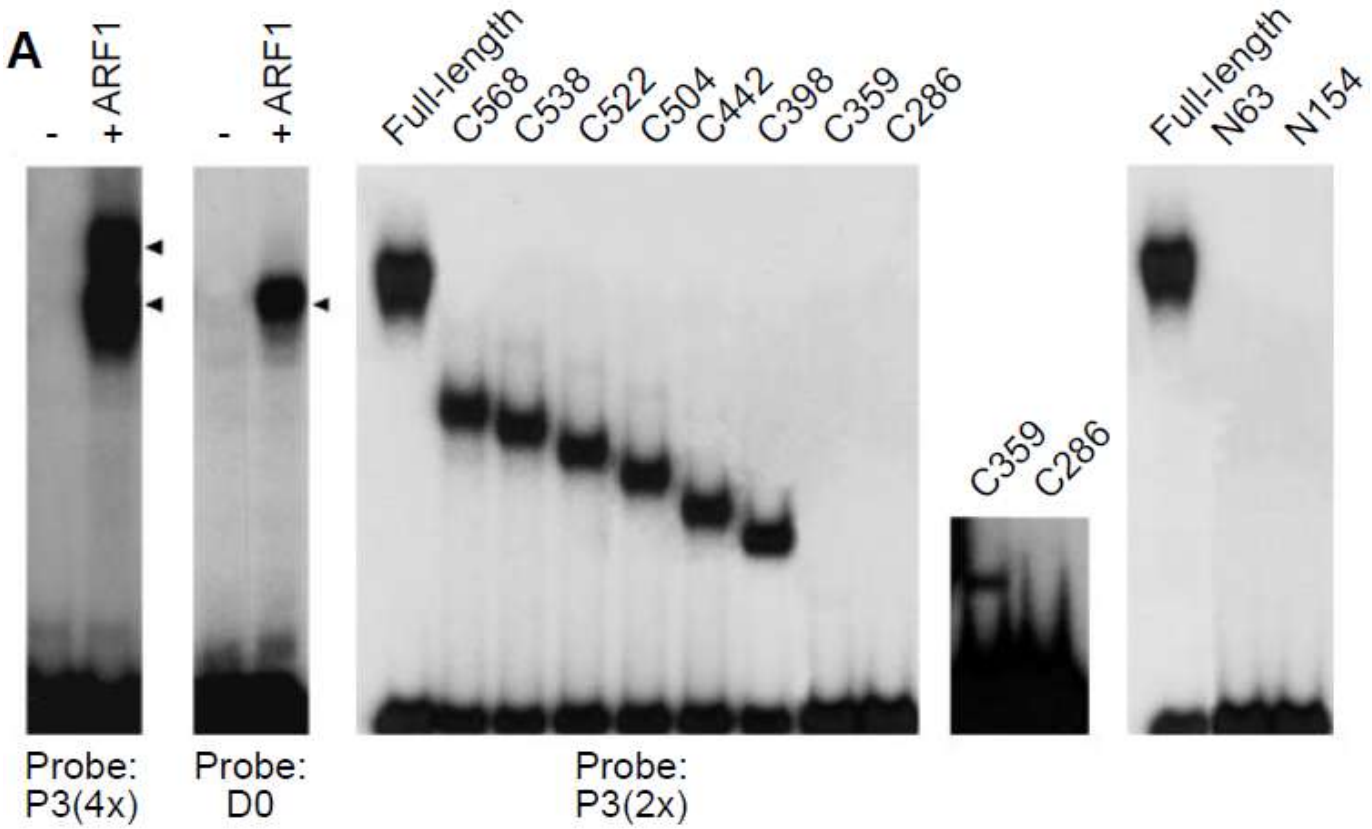


- ARF sequences related to c-terminal regions of VP1/ABI3
- Also to boxes III and IV in Aux/IAA
- Contains NLS (nuclear localization sequence)

Gel mobility shift assay

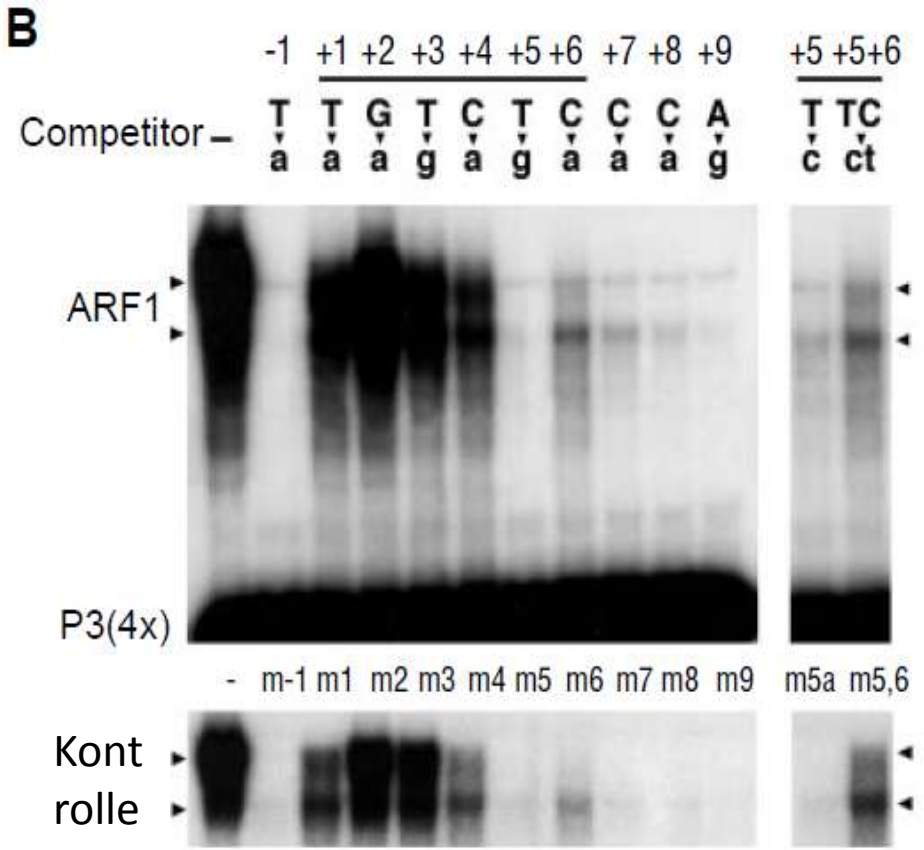


- Southern Blot
- Binding protein decreases mobility of a DNA-fragment



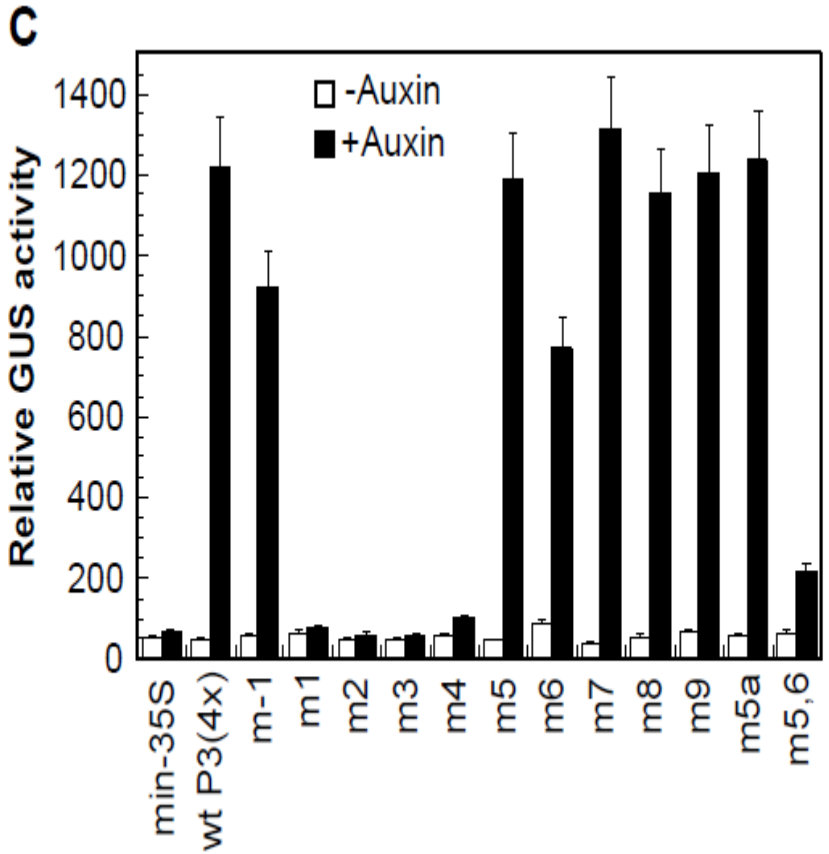
- EMSA (electrophoresis mobility shift assay)
- To detect in vitro interaction of ARF1
- Effects of boxes III and IV by truncating

Importance of the TGTCTC-sequence



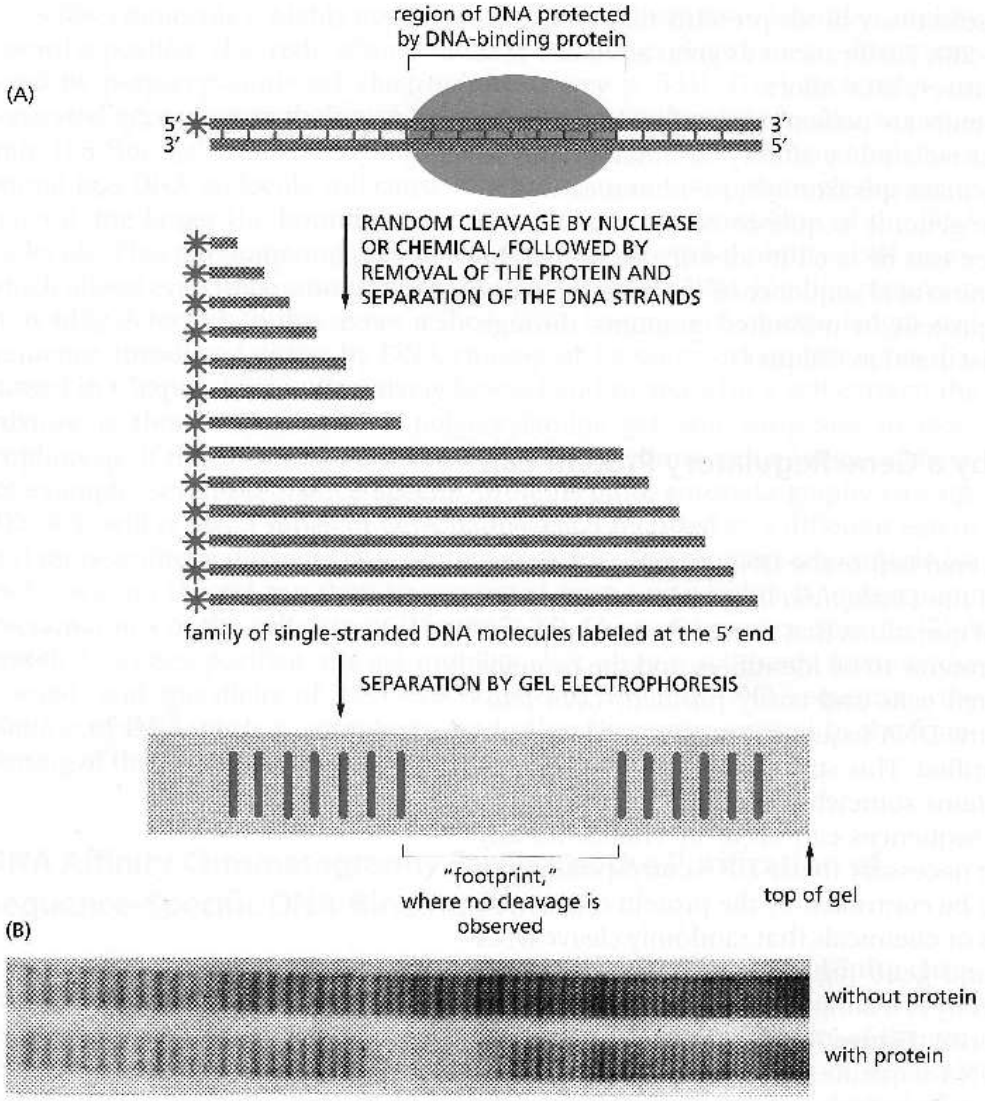
- They detected the specificity of ARF1 to the consensus sequence of the AuxRE by mutating it

In vivo assay of auxin responsiveness



- To verify the *in vitro* assays
- AuxREs were fused upstream of the minimal promotor GUS reporter gene

DNase footprinting assay



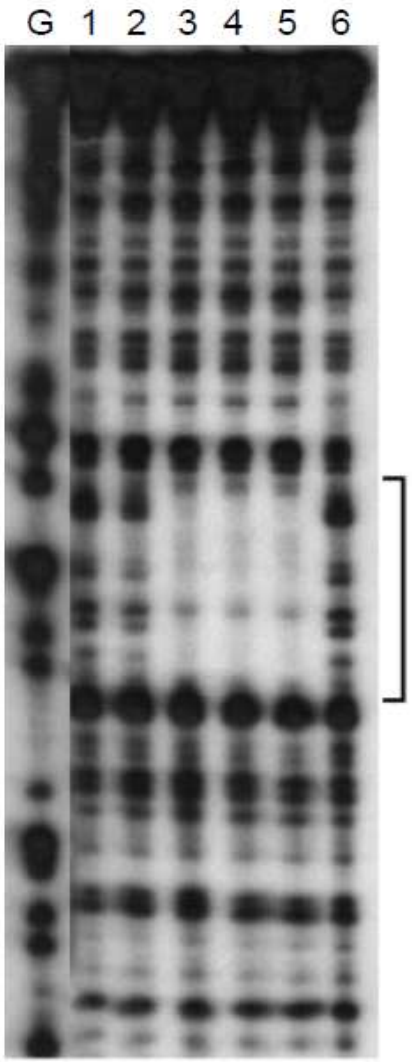
- A method to discover specific DNA-protein interactions
- You can detect the DNA binding sequence

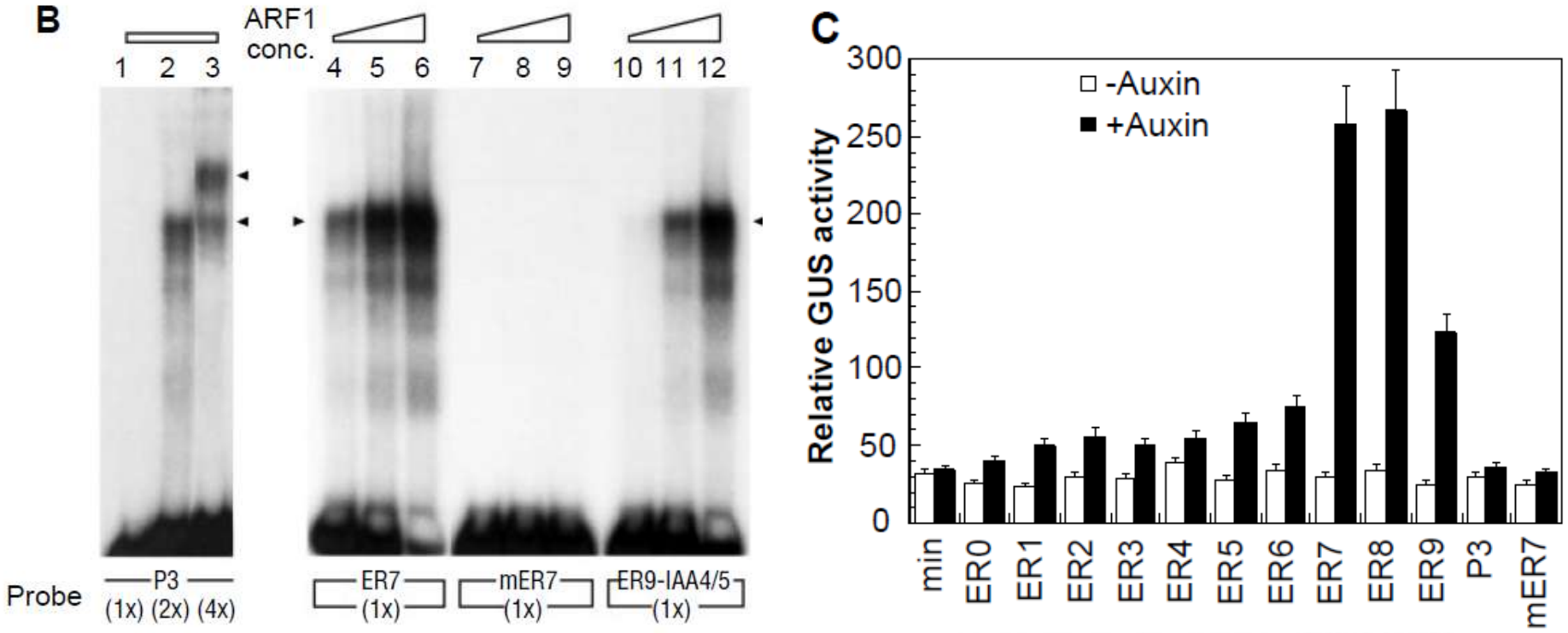
Molecular Biology of the Cell, Alberts et al, Garland Sience, 5th edition 2008, p. 430

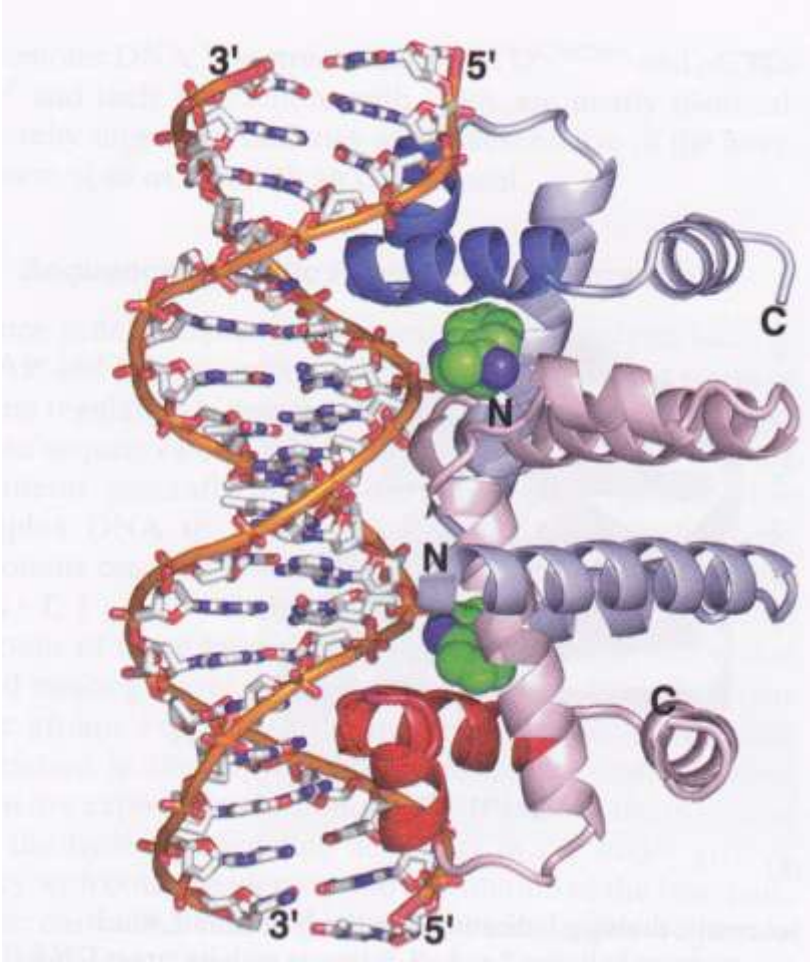
A

IR ER

cccgggcaggAGGGAGACAACTTGTCTCCCAAAGGGAGACAACTTGTCTCCCAAAGcctc

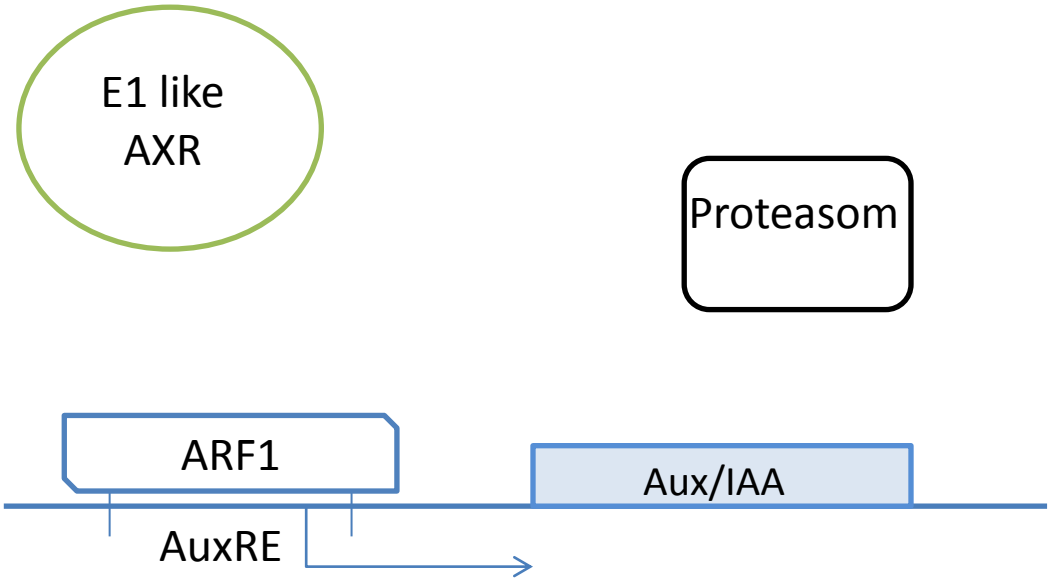


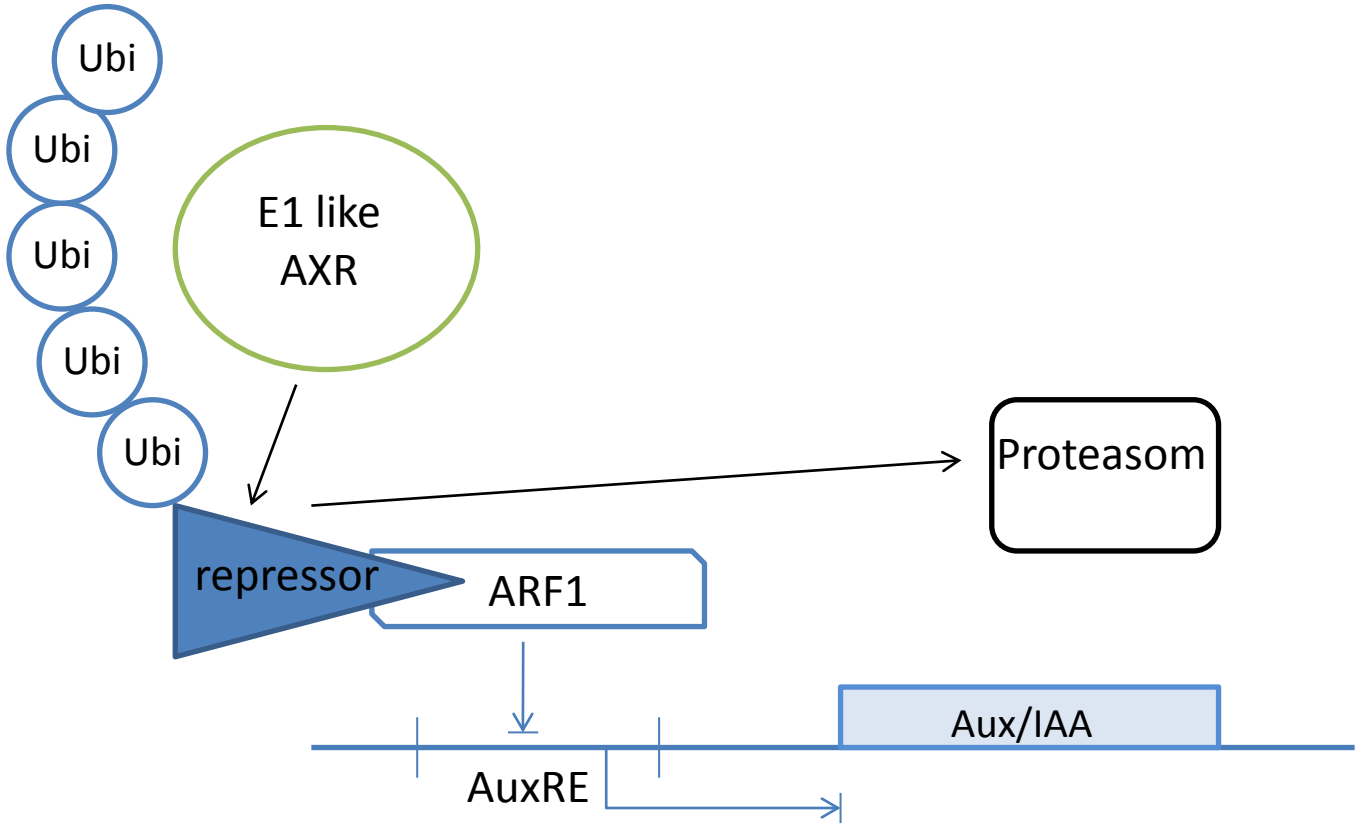

B
ARF1
conc.
1 2 3
4 5 6
7 8 9
10 11 12
Probe
P3
(1x) (2x) (4x)
ER7
(1x)
mER7
(1x)
ER9-IAA4/5
(1x)
C
Relative GUS activity
300
250
200
150
100
50
0
-Auxin
+Auxin
min
ER0
ER1
ER2
ER3
ER4
ER5
ER6
ER7
ER8
ER9
P3
mER7

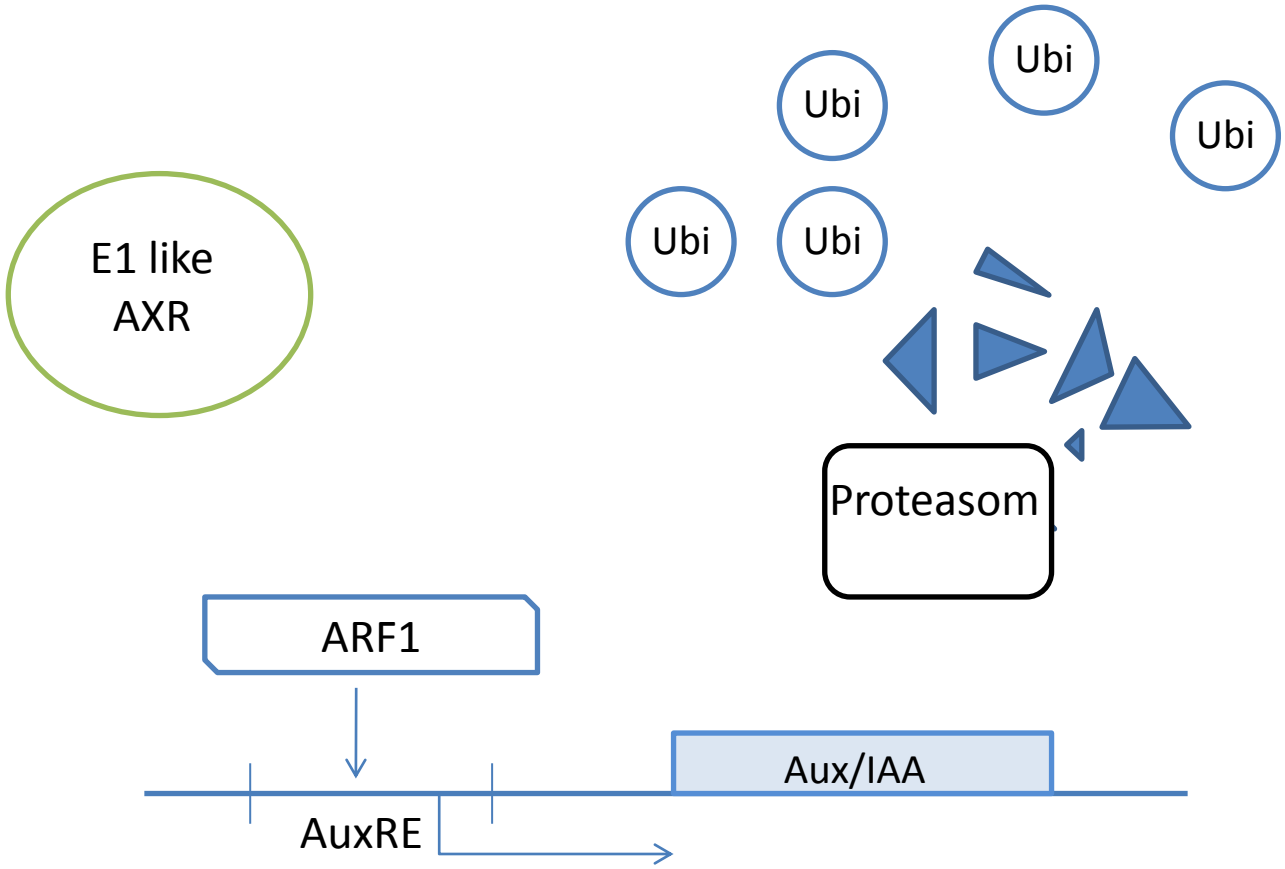


Biochemistry, Voet and Voet, Wiley VCH, 4th edition 2011, Fig. 31-34

Conclusion




Ubi
Ubi
Ubi
Ubi
Ubi
E1 like
AXR
Proteasom
repressor
ARF1
Aux/IAA
AuxRE


Ubi
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E1 like
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