

Il contenuto intracellulare di una cellula batterica è diviso tra  
due principali compartimenti  
**nucleoide** nel quale è situato il genoma  
**citoplasma** contenete l'apparato di sintesi proteica

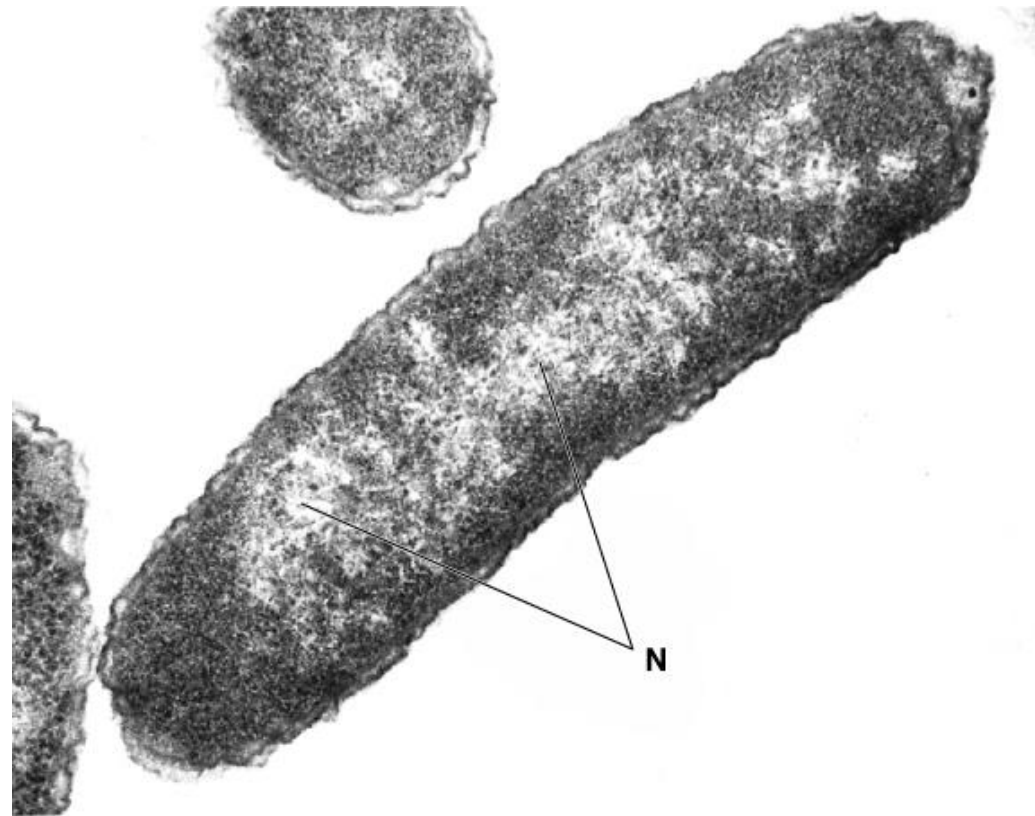
Quali sono i costituenti del  
nucleoide

**Genoma batterico**  
**RNA**

**RNA polimerasi**  
**Topoisomerasi**

**Proteine basiche**

**(definite Proteine Associate al  
Nucleoide NAP o Proteine  
Istone -simili)**



# IL NUCLEOIDE

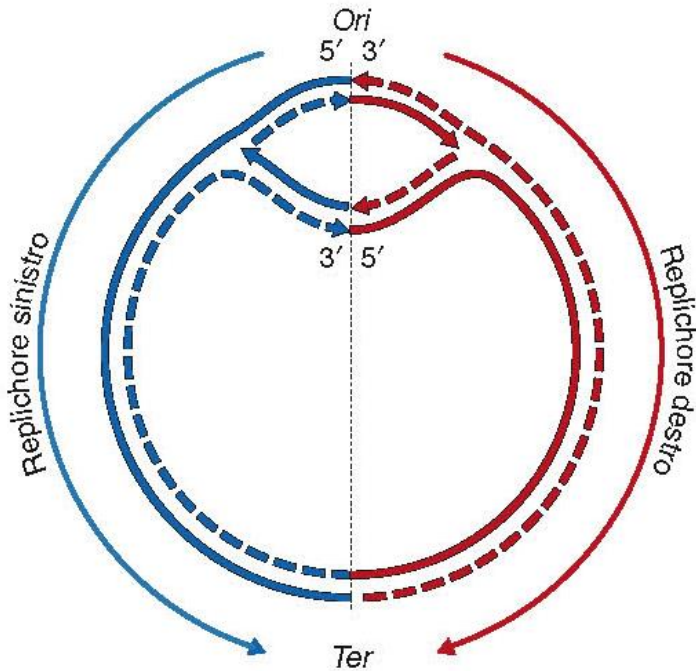
- la molecola di DNA cromosomico di E.coli è lunga circa 1.6mm
- è contenuta in una cellula di 2  $\mu\text{m}$  di lunghezza e 1 $\mu\text{m}$  di larghezza
- Un compattamento casuale della molecola determinerebbe un volume di circa 200mm<sup>3</sup> circa 400 volte superiore al volume del nucleoide
  - Il volume del nucleoide è di circa di E.coli 0.5  $\mu\text{m}$
- Il cromosoma è quindi estremamente organizzato in anse topologicamente indipendenti circa 100 da 50 kb

L'organizzazione del genoma batterico è caratterizzata dalla presenza di macrodomini funzionali , ampie regioni di DNA nel quale ogni singolo gene ha un corretto livello di espressione che dipende dal suo orientamento dalla sua posizione rispetto all'origine.

Se alcune regioni vengono invertite come orientamento sul genoma (pur rimanendo presenti i geni) si può avere la non espressione o espressione molto ridotta: queste grandi inversioni possono indurre anche morte nella cellula .

Elemento importante è anche il posizionamento rispetto all'origine: geni posizionati in posizione prossimale all'origine sono espressi di più in modo statisticamente significativo rispetto a quelli in posizione distale

# Posizionamento dei geni sul cromosoma e trascrizione



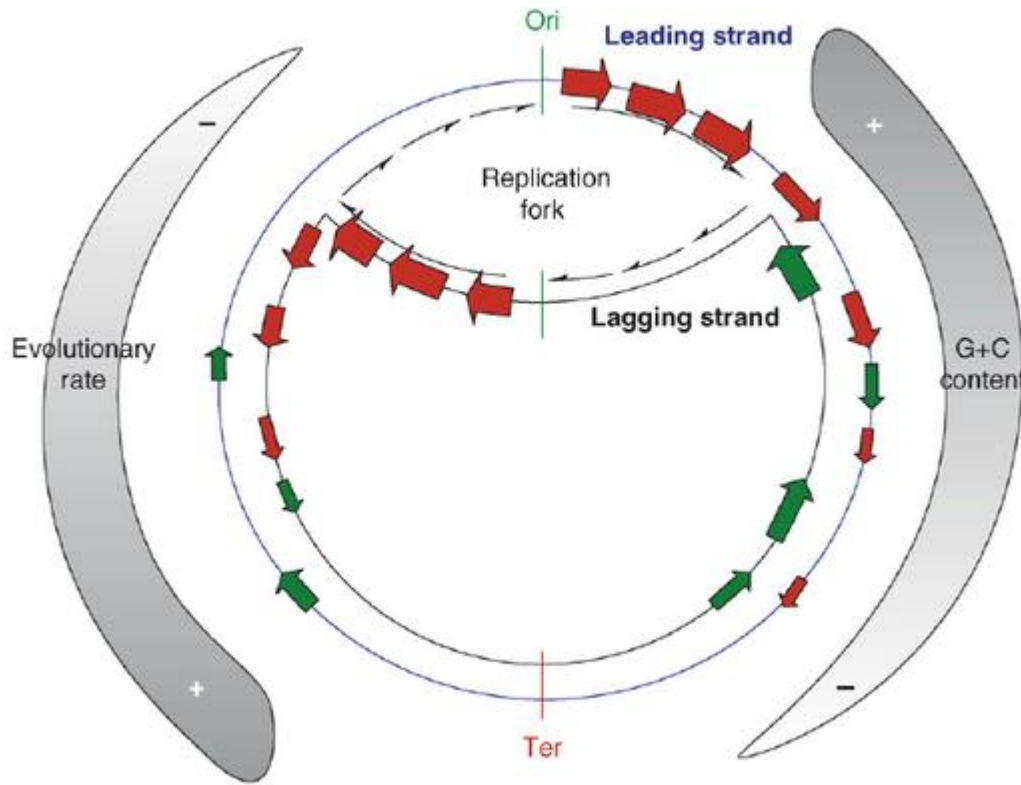
Il posizionamento dei geni sul cromosoma è in parte condizionato da come si muove l'apparato di replicazione.

Possiamo definire due regioni definite replichores che dividono il genoma in due metà corrispondenti al movimento delle due forche. In ognuno di questi replichores ci sarà un'elica leading ed una lagging strand che saranno opposte.

E' stato osservato che:

- Il numero geni trascritti nella stessa direzione in cui si muove la forza replicativa è maggiore rispetto ai geni trascritti in direzione opposta ovvero filamento leading e filamento codificante corrispondono.
- Il cromosoma nelle due opposte metà può presentare asimmetria nel contenuto in G+C, nell'orientamento dei geni nell'accumulo di mutazioni, nella biosintesi di aminoacidi essenziali.

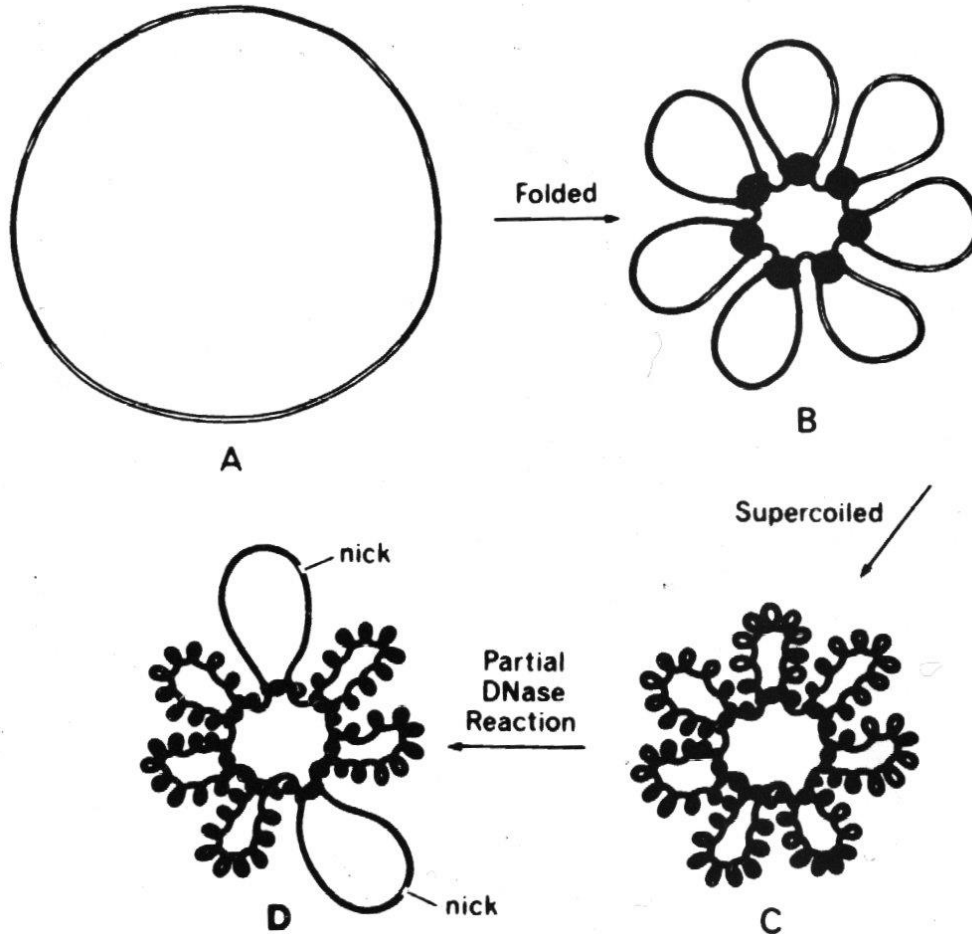




Un altro aspetto dell'asimmetria riguarda la composizione in basi delle eliche C/G e A/T dei singoli filamenti che si osserva in 185 genomi (appartenenti a 11 Phyla) e non si trova in lievito, Drosophila e uomo. Perché si origina? Essendo replicati in modo asimmetrico e si potrebbero accumulare mutazioni silenti sui frammenti codificanti

Distribuzione dei geni, orientamento e posizione sono fattori non casuali

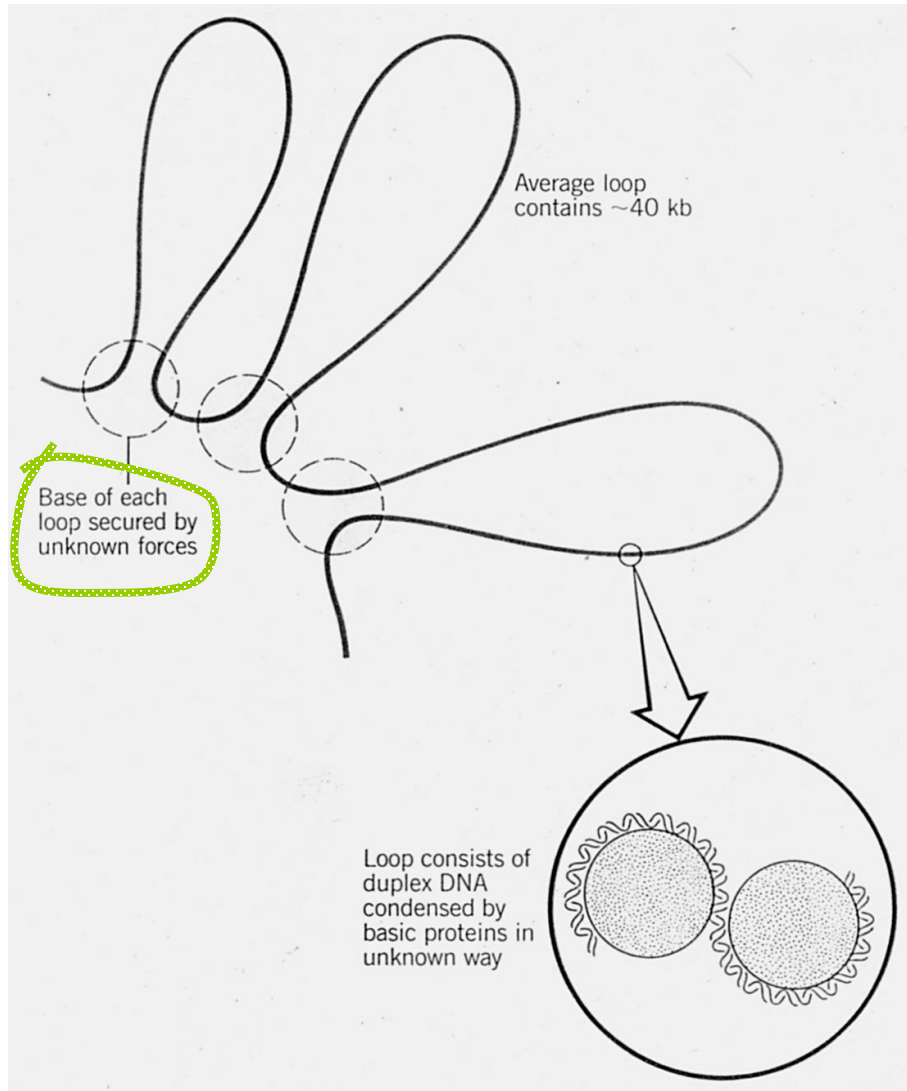
# Organizzazione del cromosoma batterico ad anse



Il cromosoma potrebbe essere organizzato in domini topologicamente indipendenti, superavvolti negativamente. Analizzando il numero di tagli necessari per rilassare completamente il DNA è stato possibile valutare la quantità di topodomini presenti in *E. coli* (100). Tenendo conto che il cromosoma di *E. coli* è di 4.600 kb la taglia media dei domini è valutata di circa 50 kb



# Ancoraggi nel nucleotide batterico



## Componenti degli ancoraggi:

### • RNA?

- 1) in vivo l'RNA nascente si osserva solo alla periferia del nucleotide
- + 2) in vitro l'RNasi rilassa il nucleotide (aumento di viscosità, diminuzione del coeff. di sedimentazione.)
- + 3) Gli inibitori della trascrizione non influenzano il numero di domini cromosomici, ma i nucleoidi si rilassano più facilmente

### • Parete -Membrana? (replicazione)

### • Proteine istone-simili? (HU, H-NS, ...)

### • DNA ancorato?

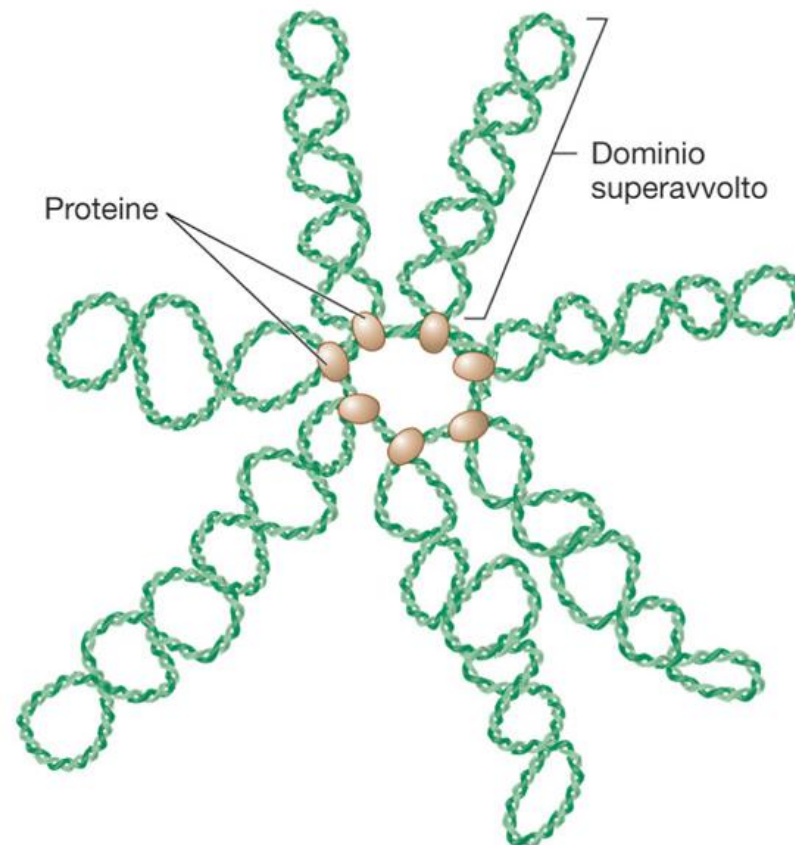


**Rilevanza funzionale in vivo?**



Il cromosoma batterico è organizzato in numerosi domini superavvolti stabilizzati dal legame con proteine specifiche alla base dell'ansa.

In *Escherichia coli*  
si calcolano circa  
100 domini



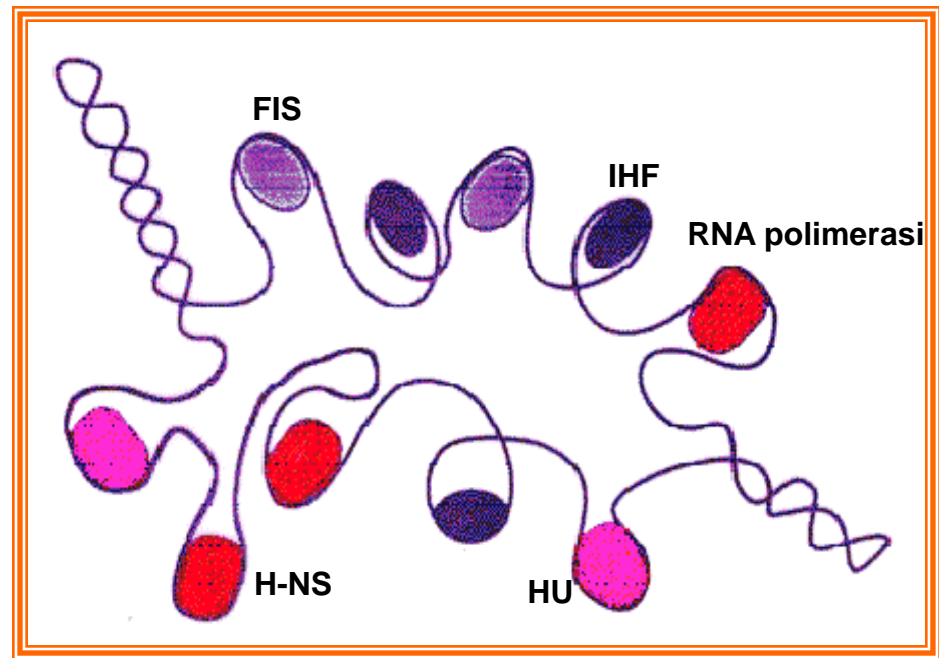
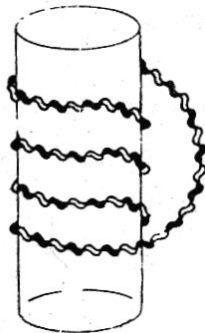
(d) Cromosoma con domini superavvolti

# Bacterial chromatin organization

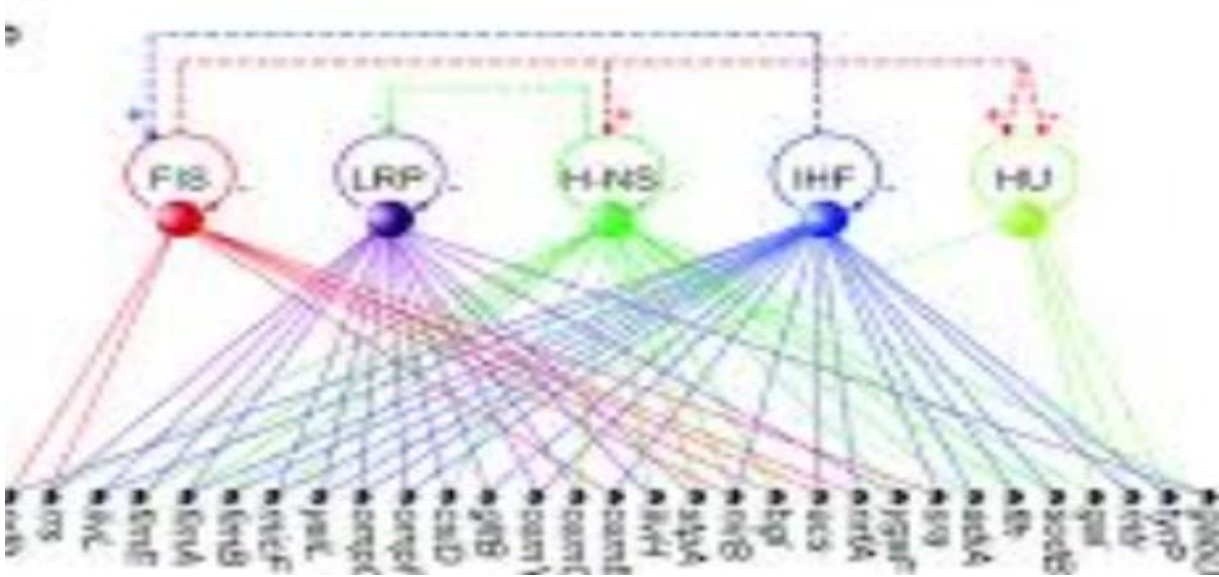
Plectonemic  
condition



Toroidal  
condition



Co-existence of  
plectonemic and toroidal conditions



# Le proteine associate al nucleotide (NAP proteins)

Anche nei batteri esistono proteine implicate nell'organizzazione strutturale del DNA.

L'isolamento e la caratterizzazione delle proteine istone simili è risultato complesso

- perchè in molti casi mostrano una stretta associazione con altre proteine prevalentemente quelle ribosomiali
- perché il loro legame è meno sensibile all'aumento della forza ionica

HU  
IHF  
FIS  
H-NS  
Dps  
Lrp



Principali proteine associate  
al nucleotide

## Struttura ed organizzazione delle principali proteine associate al nucleotide (NAP) Definite anche HLP (Histone-Like Proteins)

Structural and functional features of the major HLPs

HLP	Properties and abundance	Structure	Genes	Main functional activities
HU (heat unstable nucleoid protein)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in exponential phase (15000–30000 dimers/cell)</li> </ul>	heterodimer; HU $\alpha$ , 9.2 kDa HU $\beta$ , 9.5 kDa	<i>hupA</i> (90.4 min) <i>hupB</i> (9.9 min)	<ul style="list-style-type: none"> <li>• compacts DNA into nucleosome-like structures</li> <li>• induces DNA curvature</li> <li>• recognizes curved DNA, gapped regions, and 3/4way junctions</li> <li>• involved in DNA replication and recombination</li> </ul>
IHF (integration host factor)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in stationary phase (25000–3000 dimers/cell)</li> <li>• high amino acid identity between IHF and HU subunits</li> <li>• DNA binding preference: WATCAANNNTTR</li> </ul>	heterodimer; IHF $\alpha$ , 11.2 kDa IHF $\beta$ , 10.7 kDa	<i>htmA</i> (38.6 min) <i>htmD</i> (25 min)	<ul style="list-style-type: none"> <li>• induces very strong DNA curvature (up to 140°)</li> <li>• participates in site-specific recombination, transposition, and DNA replication</li> </ul>
FIS (factor for inversion stimulation)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in exponential phase (20000–40000 dimers/cell)</li> <li>• DNA binding preference: GNYAWWWTRNC</li> </ul>	homodimer, 2 × 11.2 kDa	<i>fis</i> (73.4 min)	<ul style="list-style-type: none"> <li>• induces strong DNA curvature (up to 90°)</li> <li>• alters DNA topology</li> <li>• participates in site-specific recombination, transposition, and DNA replication</li> </ul>
H-NS (histone-like nucleoid structuring protein)	<ul style="list-style-type: none"> <li>• non basic</li> <li>• 20000–40000 dimers/cell</li> <li>• binding form may be tetramer or higher oligomer</li> <li>• induced during cold-shock</li> </ul>	homodimer, 2 × 15.4 kDa	<i>hns</i> (27.8 min)	<ul style="list-style-type: none"> <li>• recognizes curved DNA</li> <li>• alters DNA topology</li> <li>• induces DNA curvature</li> <li>• influences recombination</li> </ul>
StpA (suppressor of <i>td</i> mutant phenotype A)	<ul style="list-style-type: none"> <li>• basic</li> <li>• 10000–15000 copies/cell</li> <li>• high amino acid identity with H-NS</li> <li>• able to form heterodimers with H-NS</li> </ul>	dimer?, n × 15.3 kDa	<i>stpA</i> (60.2 min)	<ul style="list-style-type: none"> <li>• RNA chaperon</li> <li>• recognizes curved DNA</li> </ul>

All HLPs bind DNA non-specifically (some of them show a higher affinity for a specific DNA sequence) and act as transcriptional regulators. Molecular mass data are taken from [3]. N–A,G,C or T; R–G or A; W–T or A; Y–C or T.

# La proteina HU

## Caratteristiche

- proteina basica
- molto abbondante 30000 copie/cellula
- la più abbondante tra le proteine del nucleoside
- nessuna sequenza consenso di legame al DNA

Struttura

pM

geni

eterodimero

Hua

9.2 kDa

hupA (90.4 min)

Hub

9.5 kDa

hupB ( 9.9 min)

# La proteina HU

## Funzioni

- compatta il DNA in strutture nucleosoma-simili
- induce curvatura nel DNA
- riconosce il DNA curvo
- interviene nella ricombinazione generale

Isolata nella maggior parte delle specie batteriche  
proteine simili ad HU conservate anche negli Archea

Anticorpi anti HU si legano sulla superficie del nucleoside piuttosto che al suo interno supportando l'ipotesi che HU si associ anche all'RNA e svolga un ruolo anche nella trascrizione e traduzione dei mRNA



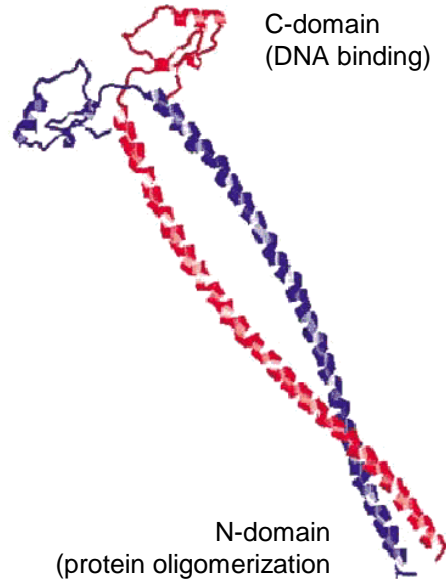
**IHF**



**HU**



**H-NS**



C-domain  
(DNA binding)

N-domain  
(protein oligomerization)

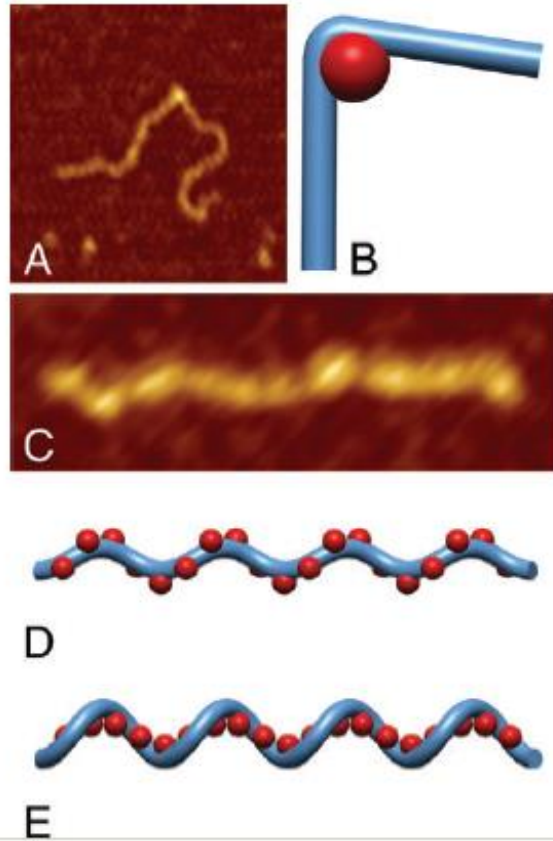
**FIS**



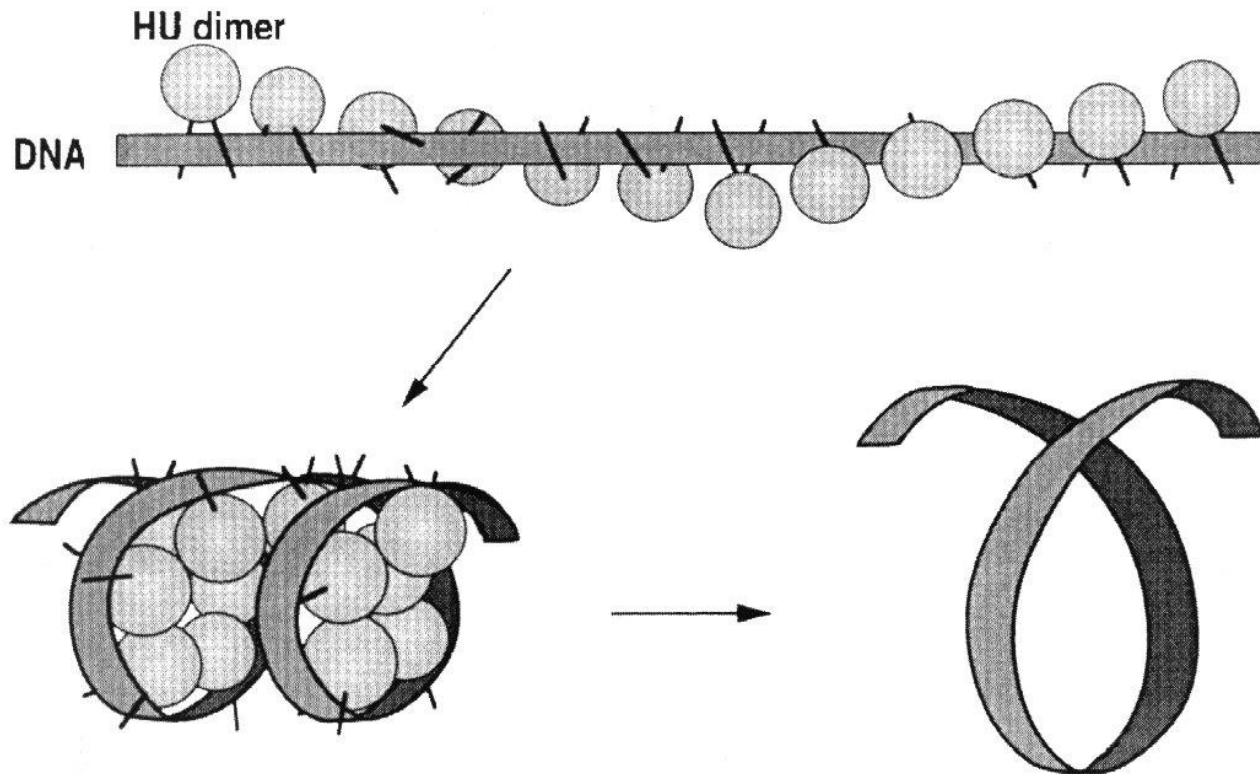
*adapted from  
Ussery et al., 2001*

Nucleoid  
proteins are  
homo- or  
heterodimers

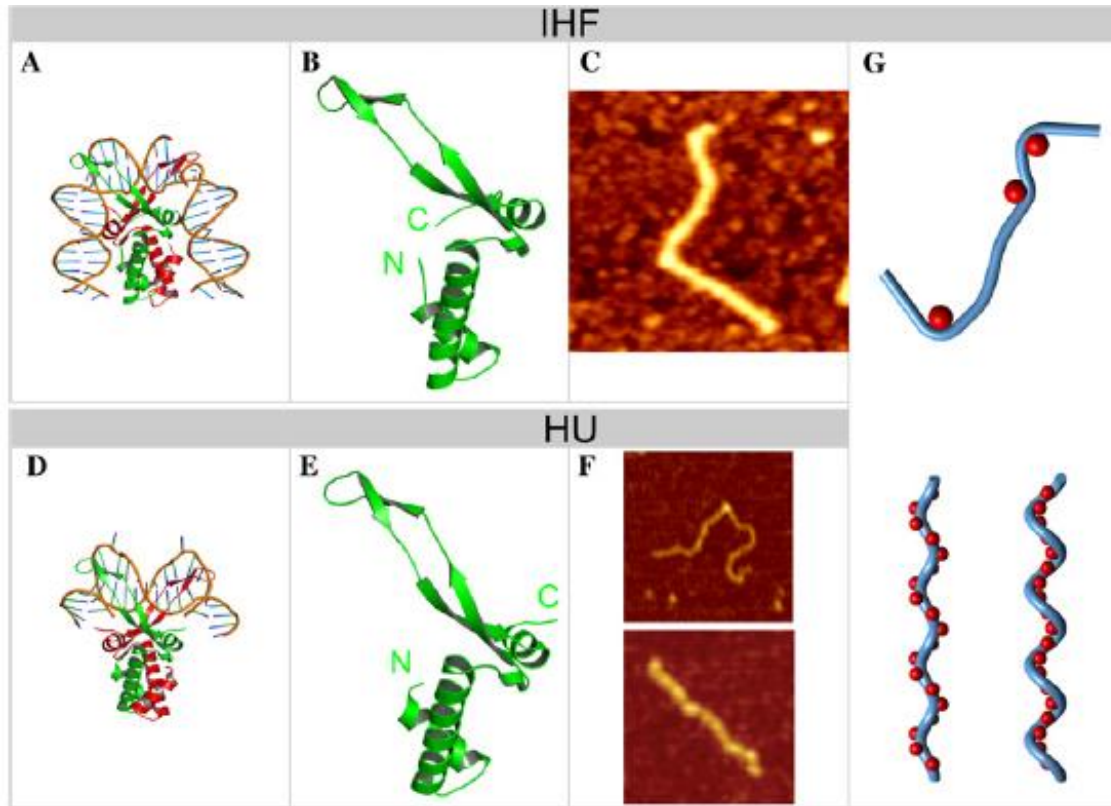
# HU lega il DNA e lo ripiega



Superavvolgimento mediato da HU. I dimeri di HU si legano in vitro a distanza di circa 9 bp. In vitro associazione di 8-10 dimeri con frammenti di 275-290 bp  
Il legame di HU ogni 9 bp prevede interazioni con i dimeri adiacenti con formazione di una struttura solenoidale che si avvolge in senso sinistrorso. Ciascun avvolgimento toroidale avverrebbe intorno a 6 dimeri di HU



# Le proteine che ripiegano il DNA



# La proteina IHF Integration Host factor

## Caratteristiche

- proteina basica
- 5-10 volte meno abbondante di HU
- abbondante in fase stazionaria
- debole specificità di sequenza per il legame al DNA  
(YAANNNTTGATW)

Struttura  
Eterodimero

pM

geni

IHF $\alpha$

11.2 kDa

himA (38.6 min)

IHF $\beta$

10.5 kDa

himD ( 25 min)

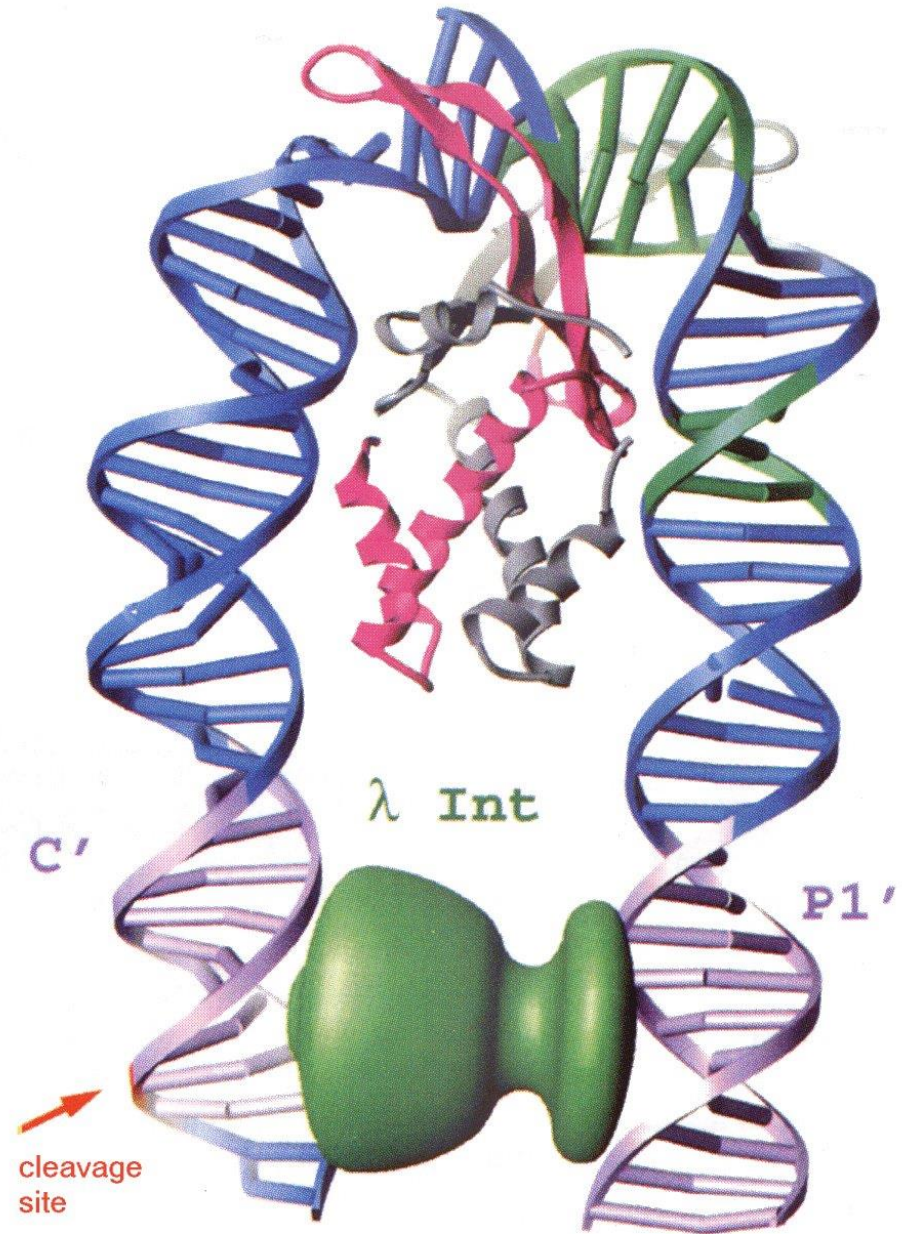
# IHF ; Caratteristiche funzionali

- Induce forte curvatura nel DNA ( fino a  $140^\circ$ )
- Interviene nella ricombinazione sito specifica
- Interviene nella trasposizione

Si lega al solco minore del DNA utilizzando due foglietti B che in opposizione l'uno all'altro prendono contatto con il DNA

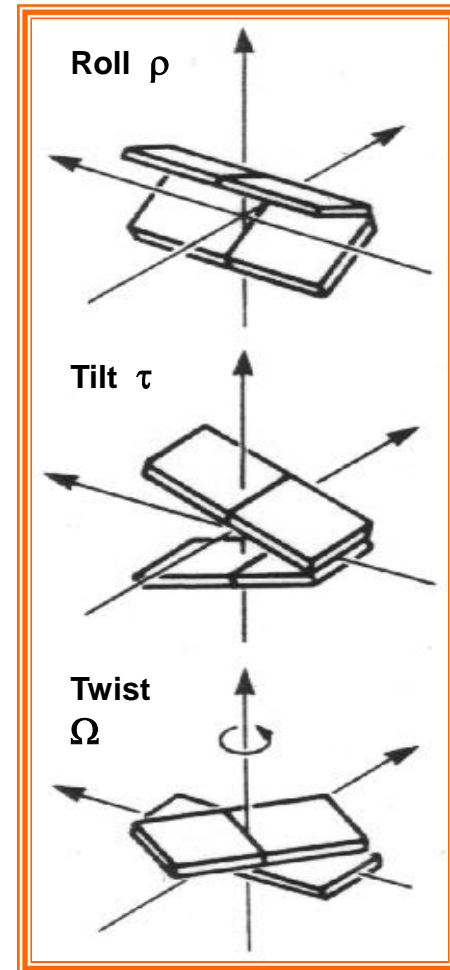
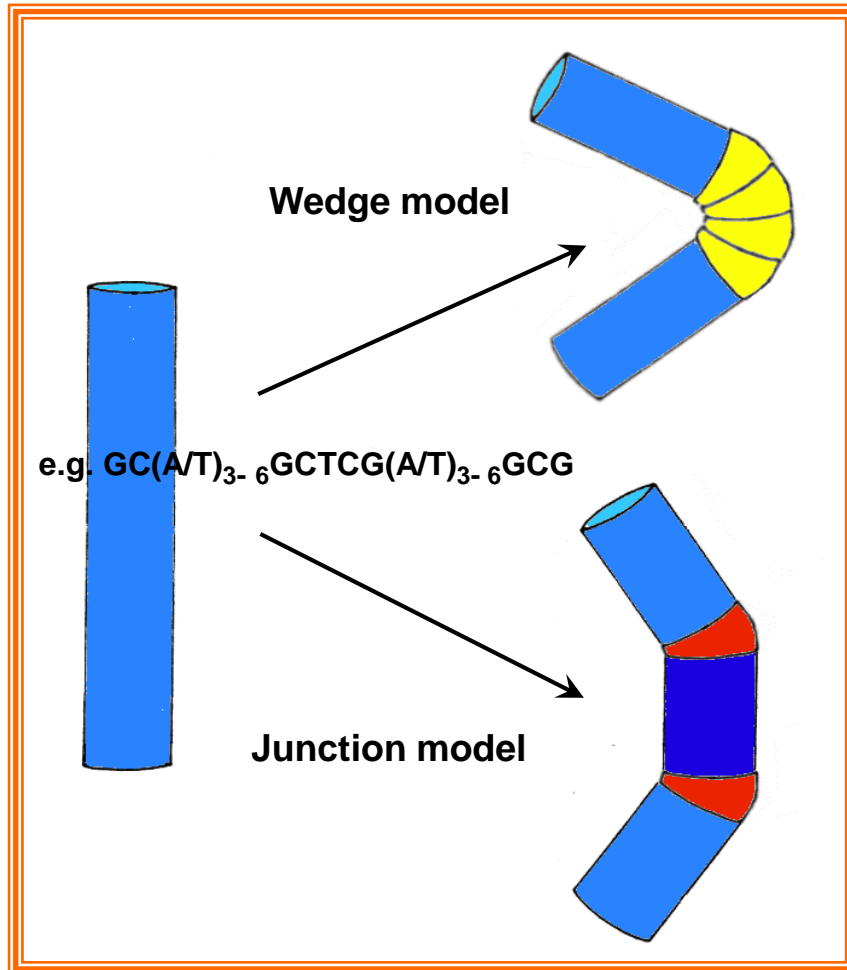
- Isolata come fattore dell'ospite per l'integrazione del fago  $\lambda$  è coinvolta in molte funzioni cellulari.
- Forte ruolo strutturale; curvando il DNA fino a  $140^\circ$  permette a siti di DNA distanti di trovarsi ravvicinati favorendo così sia processi di trascrizione che di regolazione

La curvatura mediata da IHF può facilitare il legame della Proteina Int con siti di DNA distanti tra loro.





# Intrinsically curved DNA



# FIS Factor for Inversion Stimulation

## Caratteristiche

- Proteina basica
- abbondante in fase esponenziale
- 10.000-60.000 copie
- scarsa specificità di sequenza  
(KNNYRNNWNNYRNNM)

W TA  
R GA  
K GT  
Y CT

Struttura

pM

geni

OMODIMERO

FIS

2x 11.5 kDa

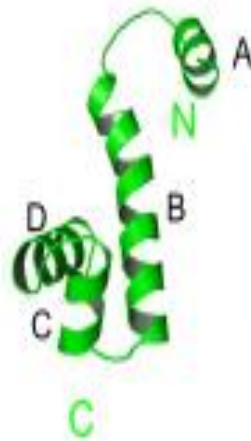
fis (27.4 min)

# FIS

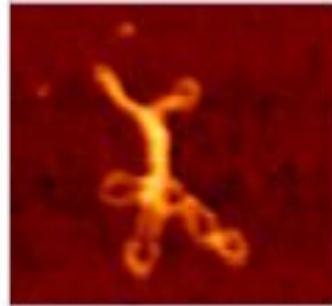
H



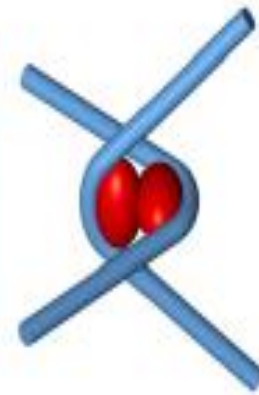
I



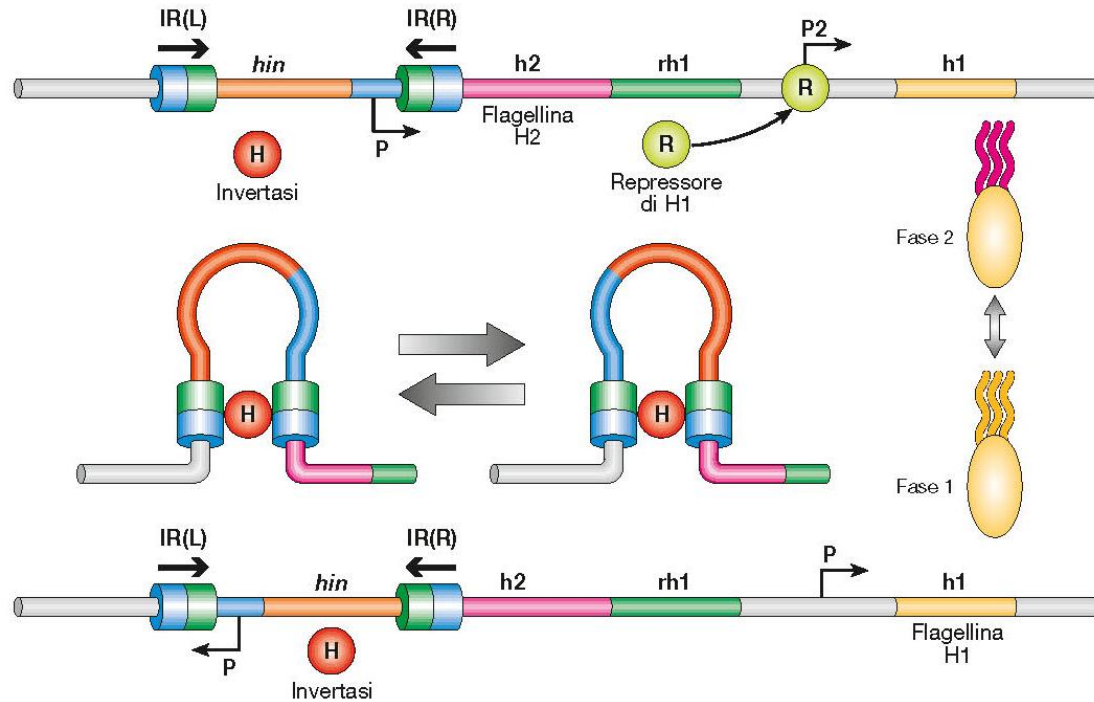
J



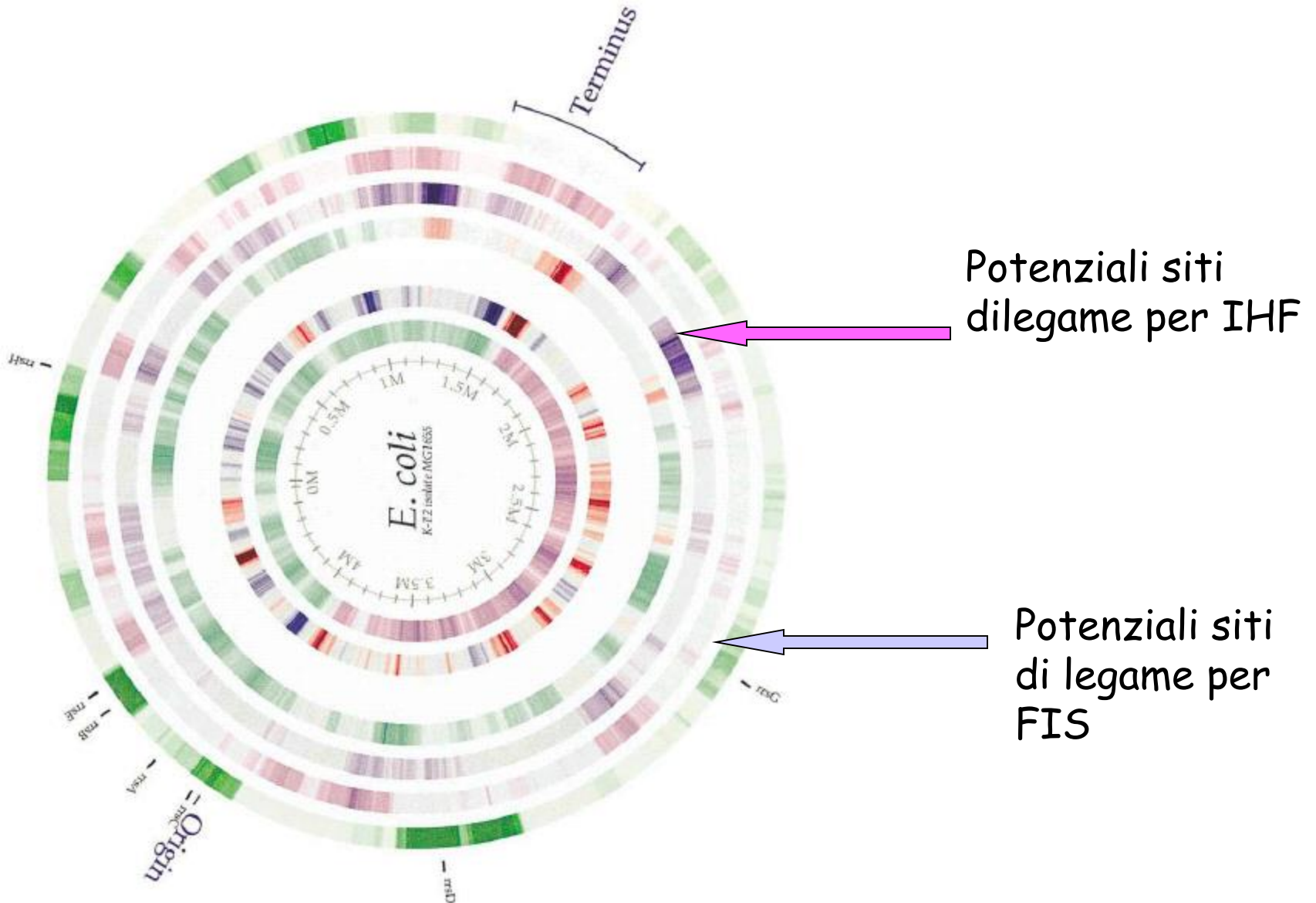
K



# L'inversione di fase in *Salmonella* è mediata dalla proteina Hin e dalla proteina Fis dell'ospite

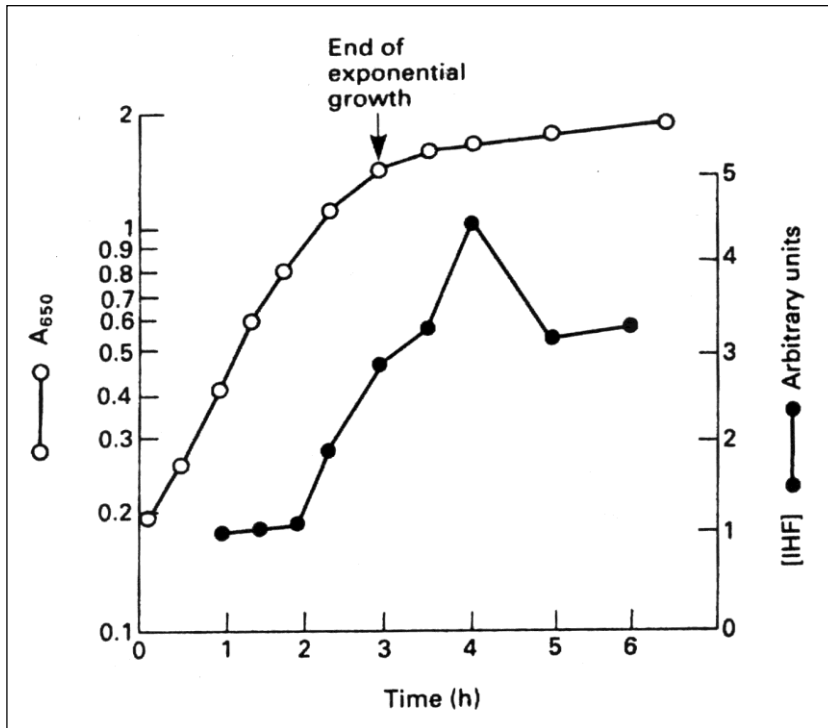


# Proteine del nucleotide come regolatori globali

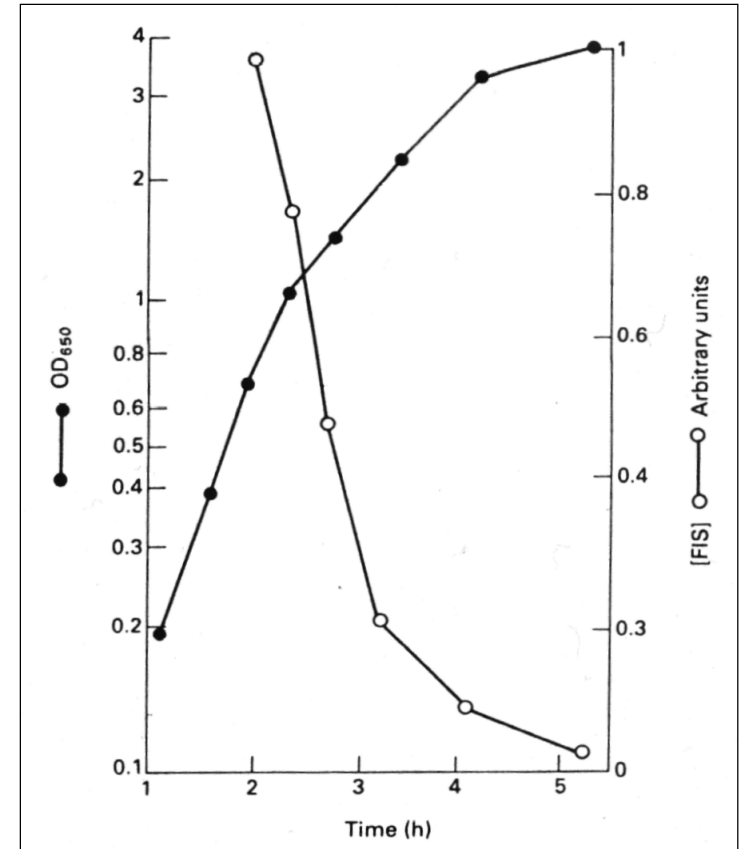


## Espressione di IHF e FIS in funzione della curva di crescita

La concentrazione di IHF aumenta in funzione della curva di crescita e raggiunge il massimo all'inizio della fase stazionaria



La proteina FIS è invece molto abbondante all'inizio della fase esponenziale



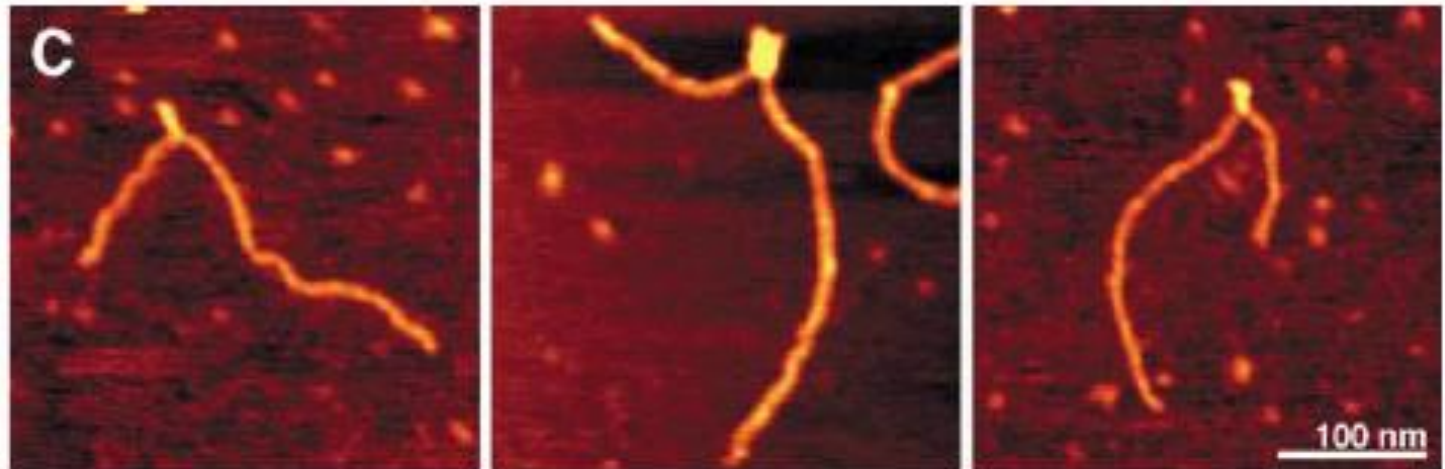
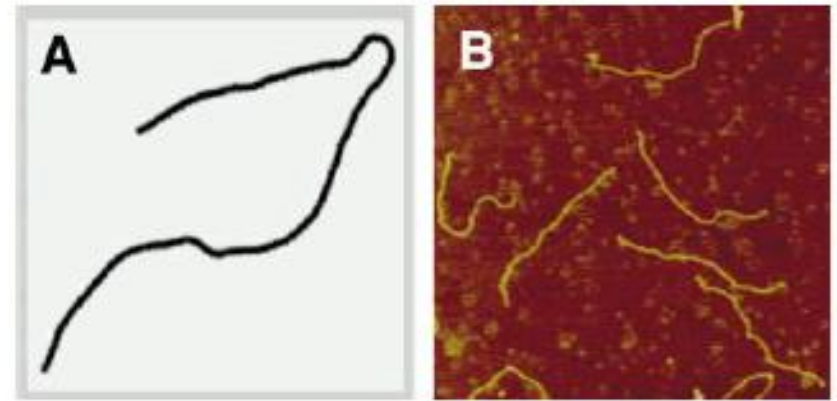
# H-NS

(Histone-like Nucleoid Structuring protein)

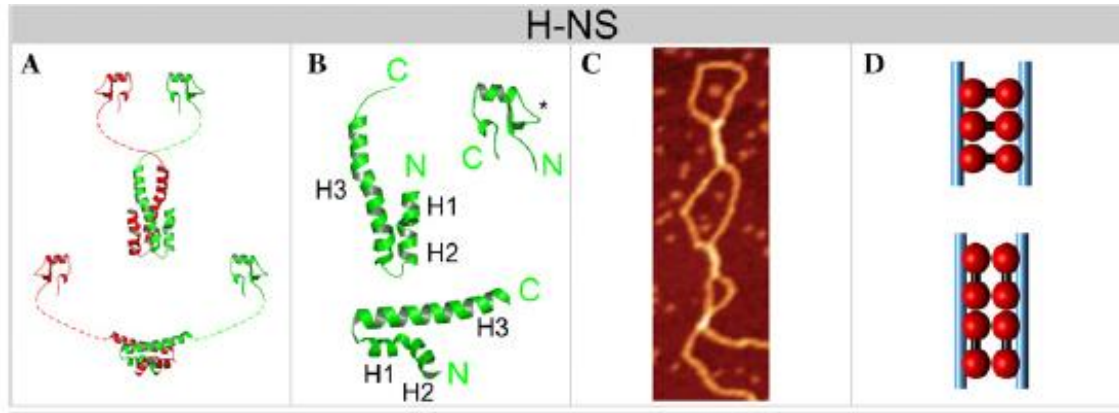
- Abundant peptide (~ 20000 copies/cell)
- Small (136 aminoacids, 15.5 kD), non-basic peptide
- Mainly acts as a homodimer or tetramer
- Able to form heterodimers with StpA or HhA
- *In vitro* binding to DNA is non-specific; induces high DNA compaction
- Higher affinity for intrinsically curved DNA; able to bend DNA *in vitro*
- Global regulator: controls 5 % of the whole *E.coli* protein coding sequences
- Generally acts as a transcriptional repressor of virulence genes outside the host



La proteina H-NS  
riconosce sequenze di  
DNA curvo ed è in grado  
di indurre curvatura nel  
DNA



# Le proteine NAP che oltre a ripiegare il DNA formano ponti : un esempio H-NS

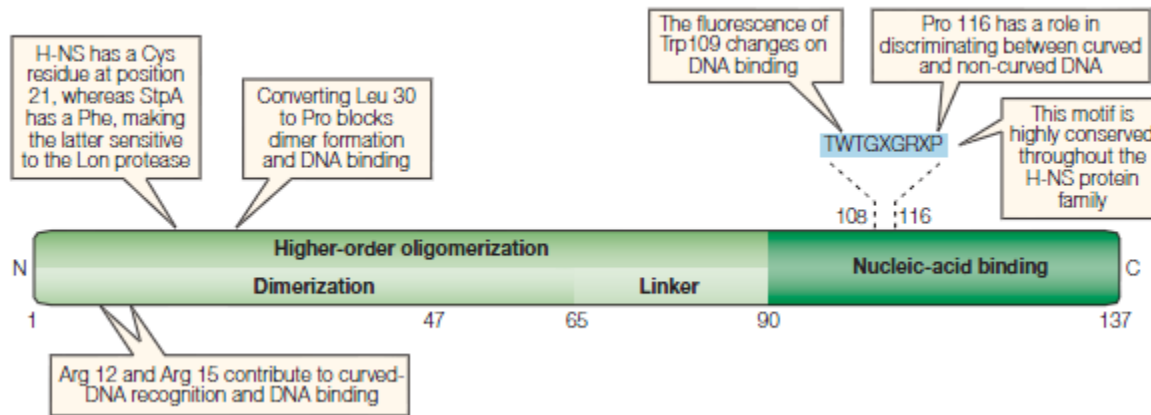


**H-NS viene considerata una proteina in grado di formare ponti**

- A) Strutture proposte per il dimero di H-NS
- B) Analisi dei monomeri di H-NS
- C) Formazione delle anse mediate da H-NS
- D) 2 diversi modelli di interazione nella formazione complessi

Altre proteine NAP che formano ponti su LRP e MuKB

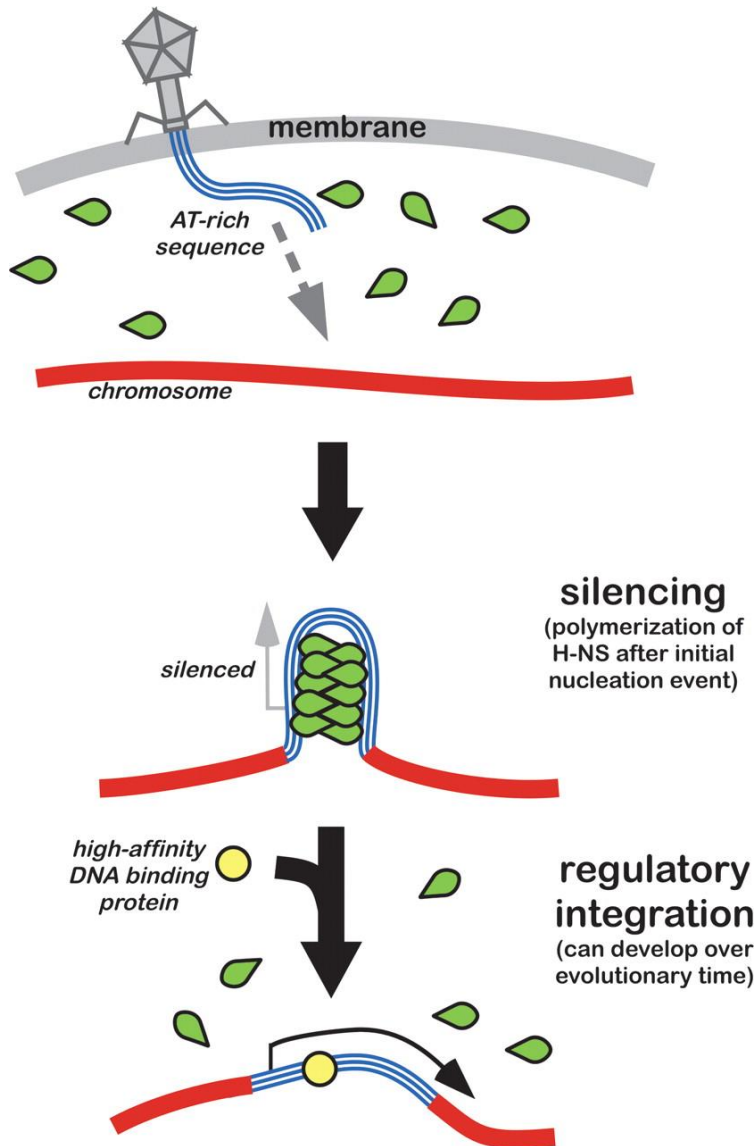
# Struttura della proteina H-NS



La proteina H-NS è costituita da soli 137 AA

Il dominio di oligomerizzazione è localizzato al N terminale ed è costituito da brevi sequenze di AA ( 1-8, 12-19, e 23-47) capaci di formare 3 strutture ad alfa elica. I linker flessibili che separano le 3 a eliche permettono alle eliche 1 e 2 di ripiegarsi facilitando la formazione di oligomeri tra i diversi dimeri

# H-NS ed il silenziamento di regione geniche acquisite per HGT



Il legame di H-NS a regioni di DNA esogeno ricche in AT silenzia l'espressione genica.

L'eventuale presenza di una proteina regolatrice sequenza specifica con un elevata affinità per il DNA può competere con H-NS per eliminare il silenziamento e permettere nuovamente l'espressione genica in condizioni specifiche.

In questo modo, la cellula ospite può tollerare la presenza di sequenze di DNA estraneo e in seguito inserire la sequenza in un network di regolazione preesistente

# H-NS e il silenziamento dei geni acquisiti tramite HGT

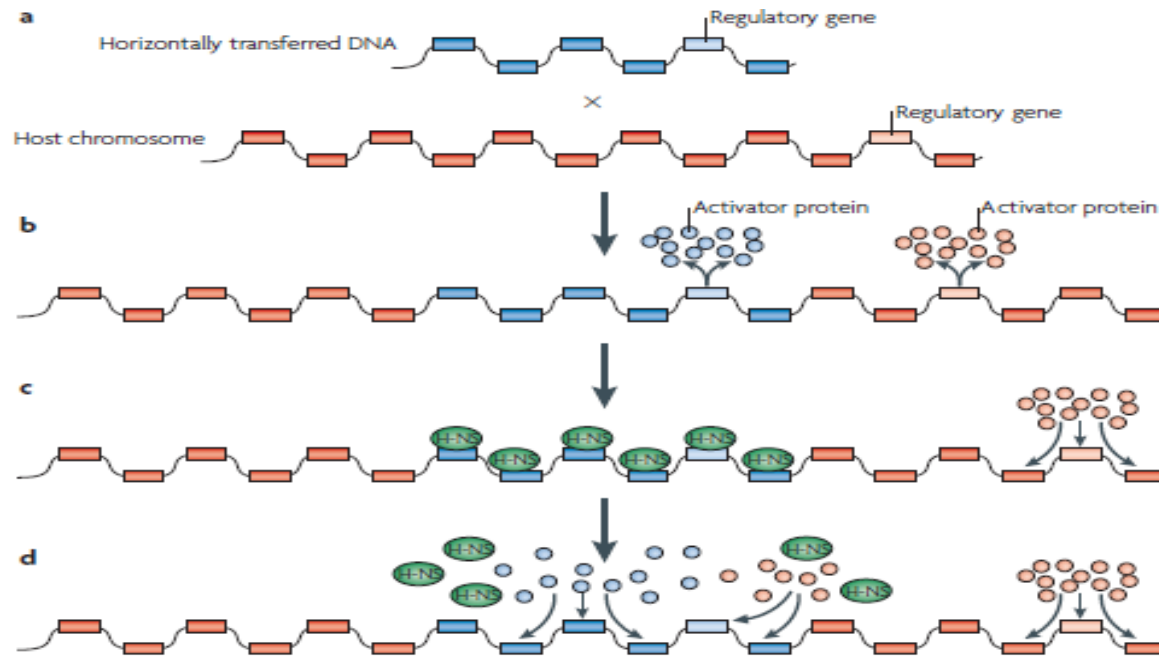
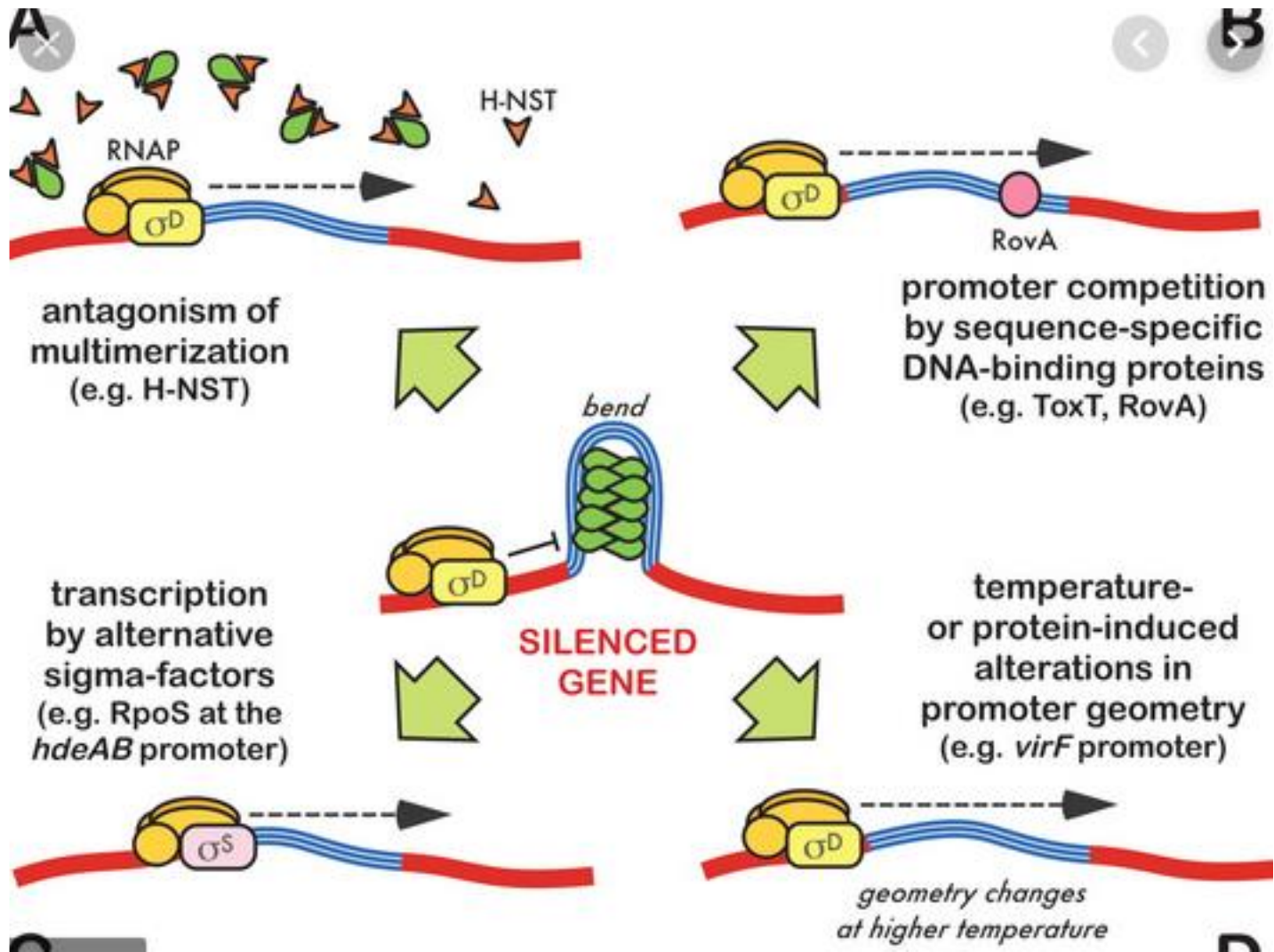
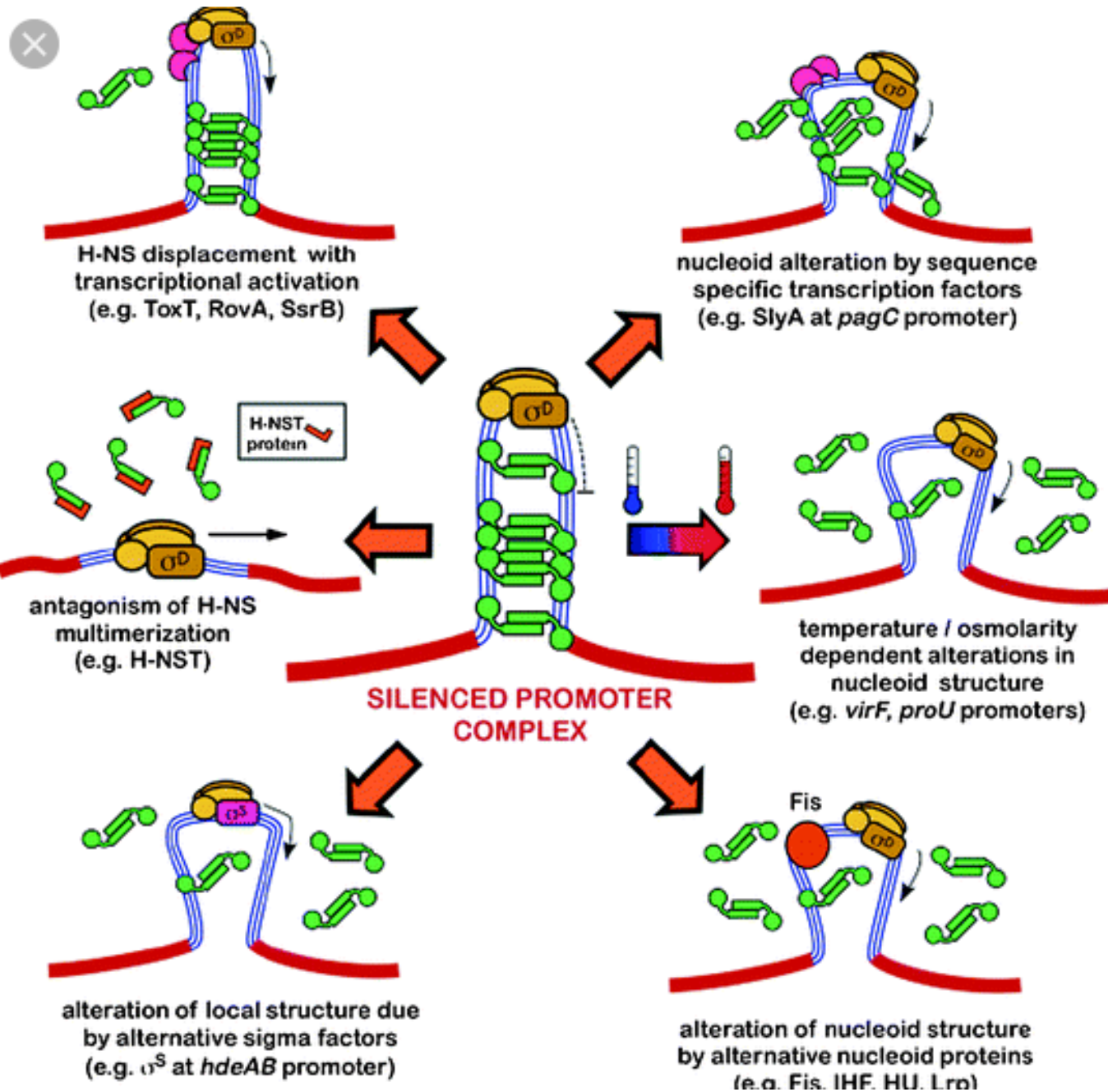


Figure 1 | **H-NS as a gene silencer.** The figure shows a model for the involvement of H-NS in the repression of horizontally transferred genes, and how this repression can be relieved. **a** | A segment of DNA carrying six genes enters the cell as a result of horizontal transfer. One of these is a regulatory gene that encodes a transcription activator that is specific for its own gene and the other five genes in the cluster. The newly arrived genes integrate into the chromosome of the host bacterium (shown in red). **b** | Once inserted, the horizontally transferred genes and the ancestral genes coexist as a contiguous DNA sequence that is distinguished by the higher AT content of the insertion (blue). **c** | The H-NS protein quickly targets and downregulates the promoters of the genes with high AT sequences. **d** | This transcription repression can be relieved in numerous ways. Changes to DNA structure, particularly the planar curvature, induced by environmental signals, such as an increase in temperature, might dislodge H-NS. The activator protein encoded by the horizontally transferred regulatory gene (blue) might displace H-NS by the same mechanism. An activator encoded by a regulatory gene in the ancestral chromosome (red) might displace H-NS by the same mechanism. A regulatory relationship between the ancestral activator and the new DNA sequences could arise by different routes: (i) suitably positioned sites for activator binding might fortuitously already exist in the horizontally transferred genes; (ii) the activator protein might evolve to bind to appropriately positioned sites; (iii) sites might evolve in the horizontally transferred DNA to which the ancestral activator can now bind; (iv) or some combination of these scenarios might apply.

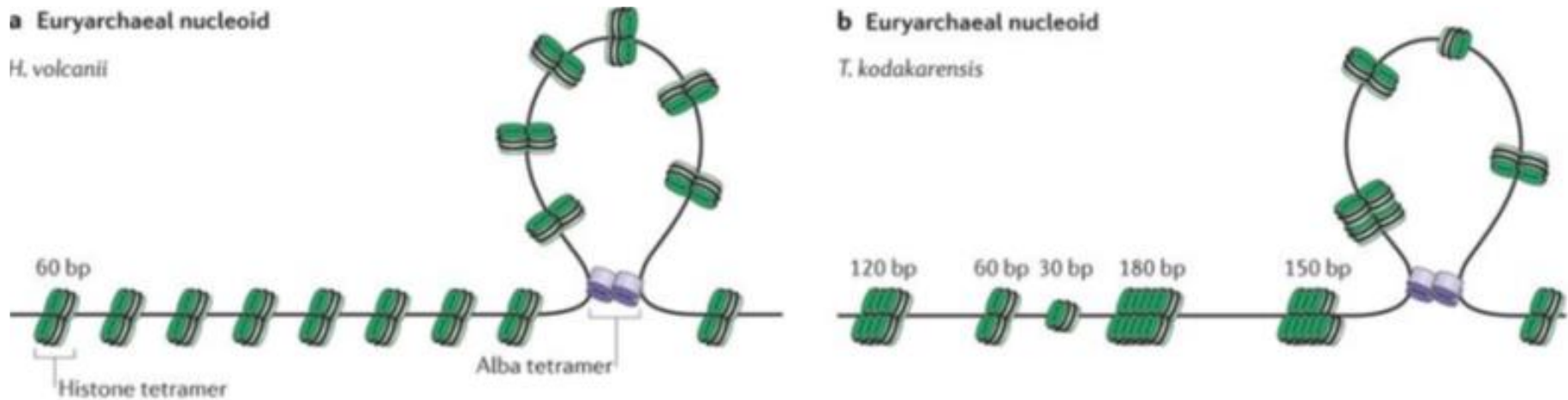








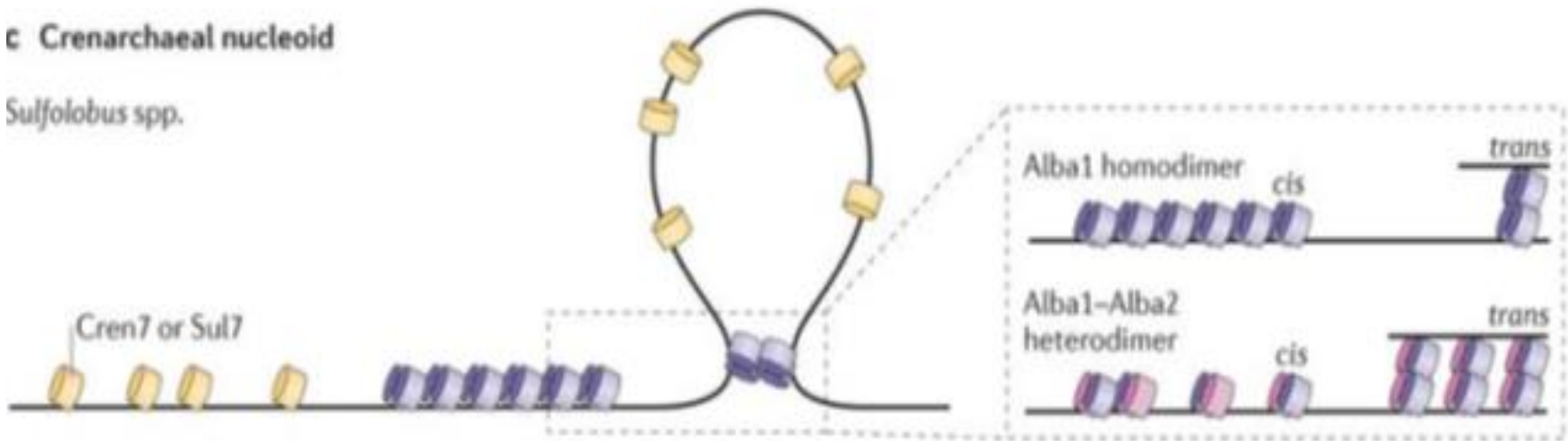
The structure of the archaeal nucleoid varies among different archaeal species depending on the chromatin proteins they express



**a,b** | The euryarchaeal nucleoid is mainly organized by histone proteins that bend or wrap DNA, as well as by Alba that binds to DNA as a homodimer or a heterodimer and that forms looped structures by bridging two DNA duplexes. In *Haloferox volcanii*, histone proteins form tetrameric nucleoprotein structures that wrap about 60 bp of DNA around their surface (part **a**). These nucleosomes form a regular 'beads-on-a-string' structure similar to eukaryotic chromatin. In *Thermococcus kodakarensis*, histone proteins assemble into multimeric forms that cover variable sizes of DNA ranging from 30 bp (indicative of a dimer binding) to 450 bp (part **b**).

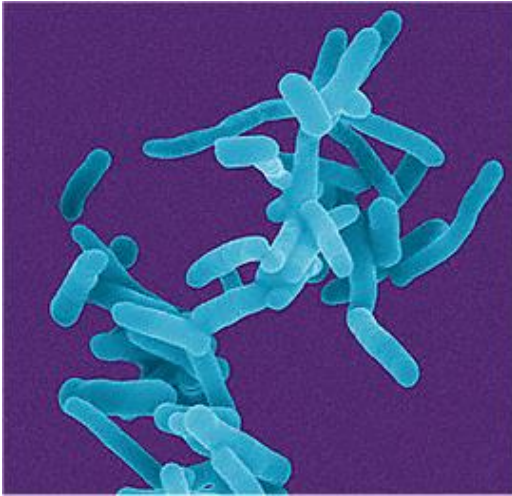
### c Crenarchaeal nucleoid

*Sulfolobus* spp.



c | The crenarchaeal nucleoid is organized by proteins that bend DNA (for example, Cren7 and Sul7 in *Sulfolobus* spp.), as well as by Alba that either forms looped structures by bridging two DNA duplexes or forms stiff filaments by binding cooperatively side by side. The best-studied chromatin proteins belong to the Alba superfamily, which is widely distributed and almost universally present in archaea<sup>15</sup>. Alba seems to have an ancient evolutionary history and considerable functional plasticity<sup>16</sup>. Most Alba proteins interact with RNA in addition to binding to double-stranded DNA (dsDNA) and have been suggested to function in RNA metabolism. In euryarchaeal methanogenic archaea, Alba proteins are low-abundance, sequence-specific dsDNA-binding proteins<sup>19</sup>, whereas in **crenarchaeal** organisms, it was shown that **Alba is a highly abundant cellular** protein that binds to dsDNA without apparent sequence specificity. Alba assembles into dimers, which are homodimeric or heterodimeric depending on whether paralogues are encoded and on their relative amounts.

# Shigella

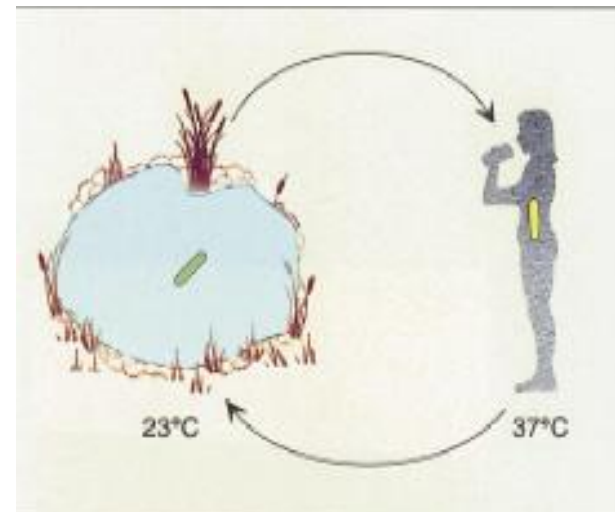


- is a Gram negative, facultative anaerobe
- is an intracellular pathogen
- is the etiological agent of bacillary dysentery, an acute diarrheal disease
- causes 160 million of episodes, determining 1.1 million deaths/year in children and infants in developing countries.

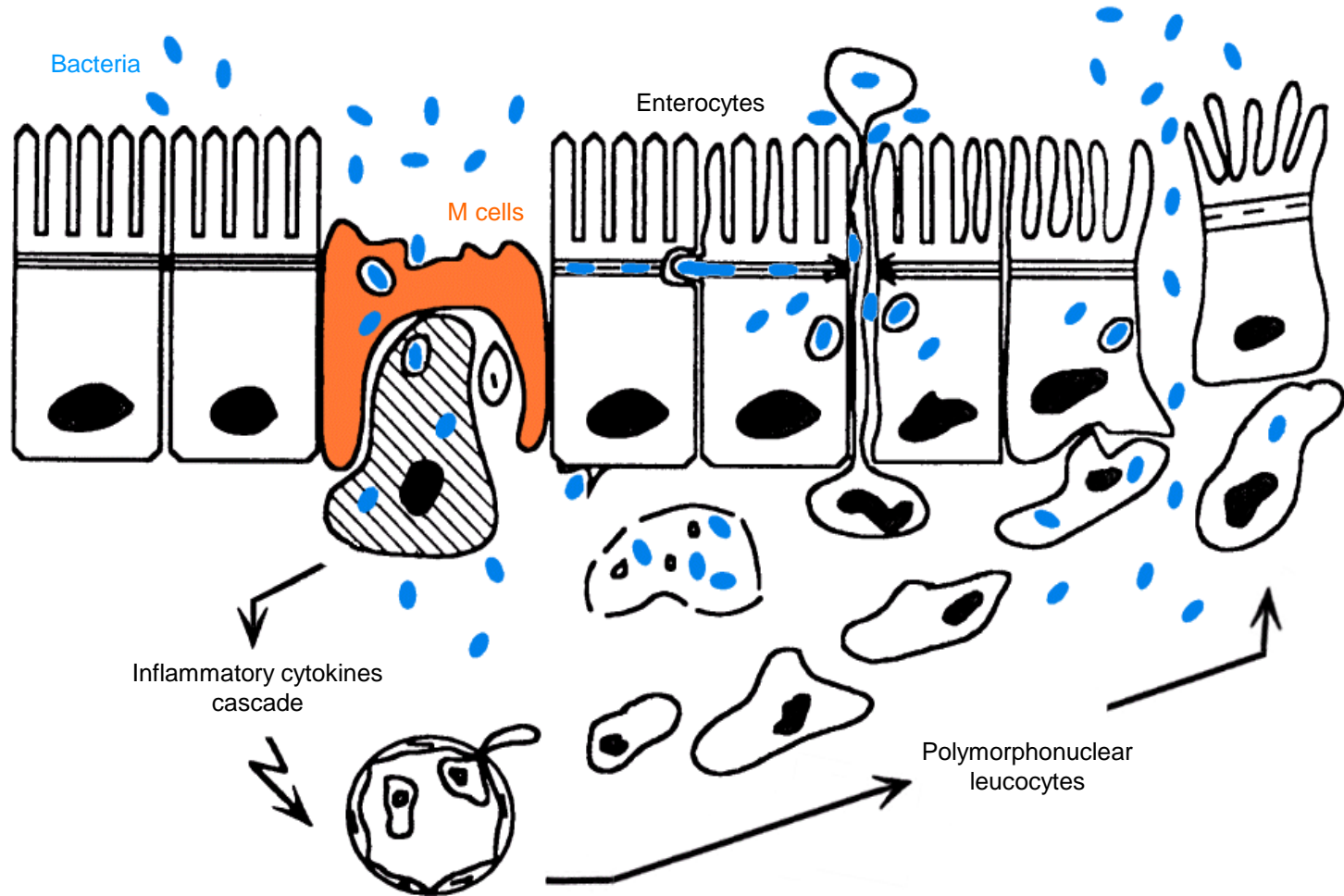
Infection is spread via fecal-oral route

Subgrouped in four "species":

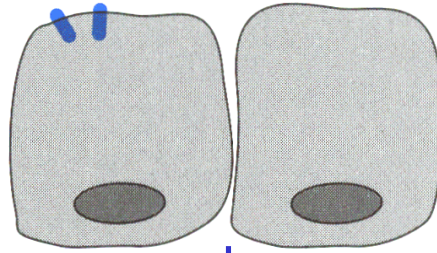
- *Shigella flexneri*
- *Shigella dysenteriae*
- *Shigella boydii*
- *Shigella sonnei*



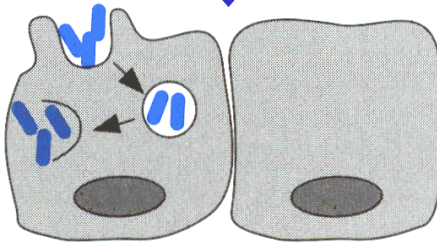
# Model for *Shigella* invasion of the colonic mucosa



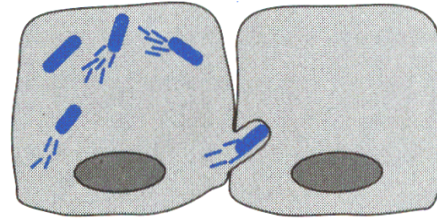
IpaD



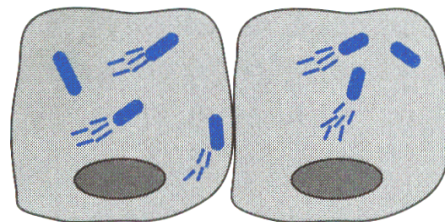
IpaB,  
IpaC




IcsA  
(virG)



IcsB



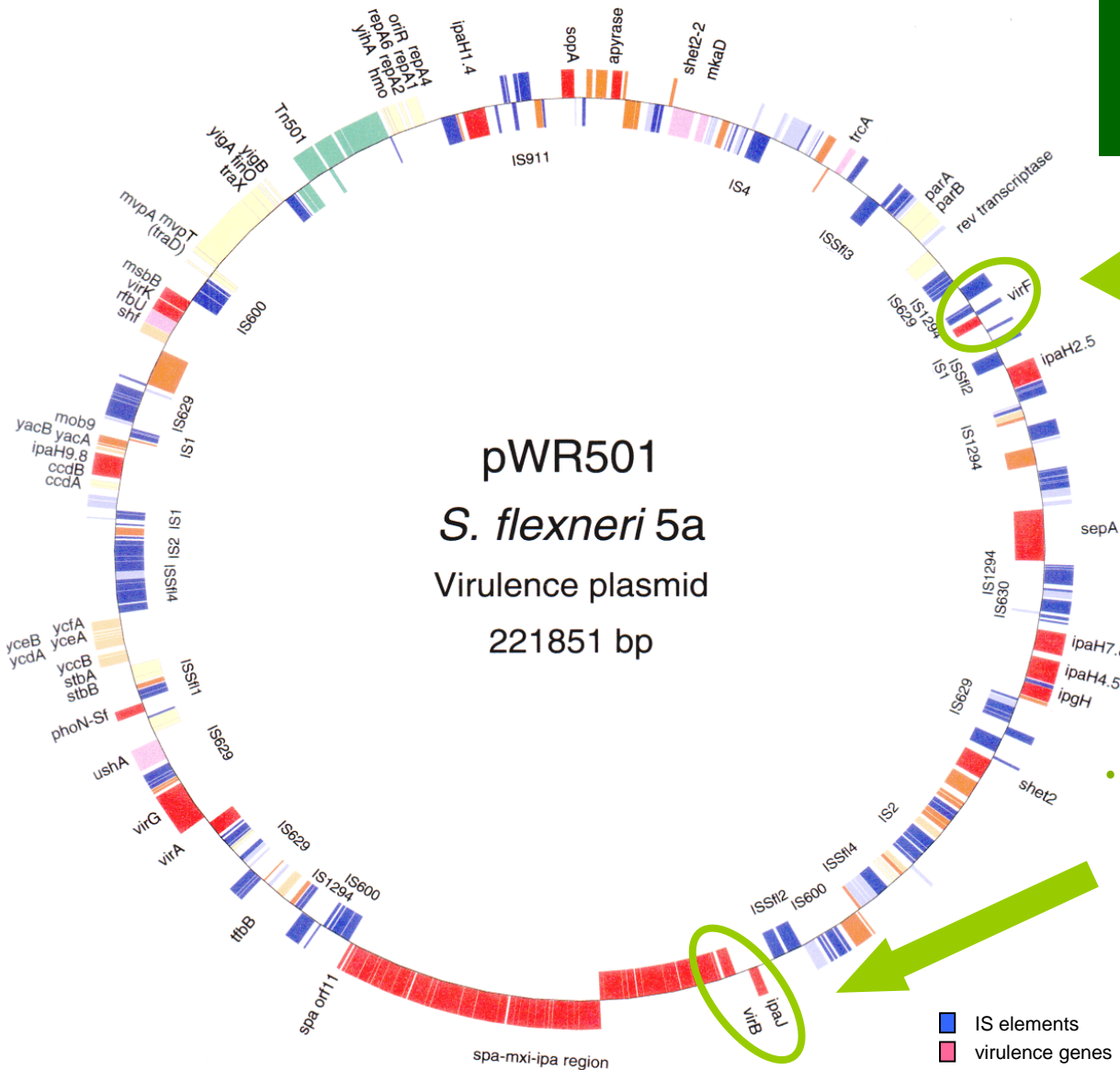
Actin  
filaments 

nucleus 

Proteins involved  
in the invasion  
process are  
encoded by  
a virulence  
plasmid (pINV)



# Genetic organization of pINV



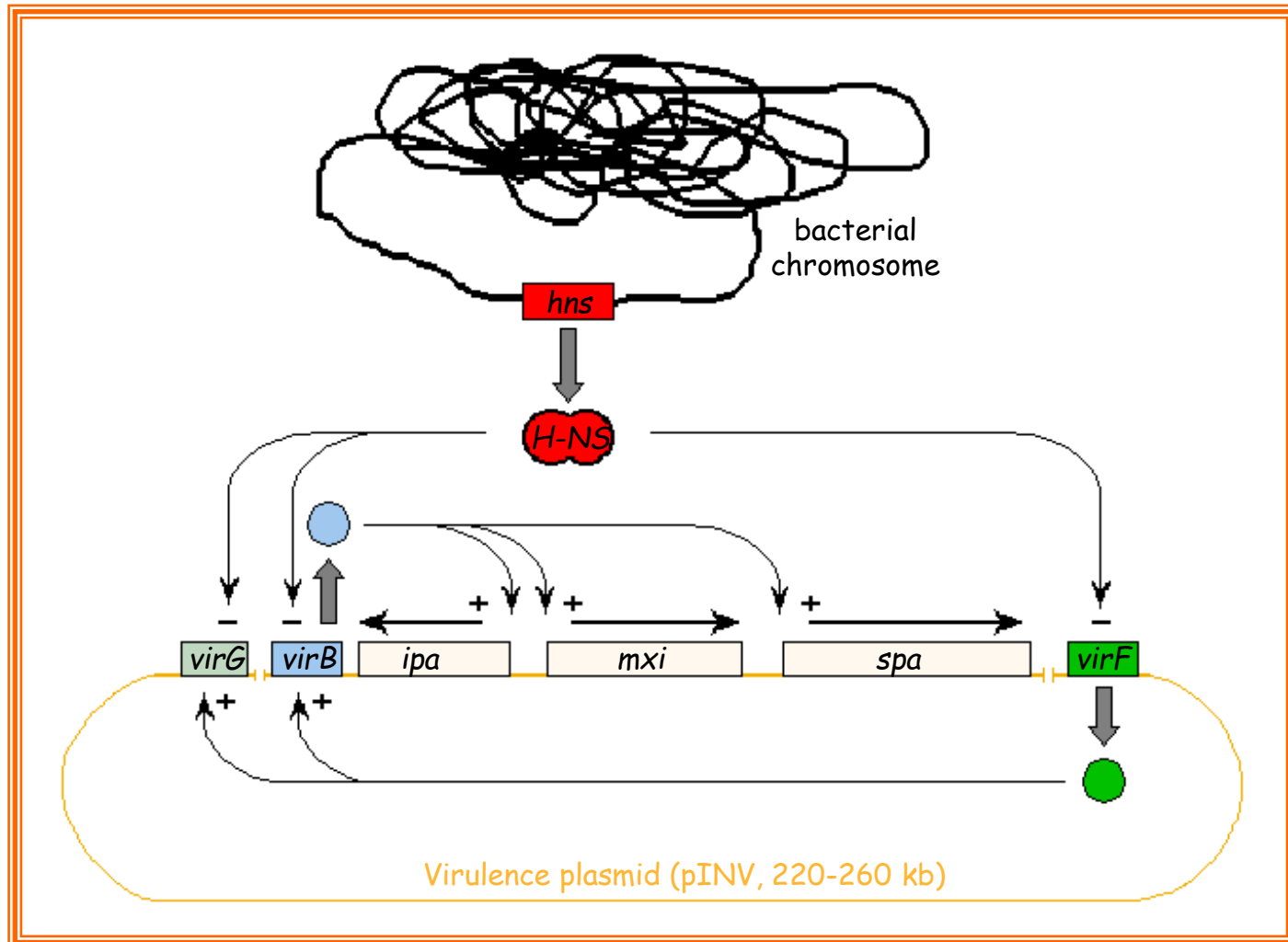
*virF* is ...

- ... located on a "desert island"
- ... the first positive activator of pINV virulence genes

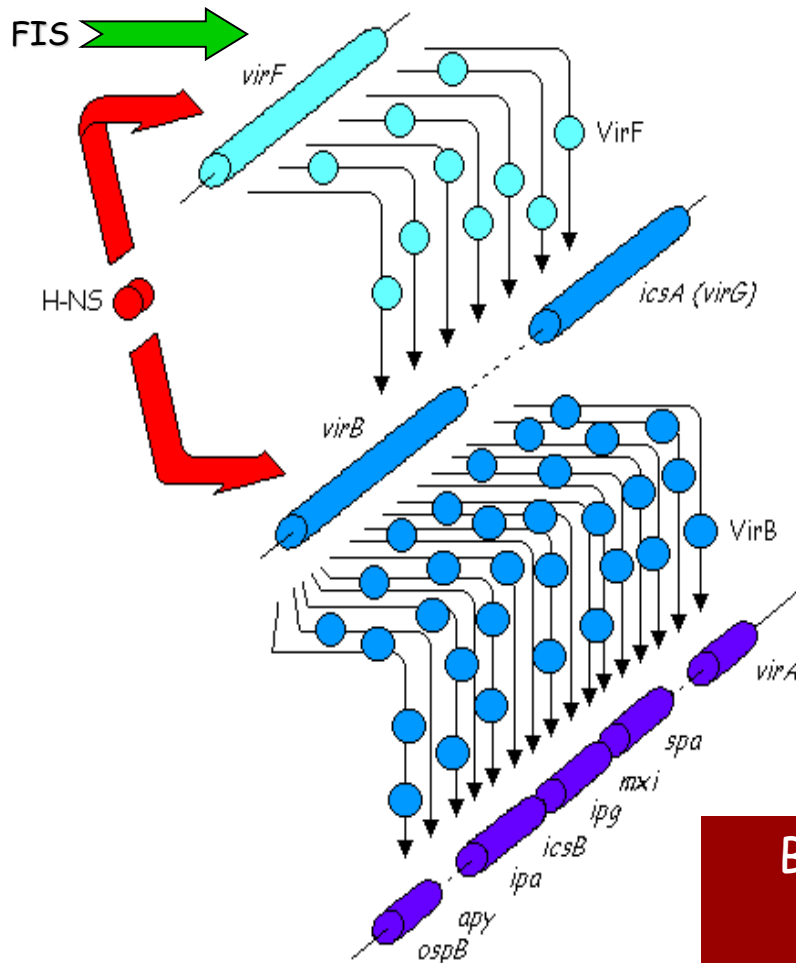
*virB* is ...

- ... located within the main Pathogenicity Island
- ... the second positive regulator of the plasmid virulence regulons

# H-NS controls the virulence regulon in *Shigella* and in *E. coli* EIEC



# The expression "cascade" of virulence genes



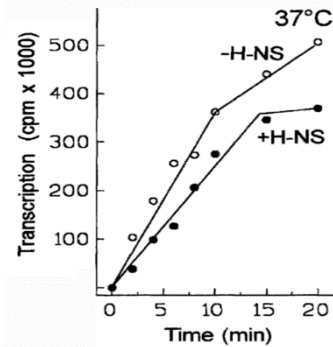
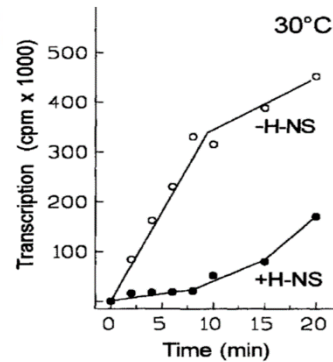
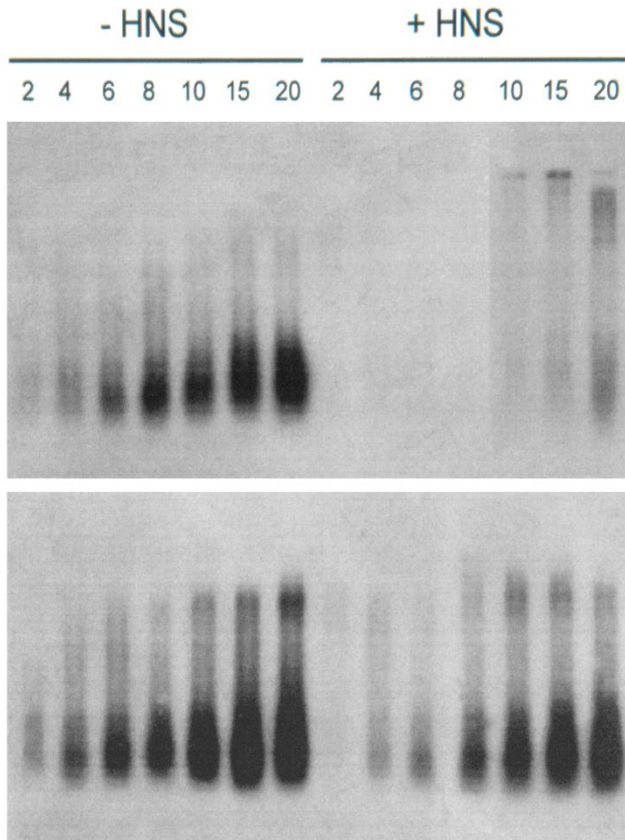
VirF is ...

- ... expressed at 37°C
- ... is controlled antagonistically by two nucleoid proteins H-NS (repressor) and FIS (activator)

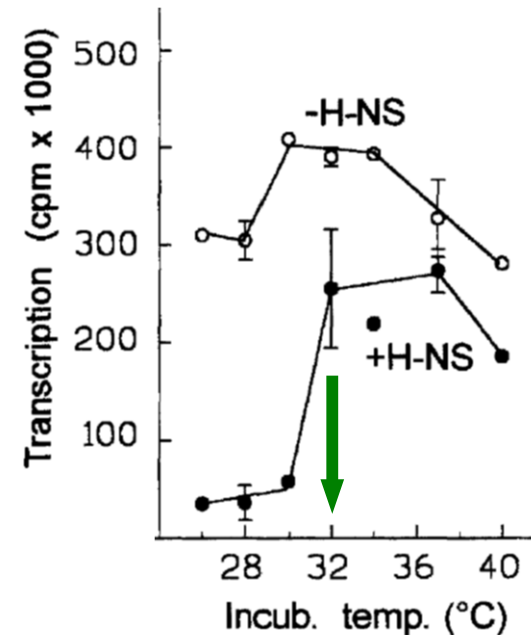
By which mechanism is the *virF* gene allowed to be expressed only at the host temperature?



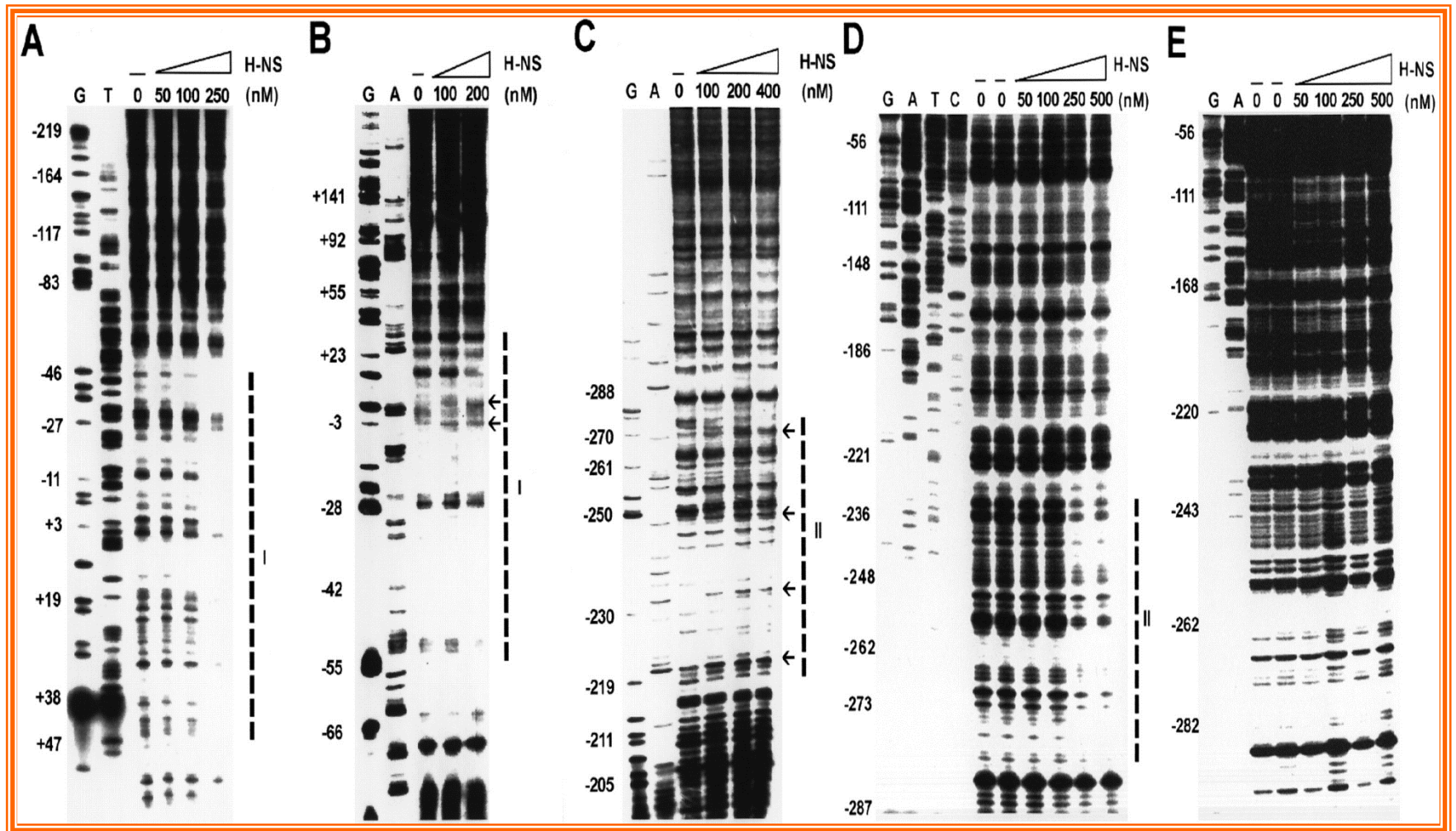
# H-NS is able to bind to and repress *virF* only at low temperature



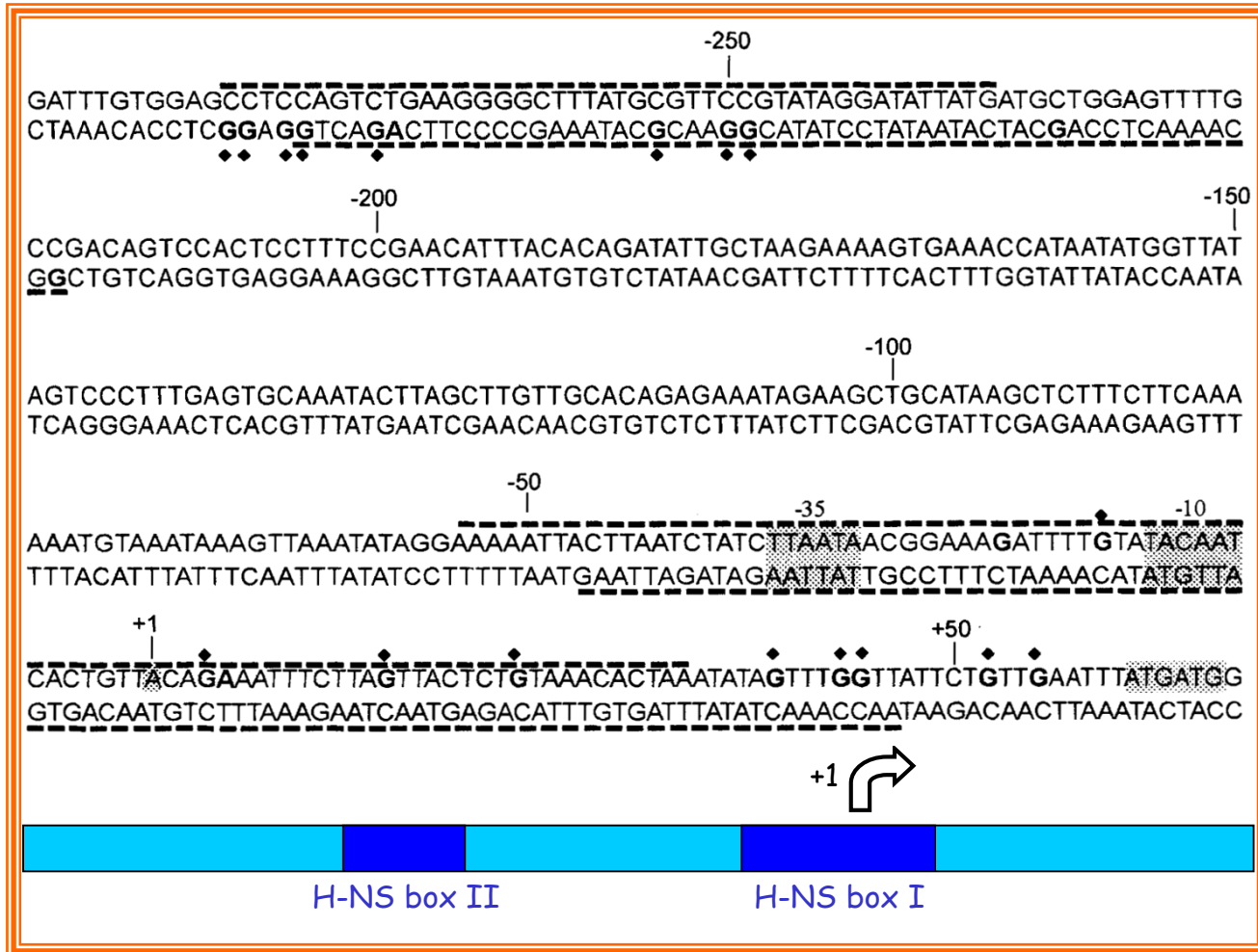
...more precisely:  
*virF* transcription is inhibited  
by H-NS only below 32°C



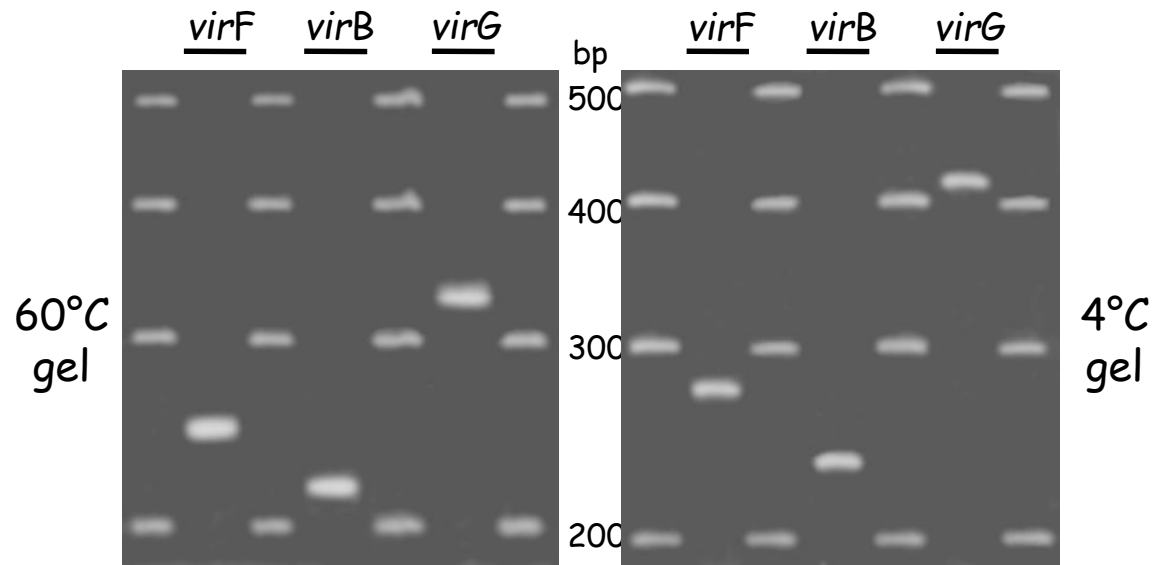
# DNaseI footprinting of the *virF* promoter region by H-NS



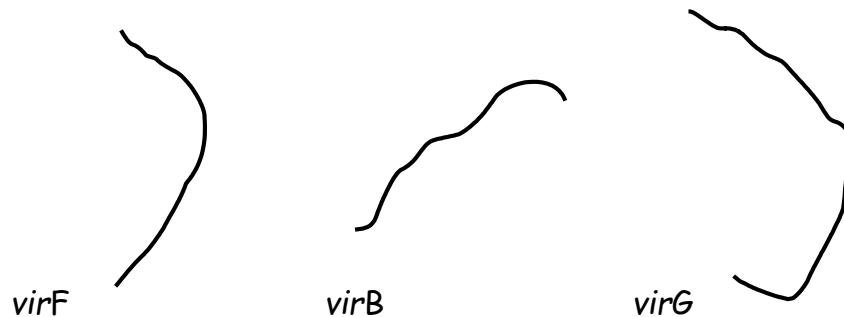
# The *virF* promoter region



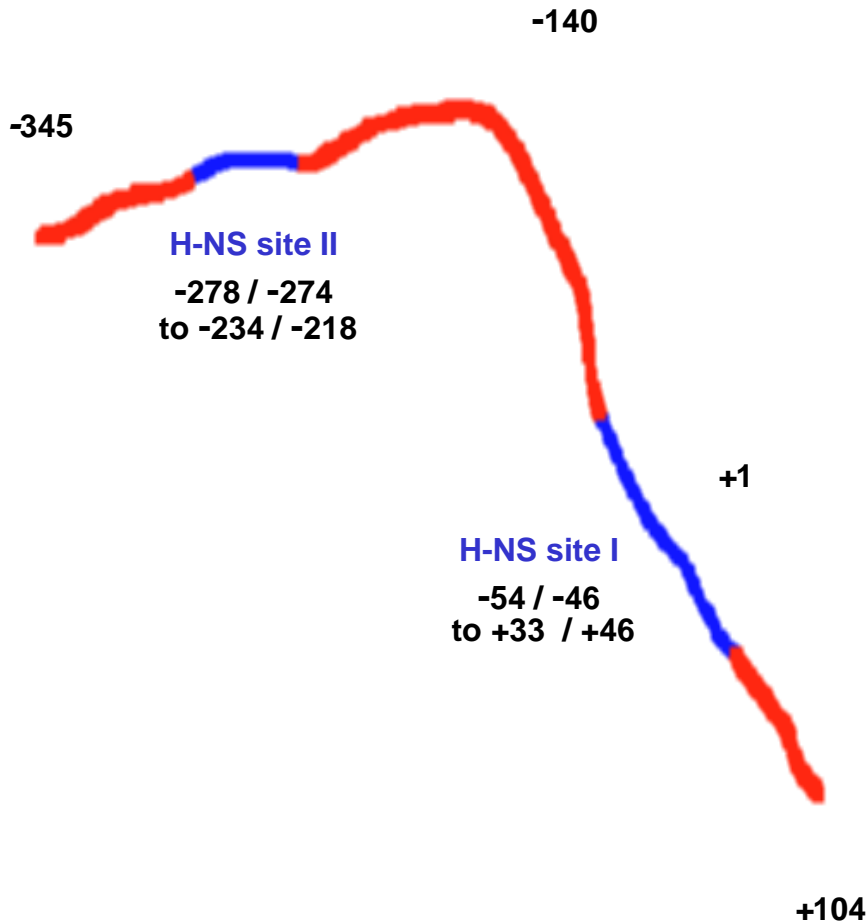
# Curvature in *H*-NS regulated *vir* promoters



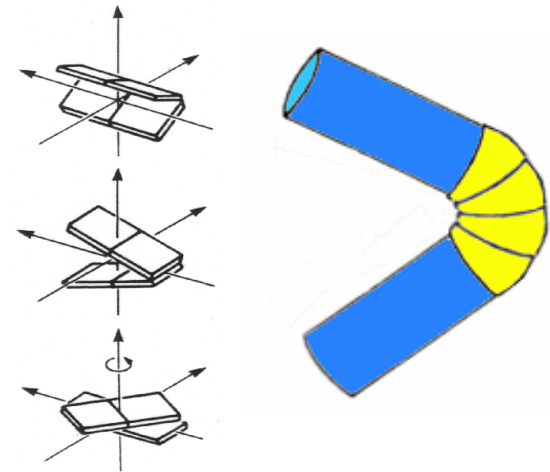
Computer-generated models



Within the *virF* promoter region H-NS recognizes two sites separated by a region endowed with significant intrinsic curvature

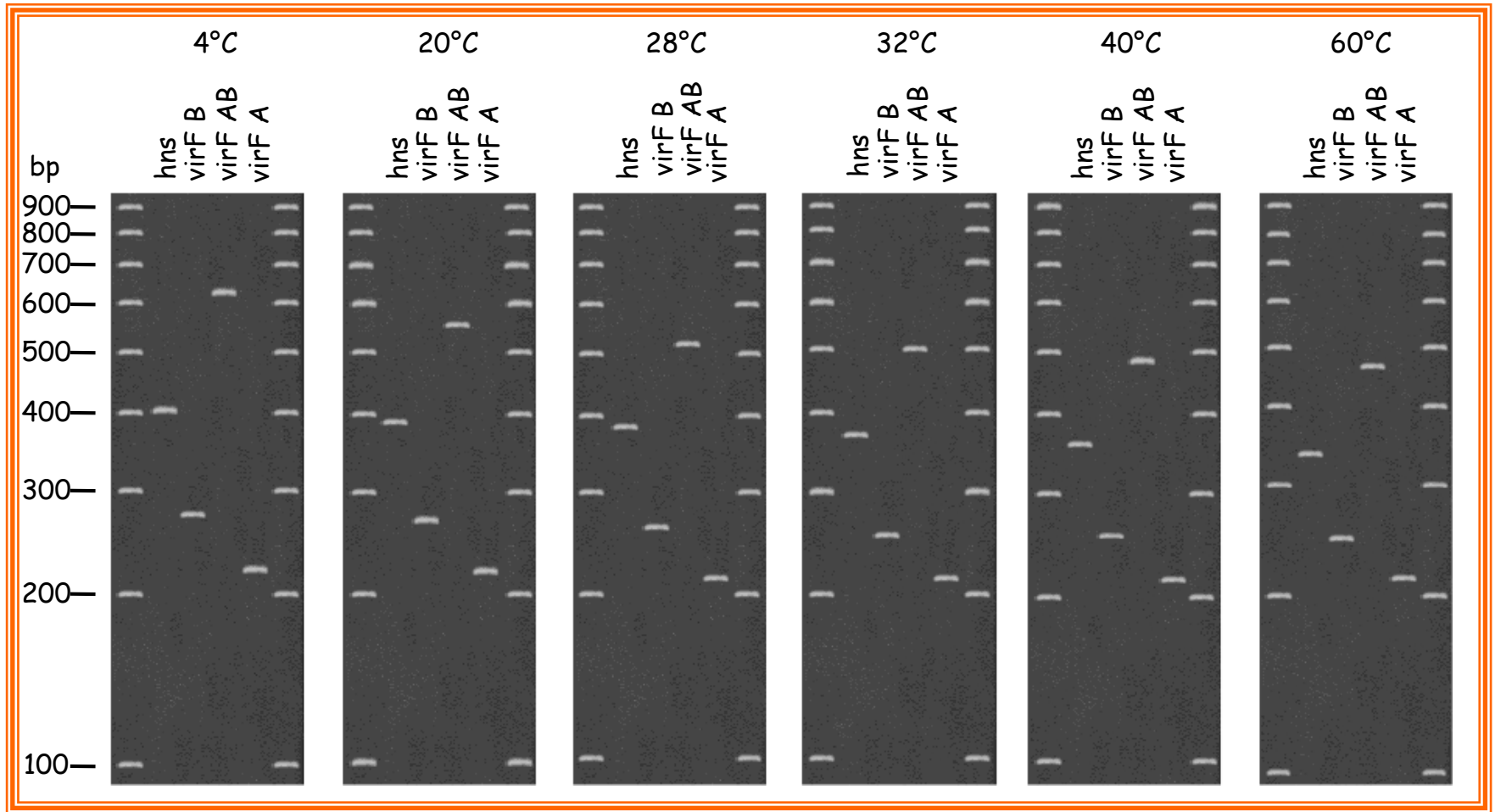


DNA bending : the **wedge model** for sequence mediated curvature



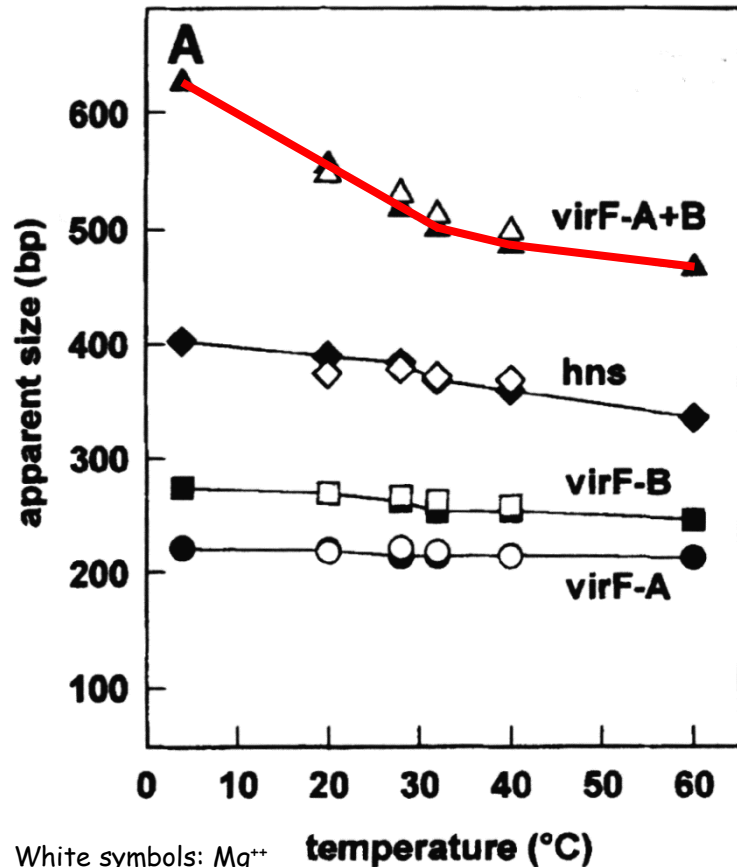
# Temperature-dependent curvature of the *virF* promoter

Acrylamide separations

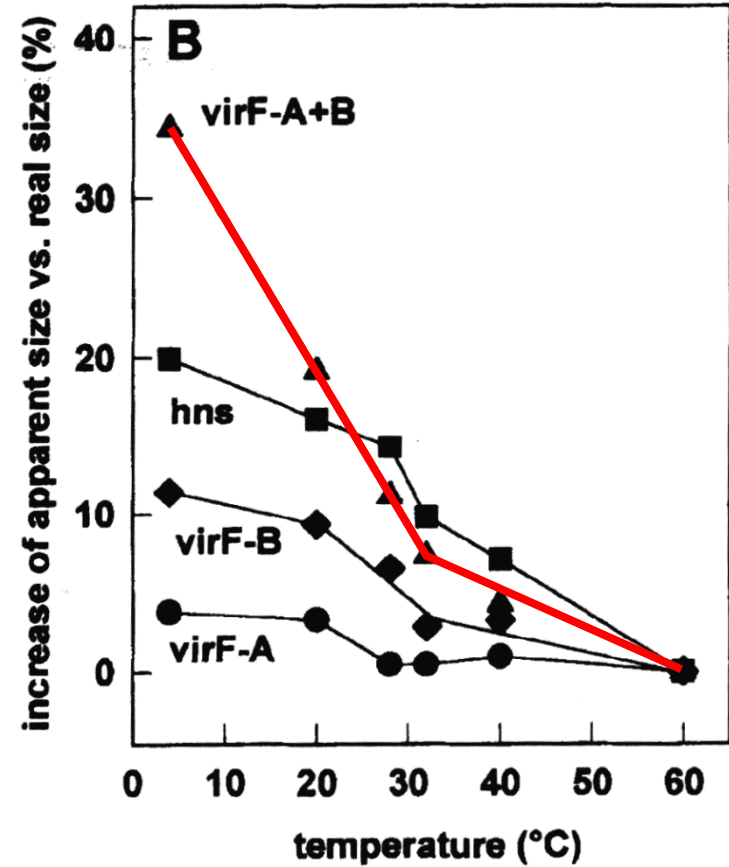




# The curvature of the *virF* promoter is strongly temperature-dependent

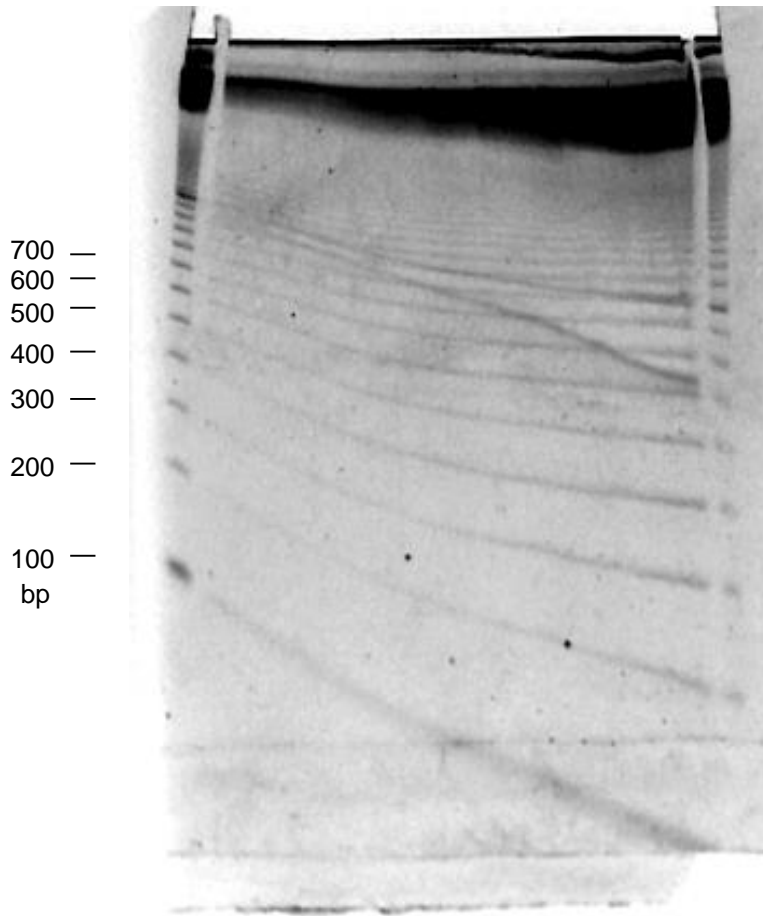


White symbols: Mg<sup>++</sup>  
Black symbols: no Mg<sup>++</sup>



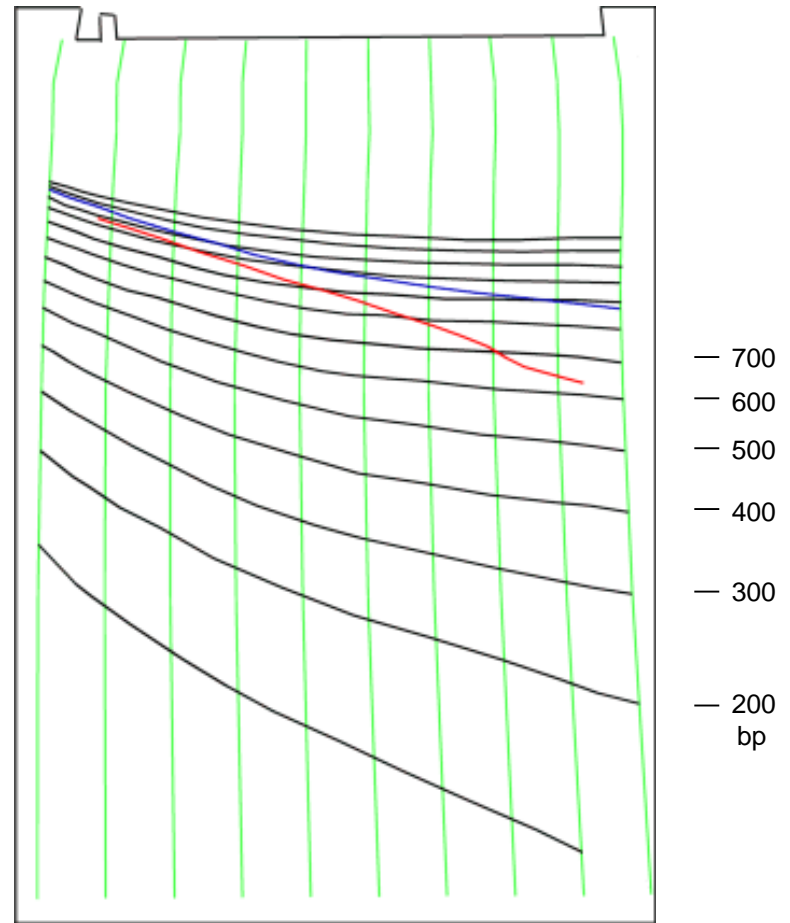
# Temperature-dependent curvature of the *virF* promoter

TGGE separation



17°C

39°C



17°C

20°C

22.5°C

24.5°C

27°C

29.5°C

31°C

33.5°C

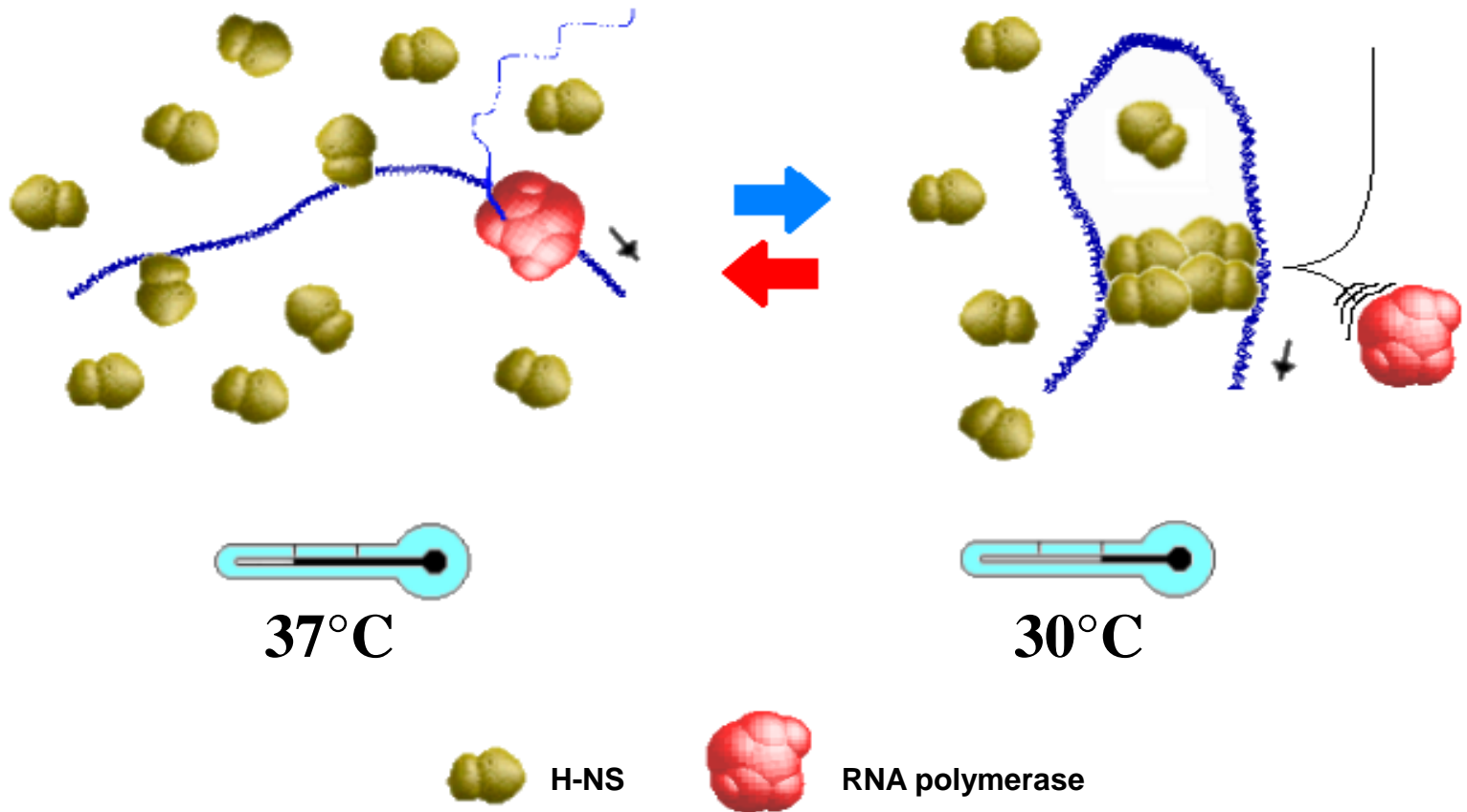
36°C

39°C



# Temperature-dependent *virF* expression

Working model

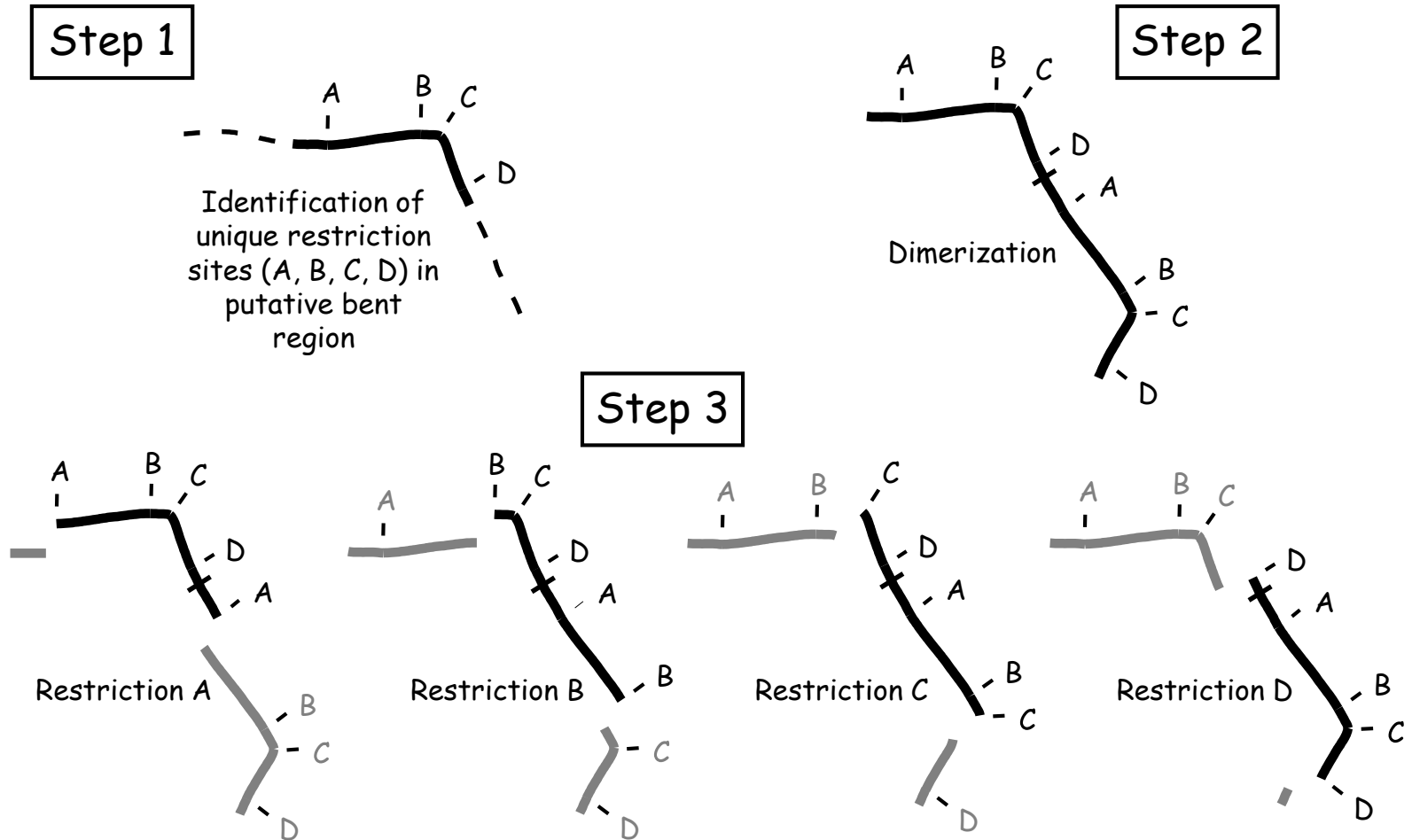


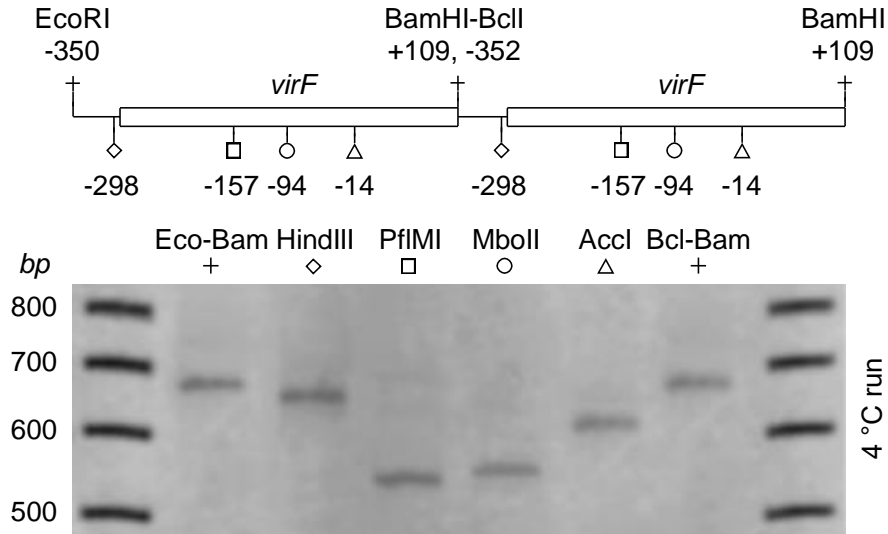
By which mechanism is the *virF* gene allowed to be expressed only at the host temperature?

Small RNAs are emerging as key regulators of virulence gene expression in bacteria. Is this true also in *Shigella*?

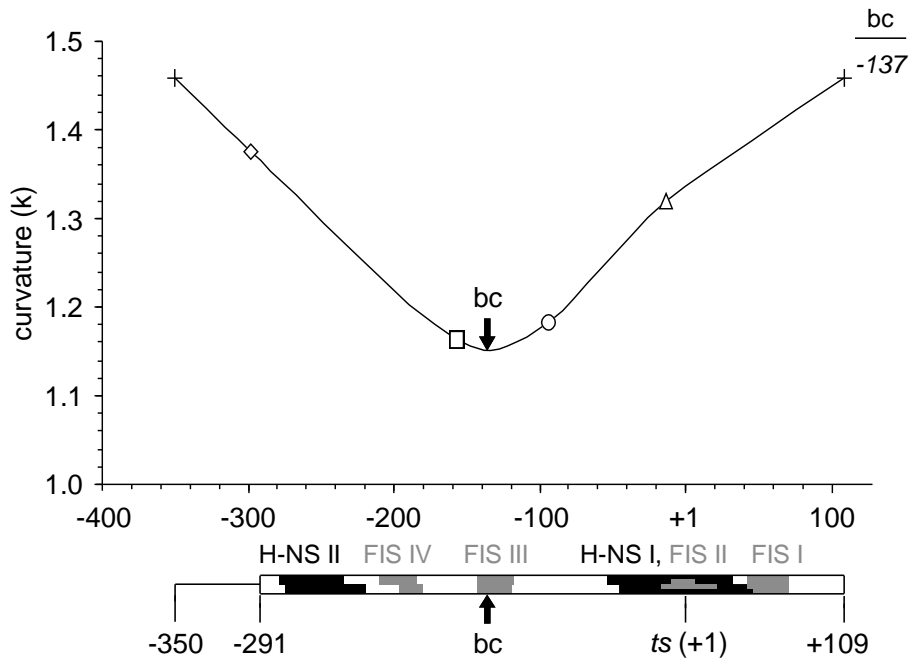
# The circular permutation assay

## Rationale





Circular  
permutation assay  
on the *virF*  
promoter region



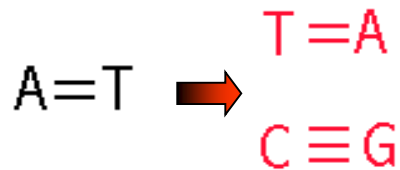
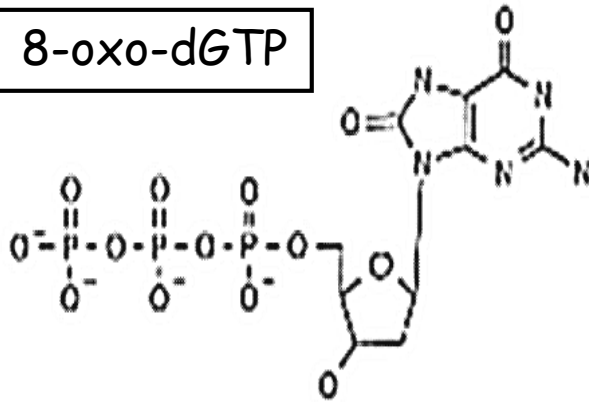
The bending centre  
maps halfway  
between the H-NS  
boxes and is located  
~140 bp upstream  
the transcription  
start site (+1)



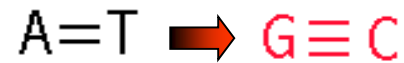
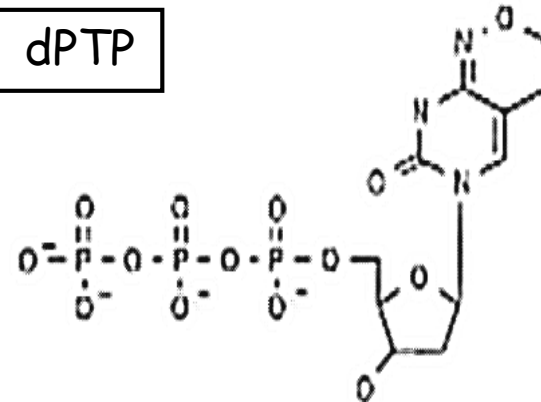
# Mutagenesis of the bent region

Mutagens used

8-oxo-dGTP



dPTP

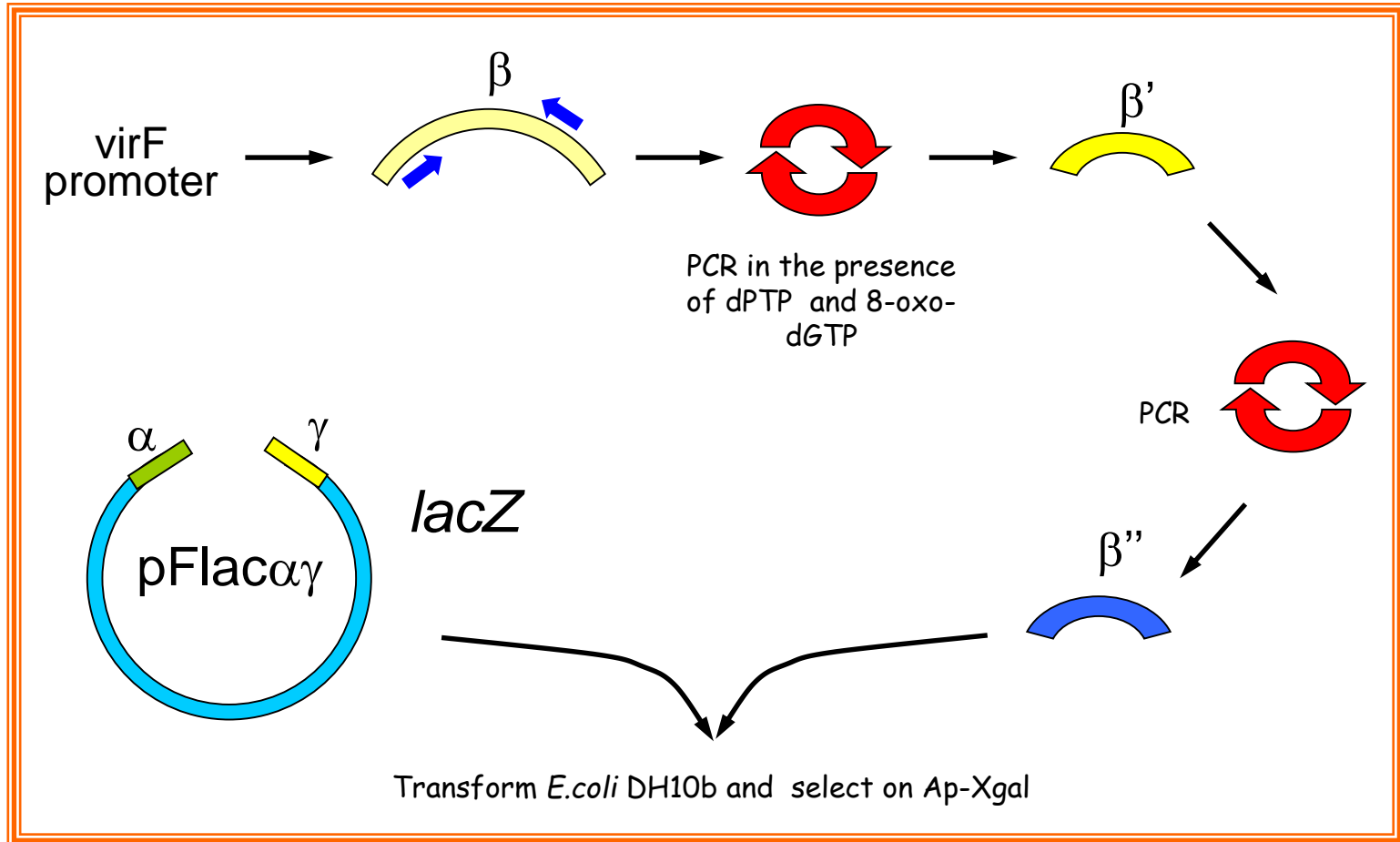


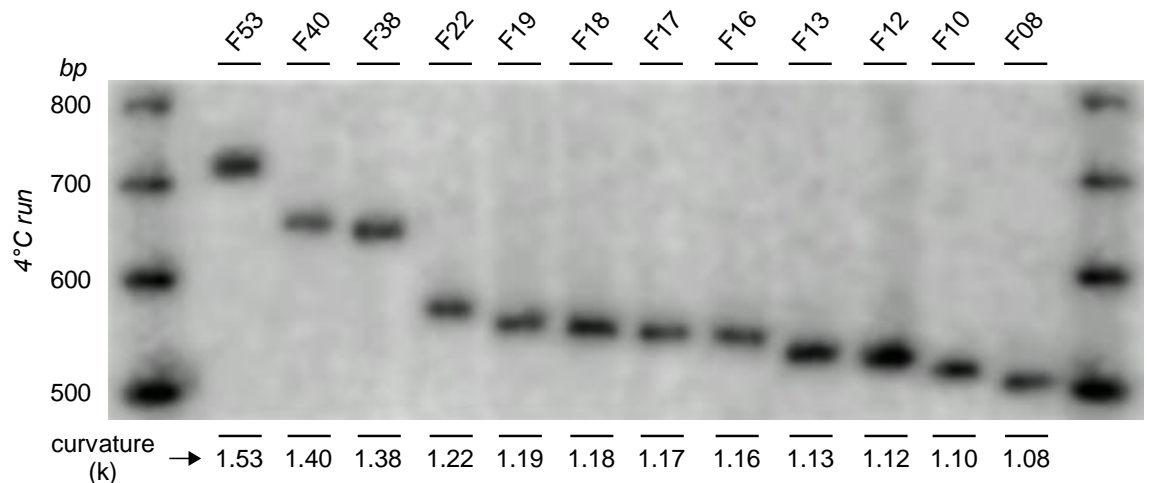
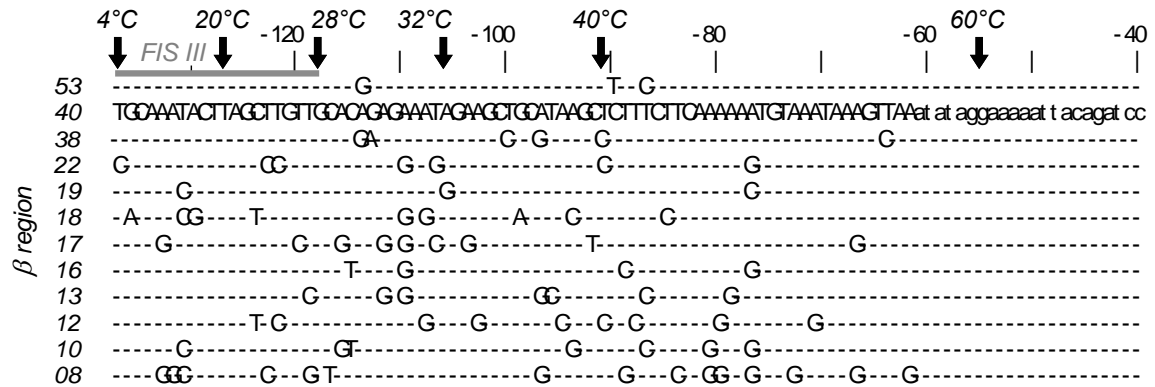
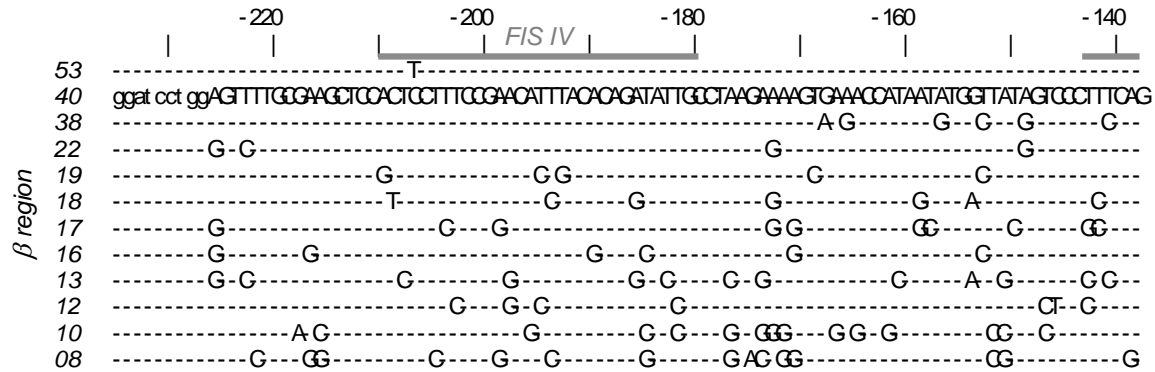
GCGCGCAAAAAGCGCGTAAAAGCGCGATAAAGC

GCGCGCAGAGAGCGCGTAGAAGCGCGACAAAGC

# Mutagenesis of the bent region

Strategy for the construction of  $\beta$ -region mutants

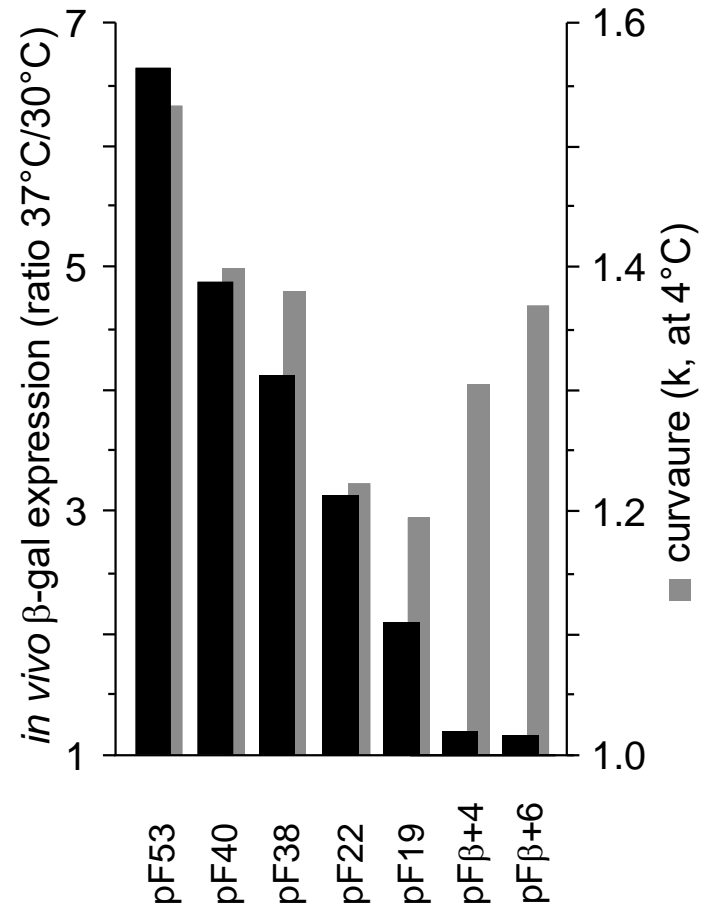
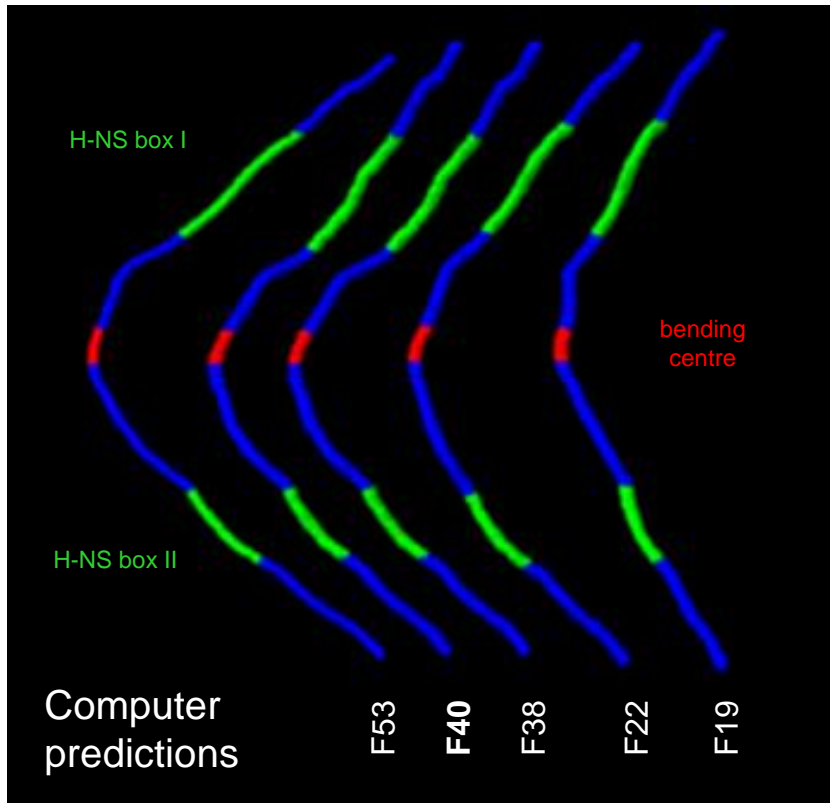




Acrilamide separation (4°C) of  $\beta$ -region mutants

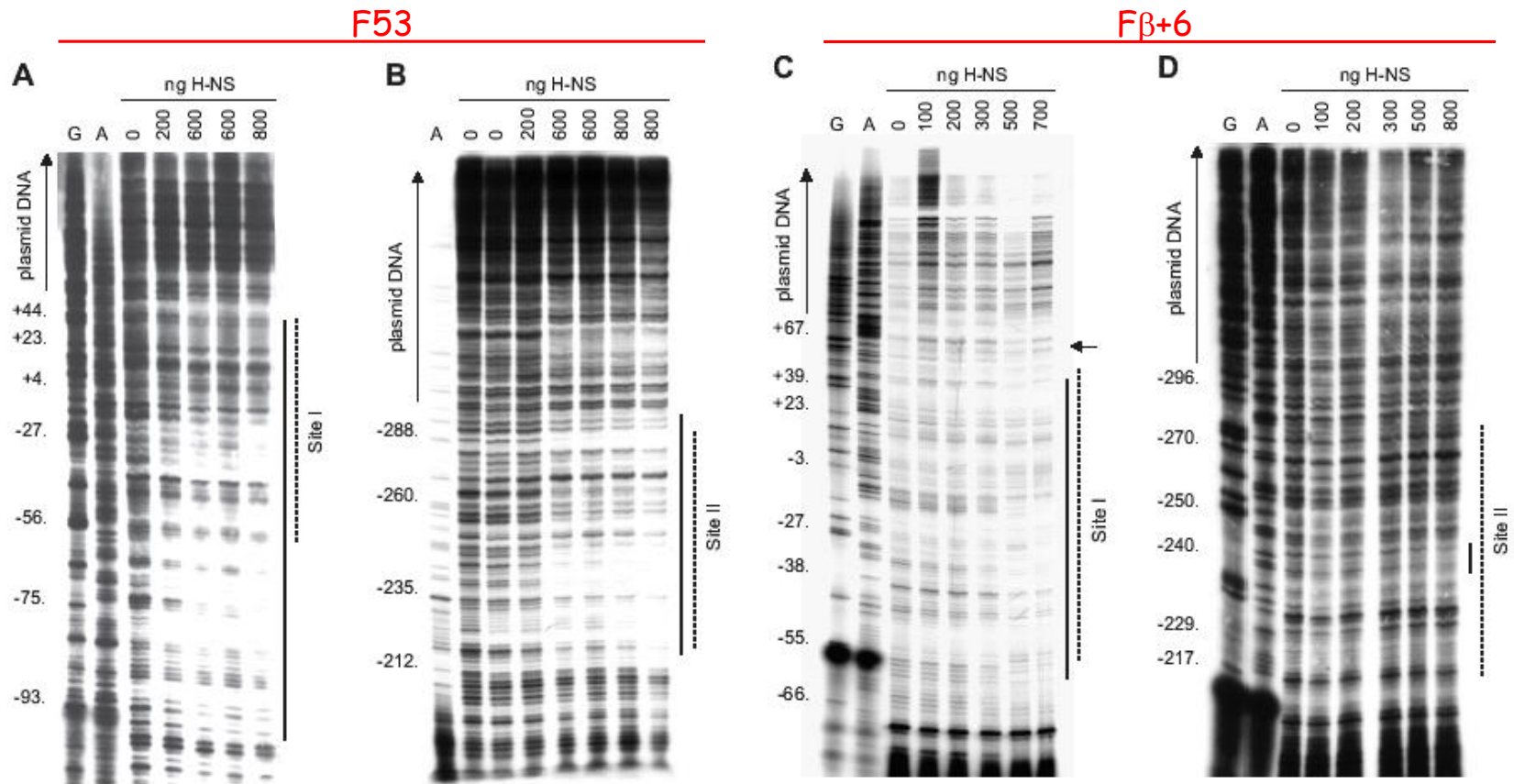


# Correlation between intrinsic curvature and thermoregulated expression of the *virF* promoter

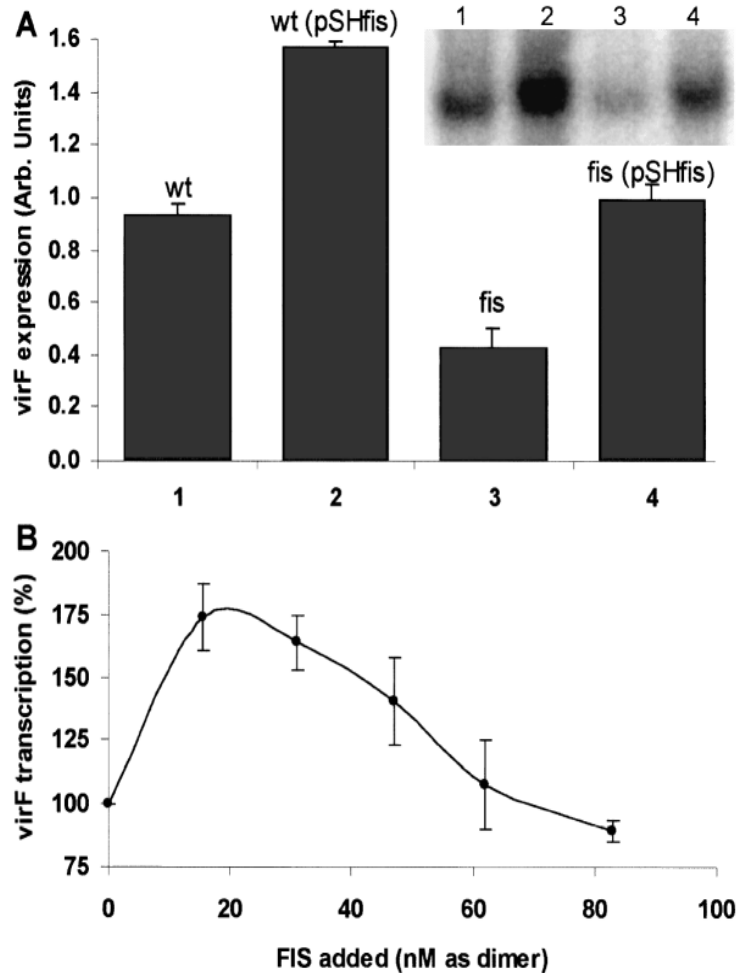


# DNaseI footprints reveal that H-NS:

- recognizes a wider site I in the strongly bent mutant (F53)
- is unable to recognize site II in the F $\beta$ +6 mutant



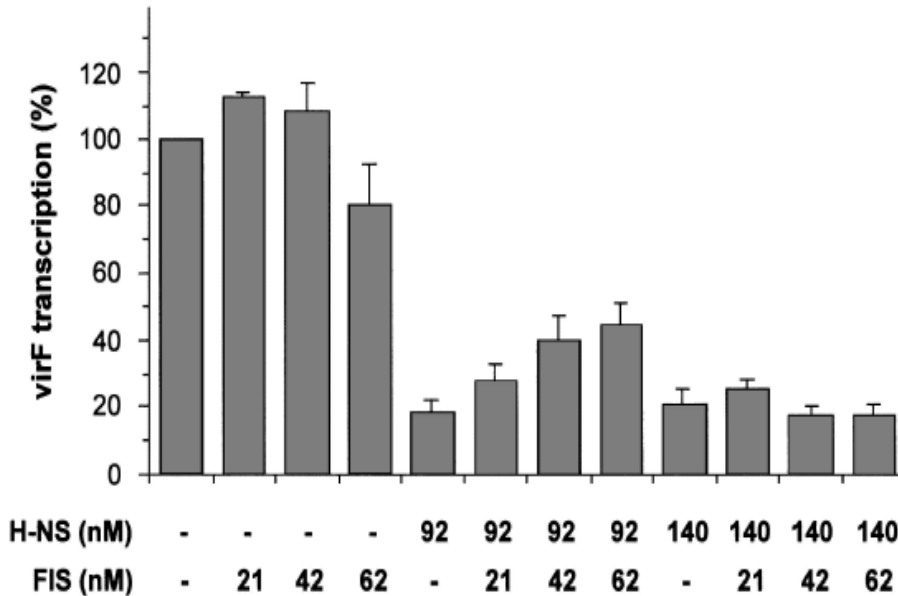
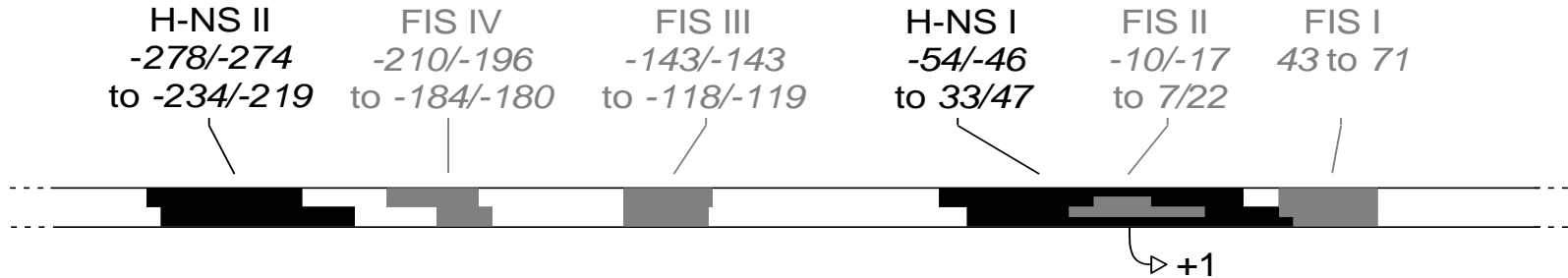
# The *virF* gene is positively regulated by FIS, another nucleoid protein



## FIS (Factor for Inversion Stimulation)

- Basic
- homodimer (2 x 11.5 kDa)
- abundant in exponential phase
- very weak DNA sequence specificity
- transcriptional regulator
- participates in site-specific recombination and transposition

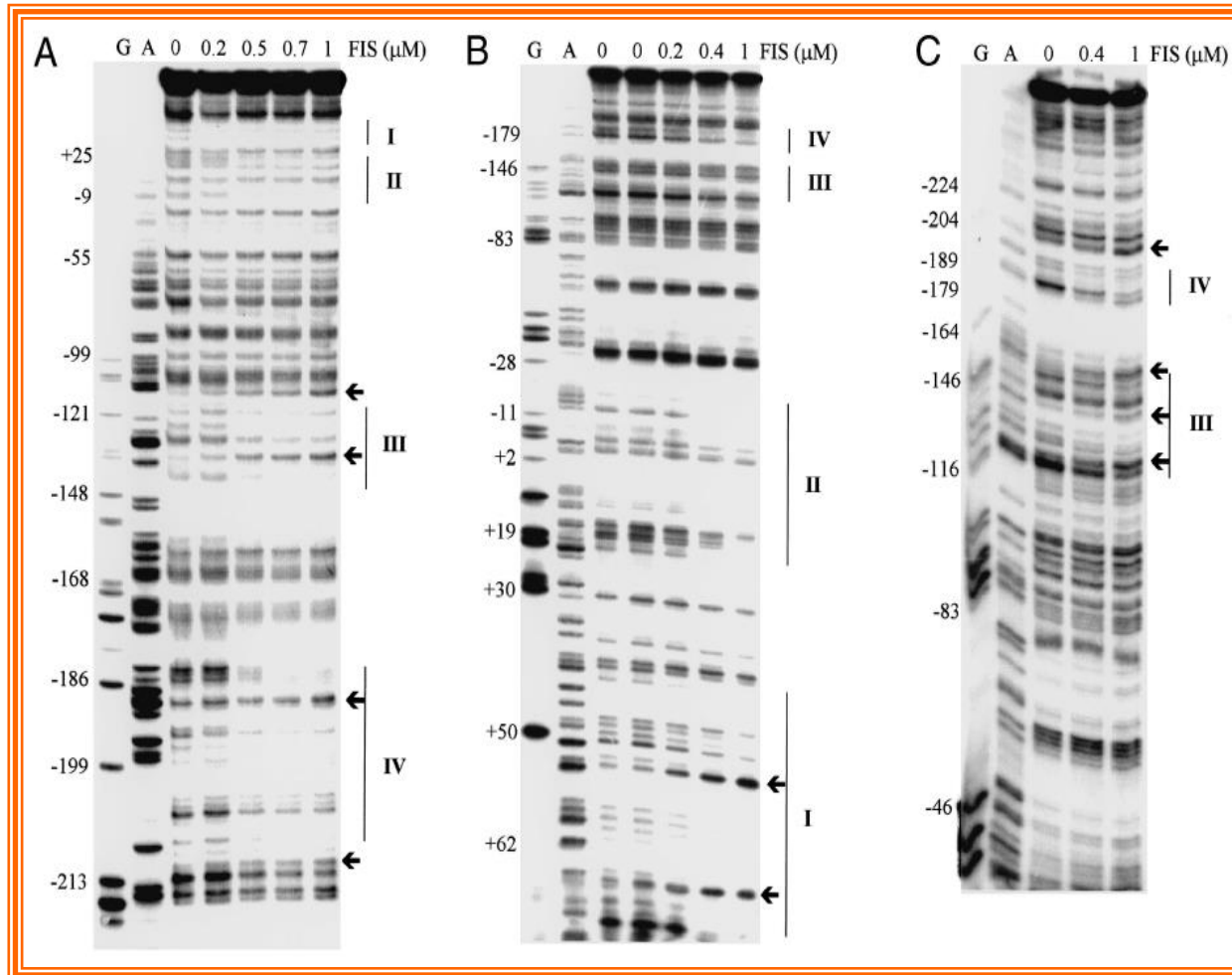
# FIS has four binding sites within the *virF* promoter ...



... and alleviates H-NS-mediated repression of the *virF* promoter at 31°C

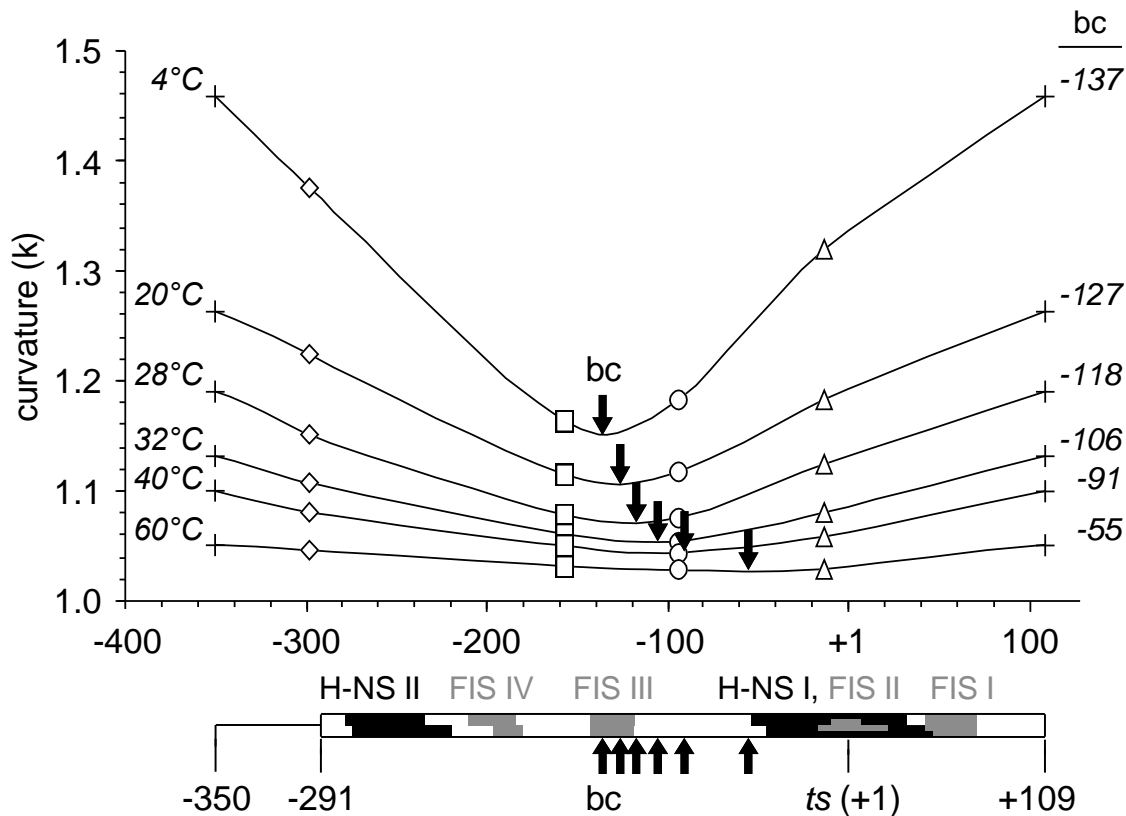
*In vitro* transcription in the presence of both, H-NS and FIS

# Identification of FIS binding sites on the *virF* promoter region



# Circular permutation assay on the *virF* promoter region

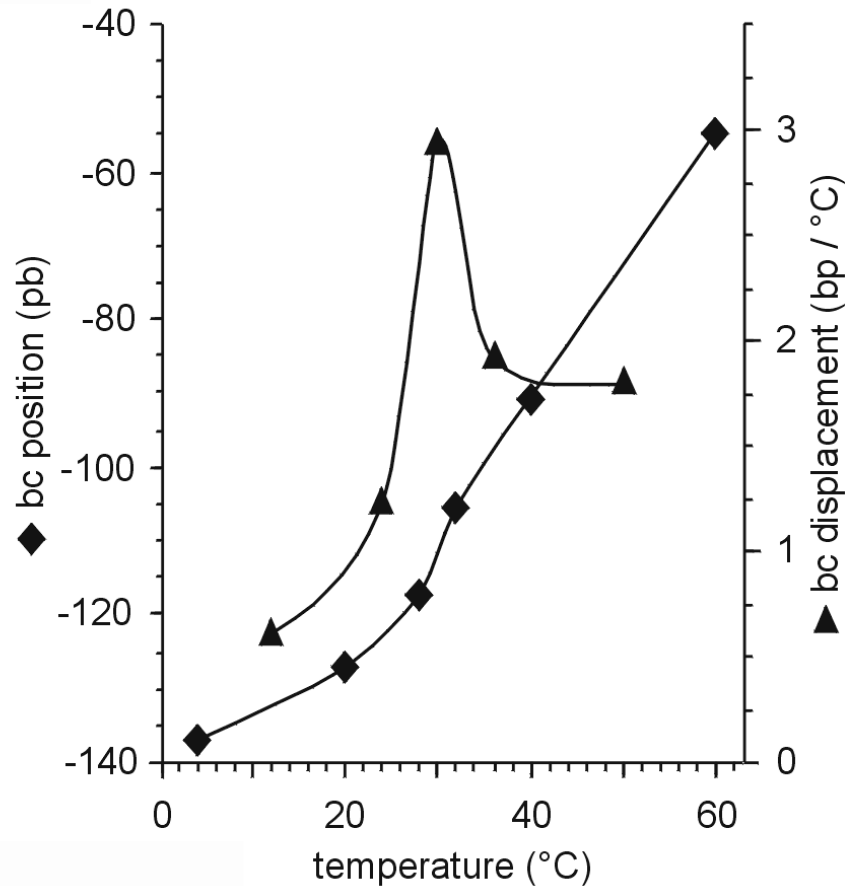
## Effect of temperature



Curvature is reduced as temperature increases

Do temperature changes alter the position of the bending centre?

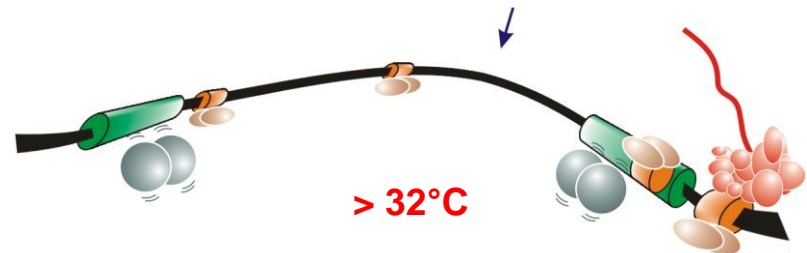
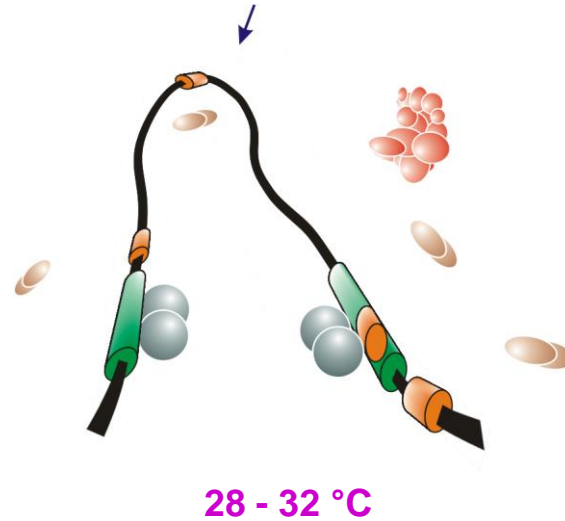
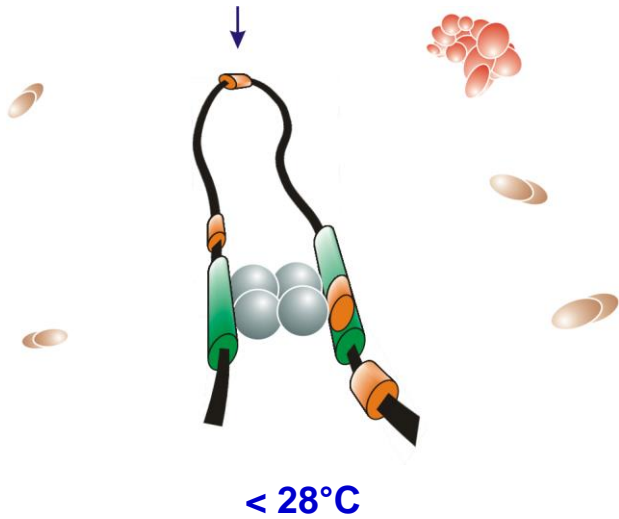
... yes, the bending centre of the *virF* promoter shifts considerably with temperature







- The displacement of the bending centre is not a linear function of temperature.
- The maximum displacement occurs between 28°C and 32°C.

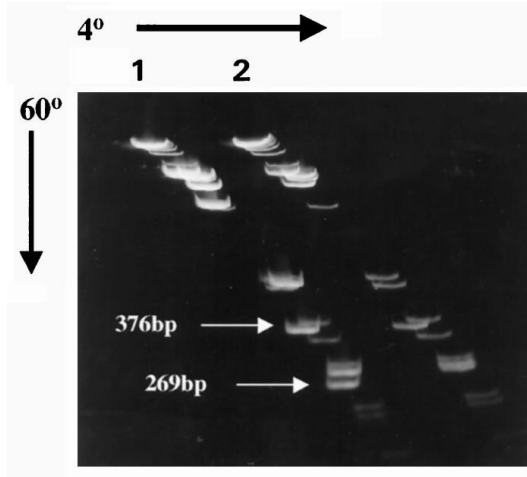


The thermodependent expression of *virF* is mediated by changes in DNA bending of its promoter



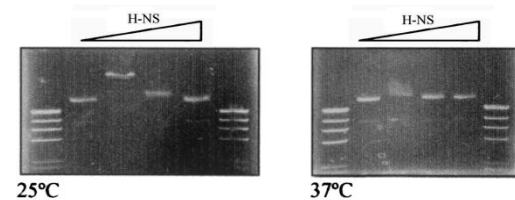
-  *H-NS and its binding sites*
-  *FIS and its binding sites*
-  *RNA polymerase*
-  *Bending center*

# Does DNA curvature regulate virulence genes as a function of host temperature also in other bacterial pathogens?



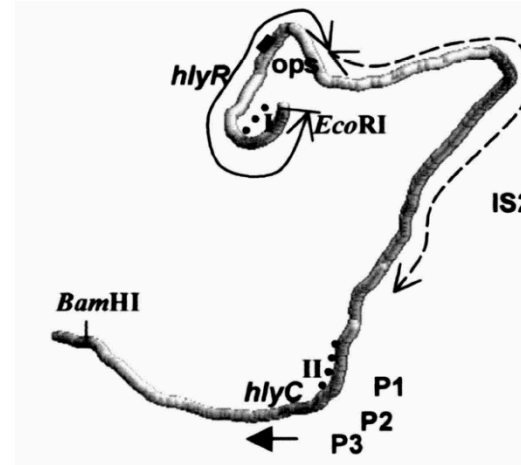
The *Yersinia enterocolitica* virulence plasmid contains DNA bends which melt at 37°C

(Rohde et al.)



In pathogenic *E. coli* the plasmid hemolysin operon is regulated by temperature-dependent binding of H-NS to curved DNA

(Madrid et al.)



From pathogenic bacteria to *E. coli* :

How far does intrinsic DNA curvature  
sustain bacterial transcription?

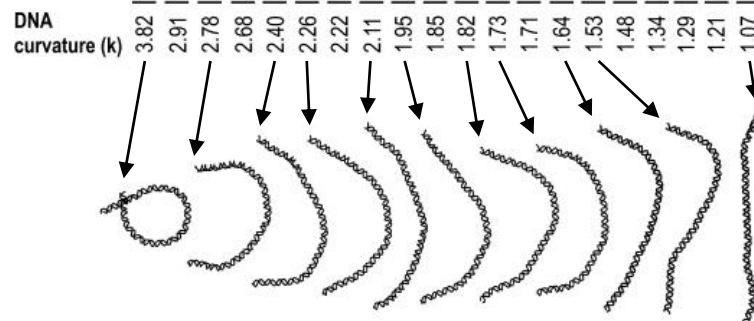
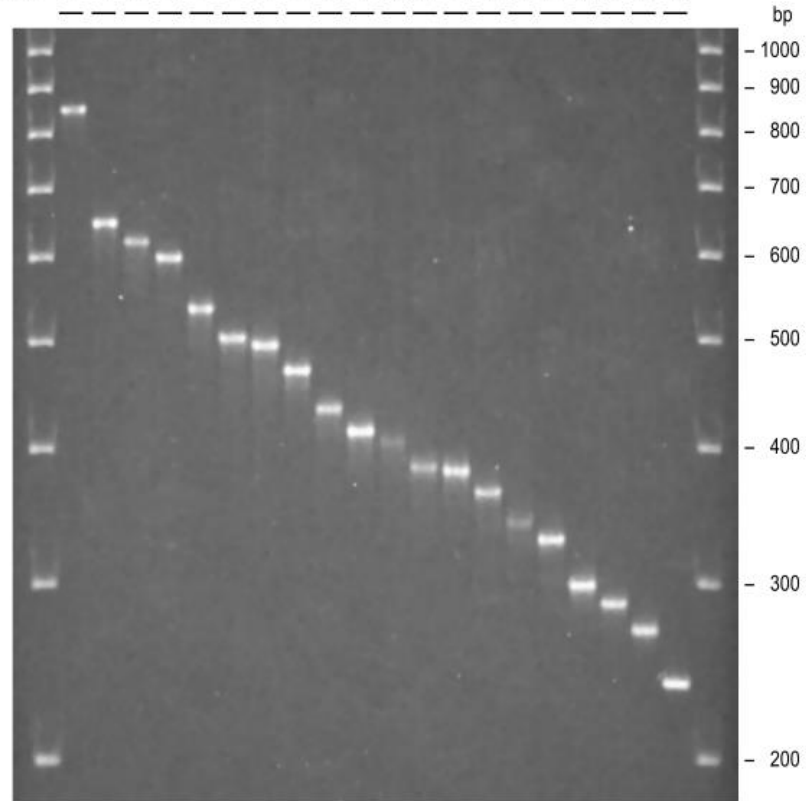
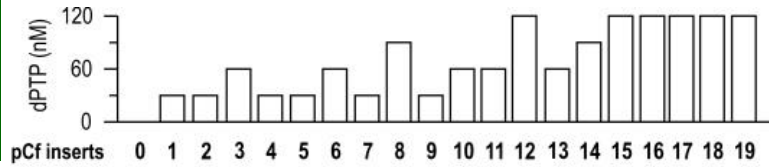
# The experimental approach

The 211 bp fragment from the kinetoplast DNA of *Crithidia fasciculata*, known to be endowed with strong curvature, has been randomly mutagenized



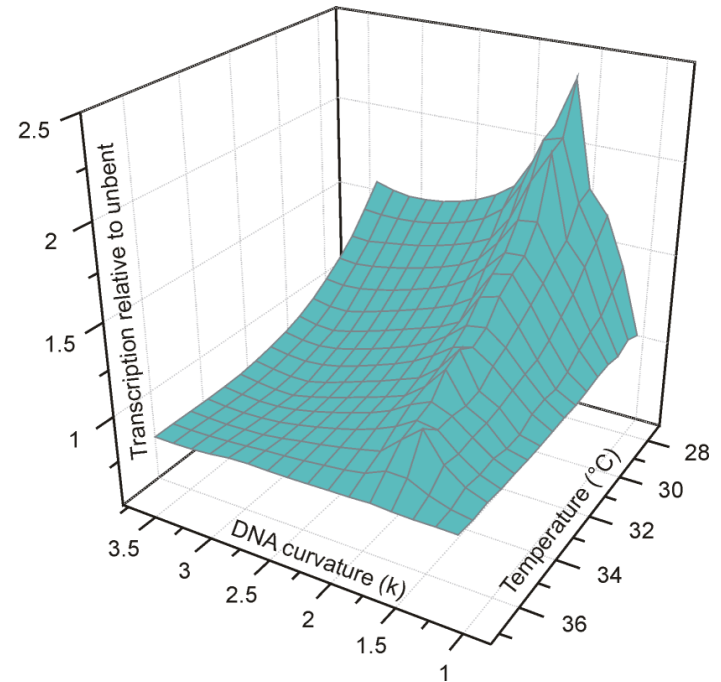
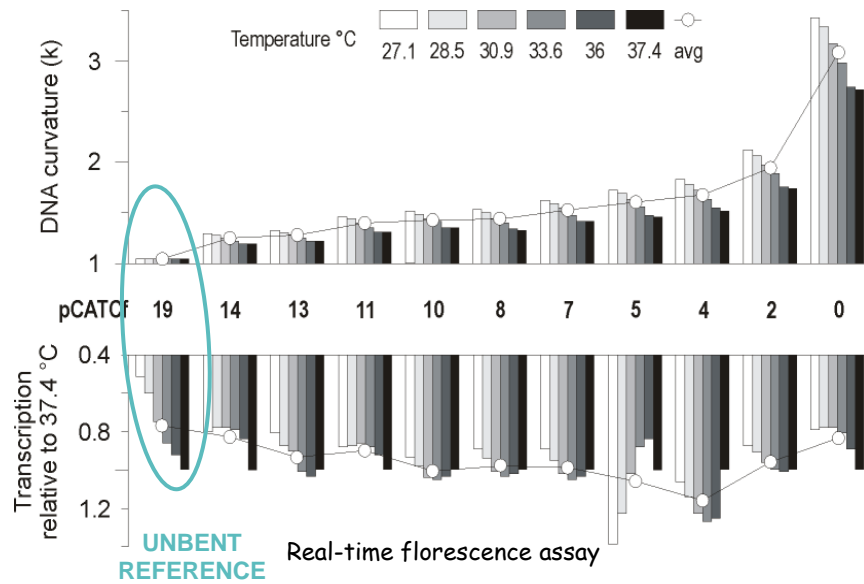
in order to obtain a spectrum of fragments covering a wide curvature range.

Then, mutagenized fragments were cloned upstream (-45) a reporter gene and ...



$$k = \frac{\text{Apparent size}}{\text{Real size}}$$

... their temperature-dependent transcription profiles were analyzed in vitro ...



## In short:

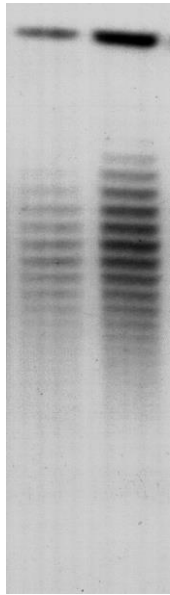
Curved DNA regions are frequently located upstream bacterial promoters.

Their marked temperature-sensitivity makes them excellent candidates as transcriptional modulators responding to environmental stimuli.

A narrow range of curvature is able to sustain bacterial transcription in vitro.

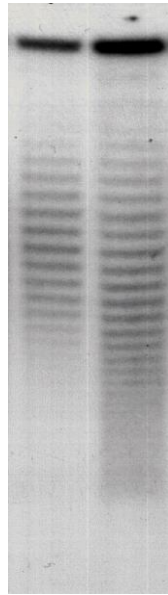


30°C 37°C



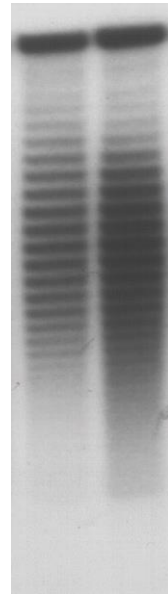
wt

30°C 37°C

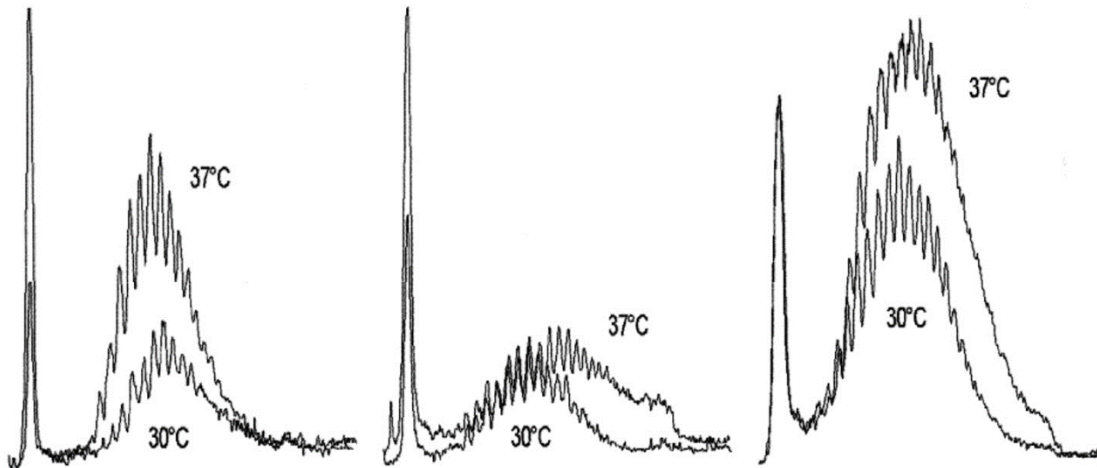


*hns118*

30°C 37°C

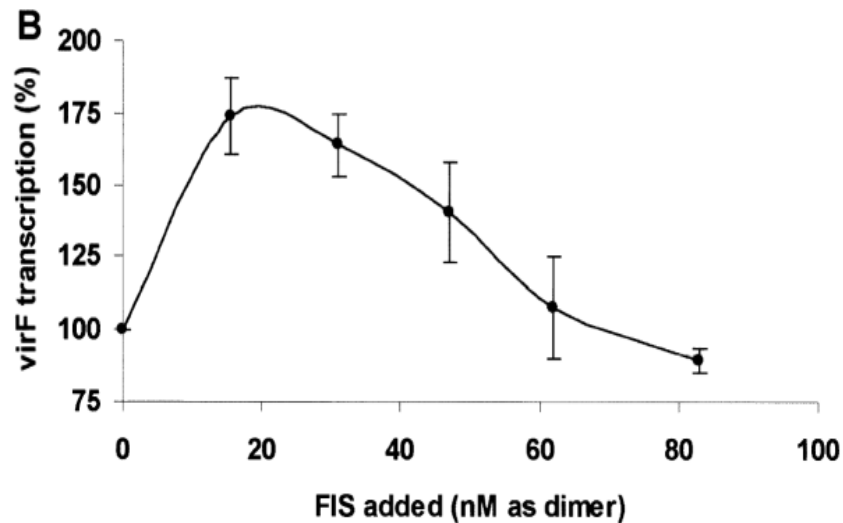
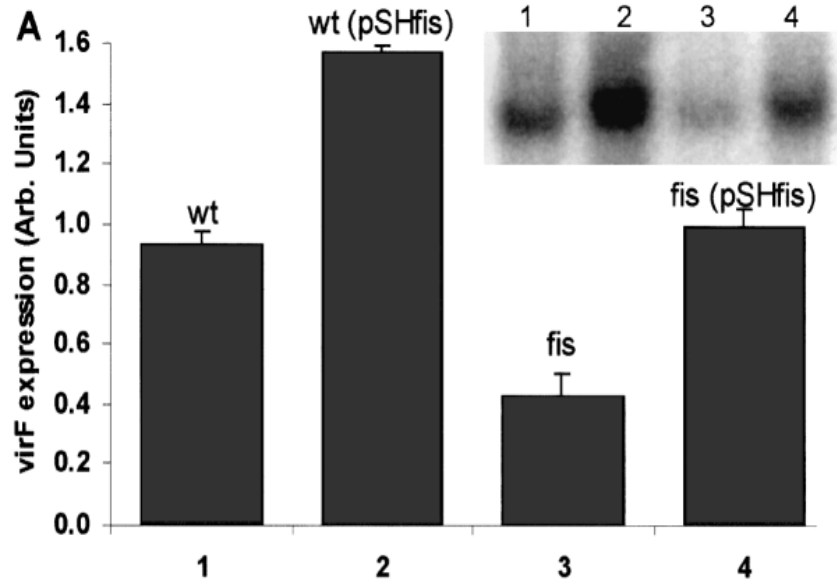


*hns2*



Distribution of  
pMYSH6504  
topoisomers in  
*hns*<sup>+</sup> and *hns*<sup>-</sup>  
strains at 30°C  
and at 37°C





*In vivo and in vitro*  
effect of FIS on  
the expression of  
*virF* at 37°C

# Influence of FIS on the H-NS mediated repression of the *virF* promoter at 31°C

