Fundamentos de Biologia Molecular

Curso de Licenciatura em Biologia
2º Ano, 1º Semestre
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Componente Teórico-Prática

Ciências ULisboa
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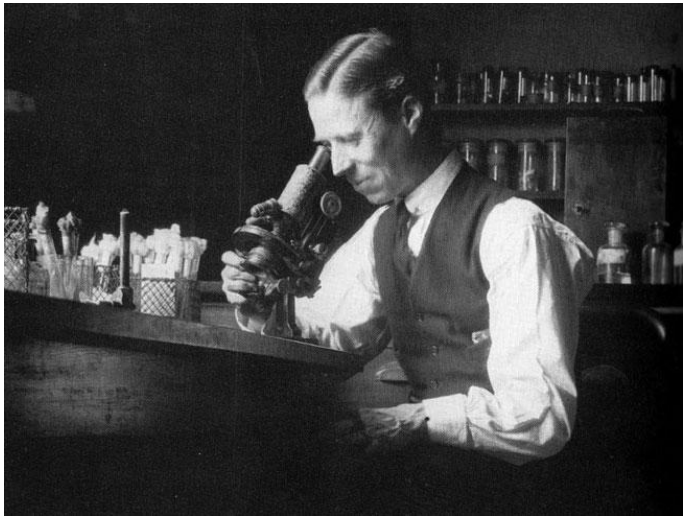
TP1: Restriction-modification Systems

- R-M systems discovery
- Nobel prizes
- R-M classification, action mechanisms and function
- R-M type I, II and III
- Other R-M mechanisms
- Practical applications

TP1: Restriction-modification Systems

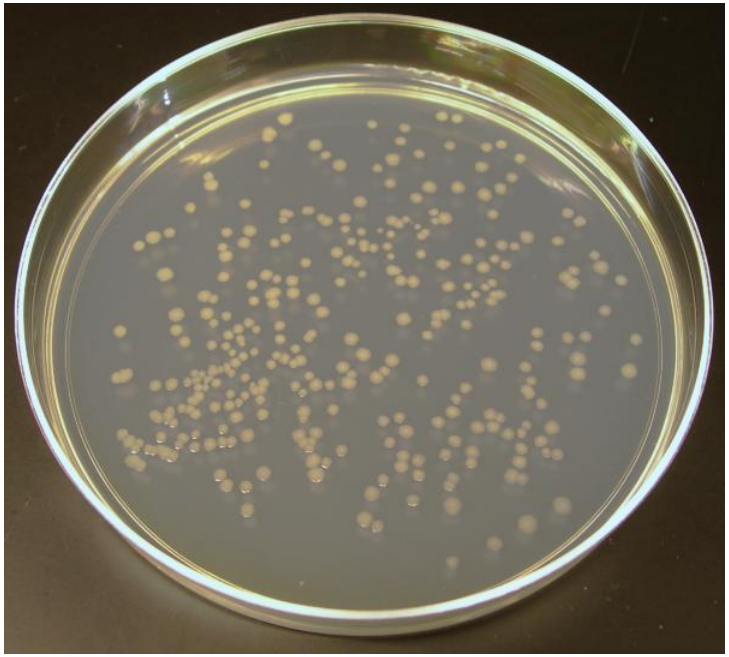
-1915 : virology was in its infancy.

- **Frederick Twort**, an English physician, was attempting unsuccessfully to propagate vaccinia virus, the primary component of the smallpox vaccine, on agar plates. Rather than vaccinia, however, the only things growing on Twort's plates were contaminating bacteria.



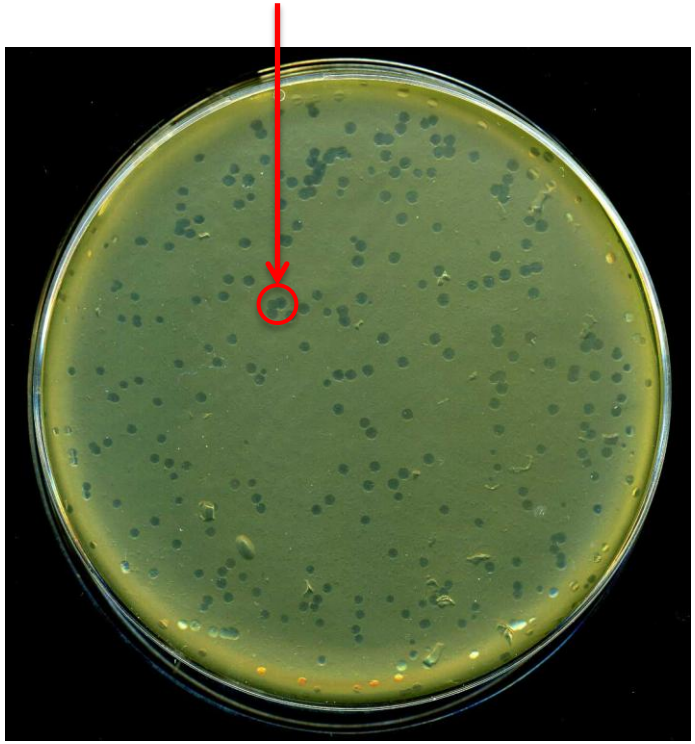
Despite the lack of progress towards his original goal, Twort soon observed that something else was happening: mysterious "glassy and transparent" spots, which turned out, upon closer examination, to be zones of dead bacteria, would occasionally materialize on his plates.

TP1: Restriction-modification Systems



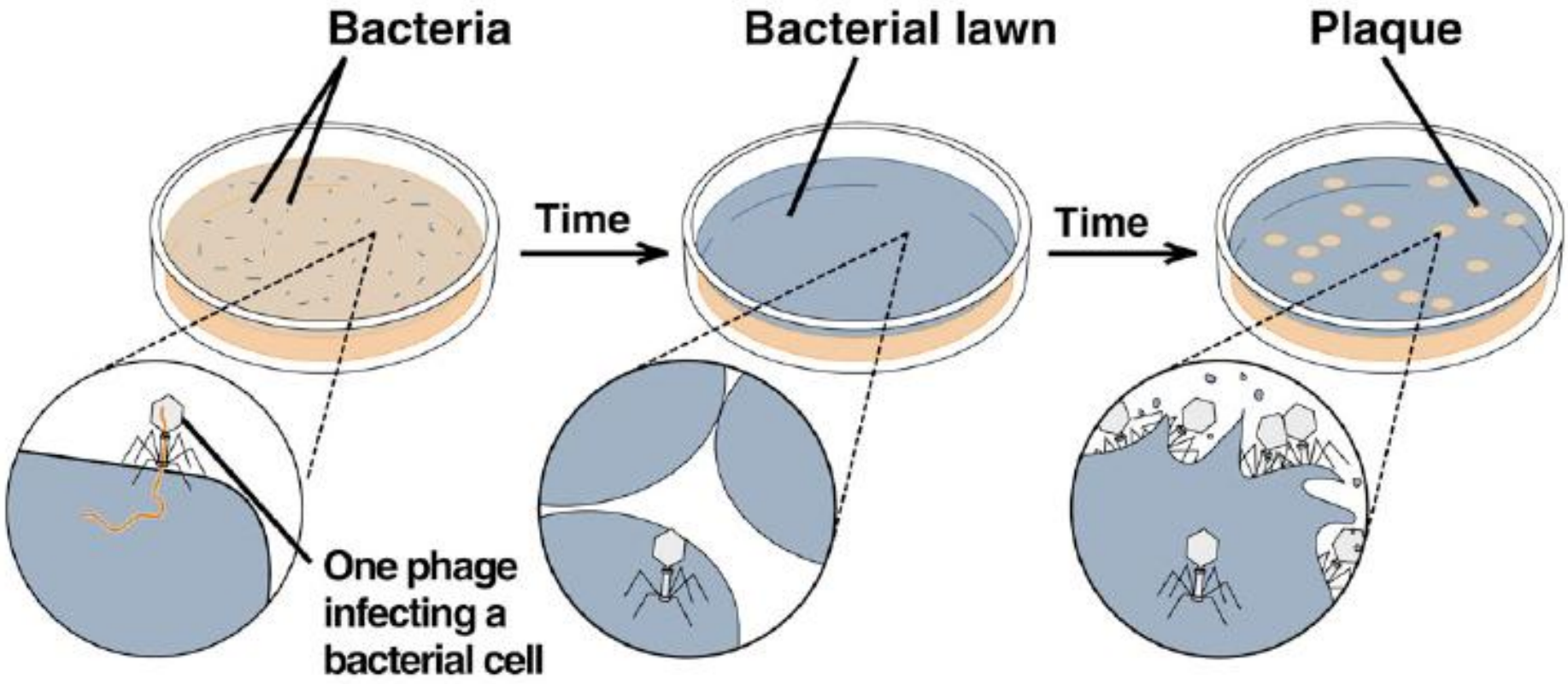
Bacterial colonies

Area where the phage is dominant. Each plaque represents the lysis of a phage-infected bacterial culture and can be designated as a plaque-forming unit (PFU)

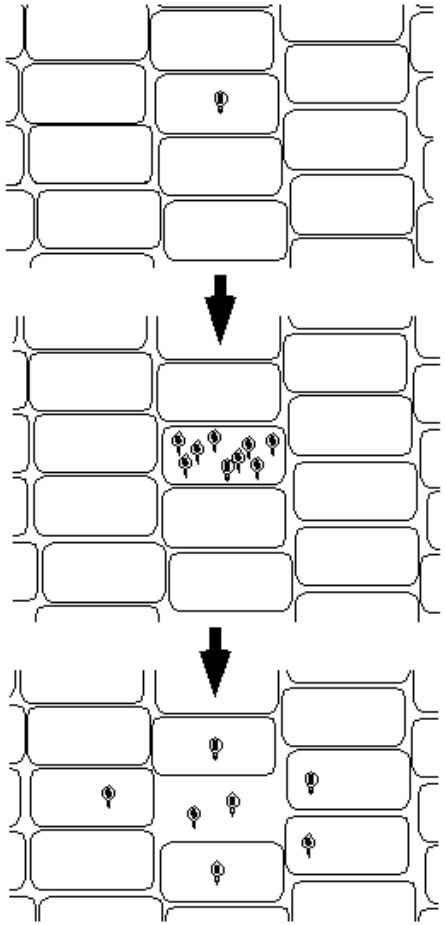


Lysis plaques

TP1: Restriction-modification Systems



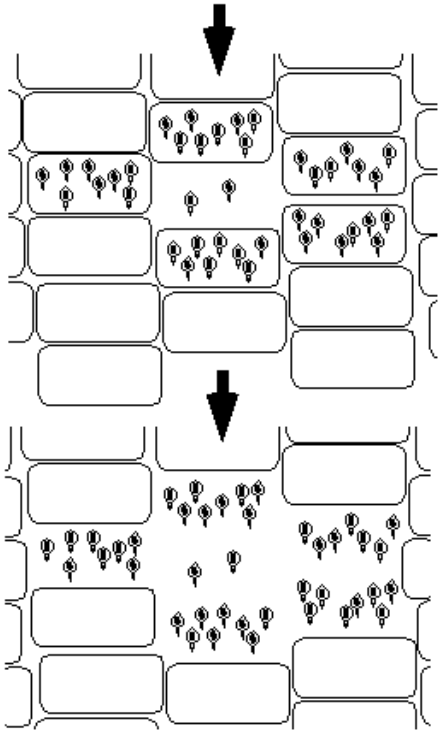
TP1: Restriction-modification Systems



A phage infects a single cell in a lawn of sensitive, growing bacteria.

Phage reproduces in the cell, typically yielding about 50 progeny phage per infected cell.

Lysis of the cell releases phage into the medium. The phage diffuses through the medium and infects adjacent cells.

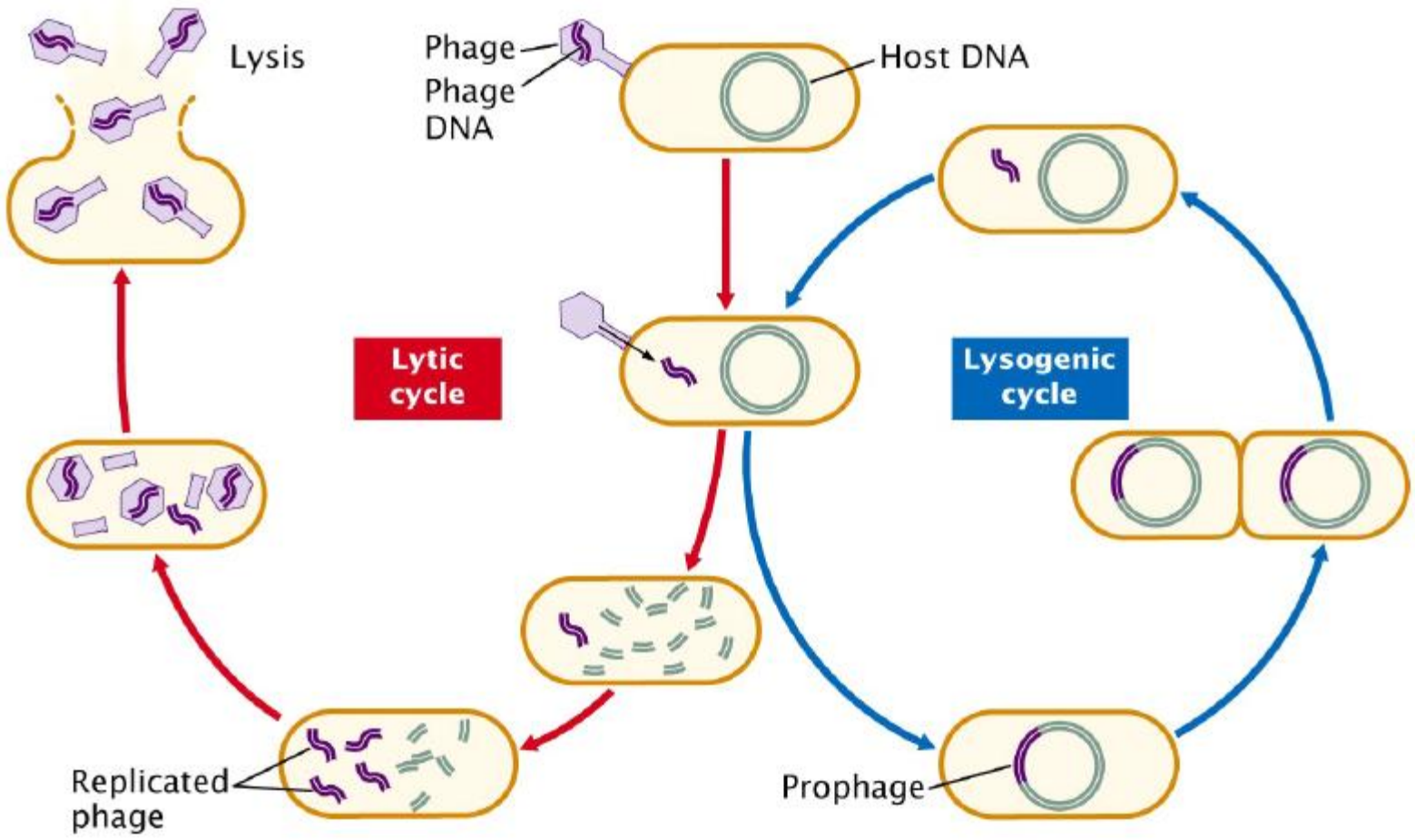


Phage reproduces in these cell, releasing 50 additional progeny phage per infected cell.

These cells lyse, releasing more phage which can then diffuse outward and infect the surrounding cells. Lysis of the cells results in a circular clearing in the lawn of bacteria. This region of visible lysis is called a plaque.

TP1: Restriction-modification Systems

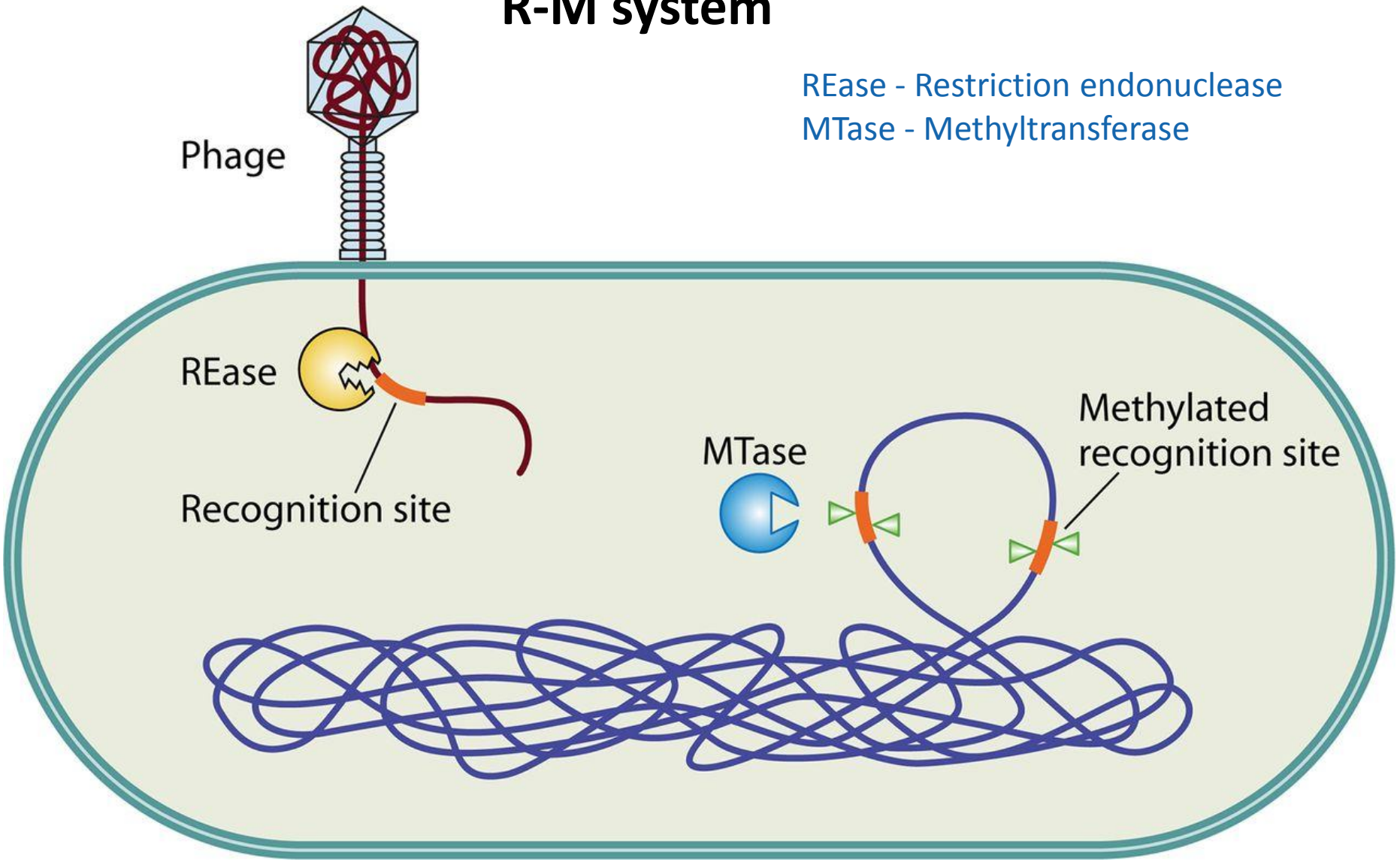
Outcomes of phage infection: Lytic cycle and lysogenic cycle



TP1: Restriction-modification Systems

R-M system

REase - Restriction endonuclease
MTase - Methyltransferase



TP1: Restriction-modification Systems


Werner Arber

- In 1962, Werner Arber's studies showed that restriction involved changes in the DNA of the phage and was accompanied by **degradation of the DNA**. It appeared that bacteria could somehow limit phage infection by breaking down the virus DNA.

Several years later, Arber's lab confirmed that the bacterial host was able to modify or restrict viral DNA, as well as foreign bacterial DNA. Arber theorized that each particular bacterial strain generated an **endonuclease** (enzyme) that could recognize specific sequences of nucleotides in foreign DNAs, and cut them there. Each bacterium would protect its own DNA from the enzyme by **adding methyl groups to the specific sites**, so the enzyme would not recognize them.

By 1968, two such enzymes had been identified in strains of *E. coli*, generating much excitement among biochemists. However, it turned out that while they recognized specific DNA sequences, they did not always cut the DNA close to those sites. Since the fragments produced were of random size and character, the enzymes (**later known as Type I restriction enzymes**) were unsuitable for mapping or sequencing.

TP1: Restriction-modification Systems

•Restriction-modification (R-M) system : consist of two distinct enzymatic activities:

- a restriction endonuclease that cleaves DNA at a specific recognition sequence
- a DNA methyltransferase that methylates DNA at the same site and thus prevents cleavage by the cognate restriction enzyme.

•Restriction enzyme and its cognate modification system constitutes the R-M system

TP1: Restriction-modification Systems

Hamilton Smith

- In 1972, he purified the first site-specific "**Type II**" restriction enzyme, known as *Hind II*. The crucial discovery came by chance: Incubating bacteria and phage together, Smith happened to notice that the phage DNA degraded over time. He and his colleagues were successful in purifying the enzyme and they were able to identify the short sequence of 6 base pairs in phage P22 that *Hind II* recognized and specifically cleavage.

Daniel Nathans

- Daniel Nathans applied this discovery within genetics. Among other applications, Daniel Nathans used different restriction enzymes on the DNA of the SV40 virus and studied its components, which allowed him to map the virus' genome.
- Smith's enzyme cut it into eleven specific fragments-consistent, manageable pieces, onto which individual genetic activities could be mapped.
- New Type II enzymes were rapidly discovered, and during the next several years, Nathans' group and used to delineate the SV40 genome. They deduced the size and physical order of the fragments in the genome, and created the first cleavage maps of a viral DNA, showing where each restriction enzyme cut it.

TP1: Restriction-modification Systems

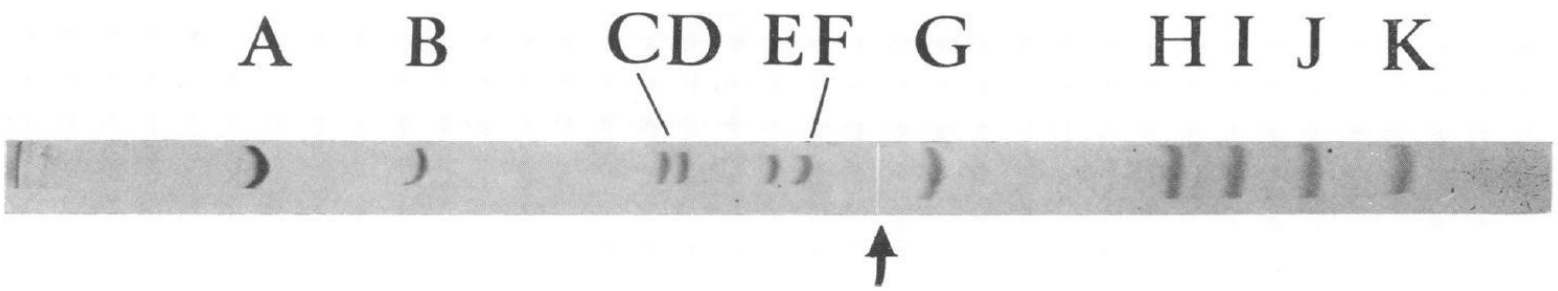


FIG. 3. Radioautographic analysis of SV40 DNA digested with *H. influenzae* restriction endonuclease. 1 μg of SV40 [^{14}C]-DNA I (3×10^4 cpm/μg) was digested (see Fig. 2) for 6 hr in a volume of 55 μl; 0.0015 unit of enzyme was added at 0 time and at 1, 2, 3, 4, and 5 hr. 20 μl of sample was electrophoresed for 12.3 hr and the radioautogram was prepared as described in *Methods*. The origin is at the *left*. The *arrow* below the radioautogram indicates a transverse cut made in the gel prior to slicing.

Danna, K., Nathans, D. (1971) Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Hemophilus influenza*. Proc. Natl. Acad. Sci. USA 68: 2913-2917.

TP1: Restriction-modification Systems

The Nobel Prize in Physiology or Medicine 1978

Werner Arber

Prize share: 1/3

Daniel Nathans

Prize share: 1/3

Hamilton O. Smith

Prize share: 1/3

"for the discovery of restriction enzymes and their application to problems of molecular genetics"

TP1: Restriction-modification Systems

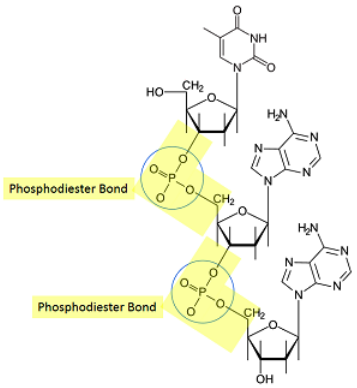
•The restriction modification (R-M) system is used by bacteria as a **way to protect themselves from foreign DNA** (eg phage DNA) – it is a part of a prokaryote "rudimental" immune system.

•This phenomenon was noticed in the 1950 decade – some bacteria strain inhibited virus growth and some didn't.

•Presence **of restriction enzymes** that cut specific sequences

•Endonucleases are restriction enzymes that cut **double DNA fragments in specific sequences**- these fragments are then destroyed by other endonucleases – protection mechanism.

•Restriction enzymes cleave phosphodiester internal, non terminal bonds originating **5'P** and **3'OH** extremities (except *Nci*I: originates 3'P and 5'OH extremities)

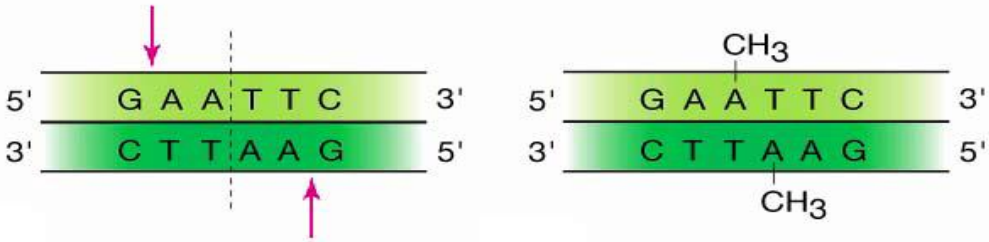


TP1: Restriction-modification Systems

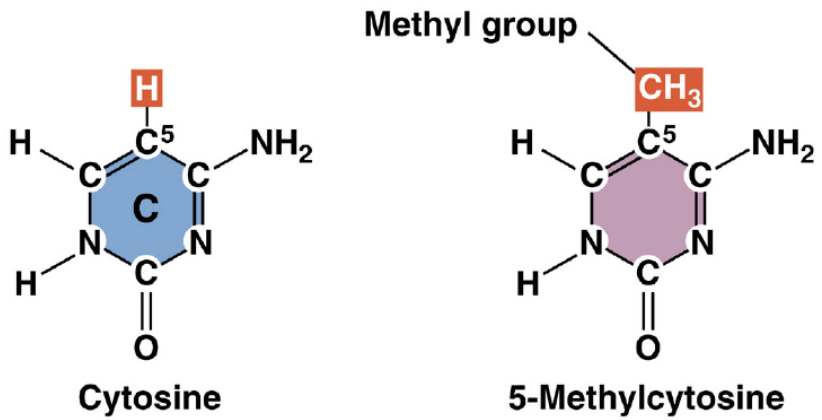
- Restriction enzymes usually cut 4-6 bp long sequences that are frequently **palindromes**[1].
- They are classified according to subunit composition, cleavage position, sequence specificity and cofactor requirements

- To prevent the restriction of its own DNA, bacteria modifies its DNA by adding methyl (CH_3) groups.

DNA methylation



Example of DNA methylation

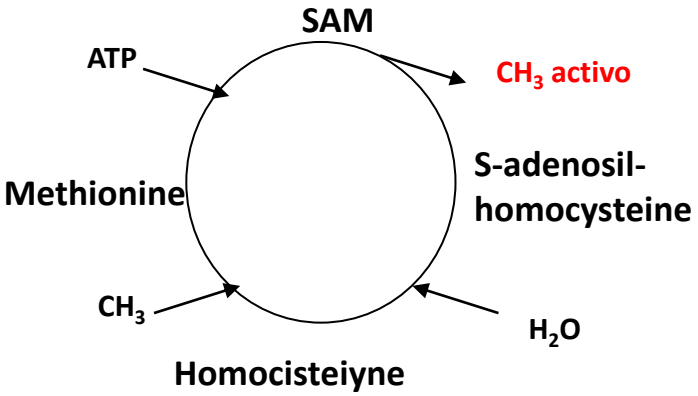


A methyl group is added to the 5-carbon position of cytosine residues in DNA

[1]A **palindrome is a word, phrase,** number, or other sequence of characters which reads the same backward or forward

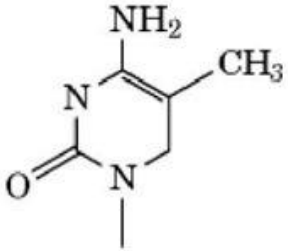
TP1: Restriction-modification Systems

Main donor of methyl groups SAM (S-adenosyl methionine)

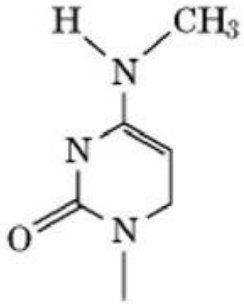


S-Adenosyl-L-methionine alternative names: SAM, AdoMet, ademetionine.

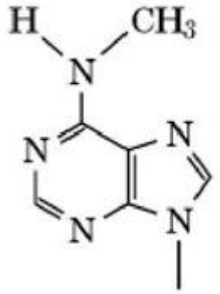
Most common DNA methylation



5-methylcytosine (5mC)



N4-methylcytosine (N4mC)



N6-methyladenine (N6mA)

- Approximately ¼ of known bacteria present R-M systems and ½ of those present more than one R-M system.

TP1: Restriction-modification Systems

R-M types : The majority of R-M systems can be classified as type I, II, or III on the basis of enzyme structure, cofactor requirements, structure of the DNA recognition site, and location of DNA cleavage relative to the recognition site.

Type	Protein structure	Restriction & methylation	RE recognition site	Ex of recognition sites	RE cleavage site	ATP required
I	Bifunctional enzyme of 3 subunits	Mutually exclusive	Bipartite and asymetric	*Eco*AI GAGNNNNNNNGTCA *Eco*KI AACNNNNNNGTGC	Non-specific, >1000 bp from recognition site	Yes
II	Separate endonuclease and methylase	Separate reactions	4-8 bp sequence, often palindromic	*Eco*RI G/AATTC *Bal*I TGG/CCA	Same as or close to recognition site	No
III	Bifunctional enzyme of 2 subunits	Simultaneous	5-7 bp, asymetric sequence	*Eco*PI AGACC *Hinf*III CGAAT	24-25 bp, dowstream of recognition site	Yes

RE- restriction enzyme
N- A, C, G ou T; **R**- G ou A

TP1: Restriction-modification Systems

Table 1. Characterization and organization of the genes and subunits of the four Types of restriction enzymes

Type	Type I	Type II	Type III	Type IV
Features	Oligomeric REase and MTase complex Require ATP hydrolysis for restriction Cleave variably, often far from recognition site 'DEAD-box' translocating REase bipartite DNA recognition domain	Separate REase and MTase or combined REase~MTase fusion Cleave within or at fixed positions close to recognition site Many different subtypes	Combined REase + MTase complex ATP required for restriction Cleave at fixed position outside recognition site 'DEAD-box' REase	Methylation-dependent REase Cleave at variable distance from recognition site Cleave m6A, m5C, hm5C and/or other modified DNA Many different types
Example	e.g. EcoKI	e.g. EcoRI	e.g. EcoP1I	No 'typical' example
Genes	*hsdR, hsdM, hsdS*	e.g. *ecorIR, ecorIM*	e.g. *ecoP1IM, ecoP1IR*	e.g. *mcrA, mcrBC, mrr*
Subunits	~135, ~62 and ~52 kDa	~31 and ~38 kDa for EcoRI	~106 and ~75 kDa for EcoP1I	Unrelated proteins
Proteins	REase: 2R + 2M + S MTase: 2M + S (±2R)	Orthodox REase: 2R Orthodox MTase: M	REase: 1 or 2 R + 2M MTase: 2M (±2R)	Varies
REBASE	104 enzymes, 47 genes cloned, 34 genes sequenced, 5140 putatives	3938 enzymes, 633 genes cloned, 597 sequenced, 9632 putatives	21 enzymes, 19 genes cloned & sequenced, 1889 putatives	18 enzymes & genes cloned, 15 sequenced, 4822 putatives

Type I and II are currently divided in 5 and 11 different subclasses, respectively. Few enzymes have been well-characterized, but based on the current avalanche of sequence information many putative genes belonging to all Types and subtypes are being identified and listed on the restriction enzyme website (http://rebase.neb.com). The modification-dependent Type IV enzymes are highly diverse and only a few have been characterized in any detail. In each case, an example is given of one of the best-characterized enzymes within the different Types I, II and III. Note that Type II enzymes range from simple (shown here for EcoRI) to more complex systems (see Table 2 for the diversity of Type II subtypes).
REBASE count is as of 16 September 2013 (http://rebase.neb.com/cgi-bin/statlist).

TP1: Restriction-modification Systems

Type I

•Restriction and modification functions in the same multisubunit enzyme (R2M2S)

•The enzymes require Mg , AdoMet, and ATP for activity,

• If the restriction sequence is fully methylated, ATP hydrolysis drives the dissociation of the enzyme from DNA.

•If the restriction site is hemimethylated (only one strand is methylated, eg DNA replication) the enzyme methylates the other strand and dissociates.

•If unmethylated, DNA cleavage occurs randomly at significant distances from an asymmetric recognition site (up to 1000bp)

TP1: Restriction-modification Systems

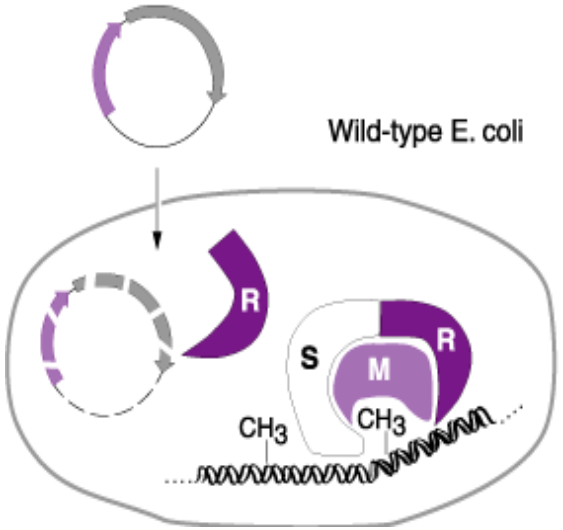
Type I

•In enteric bacteria Type I R-M systems have been demonstrated to be multifunctional protein complexes composed of three subunits encoded by the ***hsdS, hsdM*, and *hsdR* genes**

The **hsdM** subunit adds chemical markers to the bacteria´s own DNA.

The **hsdS** subunit specificity enzyme recognises these markers. However, if foreign DNA enters the cell, hsdS subunit will recognise it as foreign, unmodified DNA.

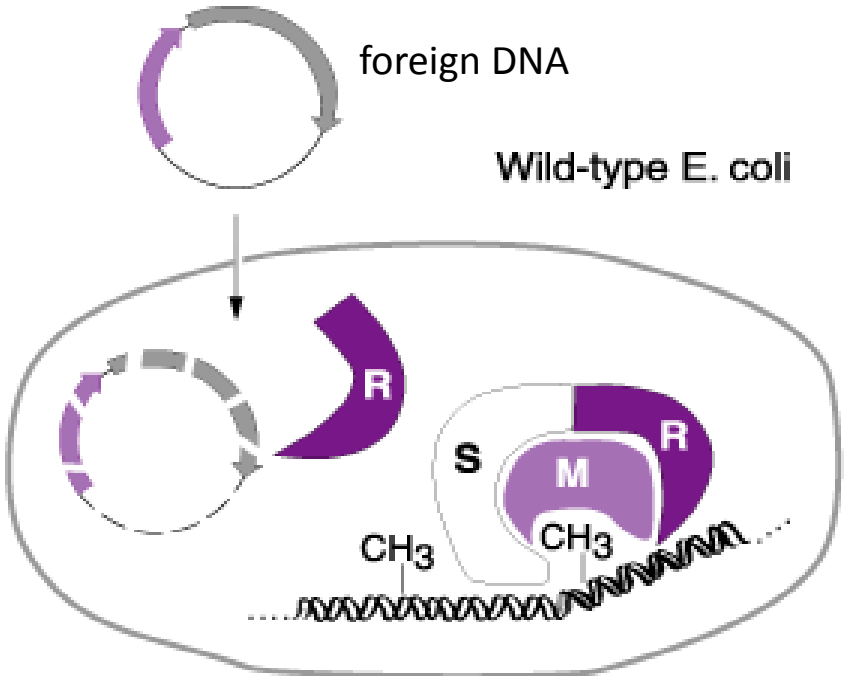
The **hsdR** subunit – endonuclease cleavage of target sequence



TP1: Restriction-modification Systems

Ex. EcoK system

*EcoK*I recognition site
5'-AAC-(N)5-GTGC-3’
5'-AACNNNNNGTGC-3'



Enzymes bind to the target sequence and cleave **or** methylate according to the methylated state of DNA:

- Cleaves non-methylated DNA
- Methylates hemi-methylated DNA

Typically foreign DNA is unmethylated and will be degraded when introduced in a strain wild type for the three genes

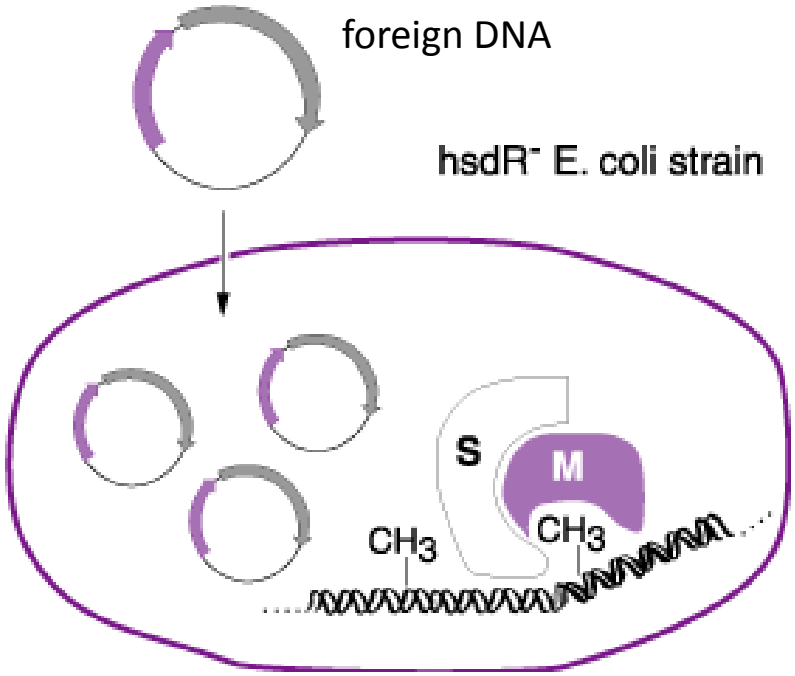
TP1: Restriction-modification Systems

Foreign DNA cloning

-Modification of some *E.coli* strains:

hsdR gene is required only for the endonuclease cleavage (*hsdS* and *hsdM* are sufficient for target sequence methylation) - *E. coli* strains mutated for *hsdR* have the penhotype restriction minus, modification plus (r-, m+)

Restriction modification systems have been removed from *E. coli* K-12 strains used for cloning



•It can be used to clone foreign DNA that is not methylated at the target sequence

•When foreign DNA is propagated in this genetic background, the target sequence becomes methylated and may then be grown in a strain wildtype for the three genes

http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture3.html

TP1: Restriction-modification Systems

Genotypes and phenotypes of *hsd* mutants in the *Eco*K system

HsdS *- determines* ***HsdM e HsdR specificity****, an hsdS minus genotype lead to a* r^-m^- *phenotype*

HsdR - ***it is required only for nuclease cleavage of target sequence***

HsdM *- it methylates at* $A^{N6}ACNNNNNNGTGC$ *ou* $GC^{N6}ANNNNNNGTT$*. DNA isolated in* $hsdM^-$ *is cleaved in HsdR host*

The two subunits encoded by hsdM and hsdS, HsdM and HsdS are both necessary and sufficient for methyltransferase activity. HsdM is important for the positioning of the domains of HsdS

GENOTYPE			PHENOTYPE
hsdS	hsdR	hsdM	
+	+	+	$r^+ m^+$
-	+	+	$r^- m^-$
+	-	+	$r^- m^+$
+	+	-	$r^- m^-$

TP1: Restriction-modification Systems

Type II

•Type II R-M systems consist of two separate proteins with independent enzymatic activities, a restriction endonuclease and a DNA methyltransferase.

•In contrast to type I systems, DNA cleavage or methylation occurs within symmetrical recognition sites.

•Most type II enzymes recognize palindromic, duplex DNA sequences, such as GAATTC, whose complementary strand has the same 5’-3’ sequence.

•The restriction endonucleases are homodimeric, require Mg , and cleave phosphodiester bonds within or immediately adjacent to the recognition sequence to leave a staggered or blunt double-strand cut.

•The methyltransferases are monomeric and require the cofactor AdoMet. Methylation takes place on both strands of the DNA duplex within the recognition sequence.

TP1: Restriction-modification Systems

Ex. EcoRI system

*Eco*RI, as most of all the other type II restriction enzymes, cleaves inside the recognition sequence :

Methylase of *Eco*RI (M.*Eco*RI) catalyzes the transfer of methyl groups from SAM to a specific A in the recognition sequence (*) of DNA

…**aact G A *A T T C tcgac**…
…**ttga C T T *A A G agctg**…

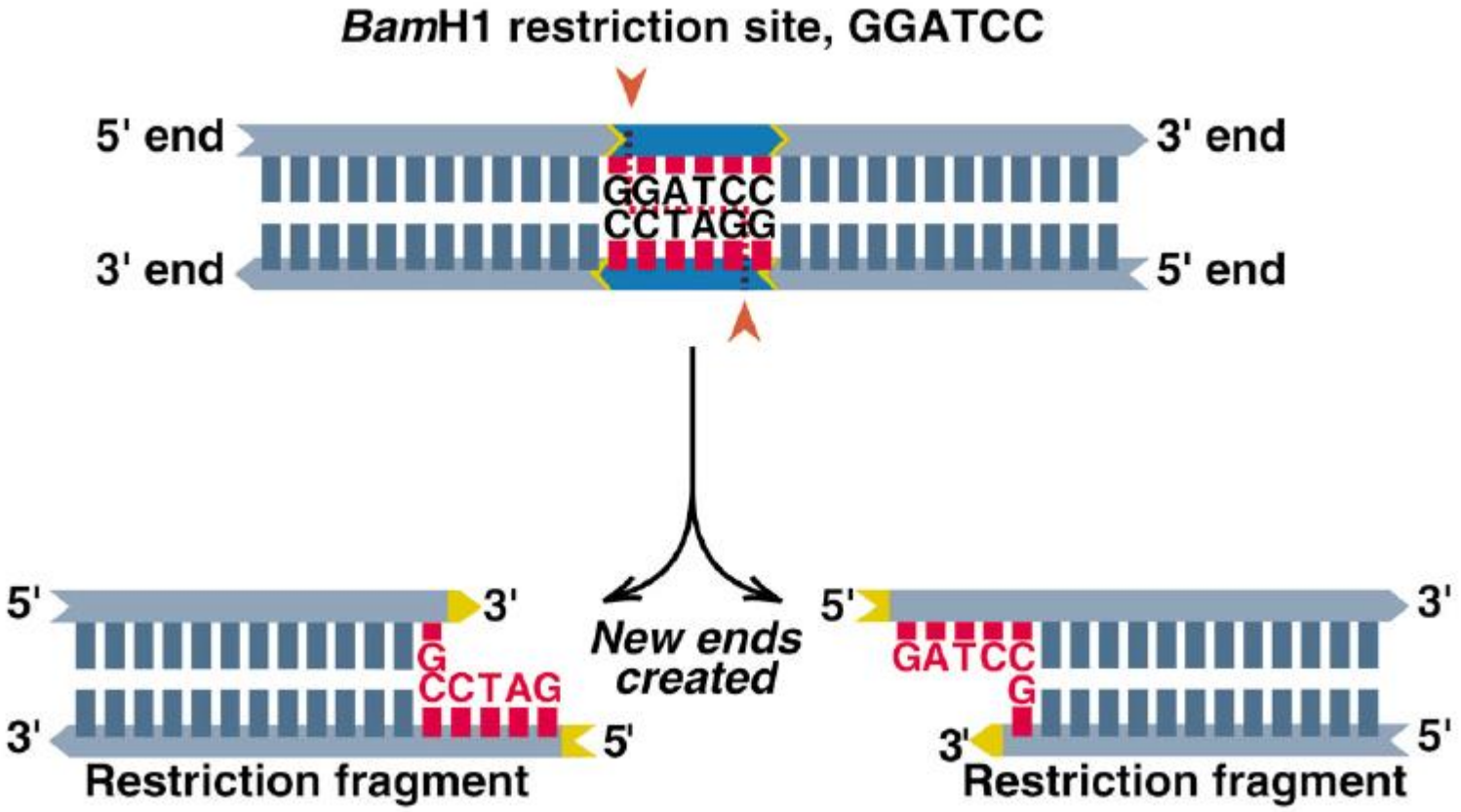
The modification of adenine (*A) to 6-methyladenine, protects DNA from *Eco*RI cleavage

TP1: Restriction-modification Systems

Ex. BamH1

Recognition sites:

- 4 to 6 nucleotides, palindrome (mostly)
- Cleavage inside the recognition site



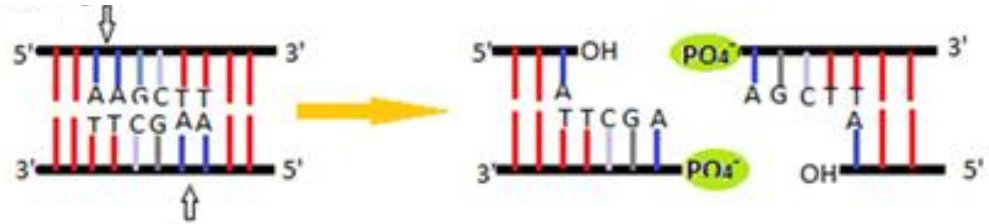
TP1: Restriction-modification Systems

Type II restriction enzymes generated extremities

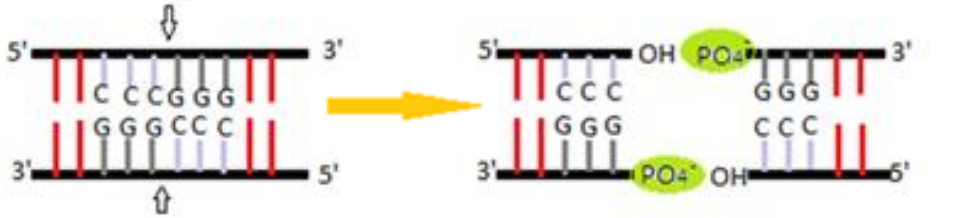
Enzymes

Recognition cleavage site

*Hin*dIII



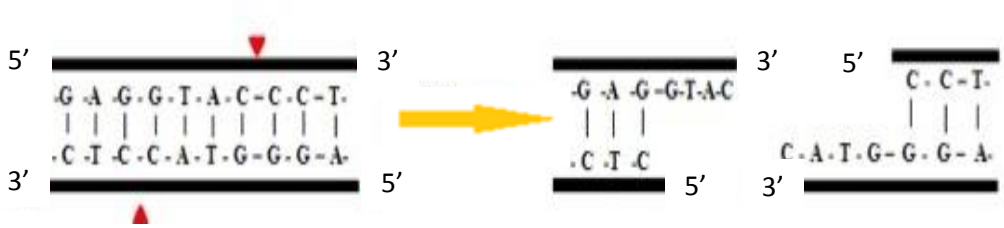
*Sma*I



Sticky ends/ extremidades coesivas
Protruding 5'P cohesive ends
or **3' recessed ends**
5' projetadas, ou 3' recuadas

Blunt ends/
extremidades cegas

*Kpn*I



Sticky ends/ extrem. coesivas
Protruding 3'OH cohesive ends
or **5' recessed ends**
3' projetadas, ou 5' recuadas

http://nptel.ac.in/courses/102103013/module2/lec1/images/4.png

http://www.fastbleep.com/assets/notes/image/7041_1.jpg

TP1: Restriction-modification Systems

Restriction enzyme nomenclature

	EcoRI	HindIII	BamHI
Genus	Escherichia	Haemophilus	Bacillus
Specie	coli	influenzae	amylo
Strain	R	D	H
Isolate	I	III	I
Recognition site	G^AATTC	A^AGCTT	G^GATGC

TP1: Restriction-modification Systems

Table 18.2 Characteristics of some common type II restriction enzymes used in recombinant DNA technology

Enzyme	Microorganism From Which Enzyme Is Isolated	Recognition Sequence	Type of Fragment End Produced
*Bam*HI	*Bacillus amyloliquefaciens*	5′–G↓GATCC–3′ 3′–CCTAG↑G–3′	Cohesive
*Cof*I	*Clostridium formicoaceticum*	5′–G↓CGC–3′ 3′–CGC↑G–5′	Cohesive
*Dra*I	*Deinococcus radiophilus*	5′–TTT↓AAA–3′ 3′–AAA↑TTT–5′	Blunt
*Eco*RI	*Escherichia coli*	5′–G↓AATTC–3′ 3′–CTTAA↑G–5′	Cohesive
*Eco*RII	*Escherichia coli*	5′–↓CCAGG–3′ 3′–GGTCC↑–5′	Cohesive
*Hae*III	*Haemophilus aegyptius*	5′–GG↓CC–3′ 3′–CC↑GG–5′	Blunt

EcoRI enzyme name is derived from *Escherichia coli,* strain RY13, 1rst identified

TP1: Restriction-modification Systems

Table 18.2 Characteristics of some common type II restriction enzymes used in recombinant DNA technology

Enzyme	Microorganism From Which Enzyme Is Isolated	Recognition Sequence	Type of Fragment End Produced
*Hin*dIII	*Haemophilus influenzae*	5′–A↓AGCTT–3′ 3′–TTCGA↑A–5′	Cohesive
*Hpa*II	*Haemophilus parainfluenzae*	5′–C↓CGG–3′ 3′–GGC↑C–5′	Cohesive
*Not*I	*Nocardia otitidis-caviarum*	5′–GC↓GGCCGC–3′ 3′–CGCCGG↑CG–5′	Cohesive
*Pst*I	*Providencia stuartii*	5′–CTGCA↓G–3′ 3′–G↑ACGTC–5′	Cohesive
*Pvu*II	*Proteus vulgaris*	5′–CAG↓CTG–3′ 3′–GTC↑GAC–5′	Blunt
*Sma*I	*Serratia marcescens*	5′–CCC↓GGG–3′ 3′–GGG↑CCC–5′	Blunt

Note: The first three letters of the abbreviation for each restriction enzyme refer to the bacterial species from which the enzyme was isolated (e.g., *Eco* refers to *E. coli*). A fourth letter may refer to the strain of bacteria from which the enzyme was isolated (the "R" in *Eco*RI indicates that this enzyme was isolated from the RY13 strain of *E. coli*). Roman numerals that follow the letters allow different enzymes from the same species to be identified. For convenience, molecular geneticists have come up with idiosyncratic pronunciations of the names: *Eco*RI is pronounced "echo-R-one," *Hin*dIII is "hin-D-three," and *Hae*III is "hay-three." These common pronunciations obey no formal rules and simply have to be learned.

TP1: Restriction-modification Systems

Type III

•**Only a few type III R-M systems are known**
•A single bifunctional enzyme catalyzes both the endonuclease and the methyltransferase restriction activities. The enzymes are composed of two nonidentical subunits: the M subunit (encoded by the mod gene) and the R subunit (encoded by the res gene).

•The R subunit must be complexed with the M subunit for restriction activity, because the M subunit provides the sequence specificity for the enzyme. The two enzymatic activities compete for the uninterrupted, asymmetric DNA recognition sequence, which is usually 5-6 bp long.

•Cleavage takes place 25-30 bp away to the 3’ side of the DNA recognition sequence. If one or both of the DNA strands is (are) methylated, no cleavage occurs. The M subunit can act independently as a methyltransferase, requiring AdoMet and methylating only one strand of the duplex recognition sequence at a time, which is sufficient to inhibit the restriction reaction.

TP1: Restriction-modification Systems

Other Restriction-Modification systems

Modification systems (site-specific methylases)

Dam	**(**N6**)G*ATC**	Dam methylase, Deoxyadenosine methylase
Dcm	**(**C5**)C*CAGG e C*CTGG**	DNA 5-cytosine methyltransferase

Only methylate DNA sequences

Restriction systems (endonucleases)

McrA	**C*CGG**	Mcr=modified cytosine restriction
McrB	**G*C**	
Mrr	**C*AC and C*AG**	

Only cleave METHYLATED **sequences**

TP1: Restriction-modification Systems

Restriction system – Dam and Dcm methylation

•The **Dam** (DNA adenine methyltransferase) and **Dcm** (DNA cytosine methyltransferase) modification systems methylate adenines and cytosines located within specific recognition sequences (**5'-GATC-3'** for Dam and the second cytosine of **5'-CCA/TGG-3´**for Dcm)

•Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm methylases if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from *dam+ E. coli is completely resistant to cleavage by MboI, which cleaves at GATC sites.*

•Prevent certain restriction enzymes from cutting their target sequence in plasmid DNA. *Eg. XbaI will not cut its recognition sequence (5'-TCTAGA-3') when the last adenine is methylated.*

TP1: Restriction-modification Systems

Modification system – Dam methylation

Dam methylation: G^{m6}ATC (methyl group at position N6 of adenine: N6-methyladenine)

1- example of Type II restriction enzyme **blocked** by Dam methylation

*Cla*I	**G*ATCGAT**
*Xba*I	**TCTAG*ATC**
*Mbo*I	**G*ATC**

2- example of Type II restriction enzyme **not blocked** by Dam methylation

*Bam*HI	**GG*ATCC**
*Pvu*I	**CG*ATCG**
*Sau*3AI	**G*ATC**

Restriction enzymes have different sensibilities to the various methylation patterns

Ex: *Sau*3AI

CLEAVES	DOESN'T CLEAVE
G^{m6}ATC	GAT^{m4}C
	GAT^{hm5}C
	GAT^{m5}C

Bold- type II restriciton enzyme recognition sequence

TP1: Restriction-modification Systems

Modification system – mcrA/mcrB/mrr complex

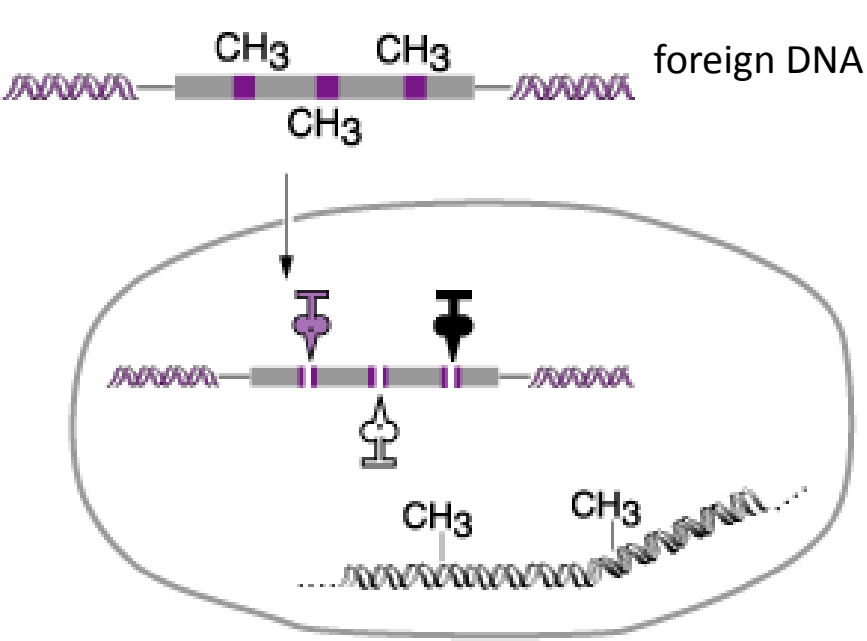
•Direct endonuclease cleavage to DNA targets that are methylated on certain sequences

•There are two mcr(methyl-cytosine restricting) systems in E. coli—mcrAand mcrBC. Another system, mrr (modified adenine recognition and restriction), restricts particular DNA sequences that include methyladenine (m6A) or methylcytosine (m5C) residues.

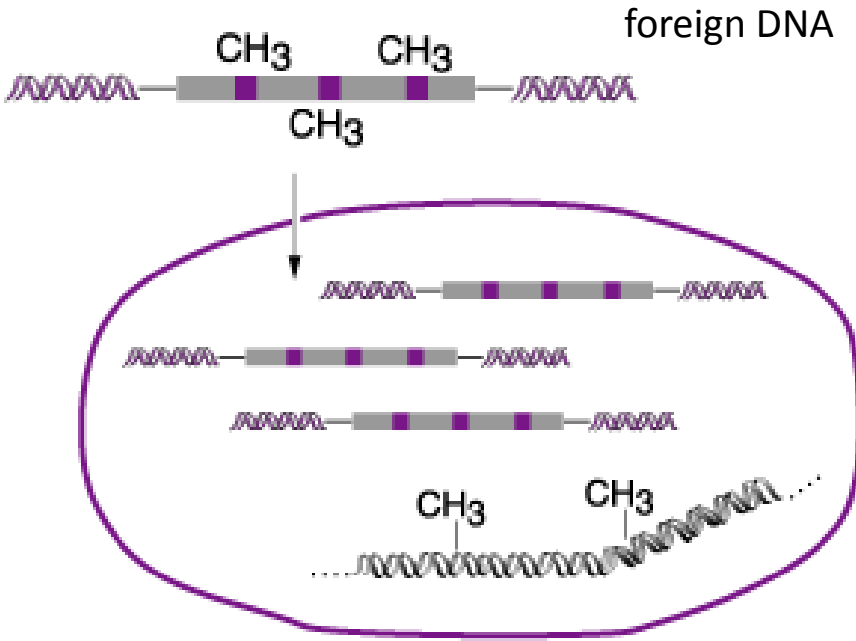
•The mcrA/mcrB/mrr complex degrades foreign DNA that is not properly methylated, such as methylated DNA obtained from mouse and human cells which contains CpG methylated DNA

TP1: Restriction-modification Systems

Only cleave METHYLATED sequences



Wild-type E. coli



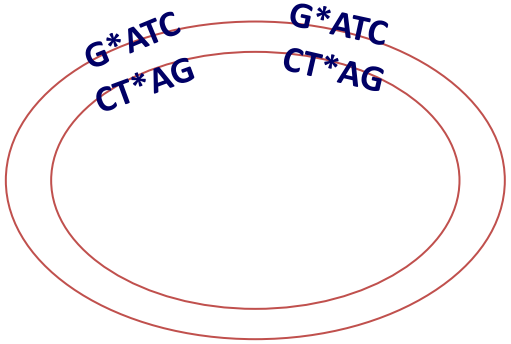
mcrA⁻/mcrB⁻ E. coli strain

Strains mutated in both McrA and McrB systems, as well as in the Mrr system should be considered when cloning DNA from other organisms as plants and mammals usually contain some degree of methylation that can be recognized by these systems.

TP1: Restriction-modification Systems

Ex. 1 – Consequences of restriction on DNA amplified in a Dam$^+$ strain:

G*ATC



***Mbo*I** GATC / CTAG — **Doesn't cleave** methylated sequences — G*ATC / CTA*G

Sau3AI GATC / CTAG — **Cleaves** methylated sequences — G*ATC / CTA*G

Ex. 2 –Cloning of human DNA, that has ***CpG islands**, should be amplified in mcrA$^-$ and mcrBC$^-$

mcrA$^-$ — **McrA- C^{m5}CGG**

mcrBC$^-$ — **McrBC- G^{m5}C**, **G^{h5}C**, **G^{N4}C**

TP1: Restriction-modification Systems

DNA methylation in prokaryotes and eukaryotes

•The extensive research on methylation was conducted on bacteria where both A and C can be methylated.

•Eukaryotic DNA methylation affects mainly C residues and is specific for CpG sequence.

•The protective function of DNA methylation is similar in eukaryotes and prokaryotes. In humans and rodents inserted viral sequences can become methylated in association with silencing of the introduced genes. Thus function of **DNA methylation machinery for recognition and/or eliminating of foreign DNA** seem to be conserved in evolution.

• eukaryotic genome - **cytosine methylation** plays a role in functional reorganization:

Low 5'-mC----------> high levels of gene expression
High 5'-mC ----------> low levels of gene expression

•Since DNA methylation is reversible and does not directly depend on the sequence context it was described as an **epigenetic mechanism of gene regulation**.

•DNA methylation is observed in most of the organisms at the different stages of evolution. Some species, **like *Drosophila melanogaster* lack DNA methylation.**

TP1: Restriction-modification Systems

Isoesquizomers

1- Recognize the same sequence and cut on the same sites

Enzyme	Sequence	Cut Site	Overhang	Properties
AccIII	TCCGGA	T/C C G G A A G G C C/T	5′ - CCGG	
BspEI	TCCGGA	T/C C G G A A G G C C/T	5′ - CCGG	dam CpG RR Yes NEB3.1 37°

2- Recognize the same sequence but cut in different positions

Enzyme	Sequence	Cut Site	Overhang	Properties
XmaI	CCCGGG	C/C C G G G G G G C C/C	5′ - CCGG	CpG RR Yes CutSmart 37°
SmaI	CCCGGG	C C C/G G G G G G/C C C	blunt	CpG RR Yes CutSmart 25°

TP1: Restriction-modification Systems

Isoesquizomers

3- Recognize the same sequence, different sensitivity to methylation

***Dpn*I** only cleaves fully-adenomethylated dam sites G*ATC

***Dpn*II** and ***Mbo*I** share methylation sensitivity - cleave dam sites which lack adenomethylation and each is blocked by complete dam methylation

***Sau*3AI** will cleave all dam sites regardless of adenomethylation

Enzyme	Sequence	Cut Site	Overhang	Properties
DpnI	GATC	G A / T C C T / A G	blunt	CpG RR Yes CutSmart 37°
BfuCI	GATC	/G A T C C T A G/	5′ - GATC	CpG Yes CutSmart 37°
DpnII	GATC	/G A T C C T A G/	5′ - GATC	dam RR Yes NEBU 37°
MboI	GATC	/G A T C C T A G/	5′ - GATC	dam CpG RR Yes CutSmart 37°
Sau3AI	GATC	/G A T C C T A G/	5′ - GATC	CpG RR Yes NEB1.1 37°

dam CpG dcm

Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by either the dam or dcm or CpG methylase.

Applications

TP1: Restriction-modification Systems

DNA cloning: bacterial strains

•*E. coli* has several mechanisms for identifying foreign DNA and destroying it - ***problem in cloning experiments***, resulting in substantially reduced recovery of desired sequences.

•EcoKI restriction, encoded by the *hsdRMS* genes, **attacks DNA that is not protected by adenine methylation at the appropriate recognition site** (AAC[N6]GTGC or GCAC[N6]GTT).

•McrA, McrBC, and Mrr, encoded by *mcrA, mcrBC, and mrr* , **are methylation-requiring systems that attack DNA only when it is methylated at specific positions**

The problem can be avoided by the use of strains in which these mechanisms are disabled by mutation.

A strain completely disabled for restriction will be defective at the *hsd*, *mcrA*, *mcrBC*, and *mrr* loci

TP1: Restriction-modification Systems

Application of restriction enzymes type II: DNA cloning

- Recombinant DNA technology was “born” with the discovery of restriction enzymes

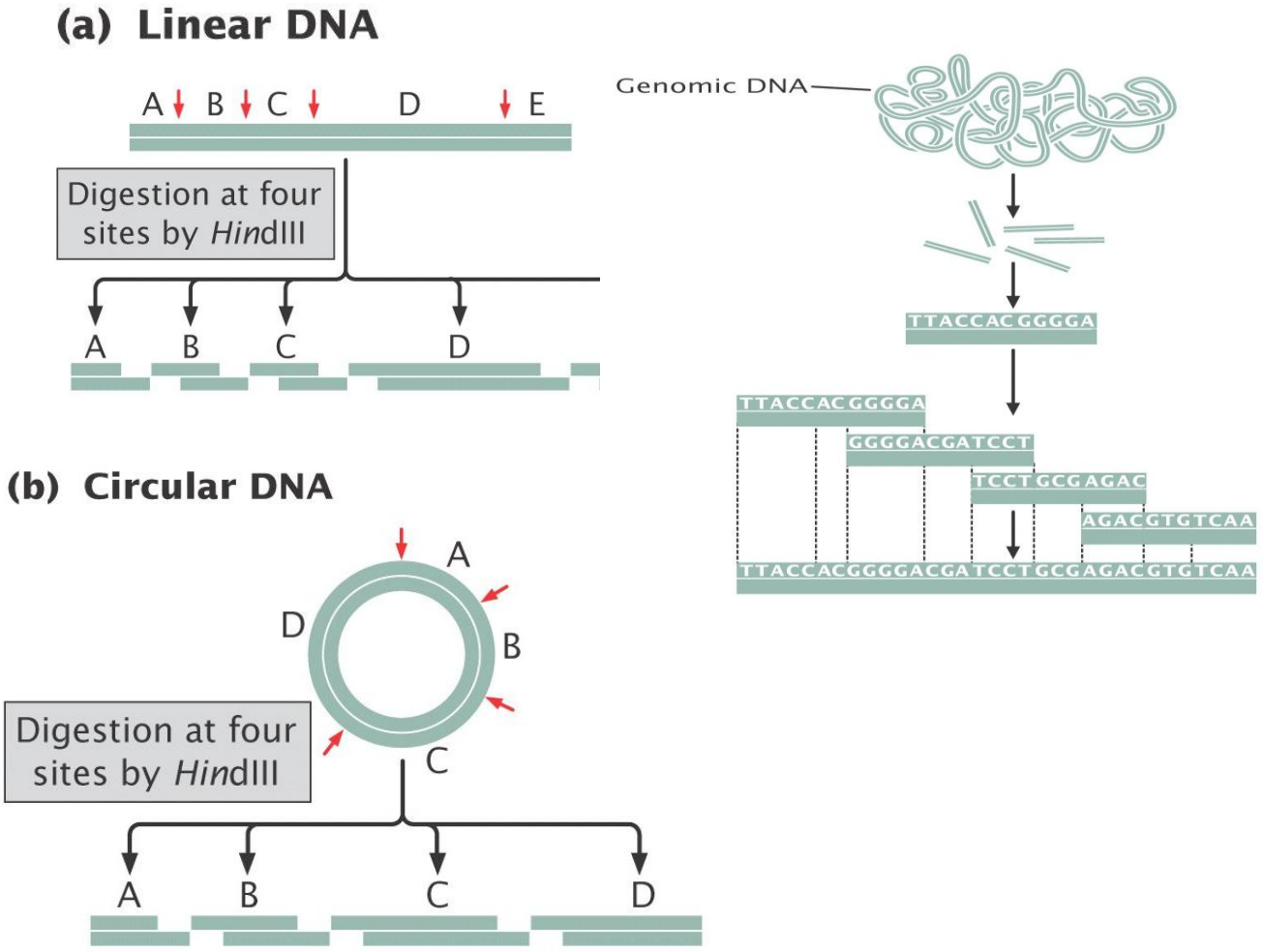
- One of the most important applications of restriction enzyme type II is **DNA cloning**, i.e., the possibility of propagating a particular gene of interest or other DNA fragment using specific DNA molecules (vectors)

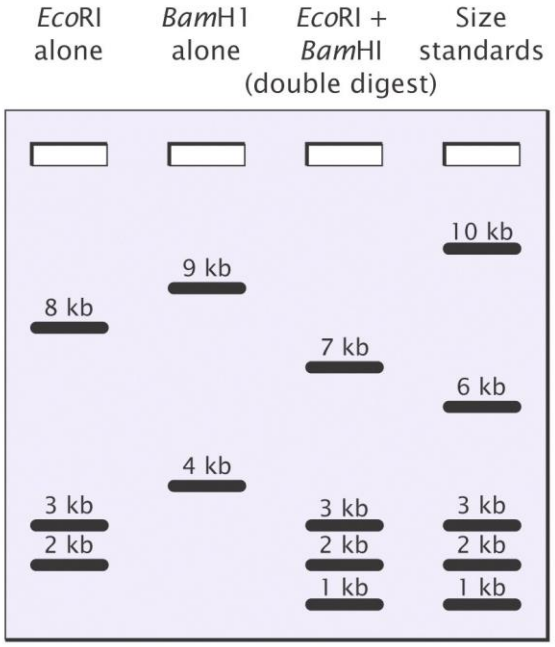
- The approach used to clone a specific gene, depends to a large degree on:
  - the gene
  - what is known about it
  - objective

- Among the several tools needed for cloning, lets consider:
  - restriction enzymes
  - vector DNA
  - DNA ligase
  - host cell

TP1: Restriction-modification Systems

Cleaving and viewing DNA after digestion with type II RE



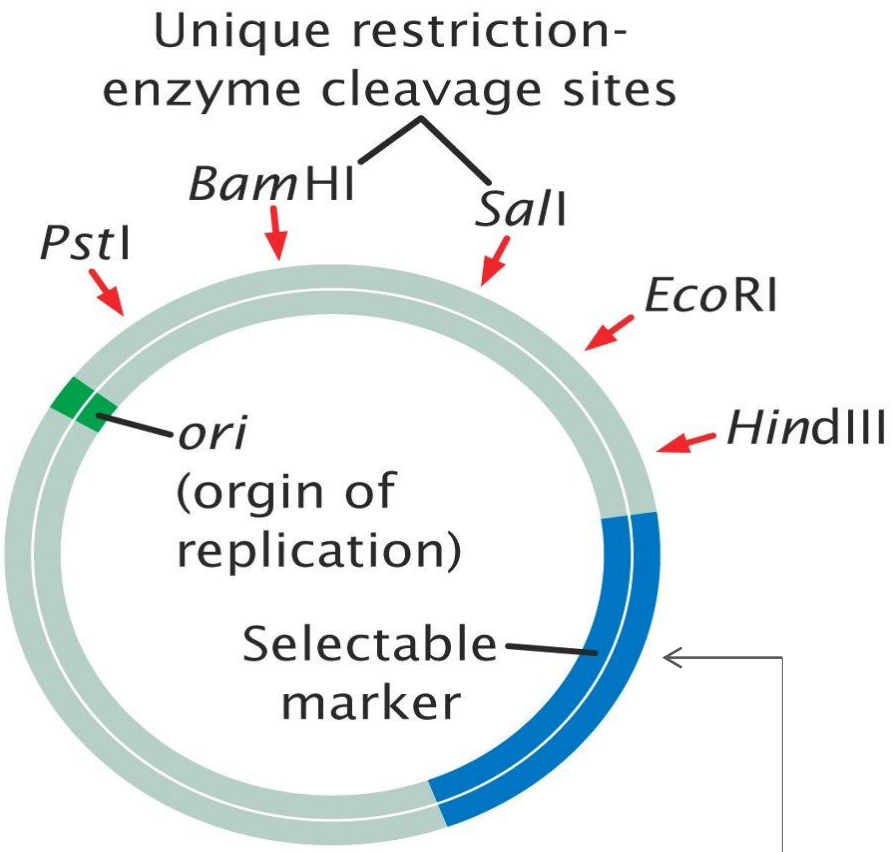


TP1: Restriction-modification Systems

Plasmid vector important markers

- Plasmids are small circular DNA molecules that are found inside some prokaryotic cells

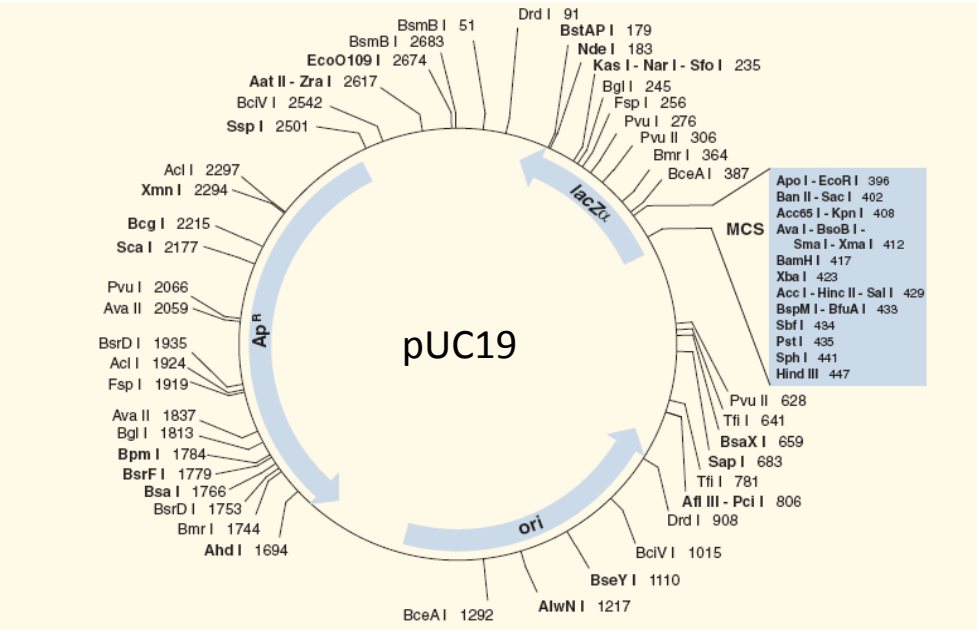
- Plasmids as cloning vectors are *in vitro* constructed and are important tools in genetic engeneering

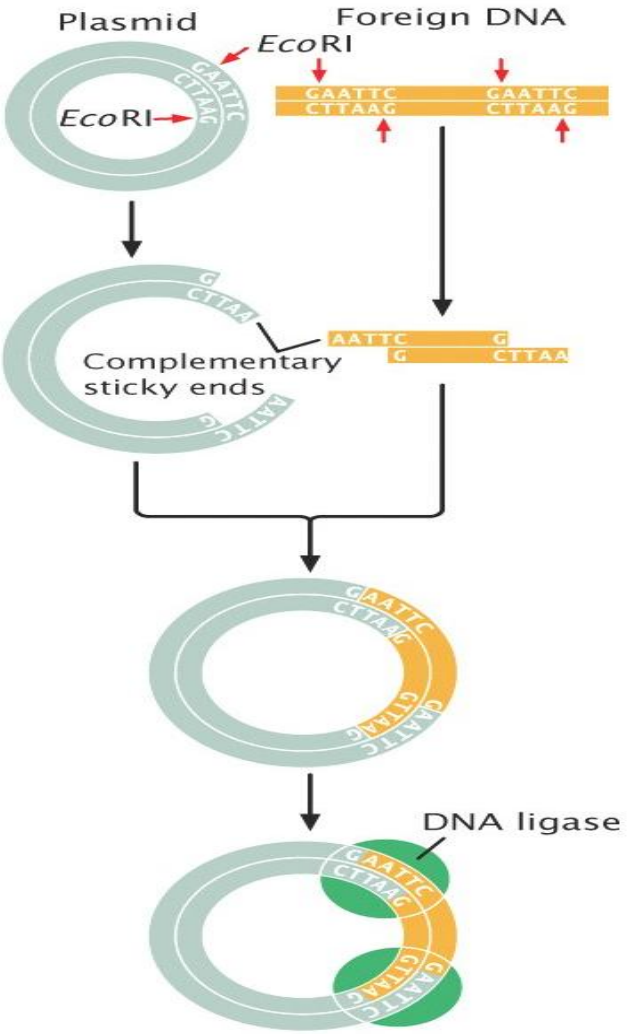


Antibiotic resistance marker
Bacterial host cell should be sensitive to the antibiotic

TP1: Restriction-modification Systems

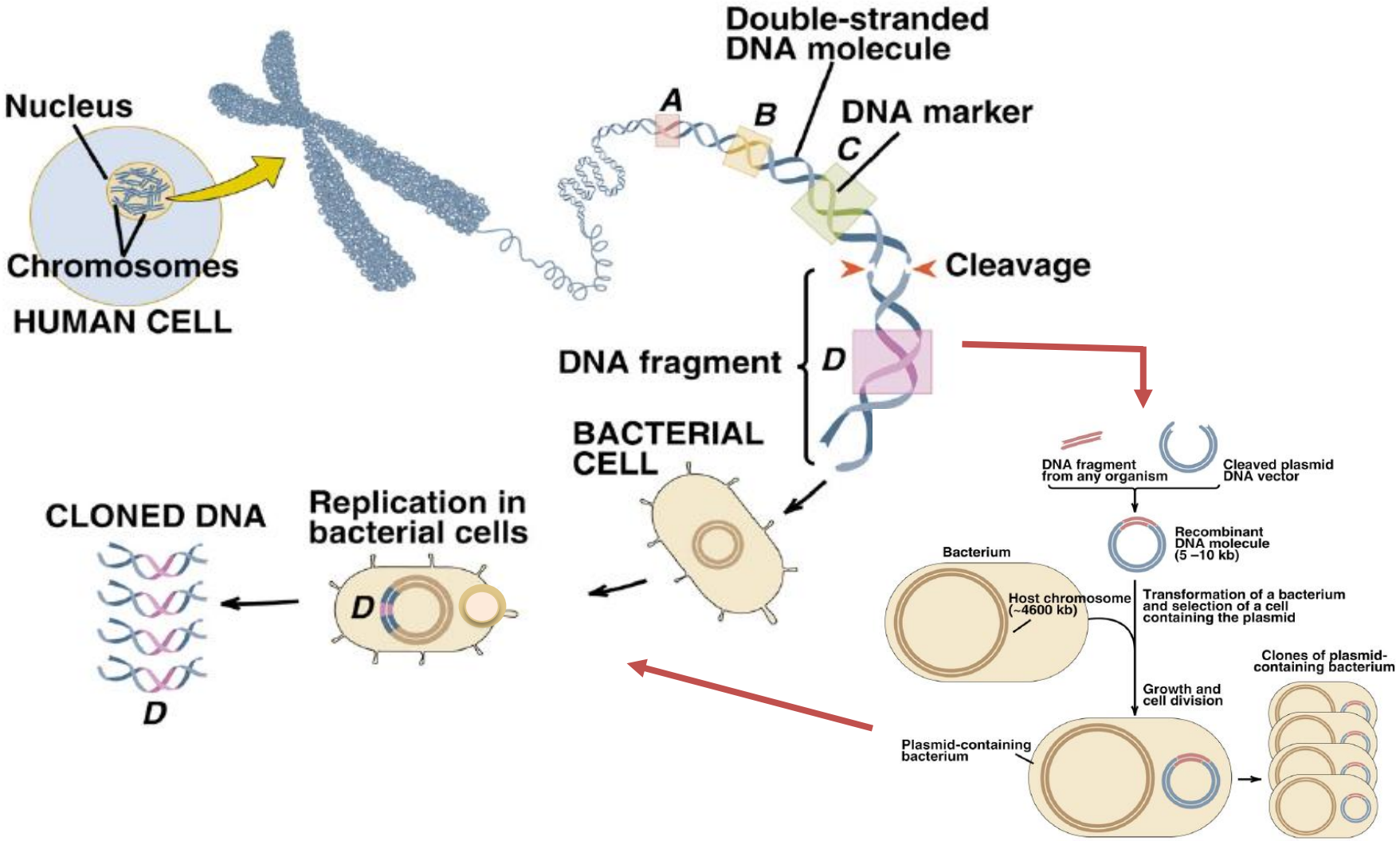
Construction of a recombinant DNA molecule





TP1: Restriction-modification Systems

Isolating and cloning a DNA fragment



Useful sites/ tools regarding R-M systems

http://rebase.neb.com/rebase/rebase.html

https://www.neb.com/tools-and-resources/interactive-tools/enzyme-finder

http://nc2.neb.com/NEBcutter2/

https://www.ncbi.nlm.nih.gov/genbank/

Type I RE: https://www.youtube.com/watch?v=uyfFLWzUjmM

Type II RE: https://www.youtube.com/watch?v=6U8bGOG9OAI