Genome Editing and Engineering

Course No: BT-637

LECTURE-3

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Introduction

- FokI (Flavobacterium okeanokoites)
- Functional domains of FokI

A brief Recap

- FokI (Flavobacterium okeanokoites)
- Type II S cleaves d.s DNA
- Recognizes nonpalindromic, pentanucleotide
- Cleaves 9/13 nucleotide downstream of recognition site GGATG
- Since 10 bp /turn
- Enzyme probably interacts with one face
- Cleaves at next helical turn
- Two separate domains

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Biochemistry

Functional domains in *Fok* I restriction endonuclease

(*Flavobacterium okeanokoites*/*Escherichia coli*/methyltransferase/restriction endonuclease/recognition and cleavage domains)

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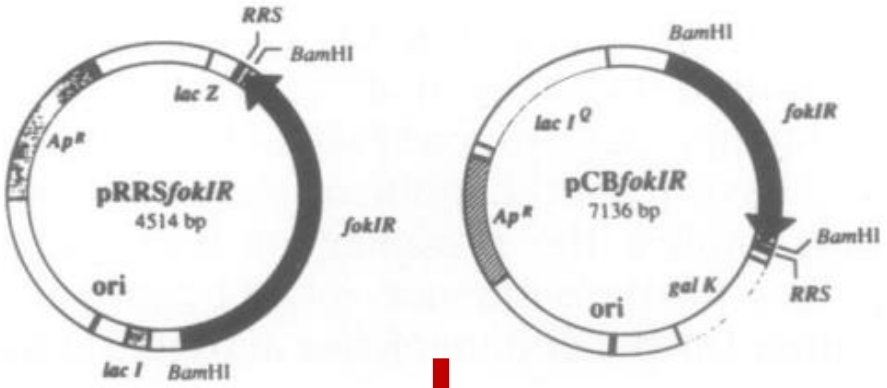
Cloning of FokI

- To study domains of FokI enzymes
- Good quantity of purified proteins
- Overproducer clone



FokIR

5′ primer: 5′ - TA (*BamHI* GGATCC) (RBS GGAGGT) (7-bp spacer TTAAAAT) ATG GTT TCT AAA ATA AGA ACT - 3′
Met Val Ser Lys Ile Arg Thr

3′ primer: 3′ - (24-bp complementary strand TTA TTG CCG CTC TAT TTG AAA ATT ACT) (*BamHI* CCTAGG) AT - 5′
Asn Asn Gly Glu Ile Asn Phe

Purified FokI from clone

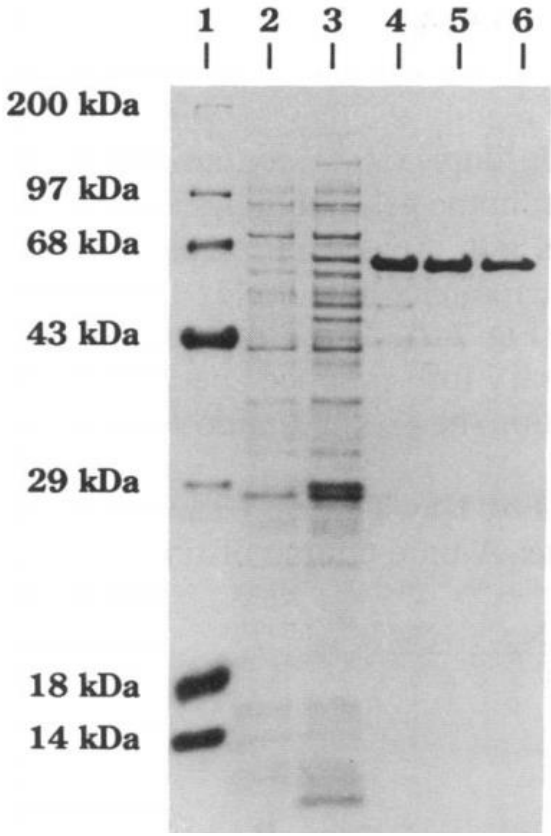
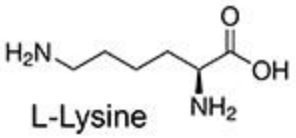
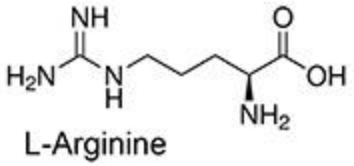


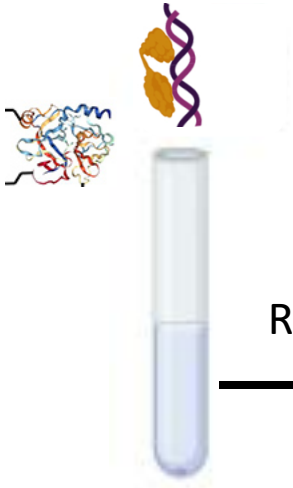
FIG. 2. SDS/PAGE profiles at each step in the purification of *Fok* I endonuclease. Lanes: 1, protein standards; 2, crude extract from uninduced cells; 3, crude extract from cells induced with 1 mM isopropyl β-D-thiogalactoside; 4, phosphocellulose pool; 5, 50–70% $(NH_4)_2SO_4$ fractionation pool; and 6, DEAE pool.

Trypsin cleavage study of FokI



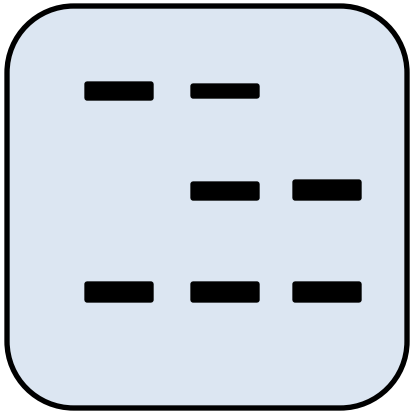


Time course experiment



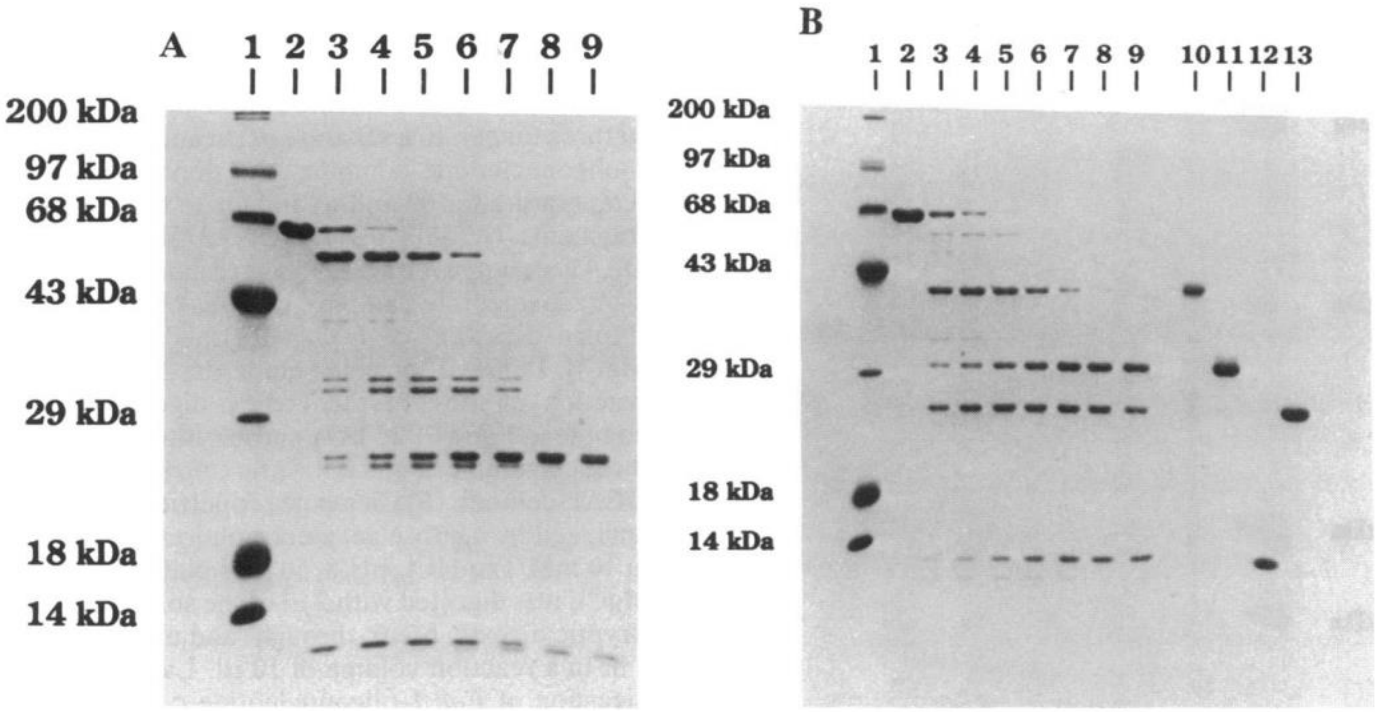
R.T. (1 hr)

2.5 min
5.0 min
10.0 min
20.0 min
40.0 min
80.0 min
160.0 min



SDS-PAGE

Trypsin cleavage study of FokI



Lanes (*A* and *B*): 1, protein standards;
2, *Fok* I endonuclease;
3, 2.5 min (of trypsin digestion);
4, 5.0 min;
5, 10 min;
6, 20 min;
7, 40 min;
8, 80 min;
9, 160 min

HPLC-purified tryptic fragments of 41 kDa (lane 10)
30 kDa (lane 11)
11 kDa (lane 12)
25 kDa (lane 13)

ein structure
nino acids
e protein.

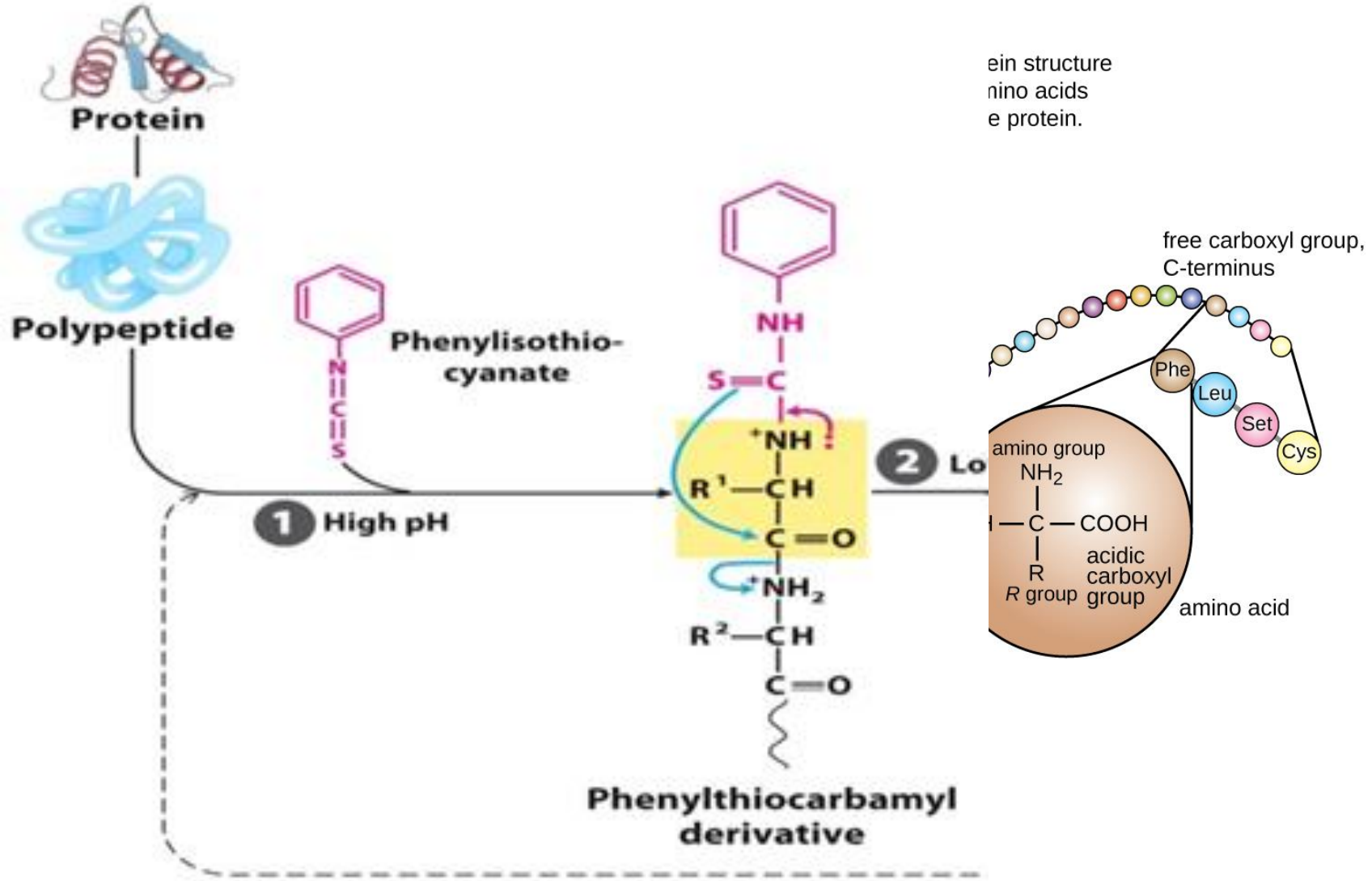


Figure 3-27
Lehninger Principles of Biochemistry, Sixth Edition

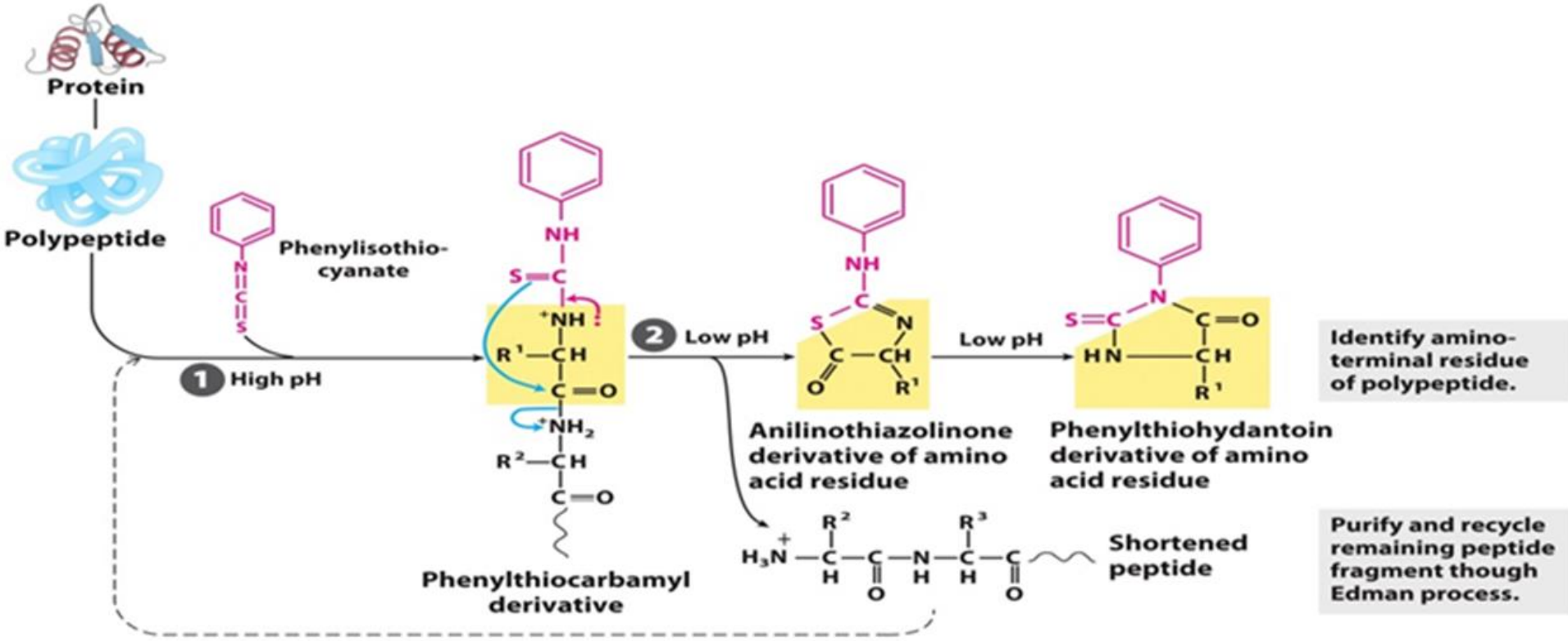


Figure 3-27
Lehninger Principles of Biochemistry, Sixth Edition

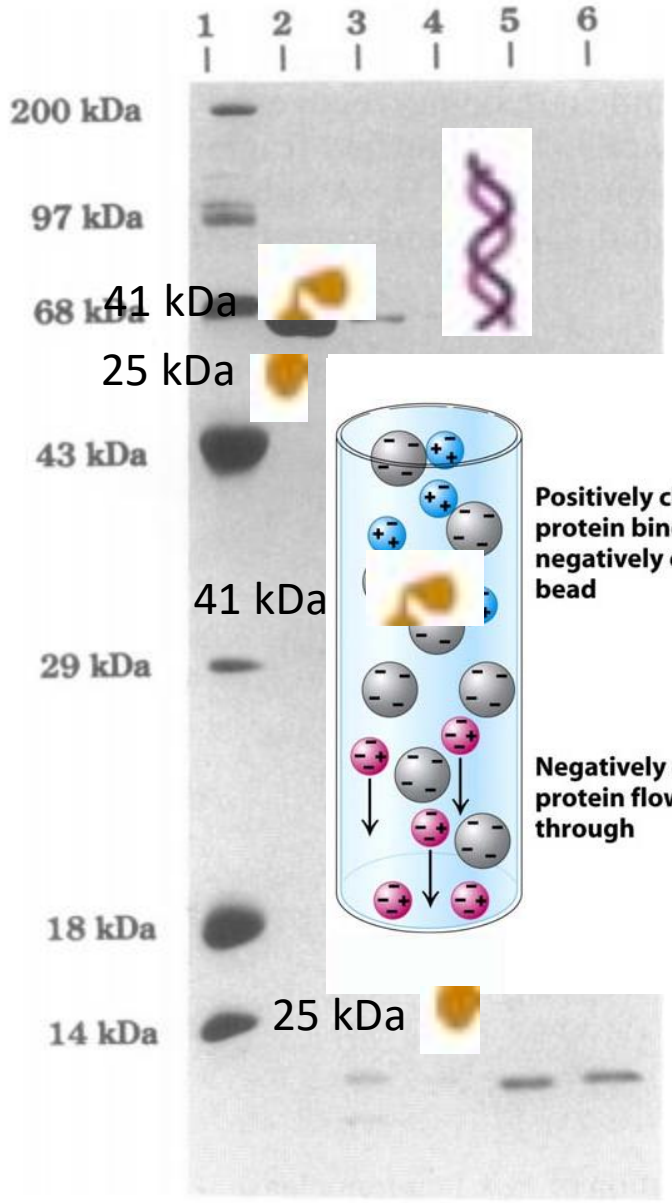
Amino terminal sequencing

Table 1. Amino-terminal sequences of *Fok* I fragments from trypsin digestion

Fragment	Amino-terminus sequence	DNA substrate
8 kDa	VSKIRTFG*VQNPGKFENLKRVVQVFDRNS	–
58 kDa	SEAPCDAIIQ	–
25 kDa	QLVKSELEEK	+
41 kDa	VSKIRTFGWV	+
30 kDa	VSKIRTFGWV	+
11 kDa	FTRVPKRVY	+

*, Unidentified amino acid.

DNA binding properties of the fragments

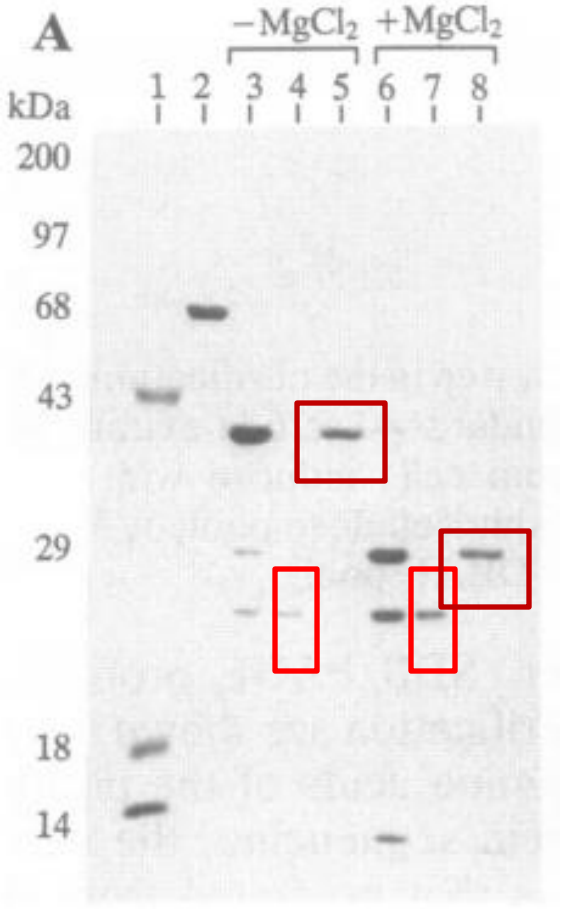


1, protein standards;
2, *Fok* I endonuclease;
3, 10-min trypsin digestion mixture of *Fok* I–oligonucleotide complex;
ryptic frag ments that bound to the oligo(dT)-cellulose column;
160-min trypsin digestion mixture of *Fok* I–oligonucleotide complex;
ryptic fragments that bound to the oligo(dT)-cellulose column.

41 and 30 kDa bind DNA

25 kDa is not retained

Cleavage properties of the fragments



1, protein standards 2, *Fok* I endonuclease

3–5 trypsin digestion of *Fok* I–oligonucleotide complex without $MgCl_2$

6–8 trypsin digestion of *Fok* I–oligonucleotide complex with $MgCl_2$

4 and 7, 25-kDa carboxyl-terminal fragment in the break-through volume

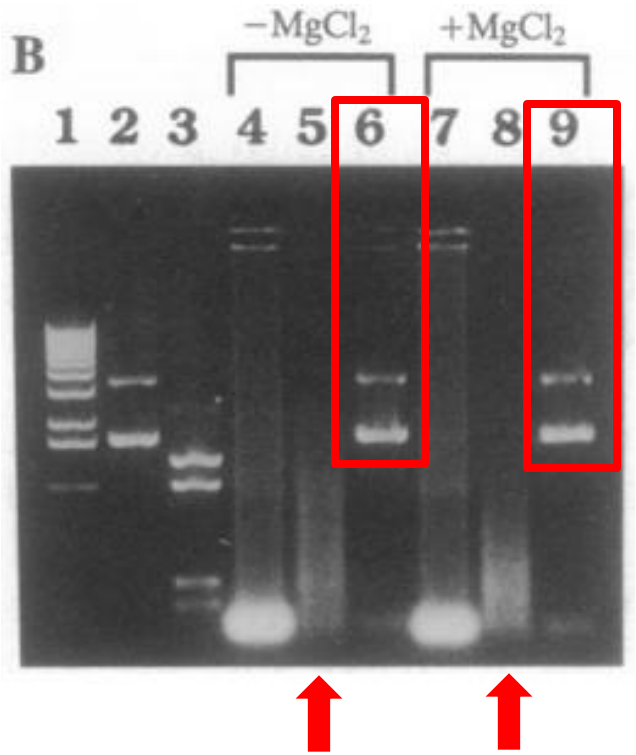
5 and 8, tryptic fragments of *Fok* I that bound to the DEAE column.

- **41 and 30 kDa bind to DEAE-Sephadex column**
- **25 kDa do not bind to DEAE column**

Cleavage properties of the fragments

41 kDa

25 kDa



1, 1-kilobase (kb) ladder

2, pTZ19R;

3, pTZ19R digested with *Fok* I endonuclease

4–6 digestion of *Fok* I–oligonucleotide complex without $MgCl_2$

7–9 digestion of *Fok* I–oligonucleotide complex with 10 mM $MgCl_2$

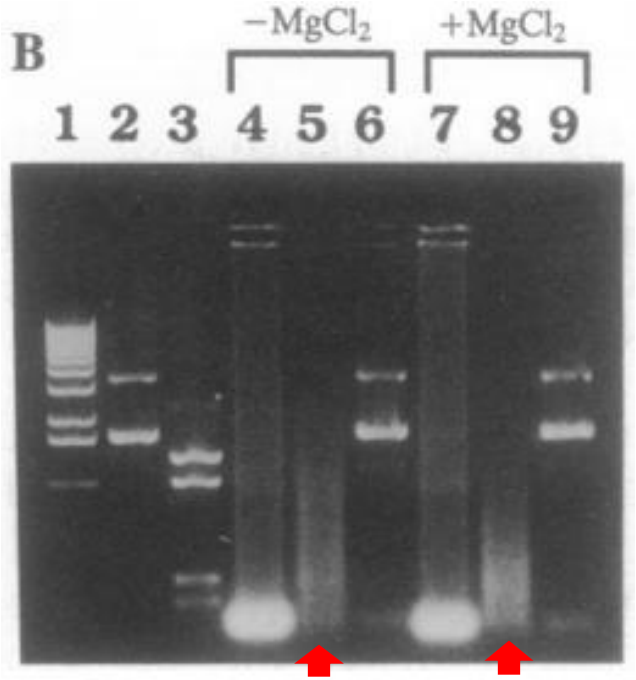
4 and 7 tryptic digests of *Fok* I–oligonucleotide complex

6 and 9 tryptic fragments of *Fok* I that bound to the DEAE column

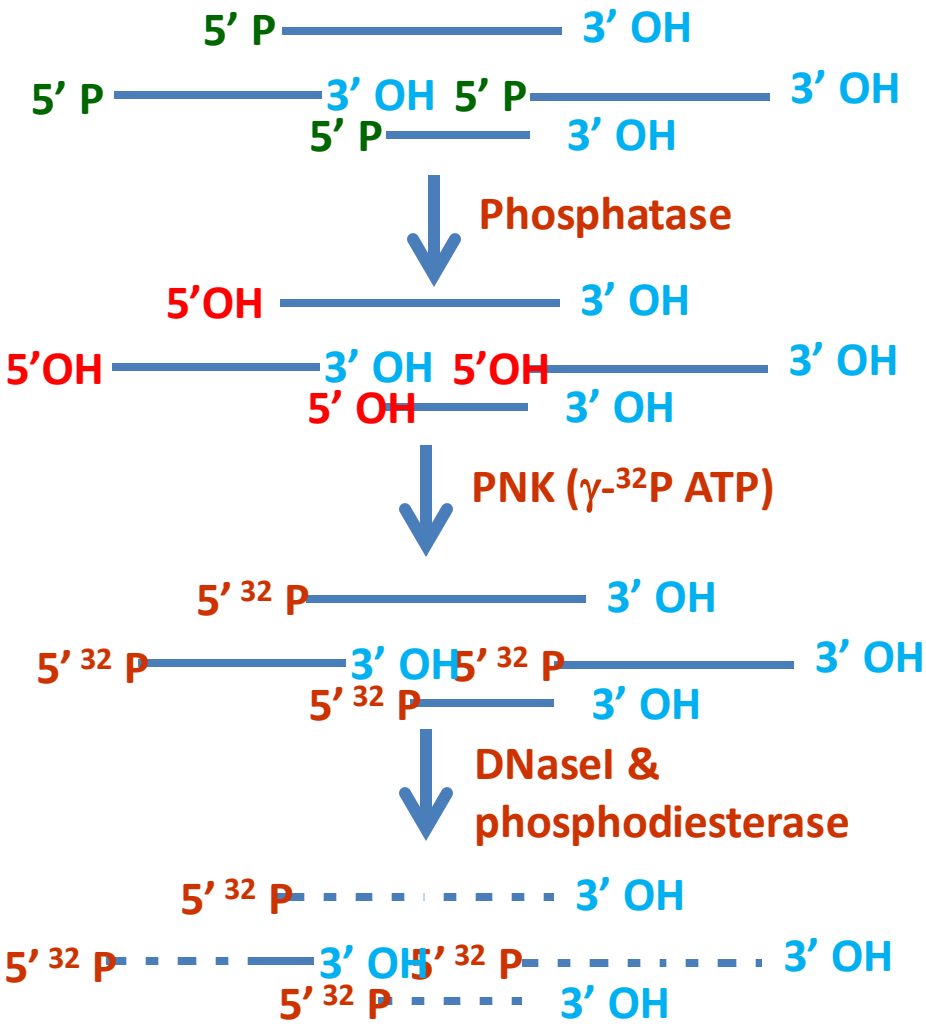
5 and 8 25-kDa carboxyl-terminal fragment in the break-through volume

- **25 kDa cleaves DNA in small pieces**
- **Nonspecific in cleavage**

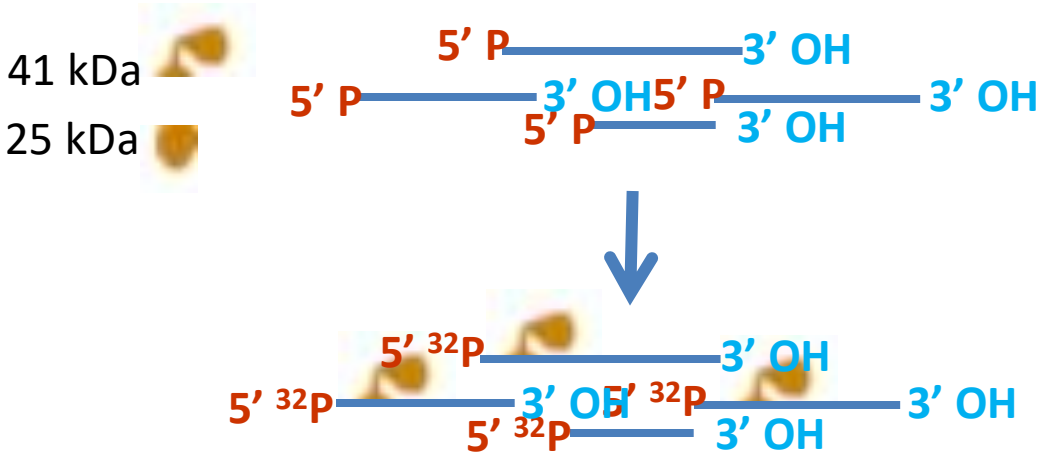
Cleavage properties of the fragments

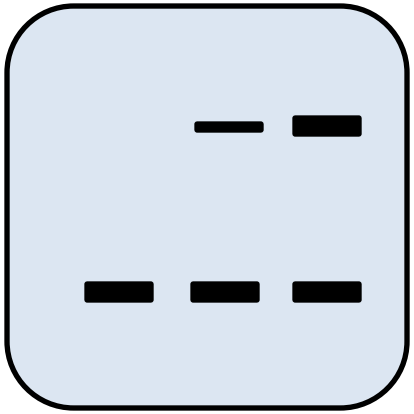


- 25 kDa cleaves preferentially
- 5’ G > A >> T ~ C
- 25 kDa = cleavage domain



Gel mobility shift assay





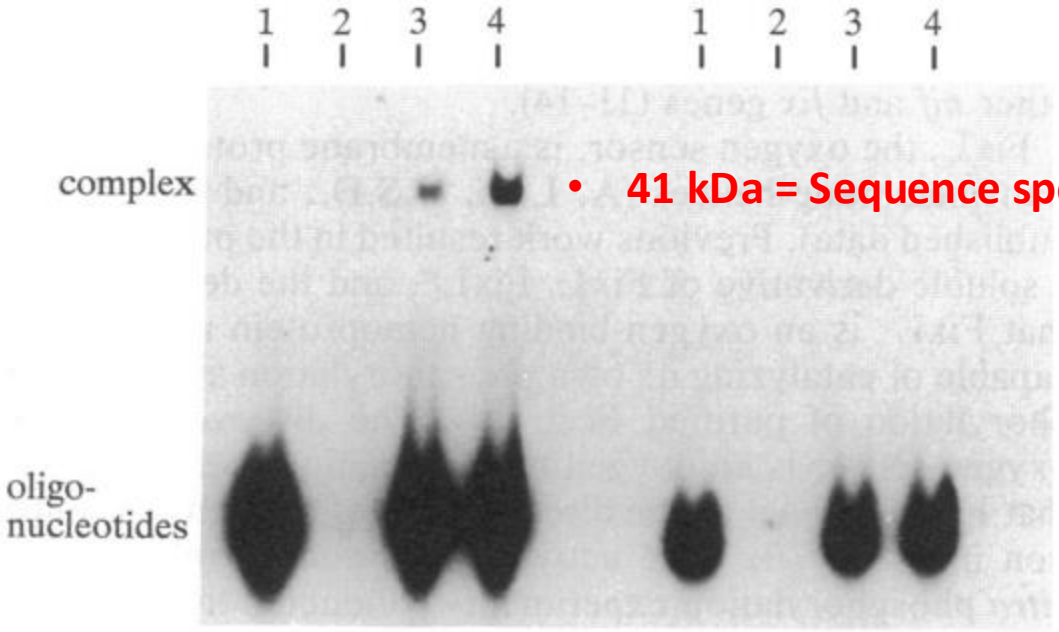
Nondenaturing-PAA

Gel mobility shift assay

1, specific oligonucleotide duplex

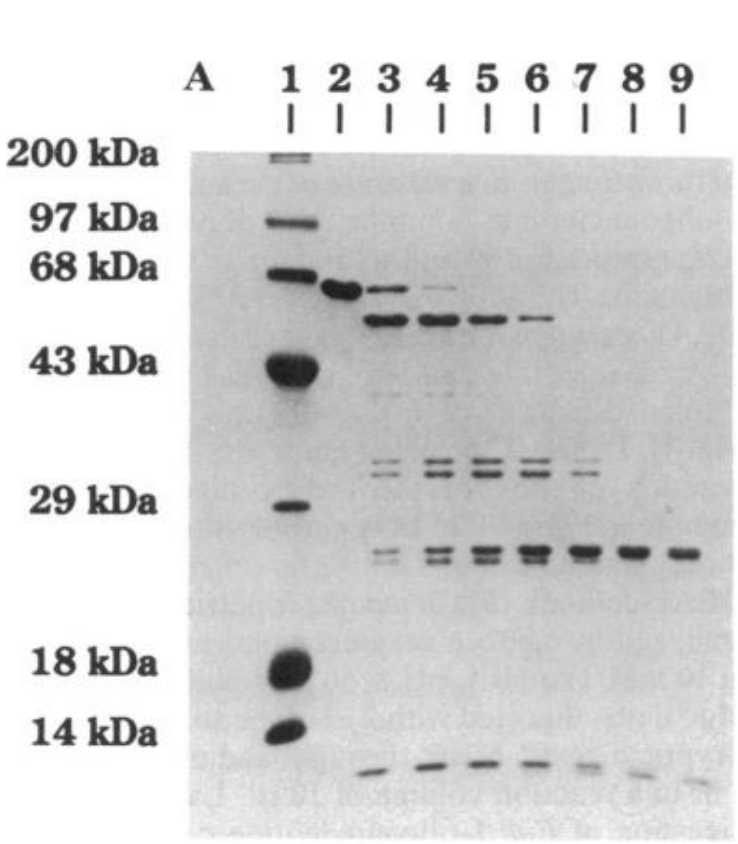
2, 41-kDa amino-terminal fragment–oligonucleotide com

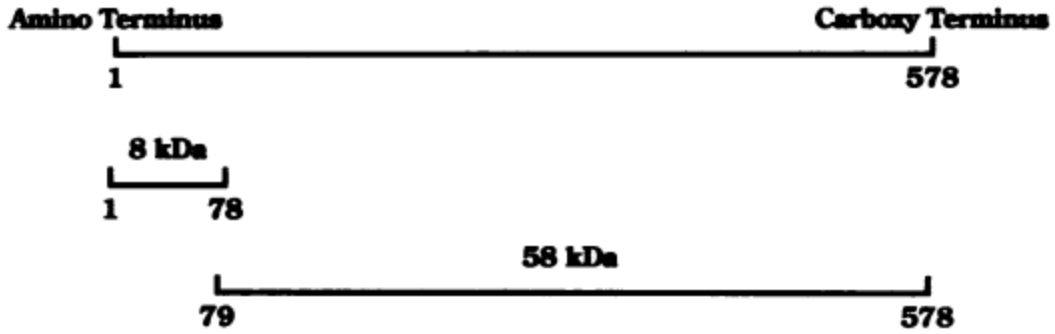
3 and 4, specific probe incubated with complex for 30 and 120



- **41 kDa = Sequence specific recognition**

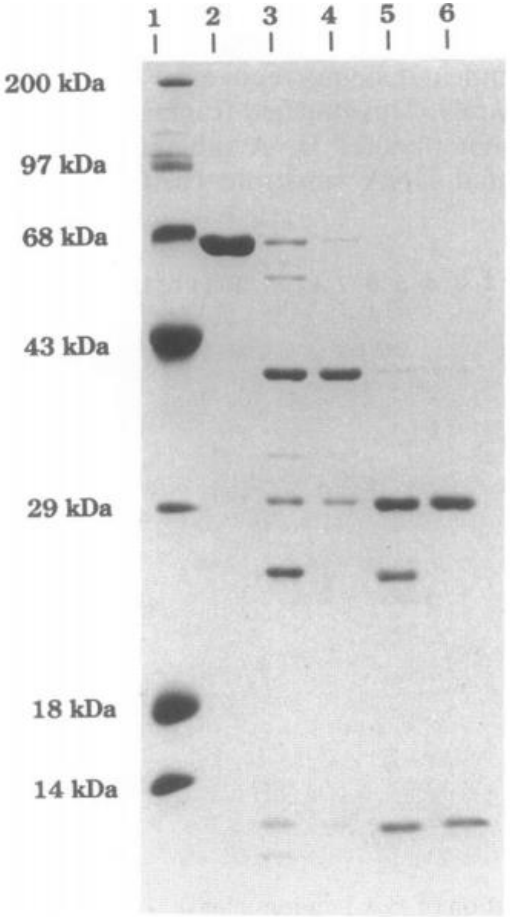
Functional domains of FokI

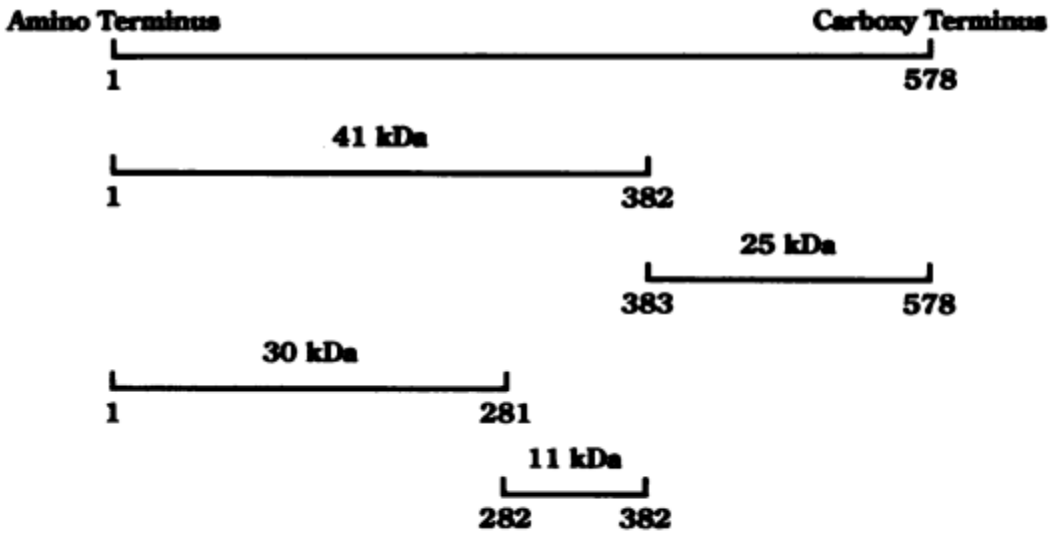




- FokI without any oligonucleotide

Functional domains of FokI





- **Two domains:**
  - **a) Seq. specific recognition (N-terminus)**
  - **b) Endonuclease activity (C-terminus)**

Future Outlook

- **Mutational analysis of the domains**
- **Modular structure**
- **Feasibility to construct chimeric endonucleases**

Conclusions of Lecture-3

- The two domains of FokI
- Recognition domain (amino-terminal)
- Cleavage domain (carboxy-terminal)
- Formation of “the complex” via Gel mobility shift assay

Thank You!