Genome Editing and Engineering

Course No: BT-637

LECTURE-4

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Introduction

- FokI (Flavobacterium okeanokoites)
- Functional domains of FokI (N and C-terminus)
- Mutate the FokI to study its modular nature

Gene, 133 (1993) 79–84
 0378-1119/93/$06.00

GENE 07304

Short Communications

C-terminal deletion mutants of the *Fok*I restriction endonuclease

(Catalytic domain; *Flavobacterium okeanokoites*; hydroxyl radical footprinting; protein-DNA interaction; recognition domain)

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C-terminal deletion mutants

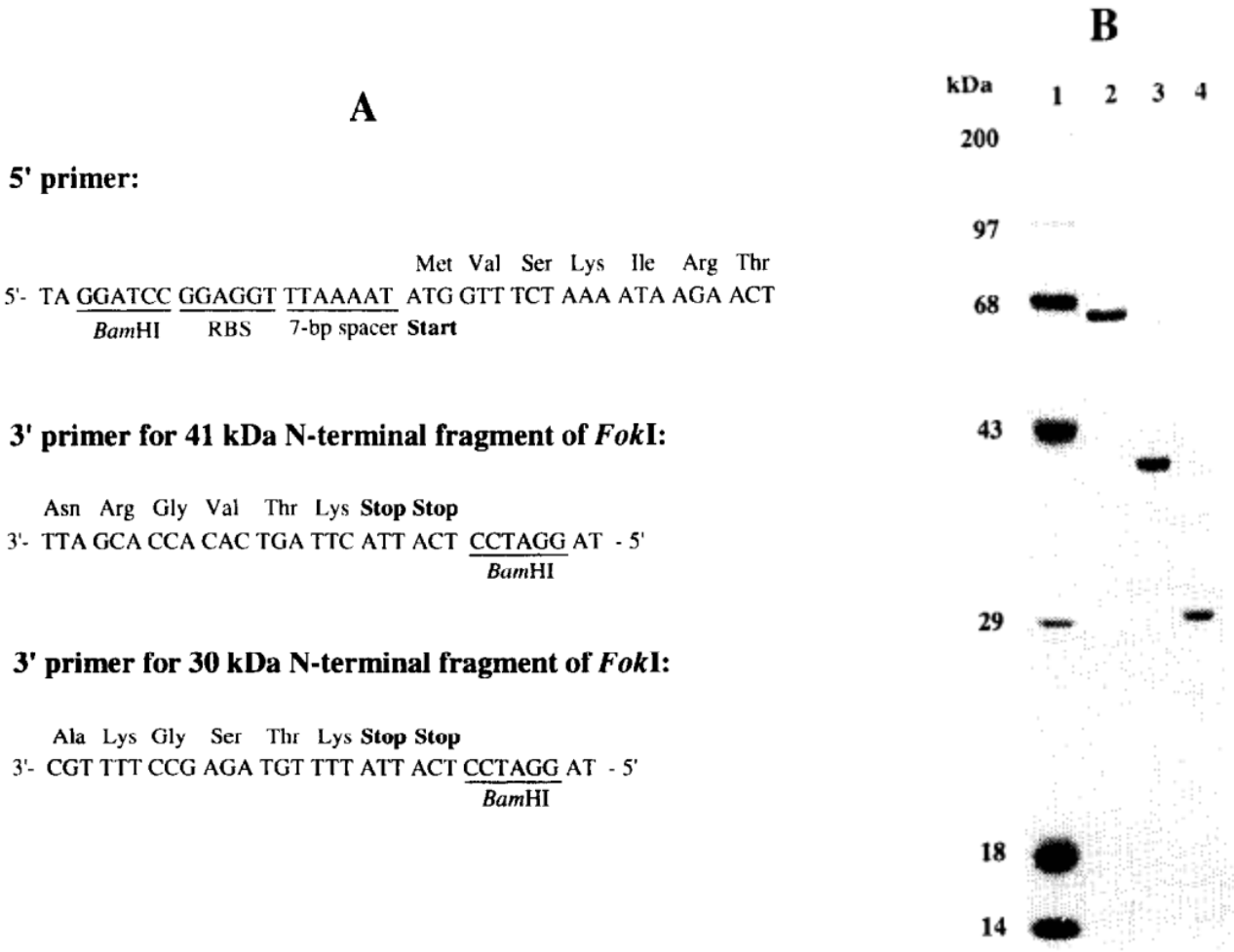
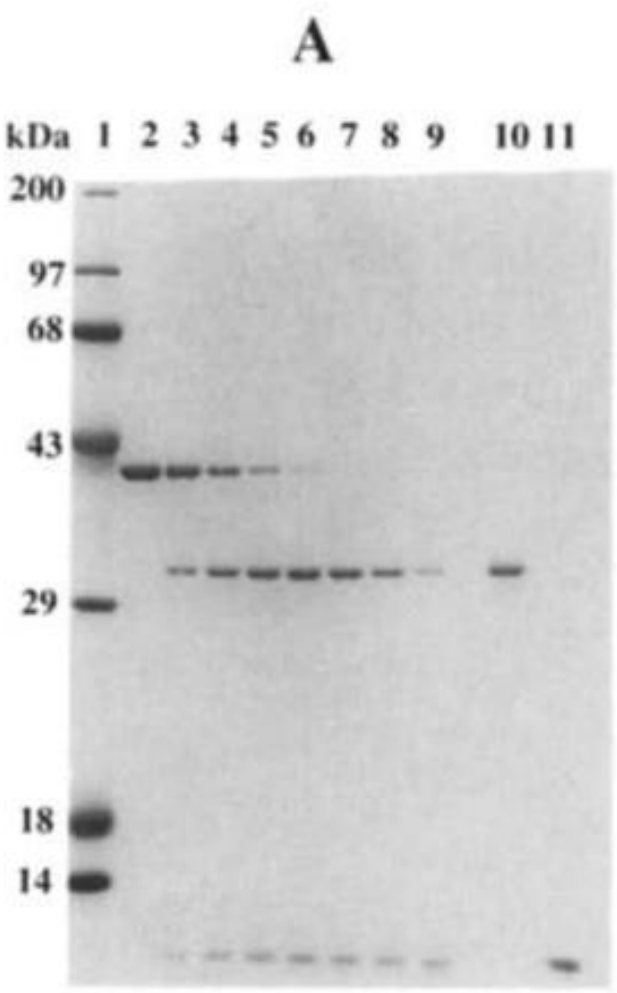
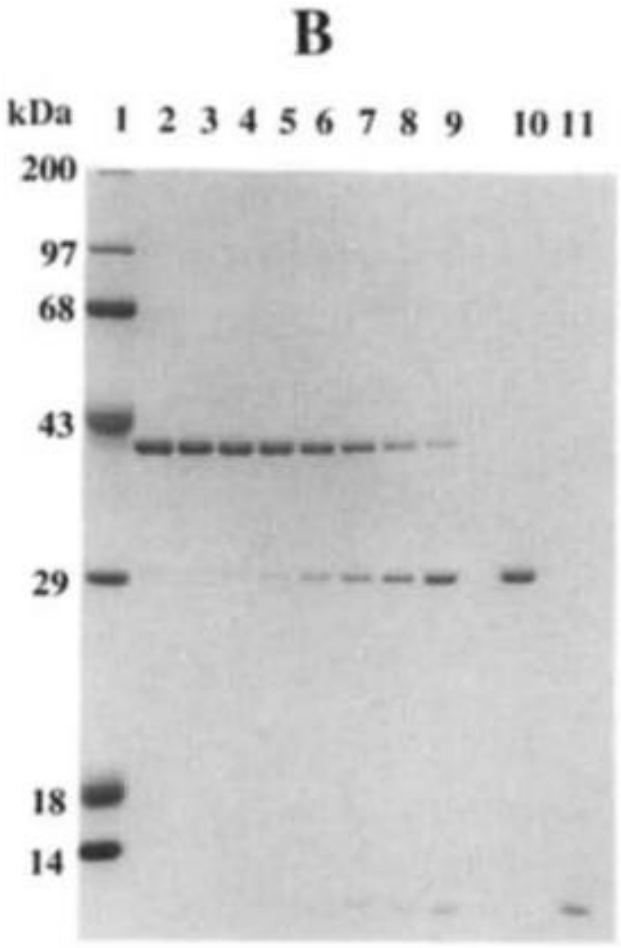


Fig. 1. Construction and analysis of MP. (**A**) Sequences of the 5′ and 3′ primers used to construct the C-terminal deletion mutants of *Fok*I. All primers are flanked by *Bam*HI sites. New translational signals were introduced to achieve high expression of the mutant proteins. A 7-bp spacer separates the RBS for *E. coli* from the ATG start codon. (**B**) 0.1% SDS-12% PAGE profile of the purified 41-kDa and 30-kDa MP. Lanes: 1, protein standards (from BRL, Gaithersburg, MD, USA); 2, wt *Fok*I ENase; 3, the 41-kDa MP; 4, the 30-kDa MP. The procedures for cell growth and purification of the MP are similar to the one described elsewhere (Li and Chandrasegaran, 1993).

Trypsin digestion pattern

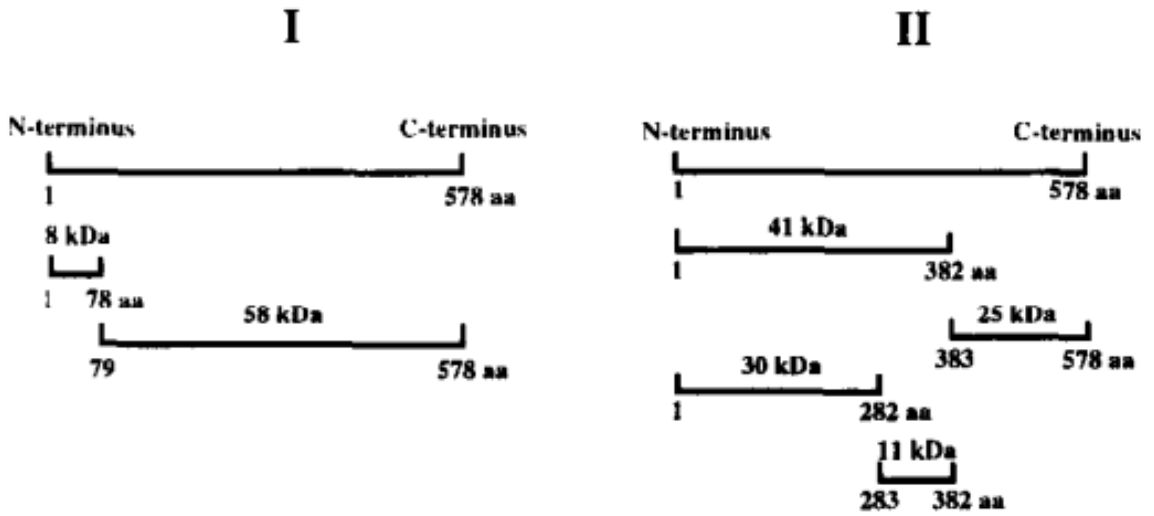


Trypsin digestion of the 41-kDa protein in the absence

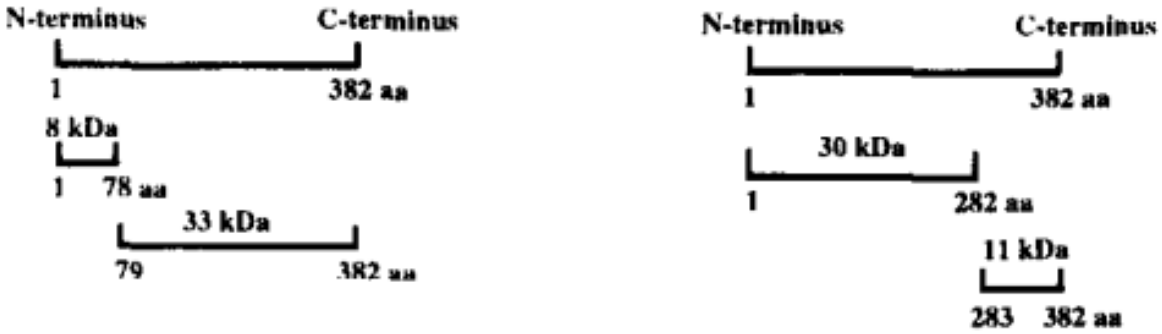


Trypsin digestion of the 41-kDa MP in the presence

Trypsin Map

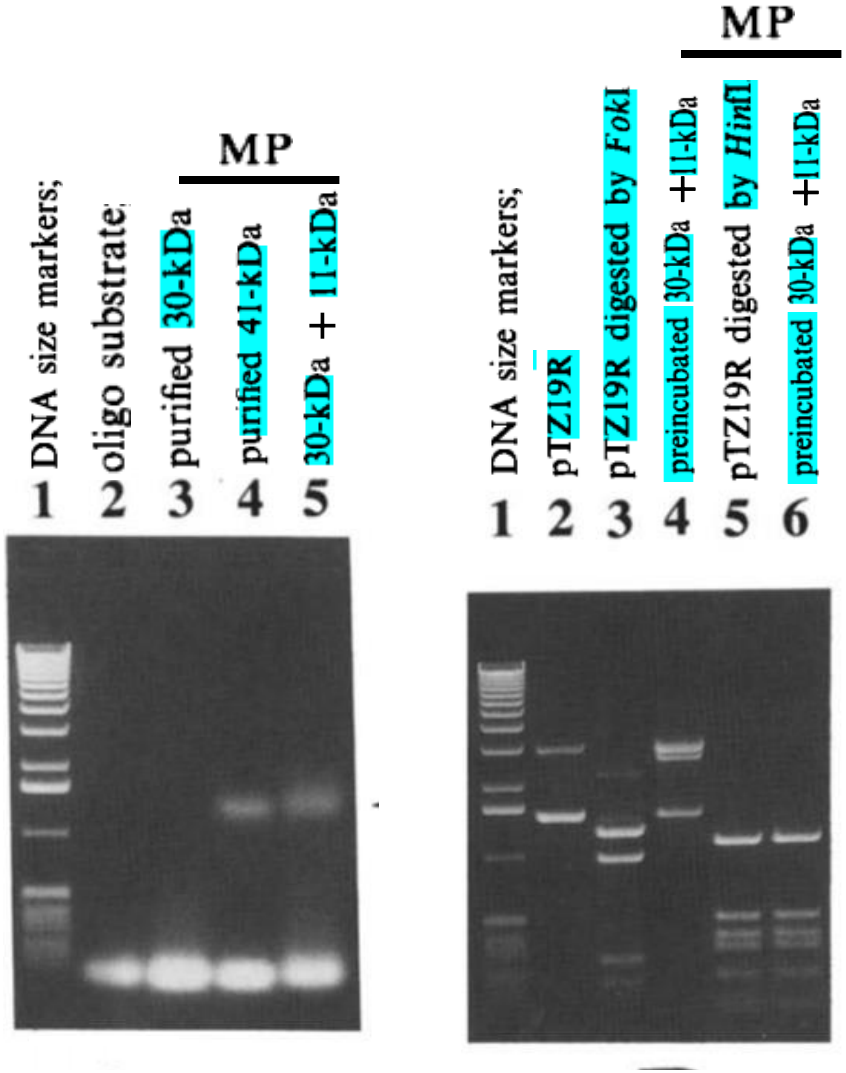


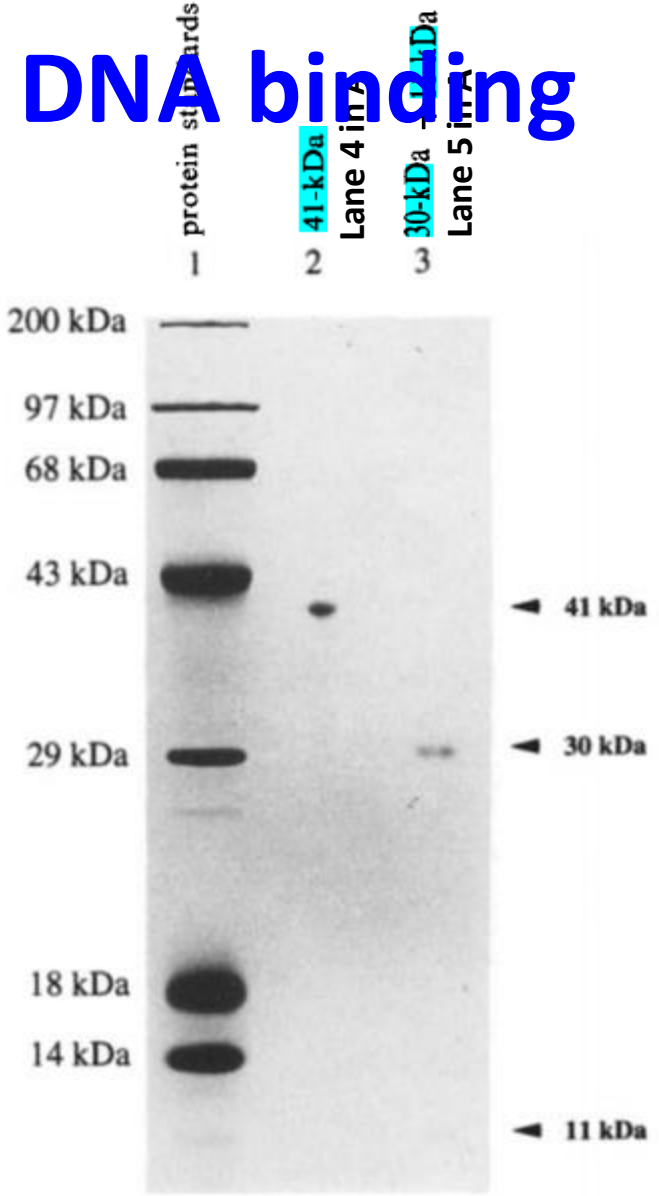
***Fok*I endonuclease**



41-kDa mutant protein

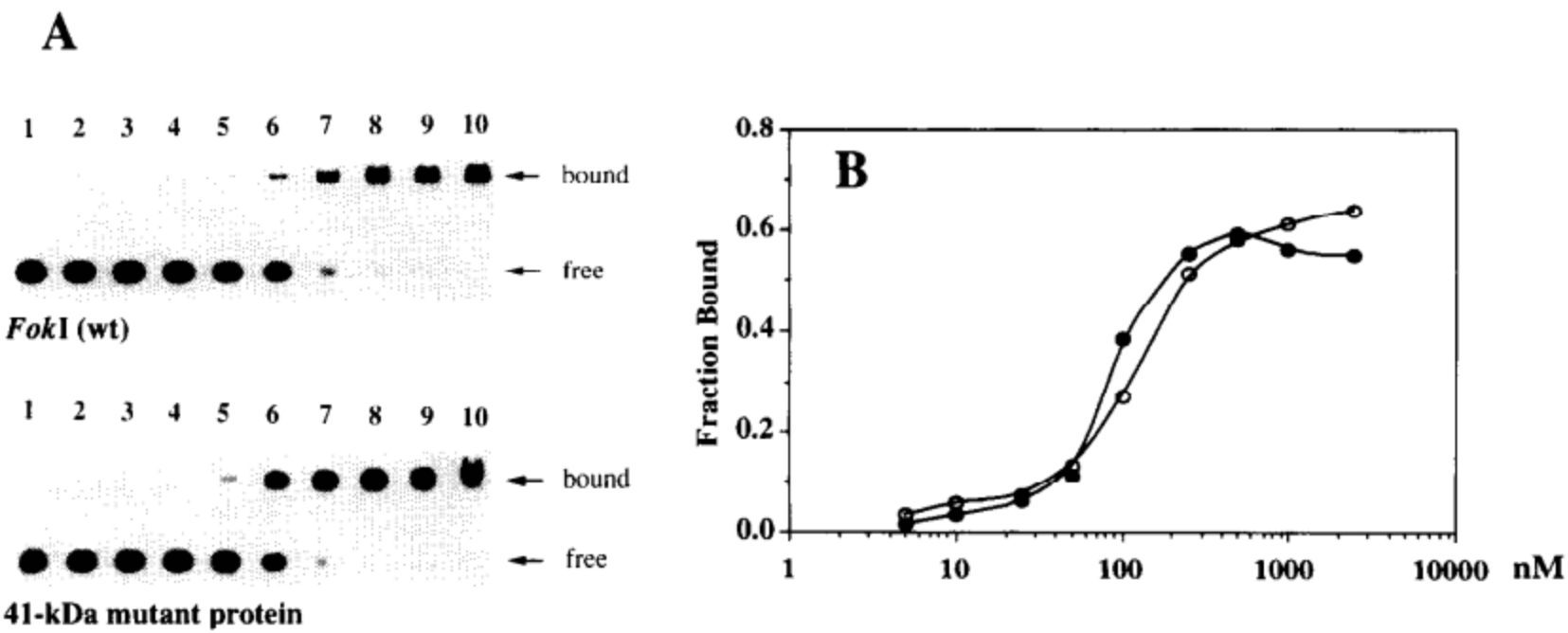
Sequence specific DNA binding





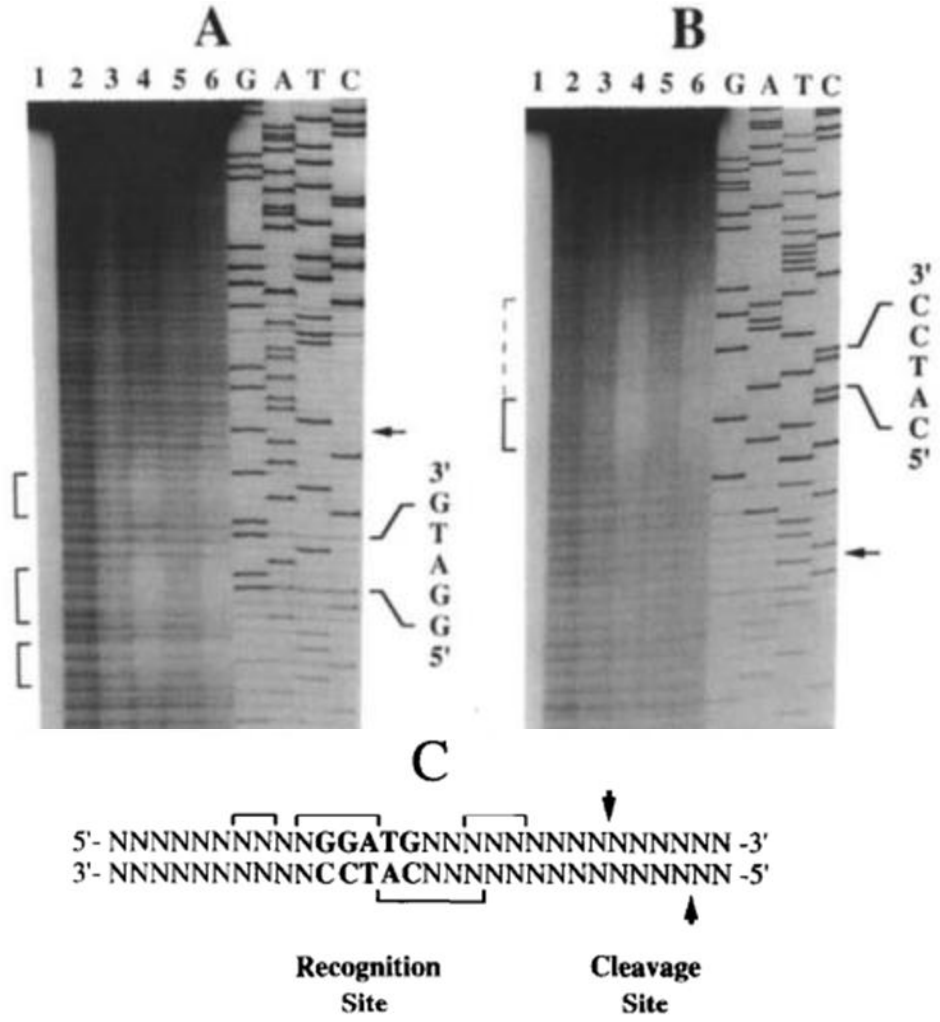
30 kDa &11 kDa associate to reconstitute the FokI recognition domain

Affinities of wt & mt for DNA



Deletion of C-terminal, 25 kDa portion has little or no effect

Mode of interaction of wt & mt



1, untreated DNA substrate

2, DNA substrate cleaved by hydroxyl radicals;

3 and 4, presence of 7.5 pmoles and 15 pmoles *FokI*;

5 and 6, presence of 9 pmoles and 18 pmoles 41-kDa MP.

- **Wt & mt interact only parts of recognition site**
- **No protection at cleavage site**

Conclusions of C-ter. article

- C-terminal mutants were generated
- 41 kDa binds seq. specific manner (wt)
- 30 kDa no longer bind DNA
- 30+11 reconstitutes to restore specificity
- No protection at the cleavage site

Proc. Natl. Acad. Sci. USA
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Biochemistry

Single amino acid substitutions uncouple the DNA binding and strand scission activities of *Fok* I endonuclease

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Communicated by Hamilton O. Smith, July 22, 1993 (received for review May 18, 1993)

D450**A**; **D**467**A**

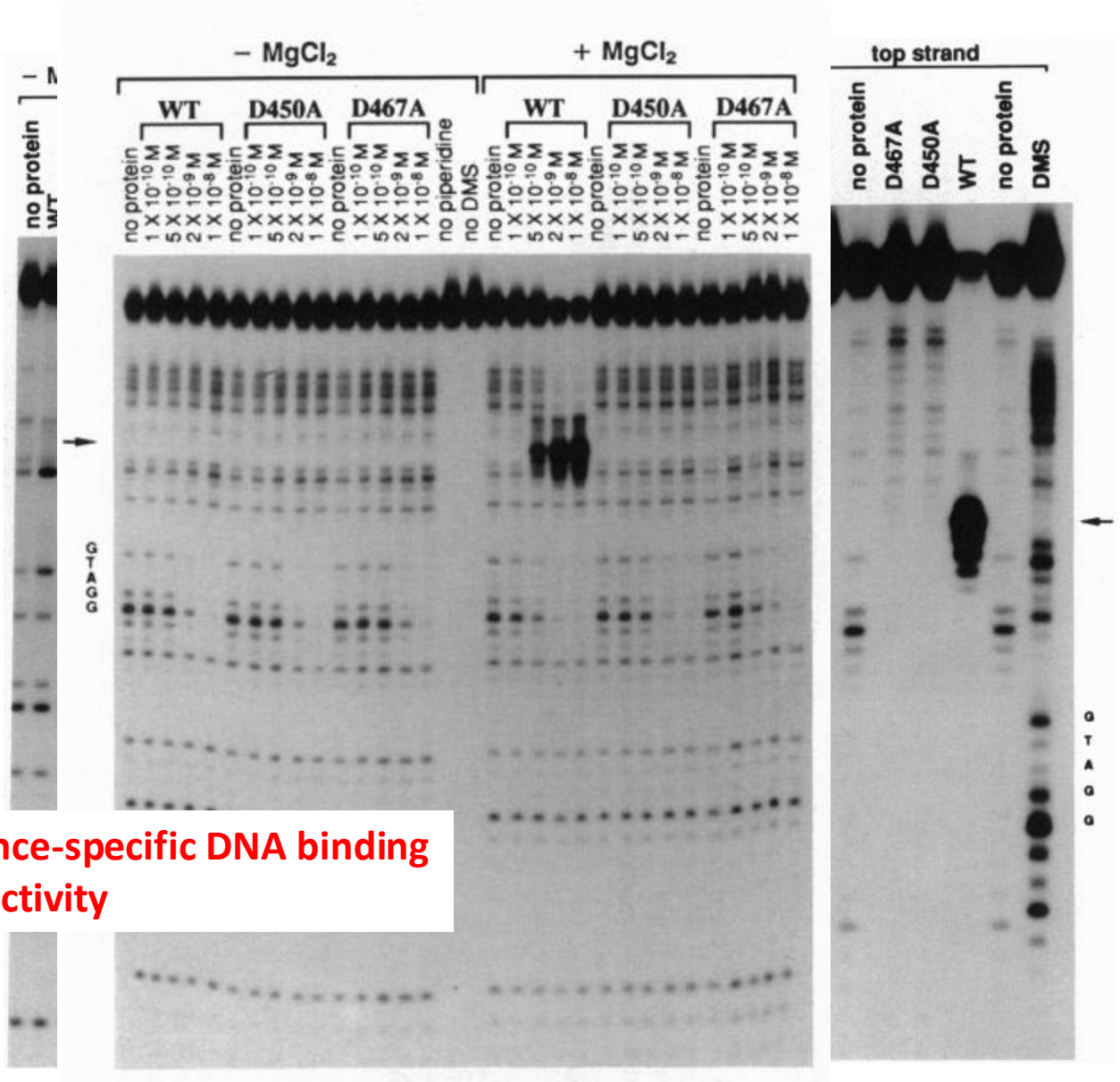
Structures comparison

(Pro-Asp-Xaa$_{15-19}$-Glu/Asp-Xaa-Lys).

D450A; D467A

FIG. 2. Segments of amino acid sequences of *Eco*RI (residues 88–113), *Eco*RV (residues 71–92), and *Fok* I (residues 447–469) endonucleases are aligned for comparison. Identical residues are indicated by solid circles; conservative substitutions are identified by open circles. Gaps are indicated by dashes. The consensus sequence of the proposed active site motif (16) is shown on the top line.

Protection Experiments



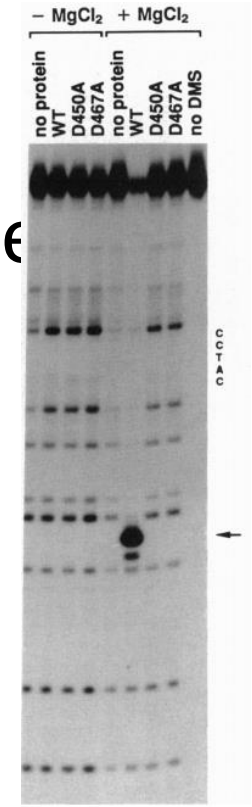
Uncouple the sequence-specific DNA binding and strand scission activity

Known facts till (1993)

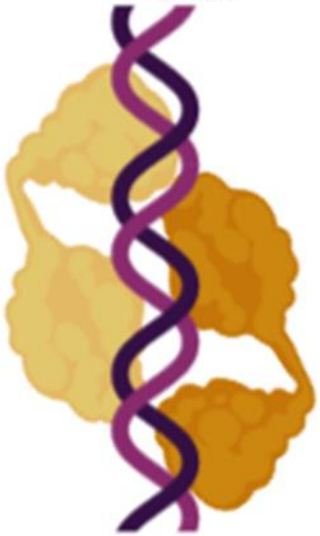
- FokI (Flavobacterium okeanokoites), Type IIS
- Recognizes the asymmetric sequence **GGATG**
- Staggered DNA cuts 9/13 bases away
- It appear to function as monomers
- By contrast other type II (EcoRI & EcoRV) acts as dimers
- **How** both strands are cleaved by FokI?

Hypothesis about cleavage

- Single molecule = two active sites
- Single molecule = one active site (use twice
- Mutational analysis = one catalytic center



- **How monomeric FokI**
- **Single catalytic center**
- **Cleave both strands** of DNA?



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Printed in U.S.A.

Insertion and Deletion Mutants of *Fok*I Restriction Endonuclease*

(Received for publication, August 5, 1994, and in revised form, October 11, 1994)

Yang-Gyun Kim, Lin Li, and Srinivasan Chandrasegaran‡

From the Department of Environmental Health Sciences, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205-2179

However, compared with the wild-type enzyme, the insertion mutants cleave predominantly one nucleotide further away from the recognition site on both strands of the DNA substrate. 9/13 10/14

The DNA binding and cleavage domains of *Fok*I appear to be linked by a relatively malleable linker. No simple linear relationship exists between the linker length and the distance of the cut site from the recognition site.

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Amino acid sequence at the insertion/deletion site[a]	amino acid insertion	Activity[b]
....QLVKSELRHKLK....	−7	−
....QLVEEKKSELRHKLK....	−4	+
....QLVKSELEEKKSELRHKLK....	wt	+
....QLV**TAEL**KSELEEKKSELRHKLK....	+4	+
....QLV**TAELTAEL**KSELEEKKSELRHKLK....	+8	+
....QLV**TAELTAELTAEL**KSELEEKKSELRHKLK....	+12	+
....QLV**TAELTAELKSELKSELEEK**KSELEEKKSELRHKLK....	+19	+
....QLV**TAELTAELTAELKSELKSELEEK**KSELEEKKSELRHKLK....	+23	+
....QL**RGGGGSGGGGSGGGGSQL**VKSELEEKKSELRHKLK....	+18[c]	+

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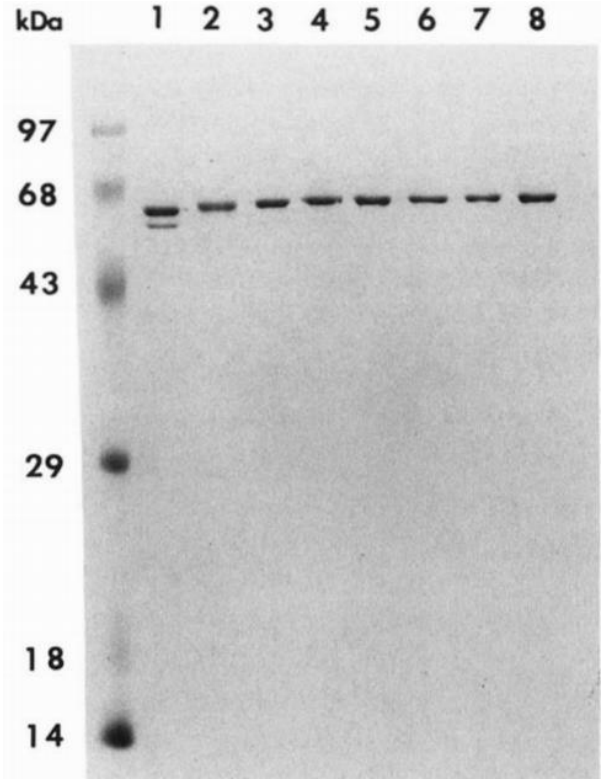

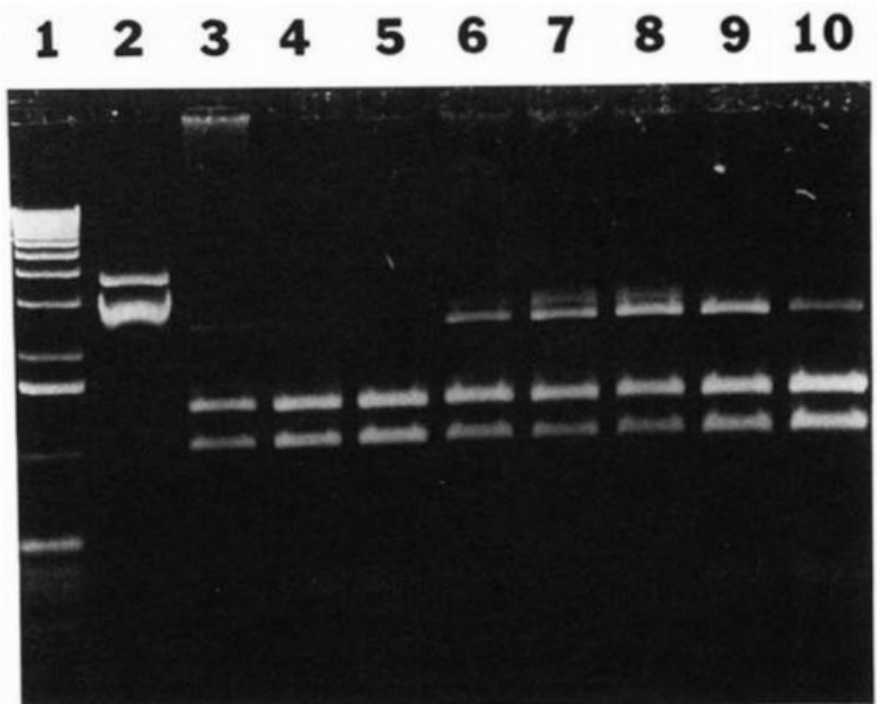

Conclusions of Lecture-4

- Uncouple the recognition & cleavage domain
- Domains protect recognition site
- Single catalytic center
- How both strands are cleaved??

Thank You!