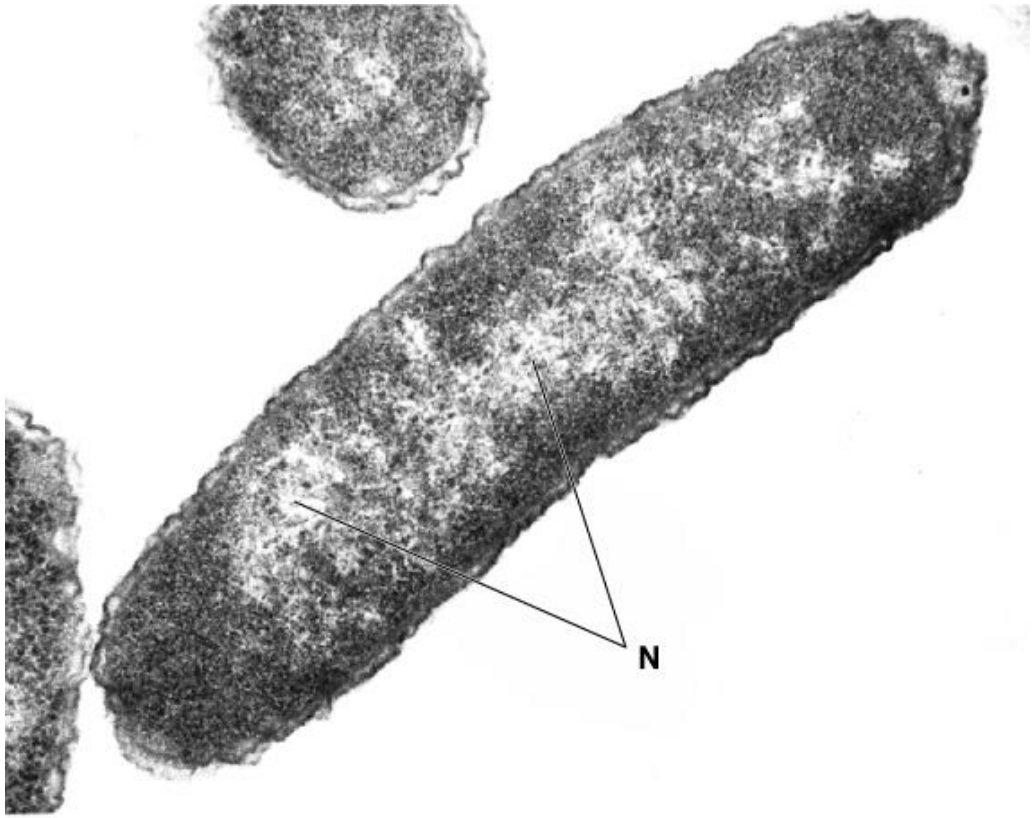
Il contenuto intracellulare di una cellula batterica è diviso tra due principali compartimenti
nucleoide nel quale è situato il genoma
citoplasma contenete l'apparato di sintesi proteica

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 μm di lunghezza e 1μm di larghezza

- Un compattamento casuale della molecola determinerebbe un volume di circa 200mm^3 circa 400 volte superiore al volume del nucleoide

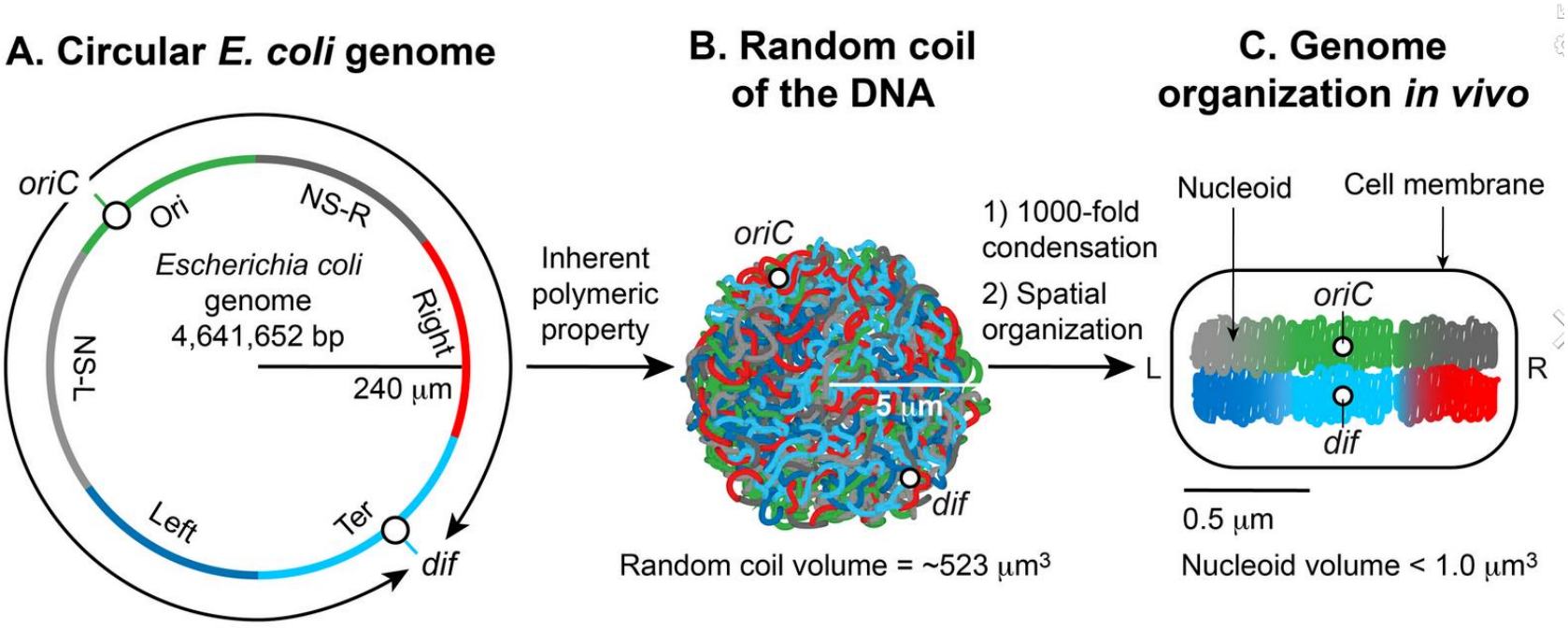
- Il volume del nucleoide è di circa di E.coli 0.5 μm

- Il cromosoma è quindi estremamente organizzato in anse topologicamente indipendenti circa 100 da 50 kb

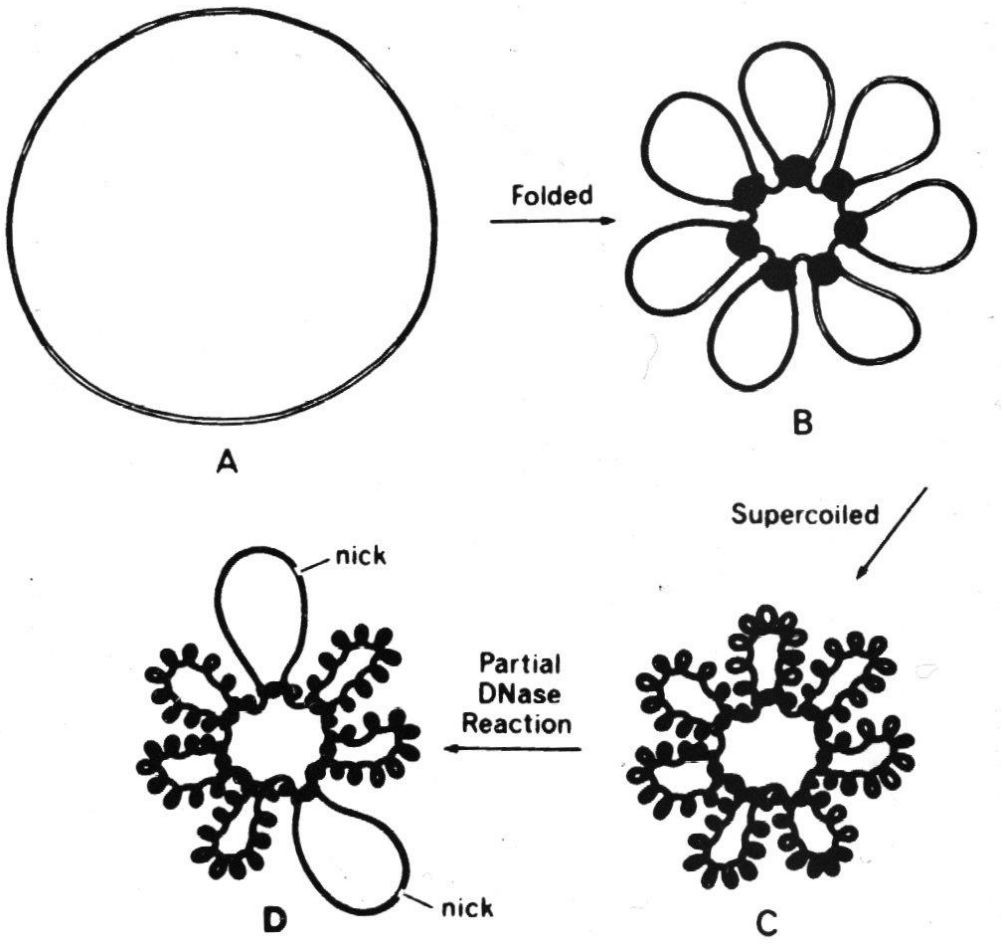
L'organizzazione del genoma batterico è caratterizzata dalla presenza di macrodomini funzionali , ampie regioni di DNA nel qule ogni singolo gene ha un corretto livello di espressione che dipende dal suo orientamento dalla sua posizione rispetto all'origine.

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

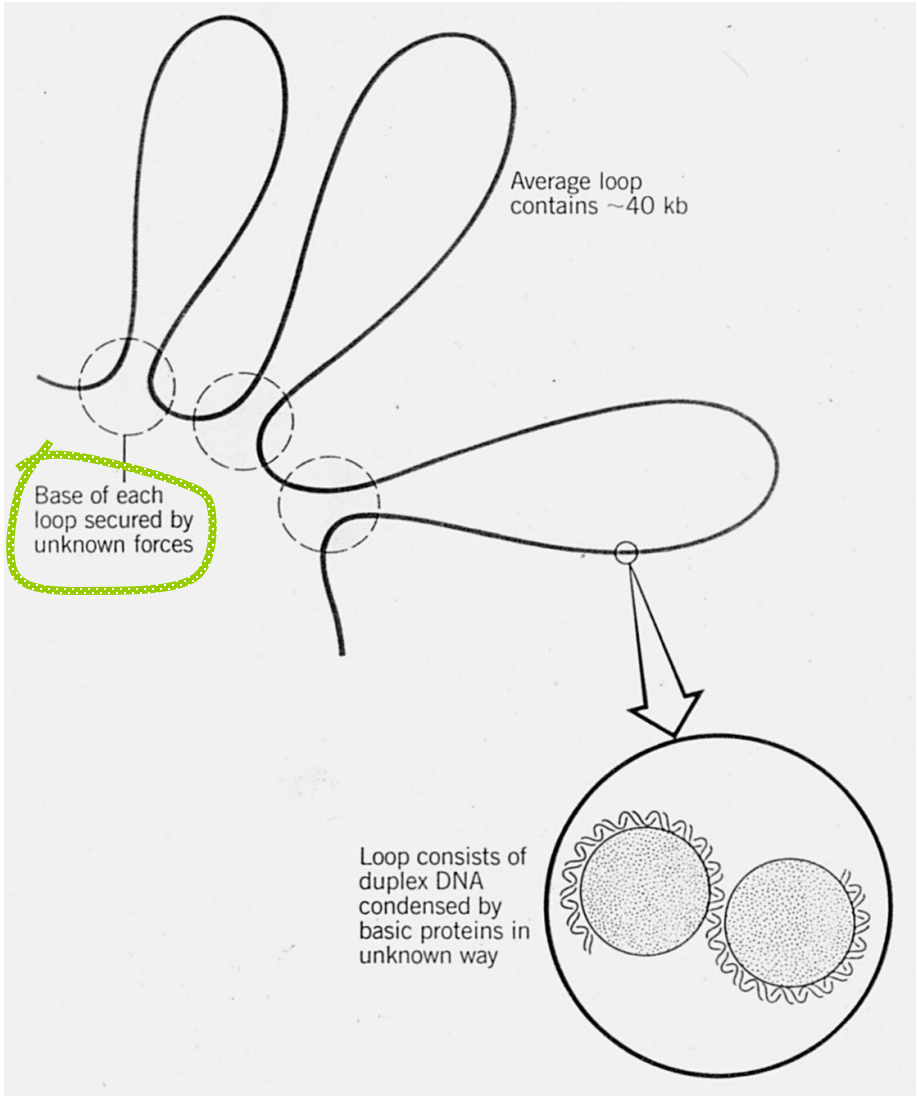

A. Circular E. coli genome
oriC
Ori
NS-R
Escherichia coli
genome
4,641,652 bp
Right
240 μm
NS-L
Left
Ter
dif
Inherent
polymeric
property
B. Random coil
of the DNA
oriC
5 μm
dif
Random coil volume = ~523 μm3
1) 1000-fold
condensation
2) Spatial
organization
C. Genome
organization in vivo
Nucleoid
Cell membrane
oriC
L
R
dif
0.5 μm
Nucleoid volume < 1.0 μm3

Organizzazione del cromosoma batterico ad anse



Il cromosoma potrebbe essere organizzato in domini topologicamante 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 nucleoide batterico



Componenti degli ancoraggi:

• ***RNA?***

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

• ***Parete -Membrana?*** (replicazione)

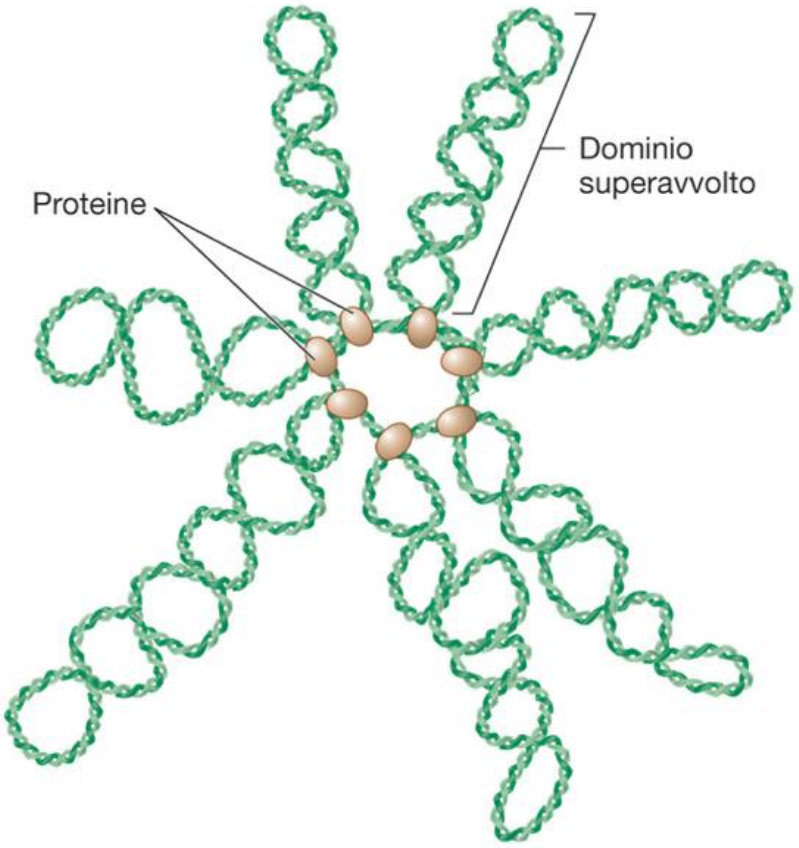
• ***Proteine istone-simili?*** (HU, H-NS, …)

• ***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

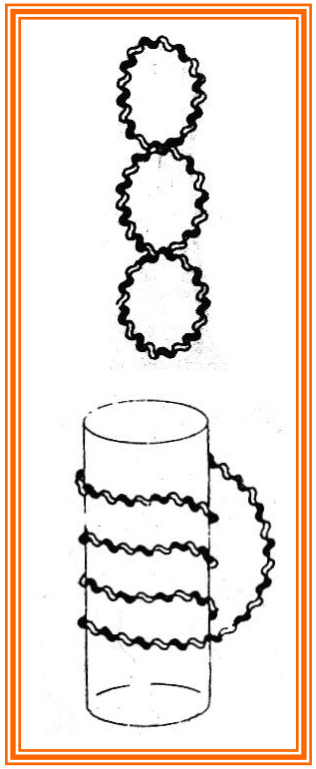


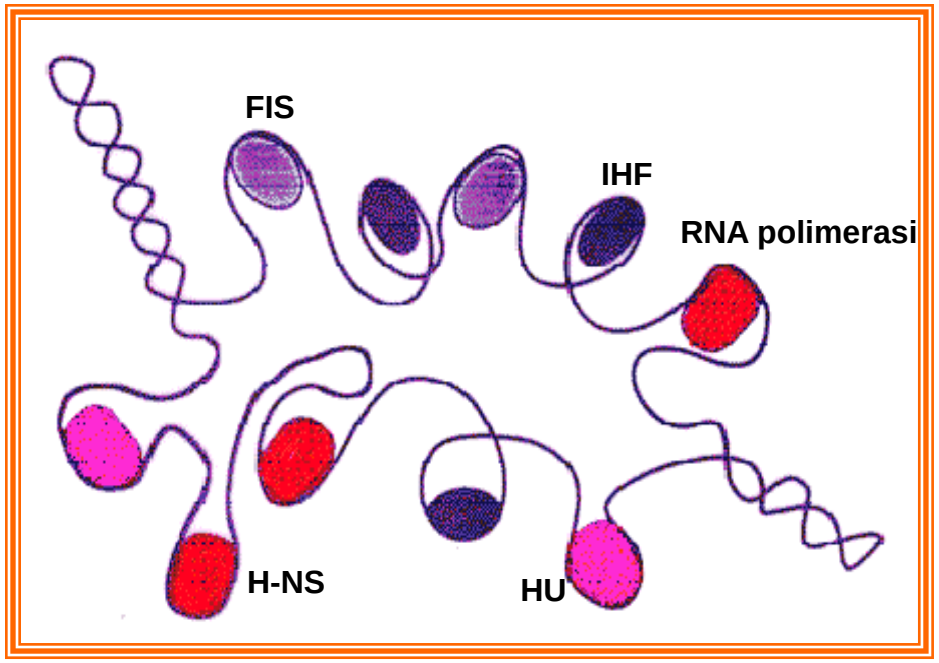
(d) Cromosoma con domini superavvolti

Bacterial chromatin organization

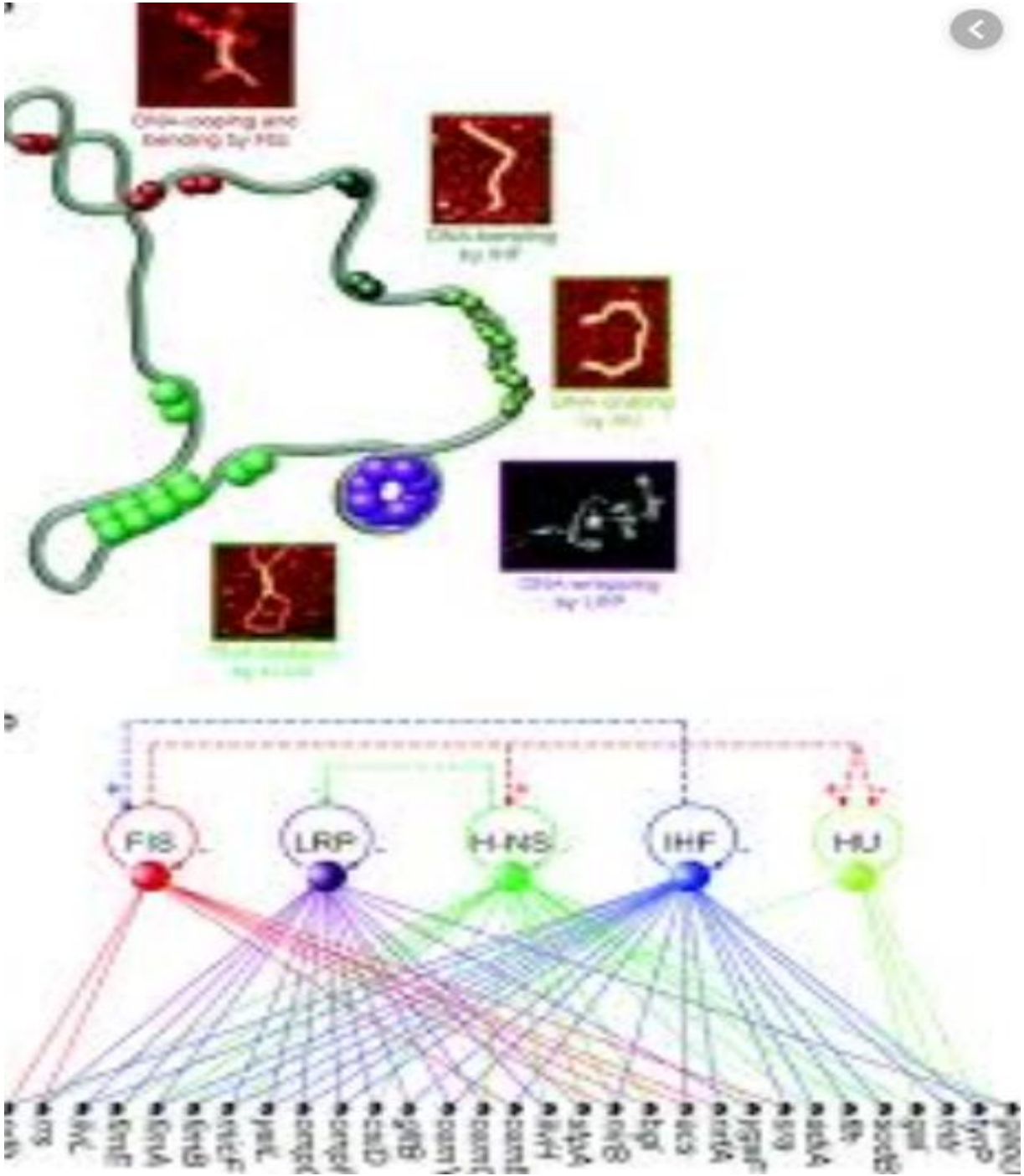
Plectonemic condition

Toroidal condition





Co-existence of
plectonemic and toroidal conditions


FIS
LRF
H-NS
IHF
HU

Le proteine associate al nucleoide (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 all nucleoide

Struttura ed organizzazione delle prinicipali proteine associate al nucleoide (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)	• basic • abundant in exponential phase (15000–30000 dimers/cell)	heterodimer; HUα, 9.2 kDa HUβ, 9.5 kDa	*hupA* (90.4 min) *hupB* (9.9 min)	• compacts DNA into nucleosome-like structures • induces DNA curvature • recognizes curved DNA, gapped regions, and 3/4way junctions • involved in DNA replication and recombination
IHF (integration host factor)	• basic • abundant in stationary phase (25000–3000 dimers/cell) • high amino acid identity between IHF and HU subunits • DNA binding preference: WATCAANNNNTTR	heterodimer; IHFα, 11.2 kDa IHFβ, 10.7 kDa	*himA* (38.6 min) *himD* (25 min)	• induces very strong DNA curvature (up to 140°) • participates in site-specific recombination, transposition, and DNA replication
FIS (factor for inversion stimulation)	• basic • abundant in exponential phase (20000–40000 dimers/cell) • DNA binding preference: GNYAWWWTRNC	homodimer, 2 × 11.2 kDa	*fis* (73.4 min)	• induces strong DNA curvature (up to 90°) • alters DNA topology • participates in site-specific recombination, transposition, and DNA replication
H-NS (histone-like nucleoid structuring protein)	• non basic • 20000–40000 dimers/cell • binding form may be tetramer or higher oligomer • induced during cold-shock	homodimer, 2 × 15.4 kDa	*hns* (27.8 min)	• recognizes curved DNA • alters DNA topology • induces DNA curvature • influences recombination
StpA (suppressor of *rd* mutant phenotype A)	• basic • 10000–15000 copies/cell • high amino acid identity with H-NS • able to form heterodimers with H-NS	dimer?, n × 15.3 kDa	*stpA* (60.2 min)	• RNA chaperon • recognizes curved DNA

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 nucleoide
- 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)

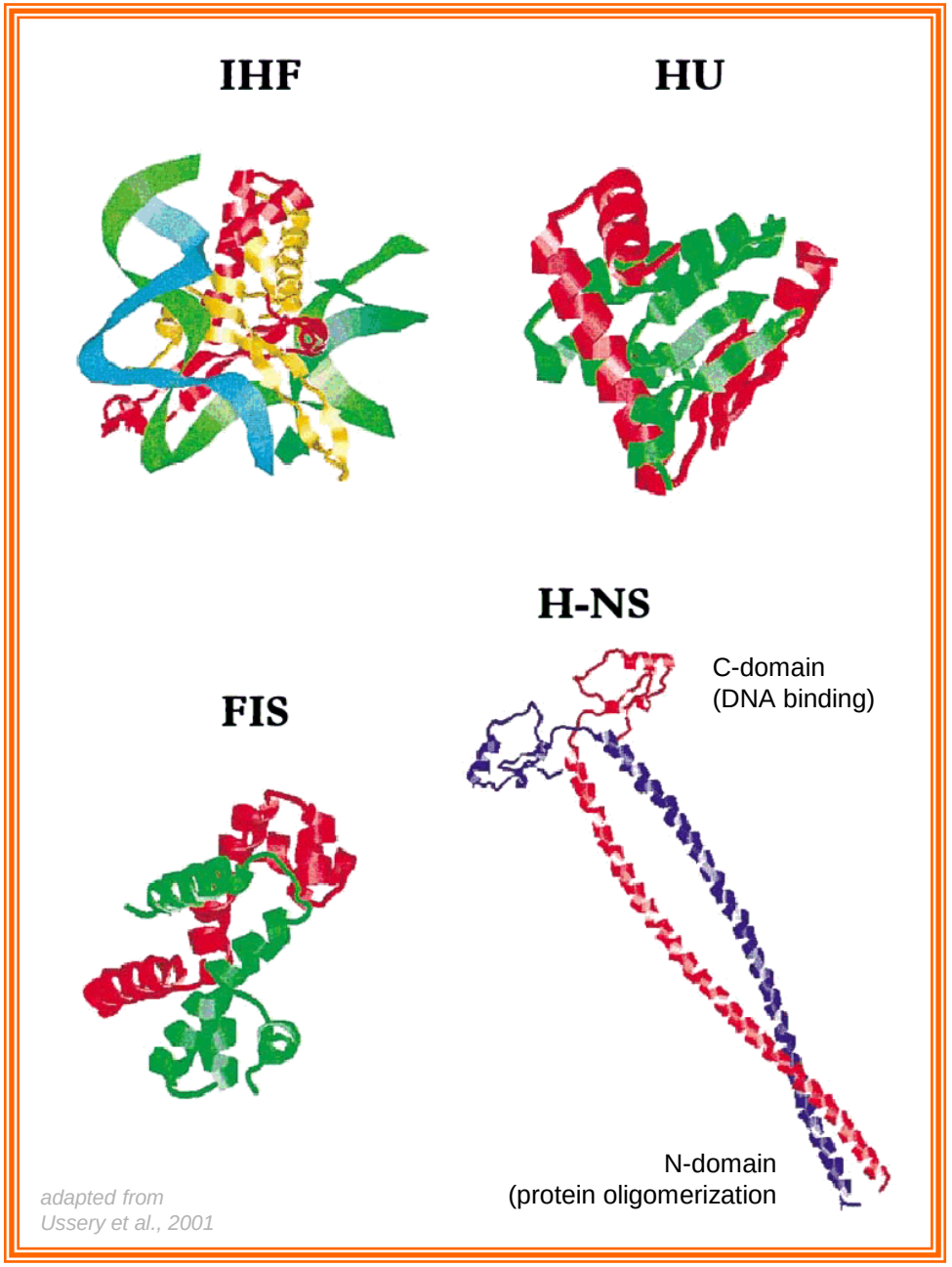
La proteina HU

Funzioni

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

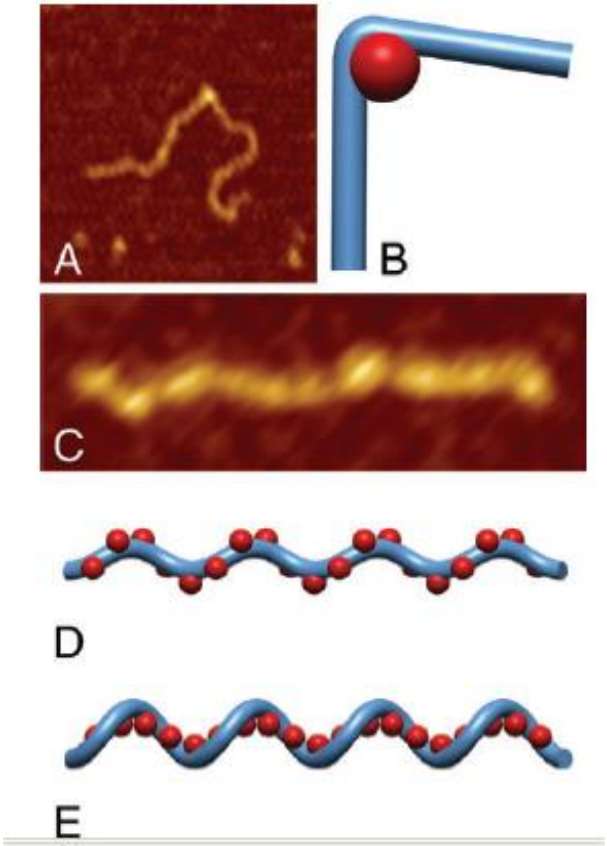
Isolata nella maggior parte delle specie batteriche
proteine simili ad HU conservate anche negli Archea

Anticorpi anti HU si legano sulla superficie del nucleoide 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



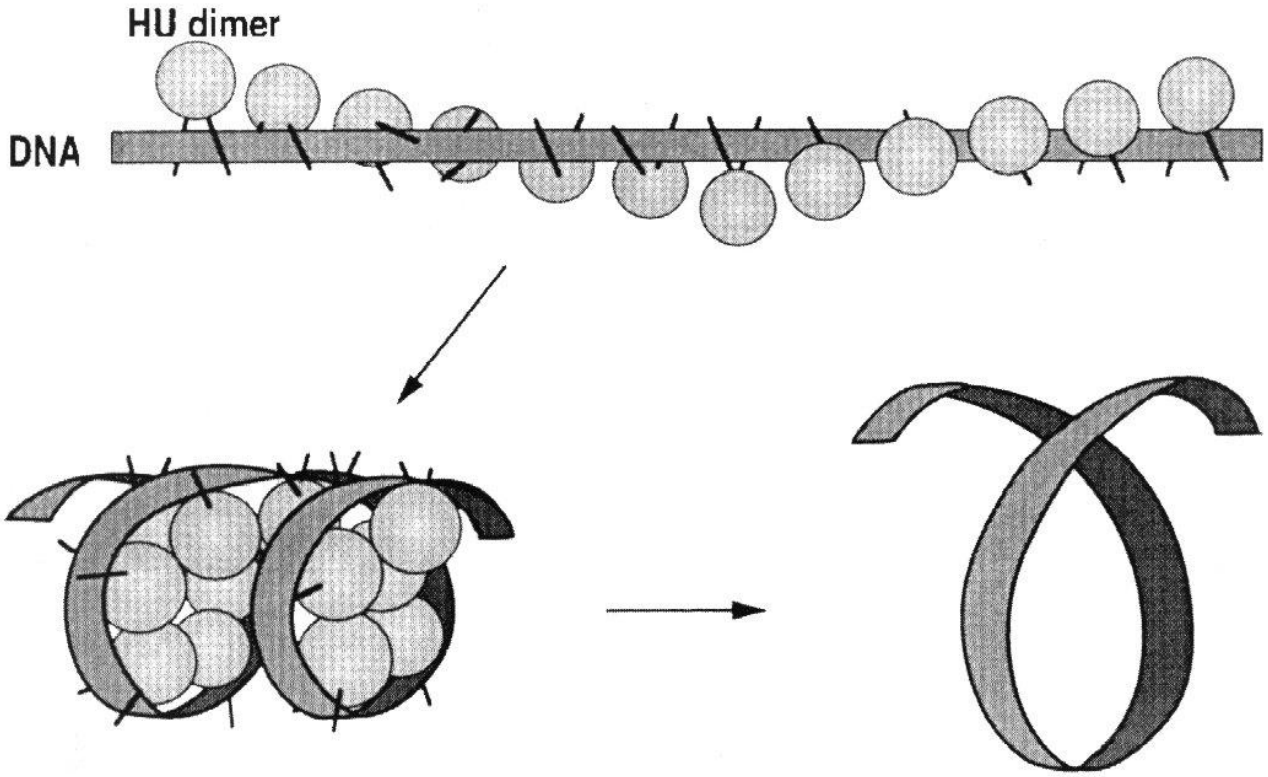
Nucleoid proteins are homo- or heterodimers

HU lega il DNA e lo ripiega

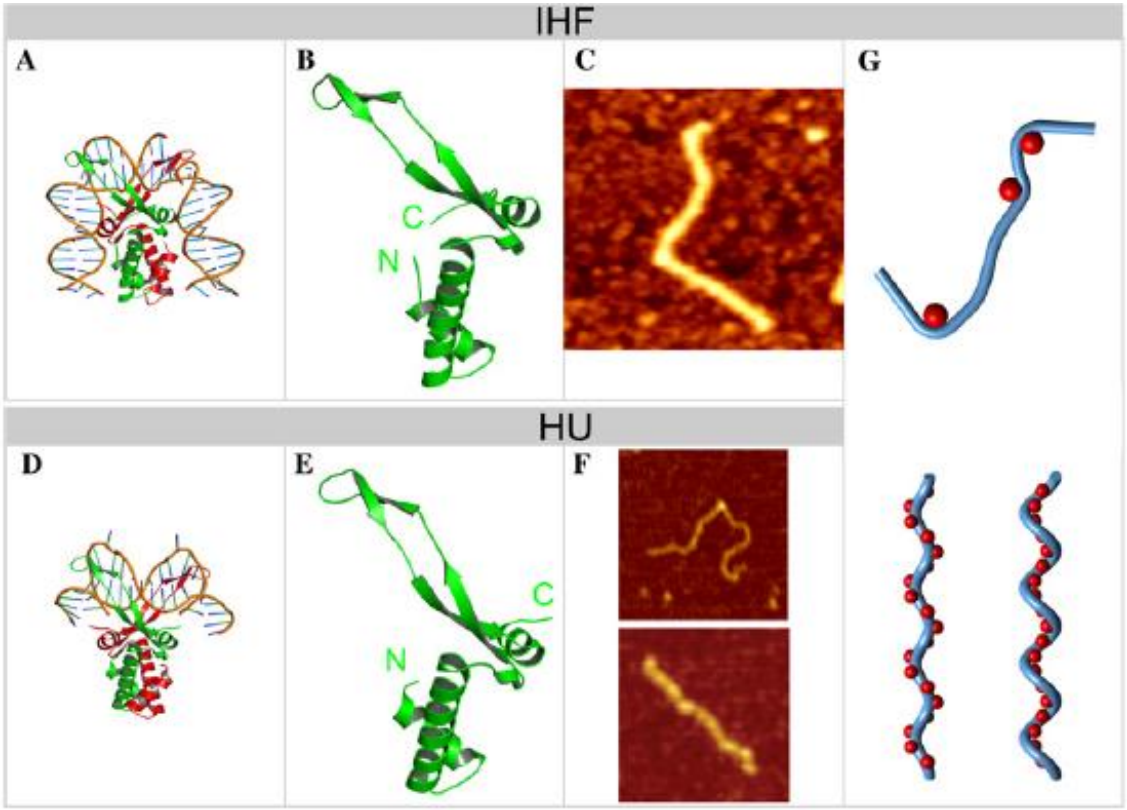


Superavvolgimento mediato da HU. I dimeri di HU si legano in vitro a distanza di circa 9 bp. In vitro associazione di 8-10 dimeri con frammenti di 275-290 bp

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 (YAANNNNTTGATW)

Struttura	pM	geni
Eterodimero		
IHFα	11.2 kDa	himA (38.6 min)
IHFβ	10.5 kDa	himD (25 min)

IHF ; Caratteristiche funzionali

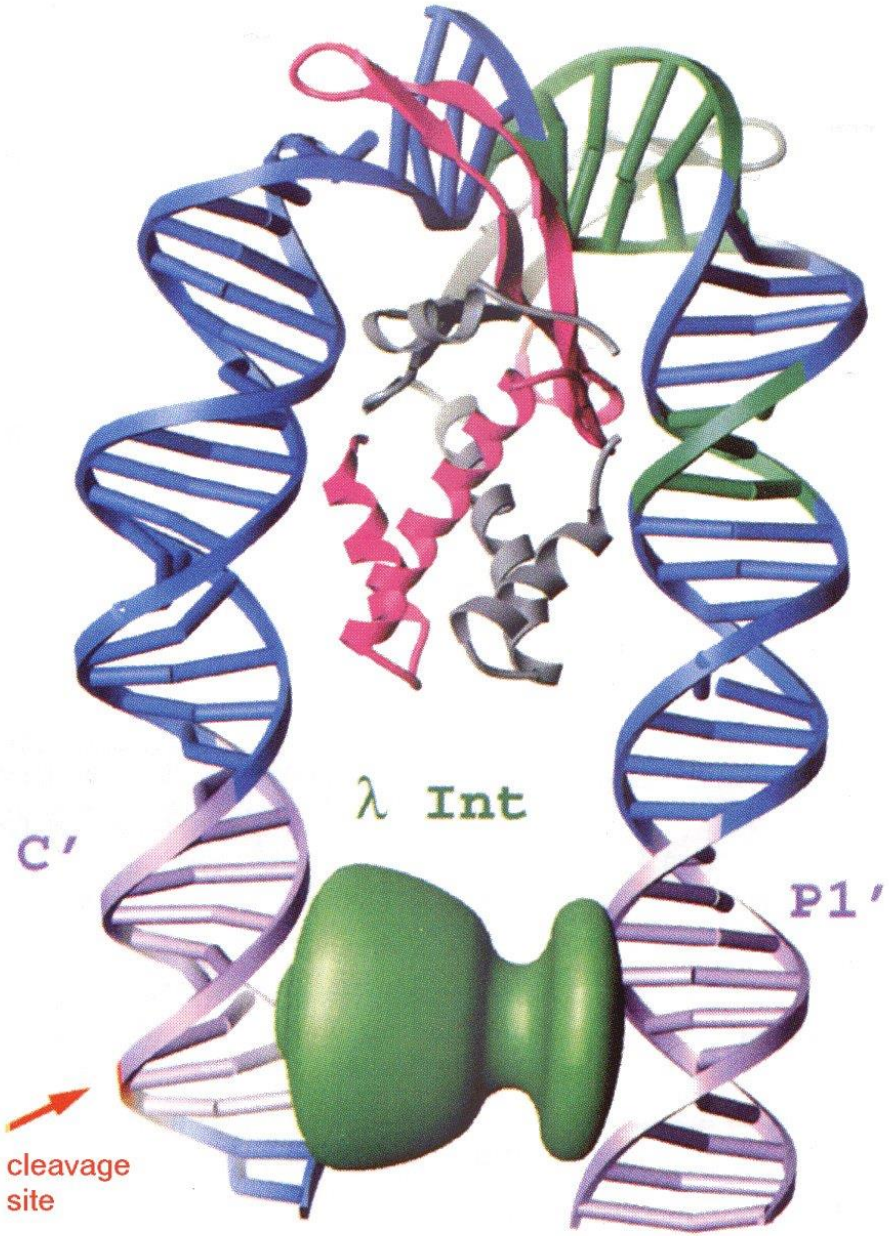
- **Induce forte curvatura nel DNA (fino a 140°)**
- **Interviene nella ricombinazione sito specifica**
- **interviene nella trasposizione**

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

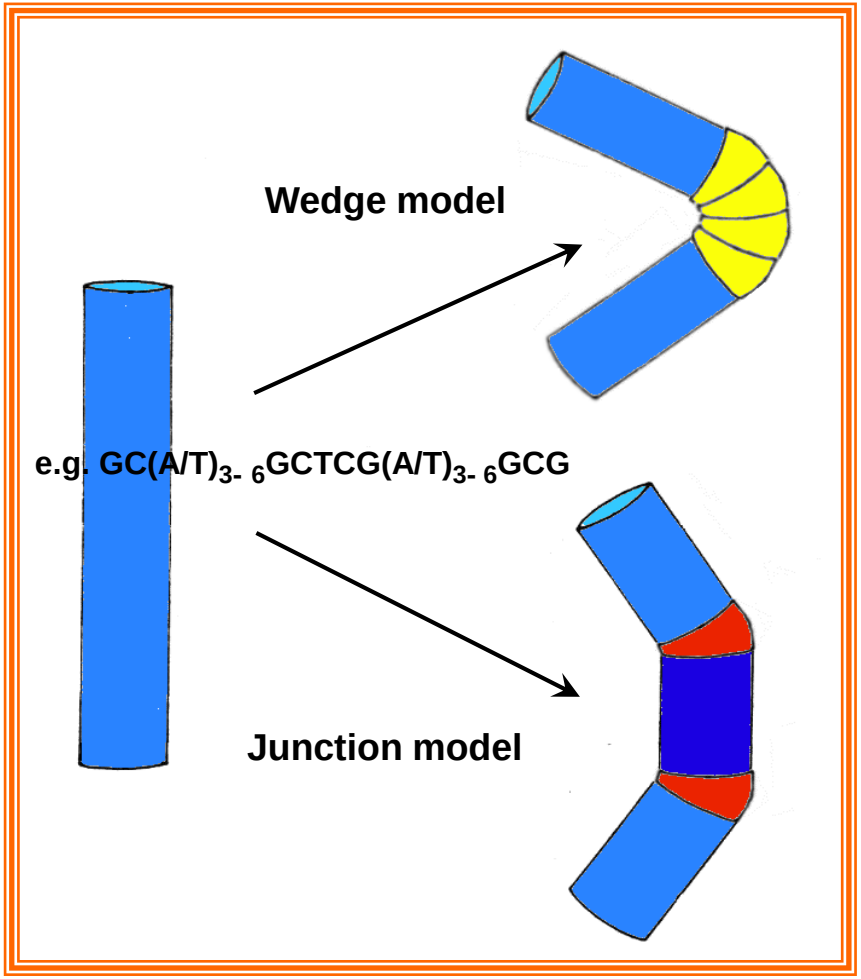
- **Isolata come fattore dell'ospite per l'integrazione del fago λ è coinvolta in molte funzioni cellulari**.

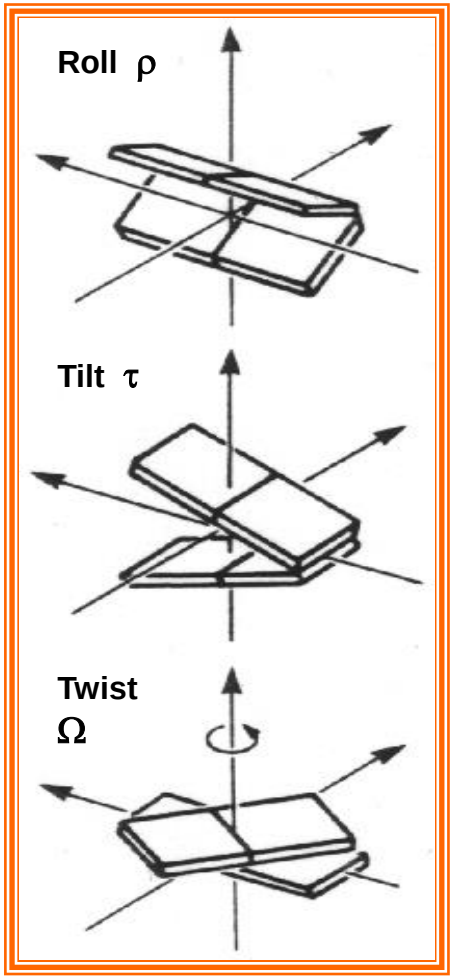
- Forte ruolo strutturale; curvando il DNA fino a 140°C permette a siti di DNA distanti di trovarsi ravvicinati favorendo cosi sia processi di trascrizione che di regolazione

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



Intrinsically curved DNA





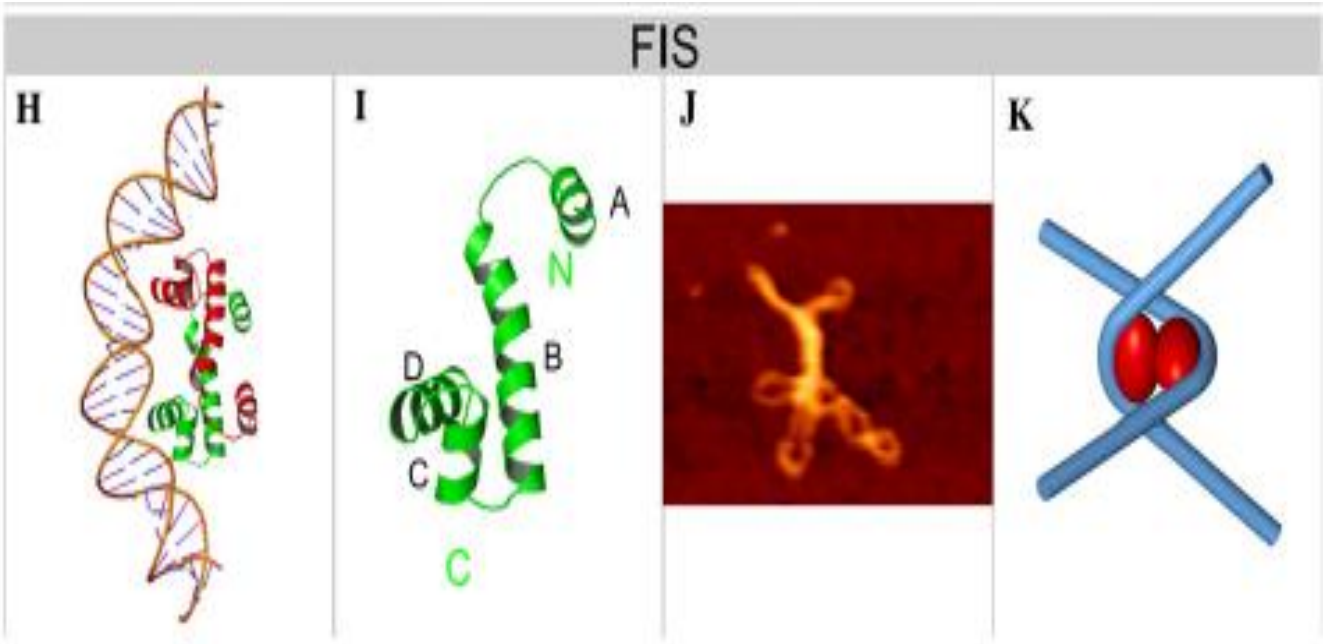
FIS Factor for Inversion Stimulation

Caratteristiche

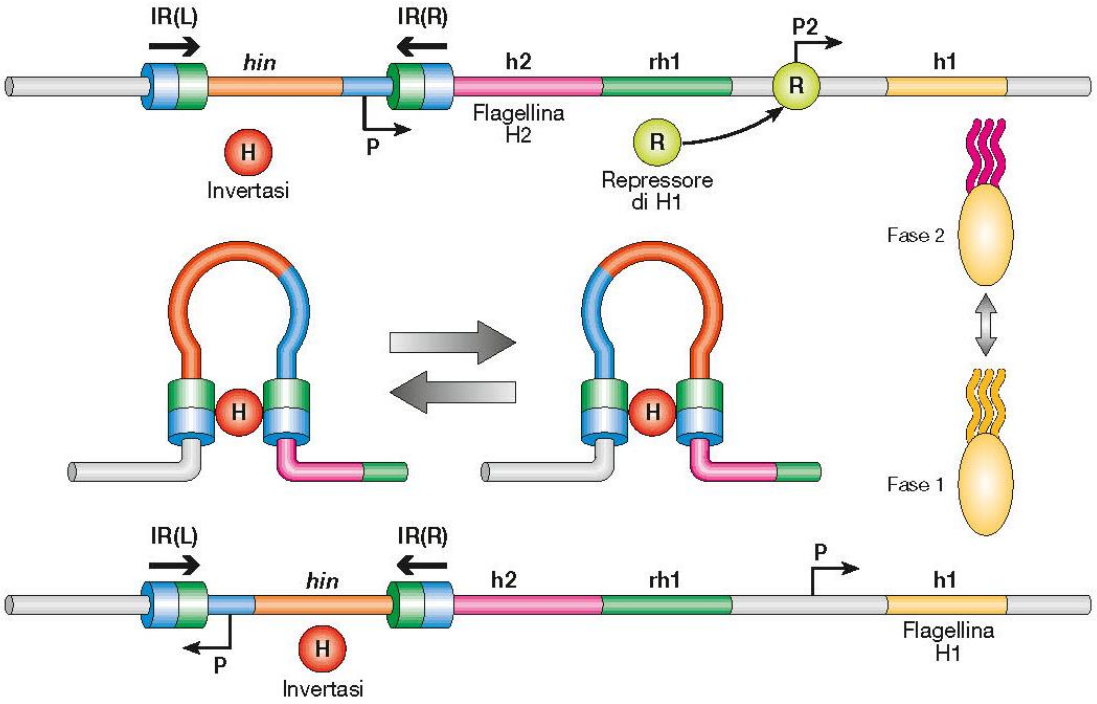
- Proteina basica
- abbondante in fase esponenziale
- 10.000-60.000 copie
- scarsa specificità di sequenza (KNNYRNNWNNNYRNNM)

W	TA
R	GA
K	GT
Y	CT

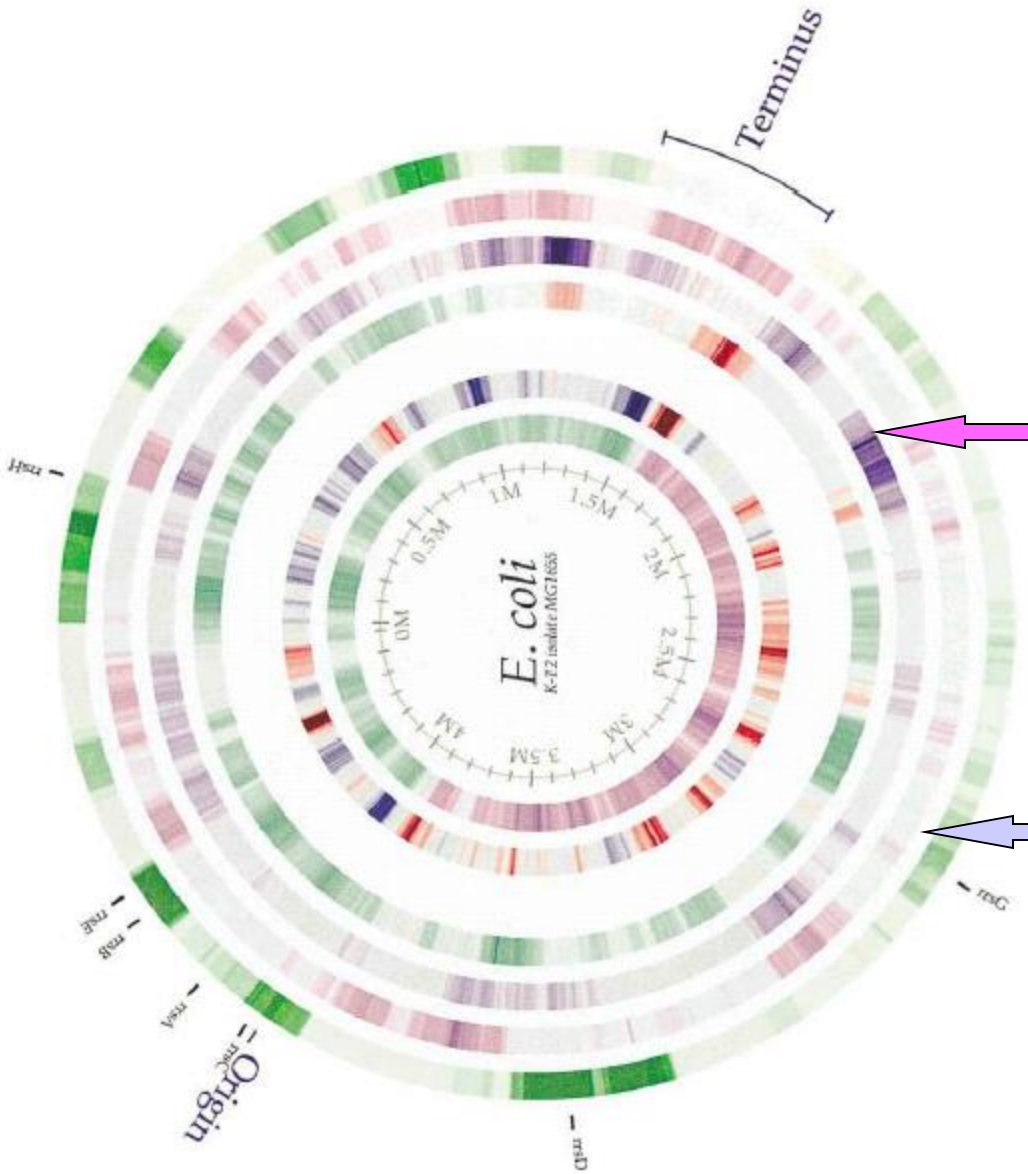
Struttura	pM	geni
OMODIMERO		
FIS	2x 11.5 kDa	fis (27.4 min)


FIS
H
I
J
K
A
N
B
D
C
C

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



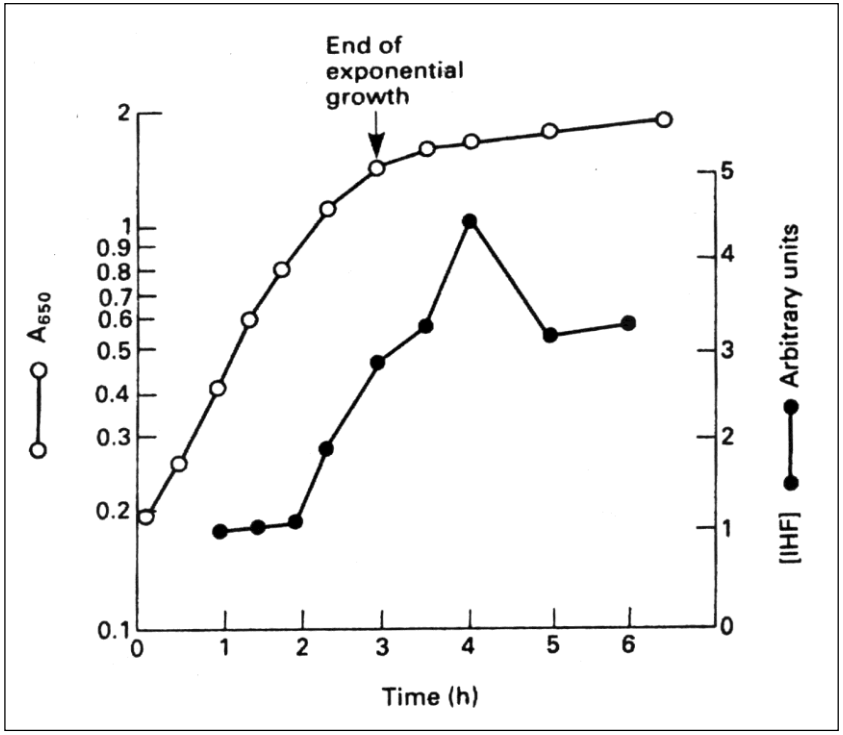
Proteine del nucleoide come regolatori globali



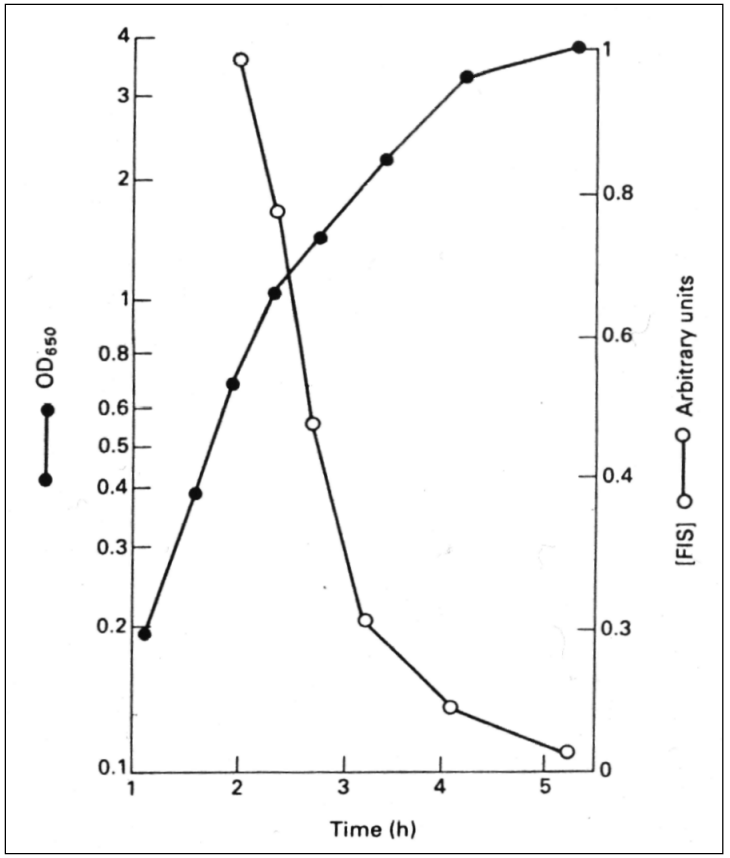
Potenziali siti dilegame per IHF

Potenziali siti di legame per FIS

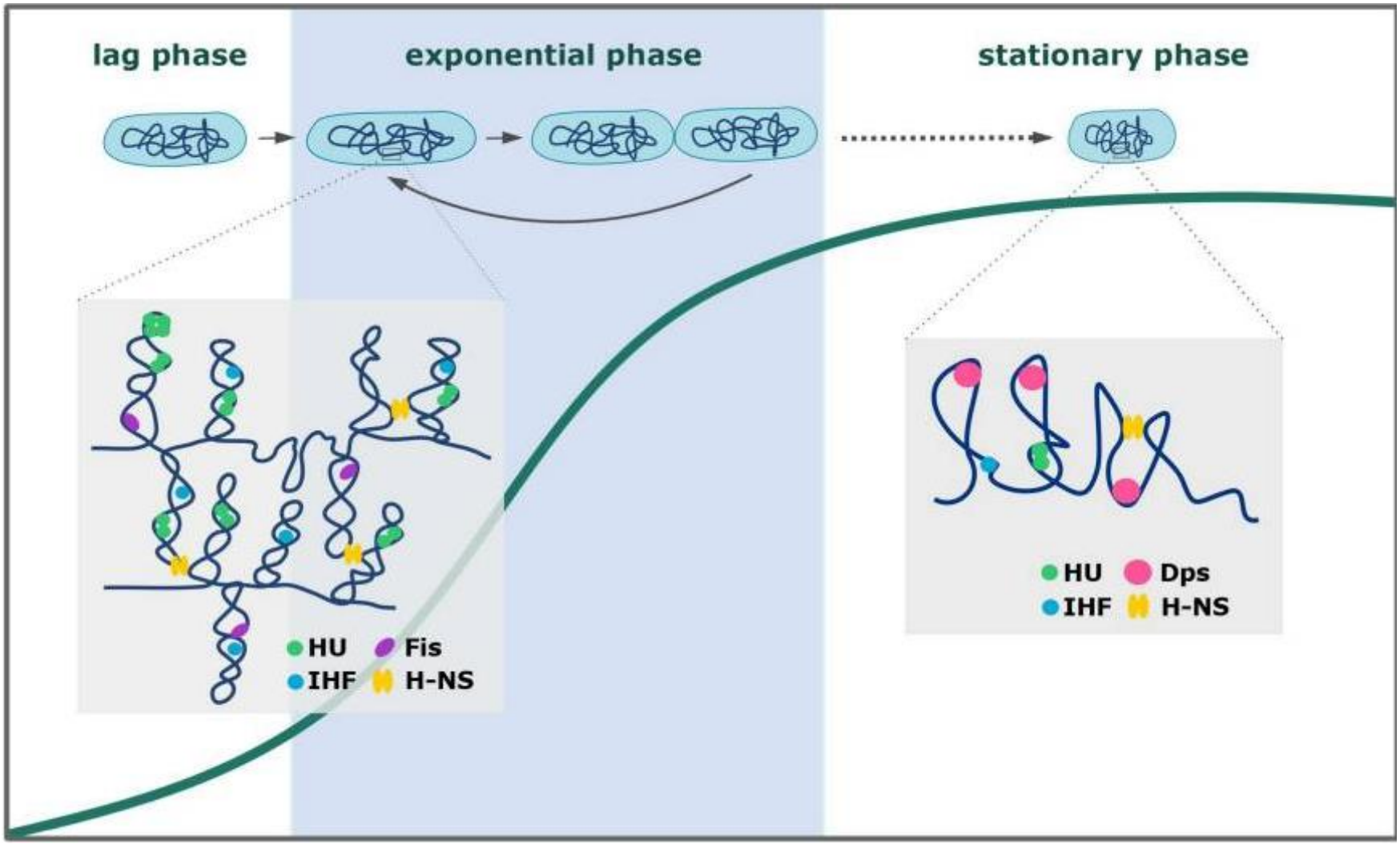
Espressione di IHF e FIS in funzione della curva di crescita



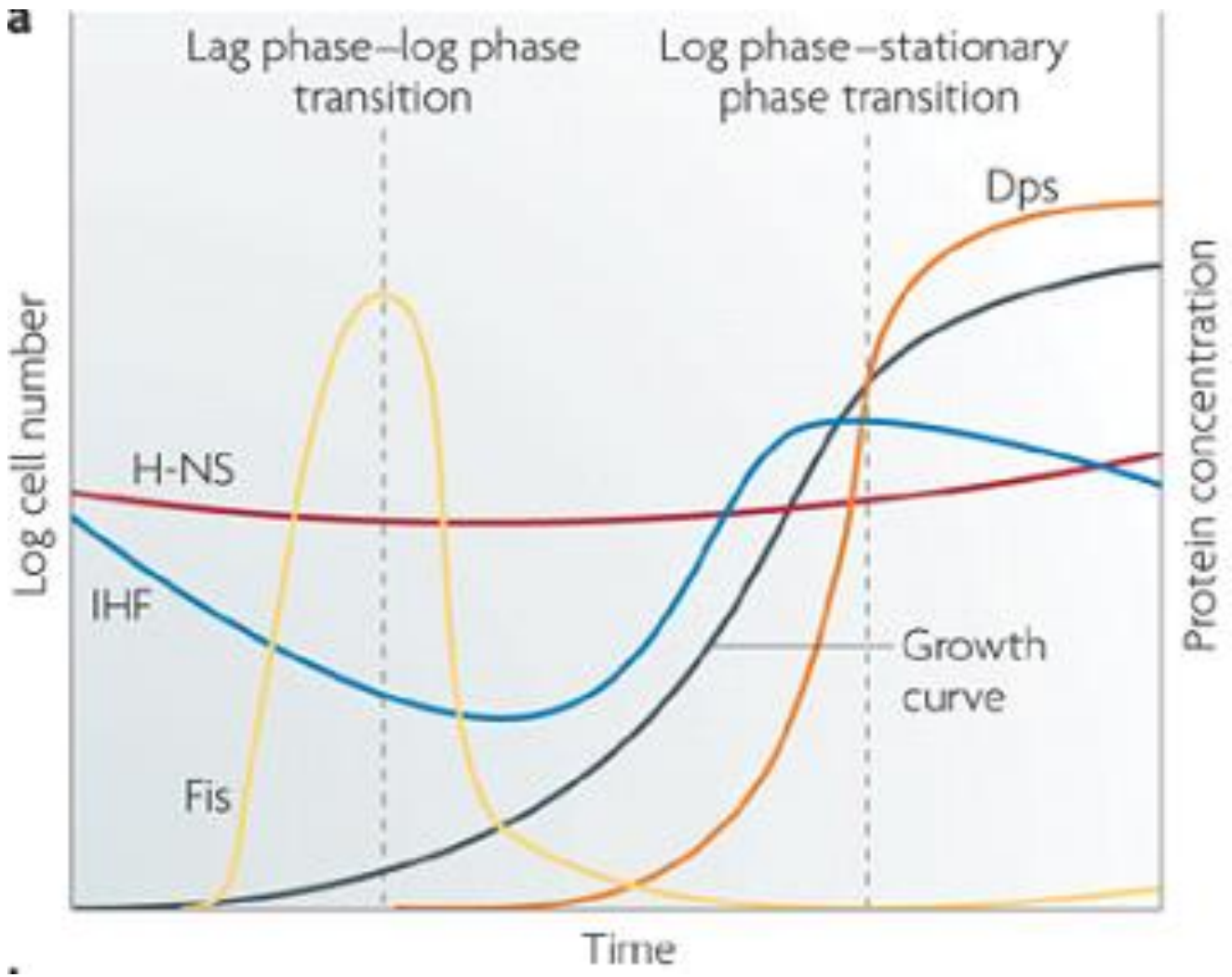
La concentrazione di IHF aumenta in funzione della curva di crescita e raggiunge il massimo all'inizio della fase stazionaria



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



Chromosome organization during the growth of *Escherichia coli*. The expression patterns of *E. coli* NAPs reflect the chromosome compaction level (higher in the stationary than in the exponential phase) and cellular processes that involve certain NAPs

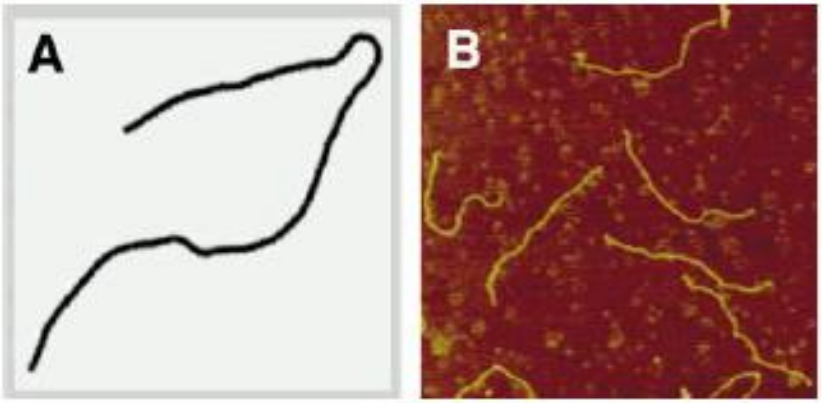


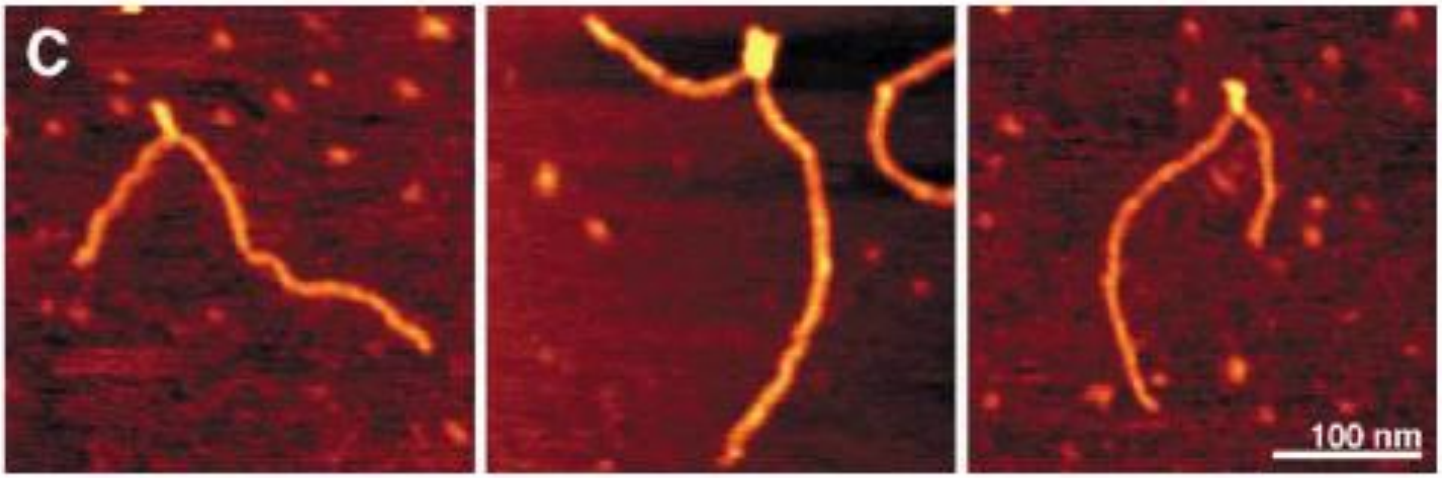
The expression patterns of the four nucleoid-associated proteins (NAPs), DNA protection from starvation protein (Dps), factor for inversion stimulation (Fis), histone-like nucleoid-structuring protein (H-NS) and integration host factor (IHF). A typical bacterial growth curve is shown, with the lag phase–log phase and log phase–stationary phase transitions indicated

H-NS
(Histone-like Nucleoid Structuring protein)

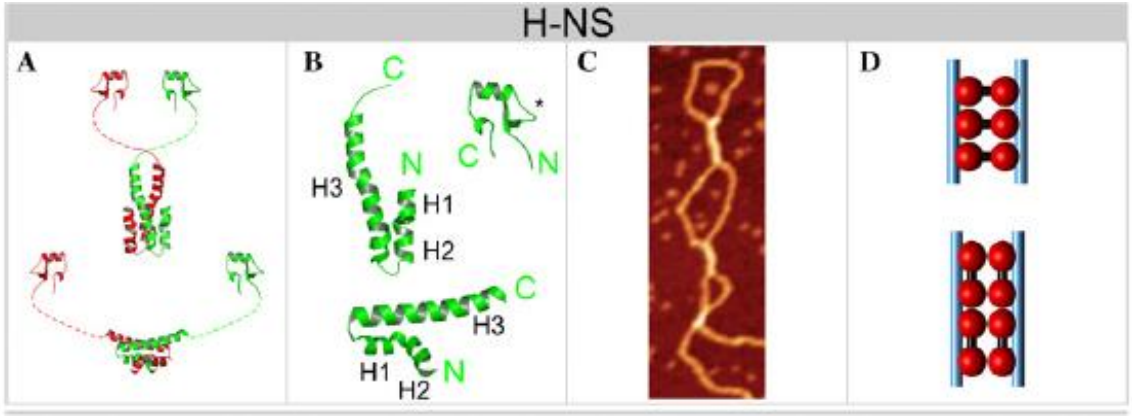
- Abundant peptide (~ 20000 copies/cell)
- Small (136 aminoacids, 15.5 kD), non-basic peptide
- Mainly acts as a homodimer or tetramer
- Able to form heterodimers with StpA or HhA
- *In vitro* binding to DNA is non-specific; induces high DNA compaction
- Higher affinity for intrinsically curved DNA; 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

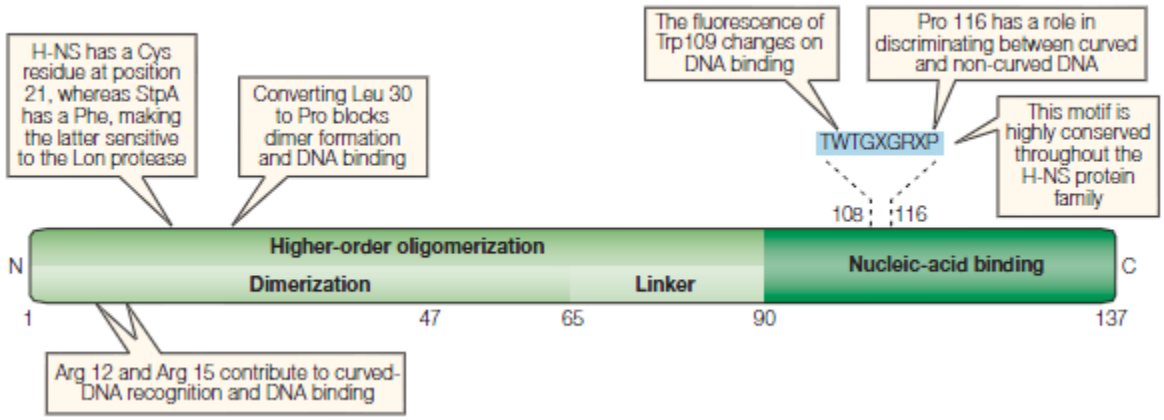


H-NS viene considerata una proteine in grado di formare ponti

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

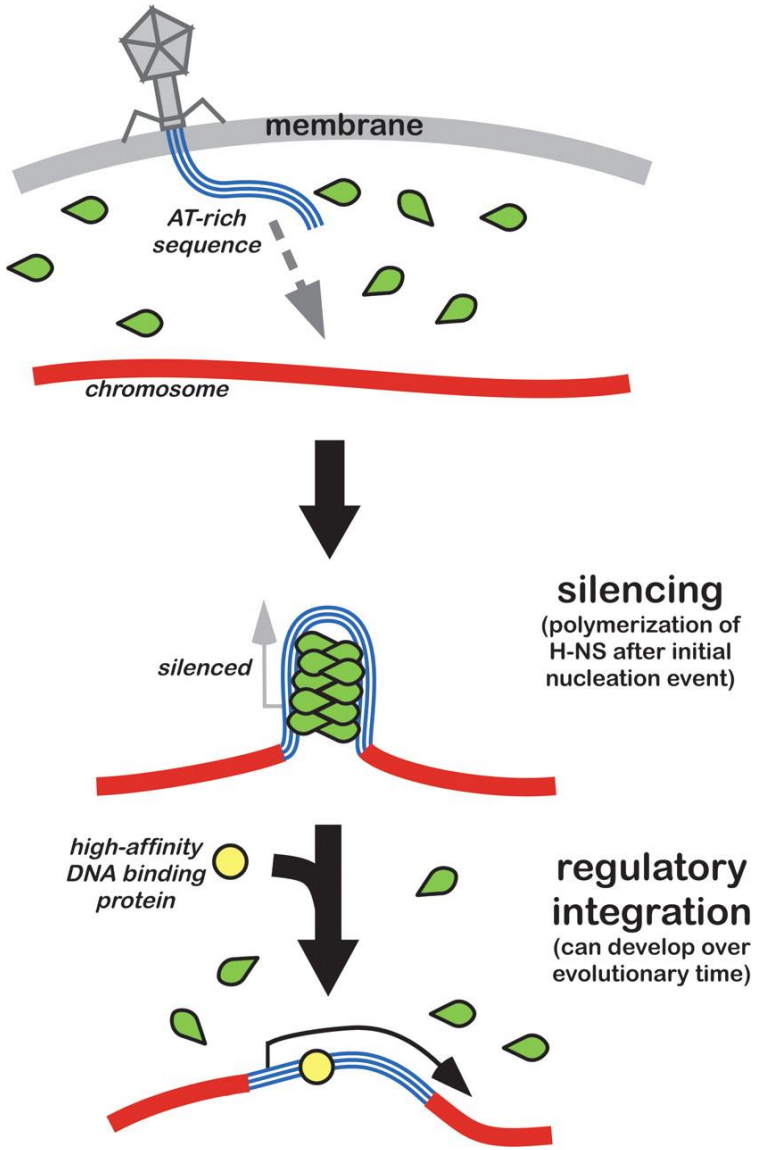
Altre proteine NAP che formano ponti su LRP e MuKB

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 flesibili 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

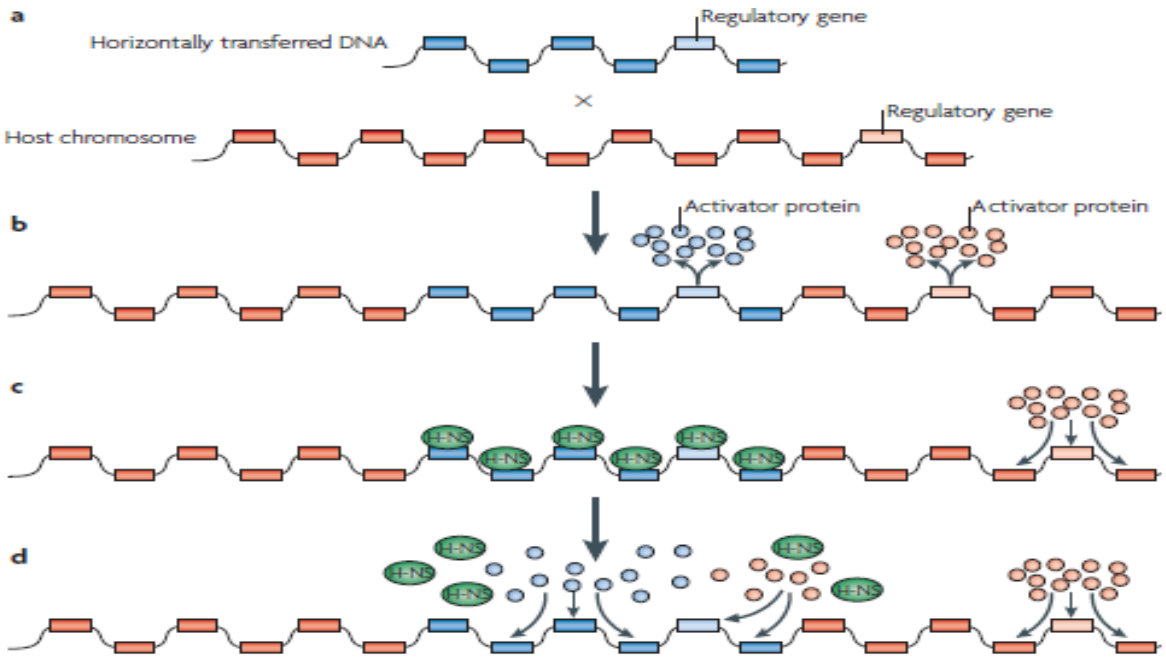
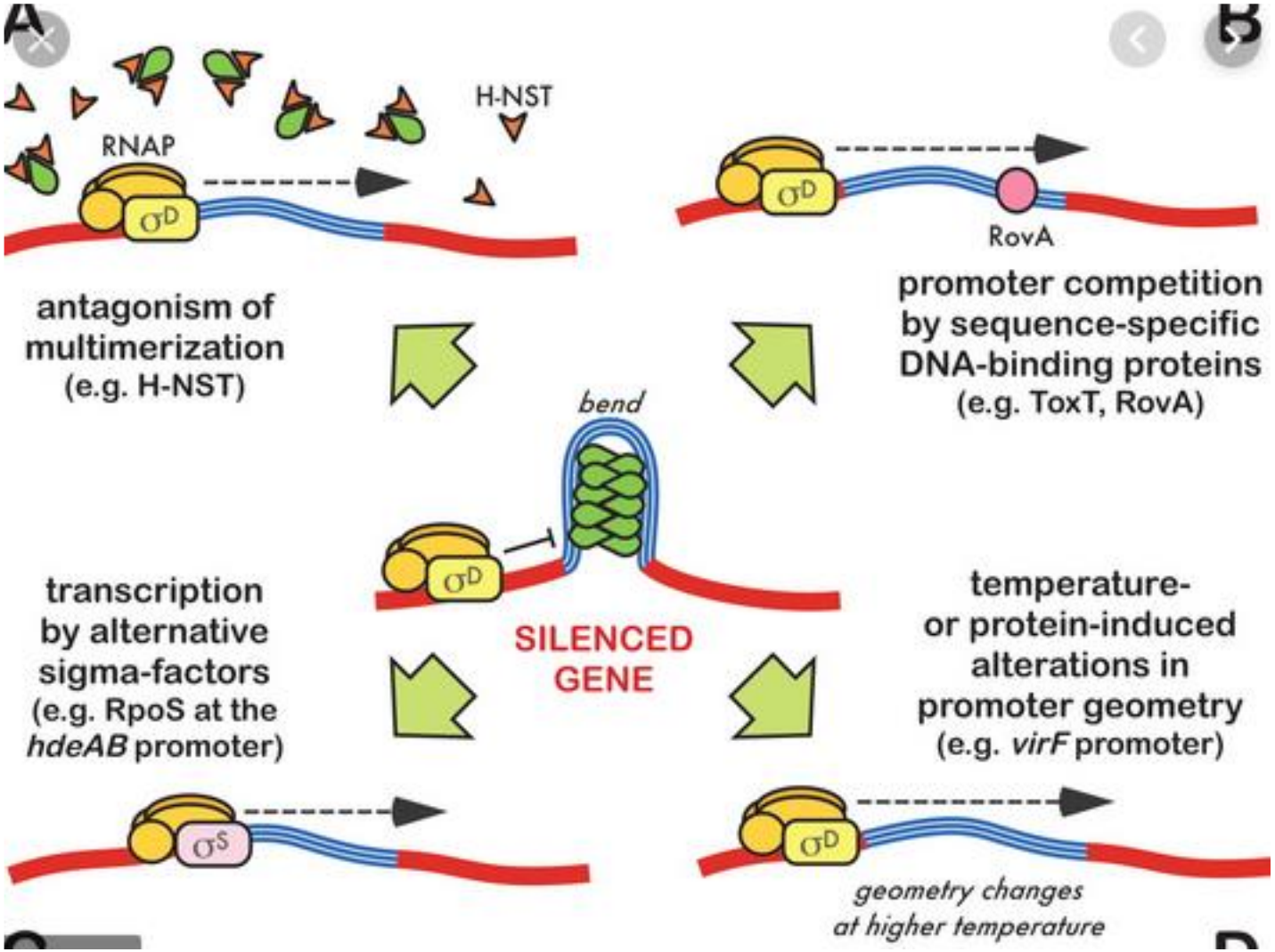
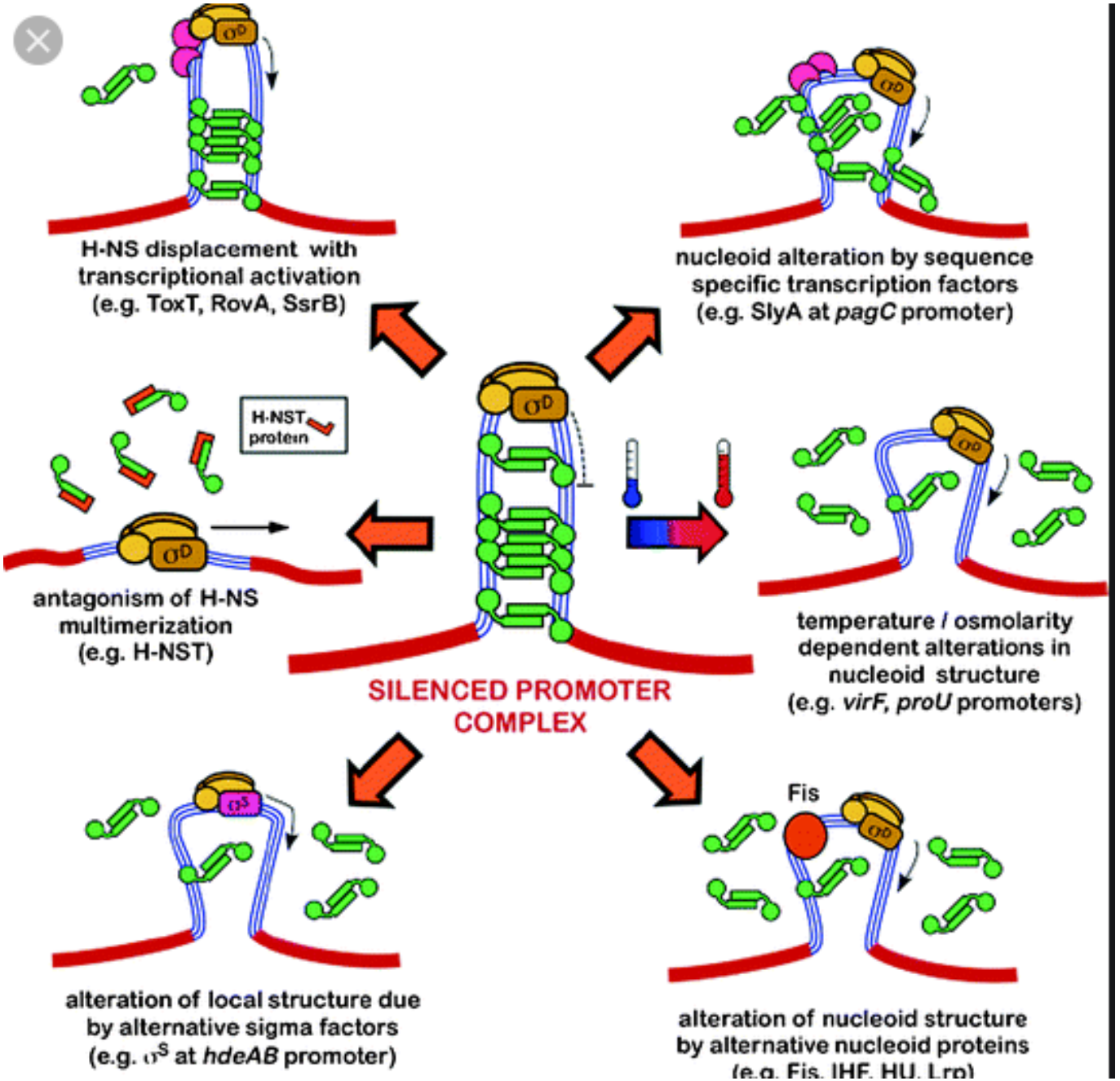


Figure 1 | **H-NS as a gene silencer.** The figure shows a model for the involvement of H-NS in the repression of horizontally transferred genes, and how this repression can be relieved. **a** | A segment of DNA carrying six genes enters the cell as a result of horizontal transfer. One of these is a 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 remodelling the DNA near its binding sites through structural perturbations, such as DNA bending. 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.


A
B
H-NST
RNAP
σD
RovA
antagonism of multimerization (e.g. H-NST)
promoter competition by sequence-specific DNA-binding proteins (e.g. ToxT, RovA)
bend
SILENCED GENE
transcription by alternative sigma-factors (e.g. RpoS at the hdeAB promoter)
temperature- or protein-induced alterations in promoter geometry (e.g. virF promoter)
σS
geometry changes at higher temperature


H-NS displacement with transcriptional activation (e.g. ToxT, RovA, SsrB)
nucleoid alteration by sequence specific transcription factors (e.g. SlyA at pagC promoter)
H-NST protein
antagonism of H-NS multimerization (e.g. H-NST)
temperature / osmolarity dependent alterations in nucleoid structure (e.g. virF, proU promoters)
SILENCED PROMOTER COMPLEX
Fis
alteration of local structure due by alternative sigma factors (e.g. σS at hdeAB promoter)
alteration of nucleoid structure by alternative nucleoid proteins (e.g. Fis, IHF, HU, Lrp)

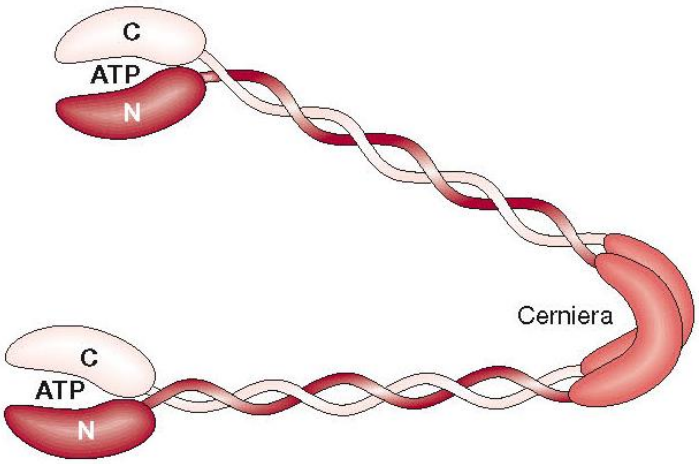
La proteina MukB una proteina SMC like

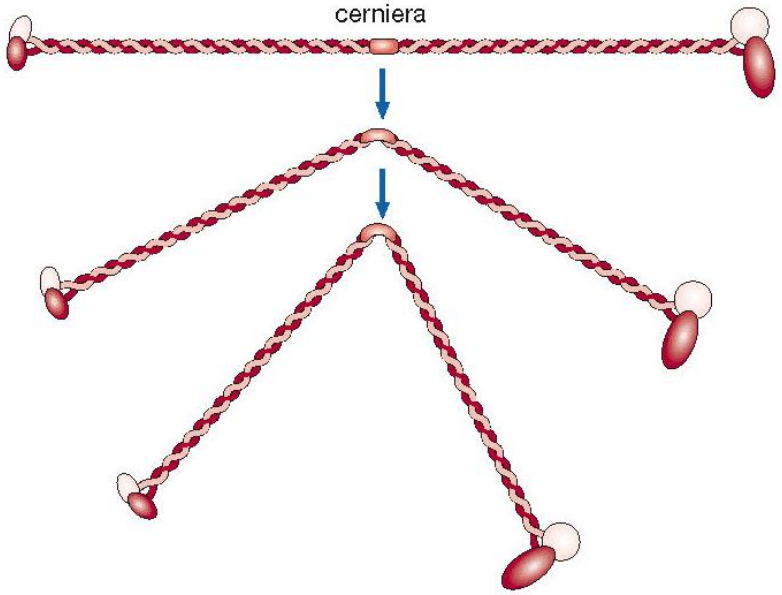
La proteina MukB è una proteina SMC like (Structural Maintanance of Chromosome) presente in molti batteri.

In assenza della proteina MukB i batteri diventano termosensibili e a temperatura permissiva hanno una crescita ridottissima : si osserva una decondensazione del DNA ed una perdita del nucleoide ad alta frequenza.

La proteina MukB svolge un ruolo importante anche nella segregazione dei cromosomi in seguito a divisione cellulare.

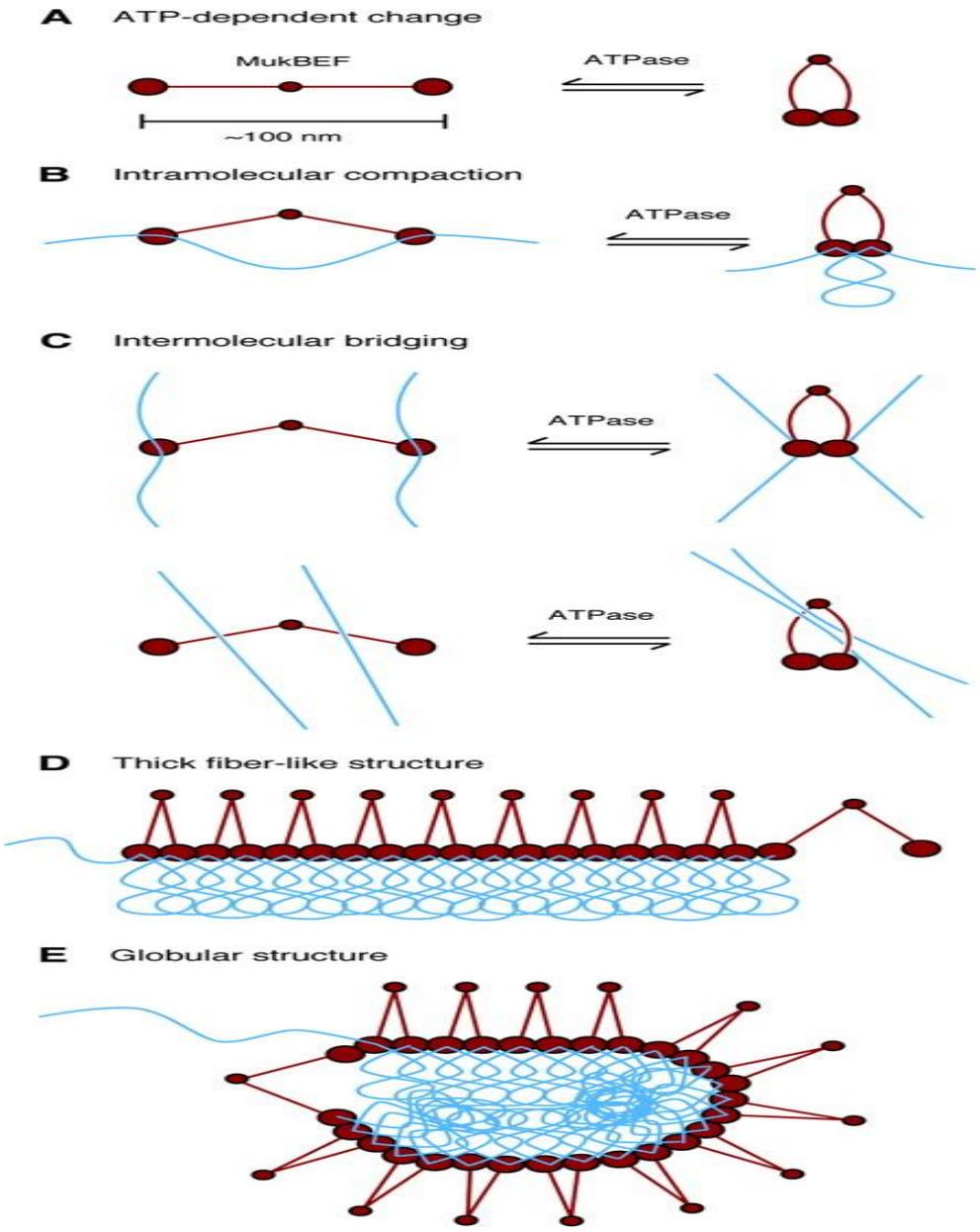
La perdita di MukB e della proteina del nucleoide HU è letale per la cellula.





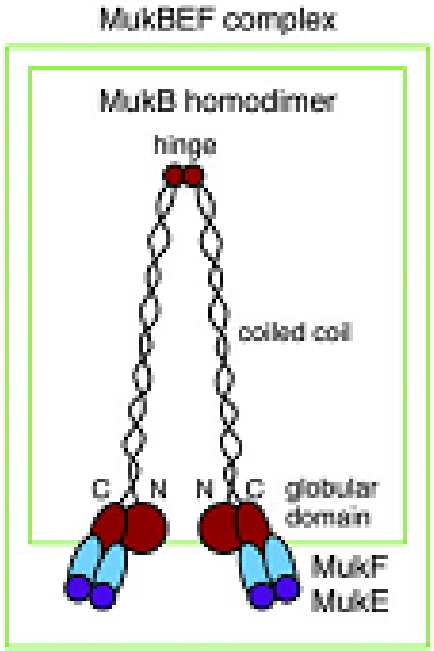
La proteina Muk B come molte proteine della famiglia SMC ,è costituita da due domini globulari N- e C- terminali (teste) separati da 2 regione coiled-coil intervallate da una terza regione globulare che costituisce una cerniera flessibile.

Le proteine MuKB sono omodimeri ed hanno nelle regioni C- e N-terminali dei domini ATPasici conservati.

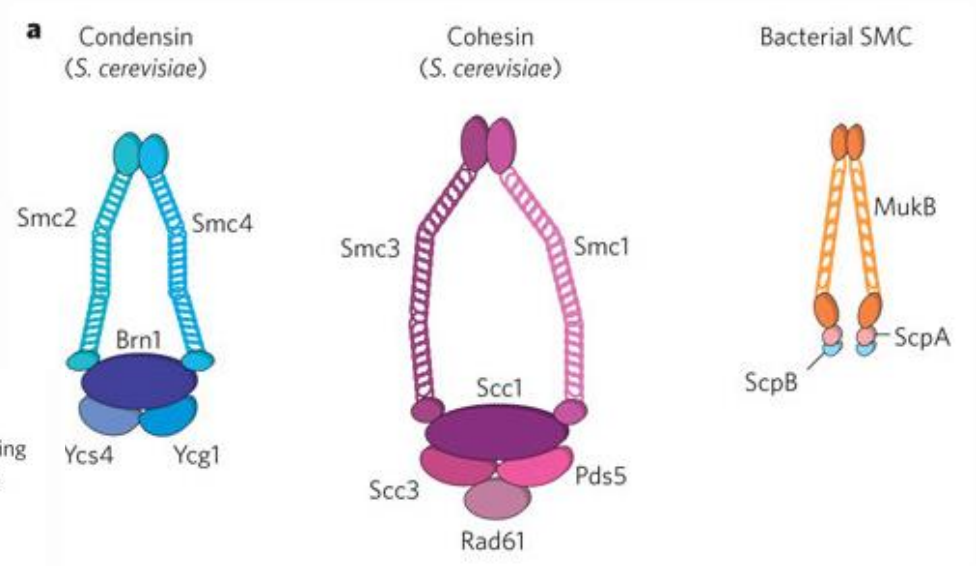


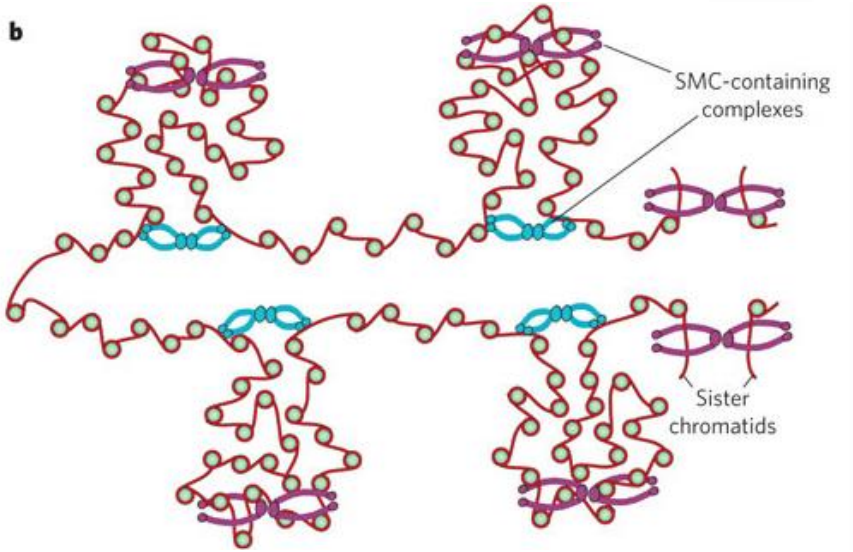
Nella forma chiusa la proteina scorre sul DNA ed è in grado di indurne il ripiegamento.

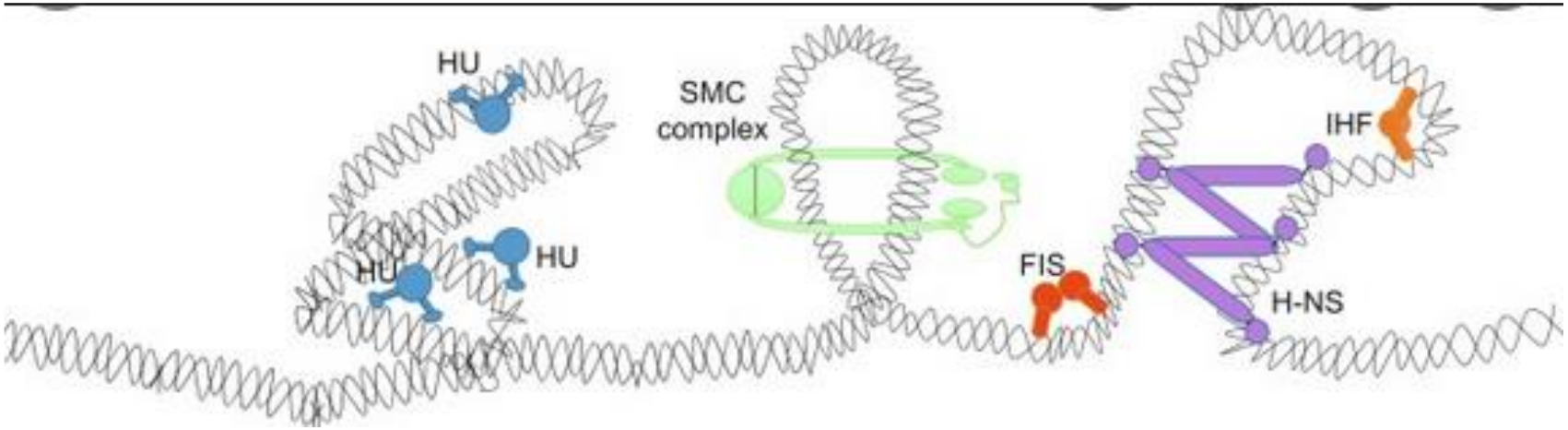
La proteina MukB potrebbe legare il DNA in due punti interagendo con i domini testa e poi grazie alla flessibilità della regione cerniera provocarne il ripiegamento e la condensazione



La condensina Batterica costituita dalla proteina MukB che appartiene alla famiglia delle SMC proteins (Structural Maintance of Chromosome) e dalle proteine MukFE è in grado di compattare il DNA in presenza di ATP con un meccanismo simile a quello osservato negli euc






HU
HU
HU
SMC
complex
IHF
FIS
H-NS

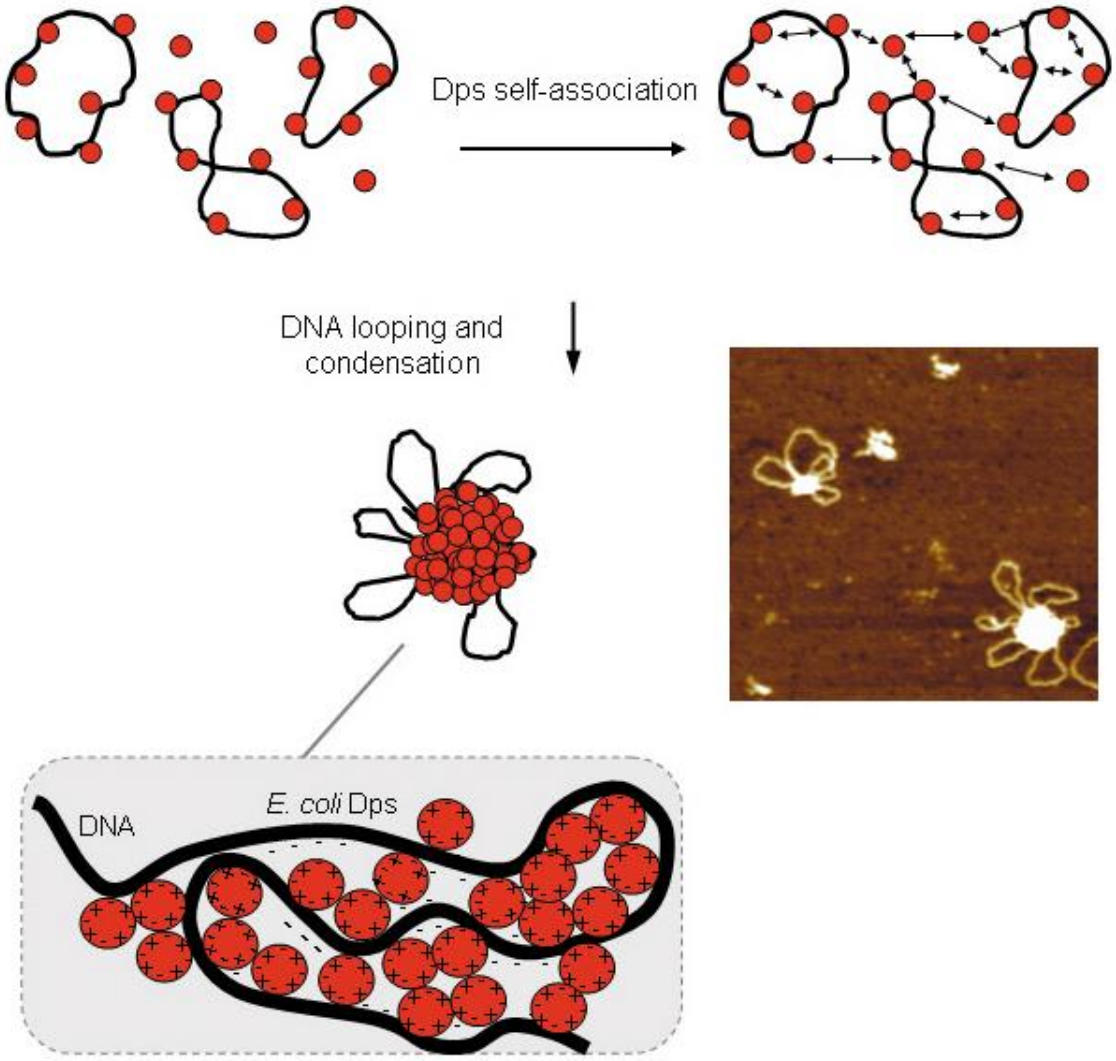
La proteina Dps (Dna Binding protein from starved cells)

forma un complesso costituito da 12 monomeri di 19 KDa

Il complesso Dps contiene uno ione Fe e rende il DNA resistente allo stress ossidativo

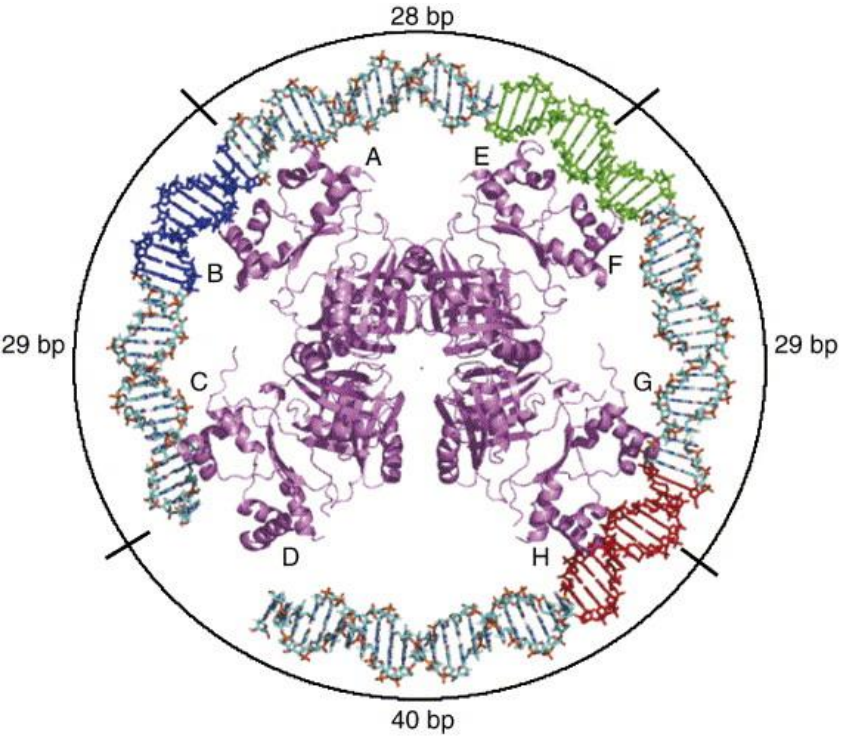
E' presente in alto numero di copie circa 20.000

Si lega al DNA a livello di sequenze non specifiche



I residui di lisina localizzati all'estremità N terminale di Dps carichi positivamente promuovono la condensazione del DNA in quanto interagiscono sia con il DNA che con le regioni cariche negativamente delle molecole adiacenti di Dps

Lrp Leucine responsive regulatory protein



Ottamero di LRP

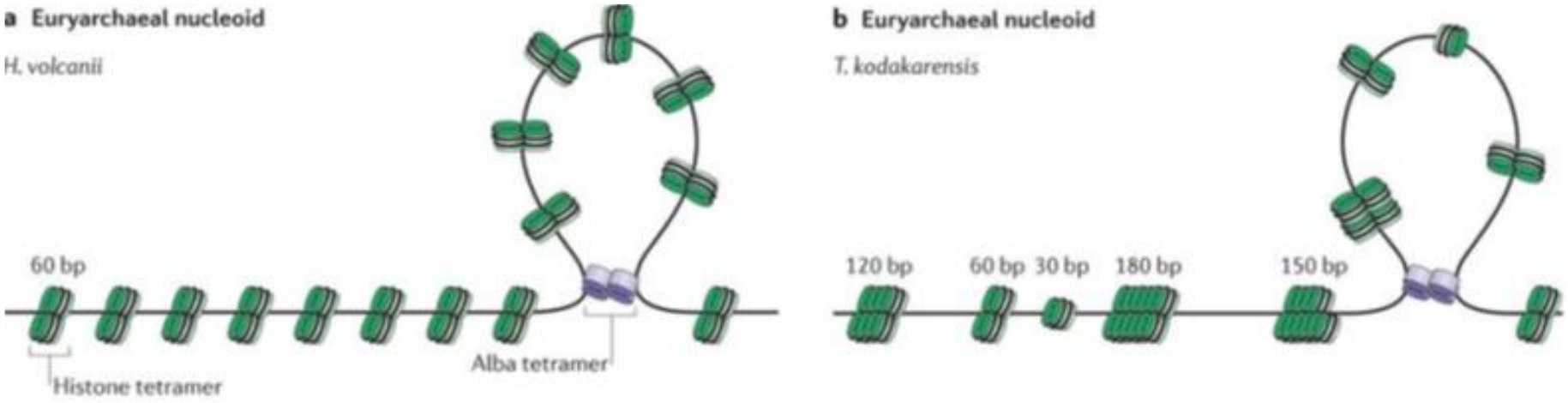
influenza ala trascrizione del 10% dei geni di E.coli e a seconda del target il suo effetto può essere potenziato o meno dalla presenza di leucina.

I geni regolati comprendono geni coinvolti nell'acquisizione e metablismo degli AA oltre a geni di virulenza quali quelli coinvolti nella sintesi di alcuni pili.

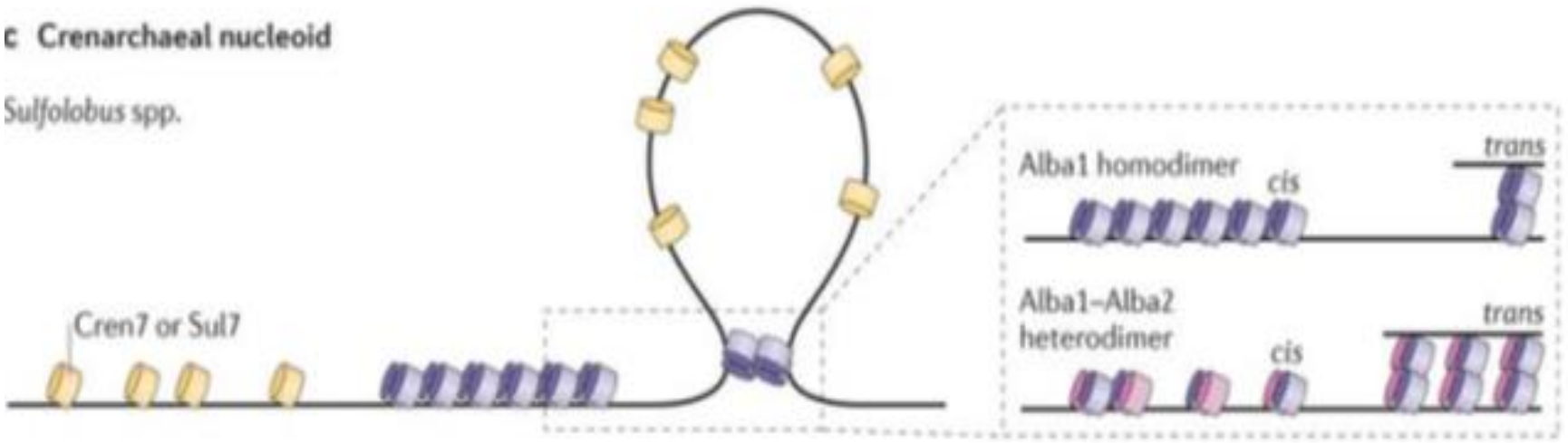
Lrp riconosce una sequenza consenso degenerata sul DNA e modifica la struttura del DNA con il suo legame.

Esiste in diversi stati oligomerici , dimero, ottamero o esadecamero.

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

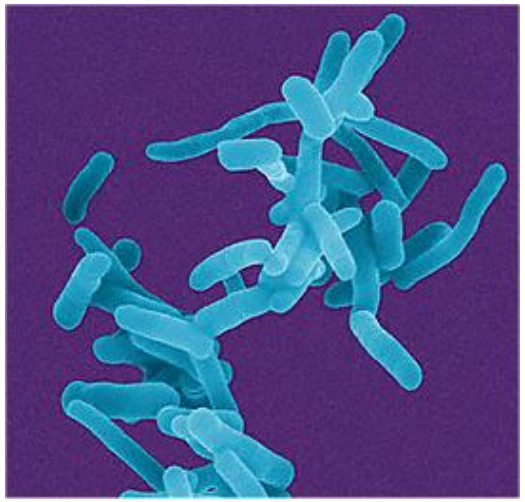


a,b | The euryarchaeal nucleoid is mainly organized by histone proteins that bend or wrap DNA, as well as by Alba that binds to DNA as a homodimer or a heterodimer and that forms looped structures by bridging two DNA duplexes. In *Haloferax volcanii*, histone proteins form tetrameric nucleoprotein structures 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 | 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[15]. Alba seems to have an ancient evolutionary history and considerable functional plasticity[16]. 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[19], 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

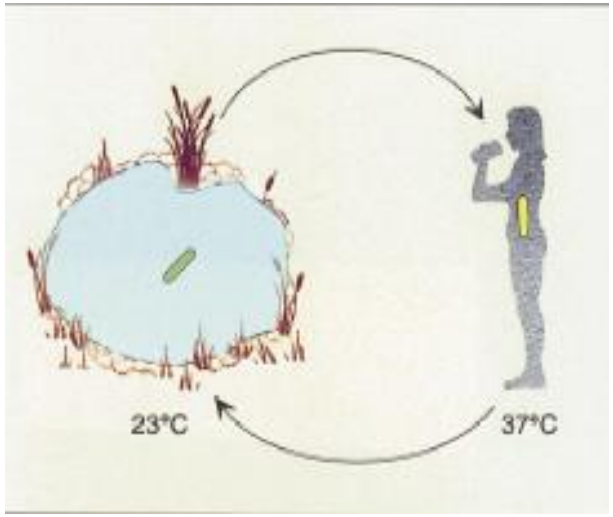


- is a Gram negative, facultative anaerobe
- is an intracellular pathogen
- is the etiological agent of bacillary dysentery, an acute diarrheal disease
- causes 160 million of episodes, determining 1.1 million deaths/year in children and infants in developing countries.

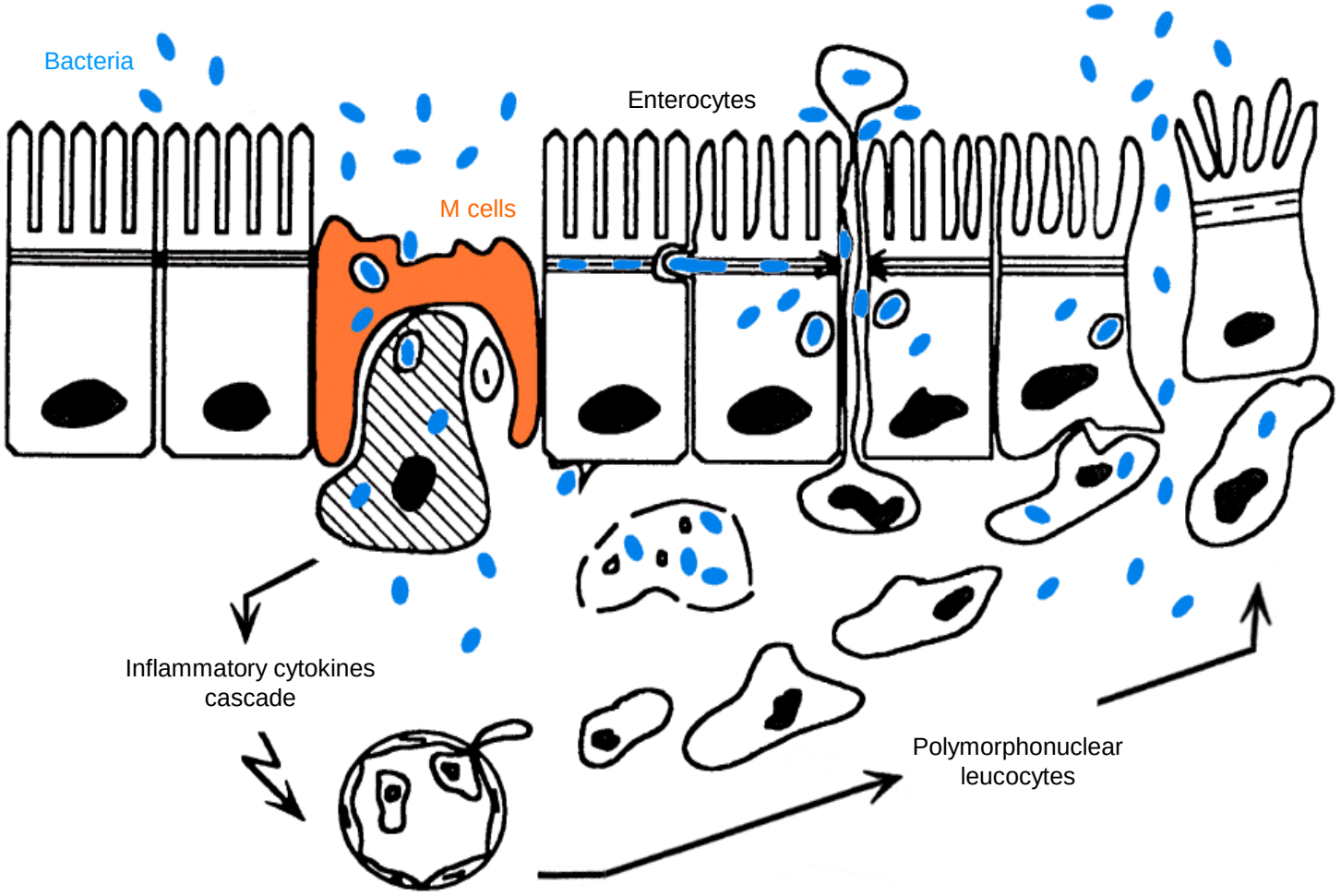
Infection is spread via fecal-oral route

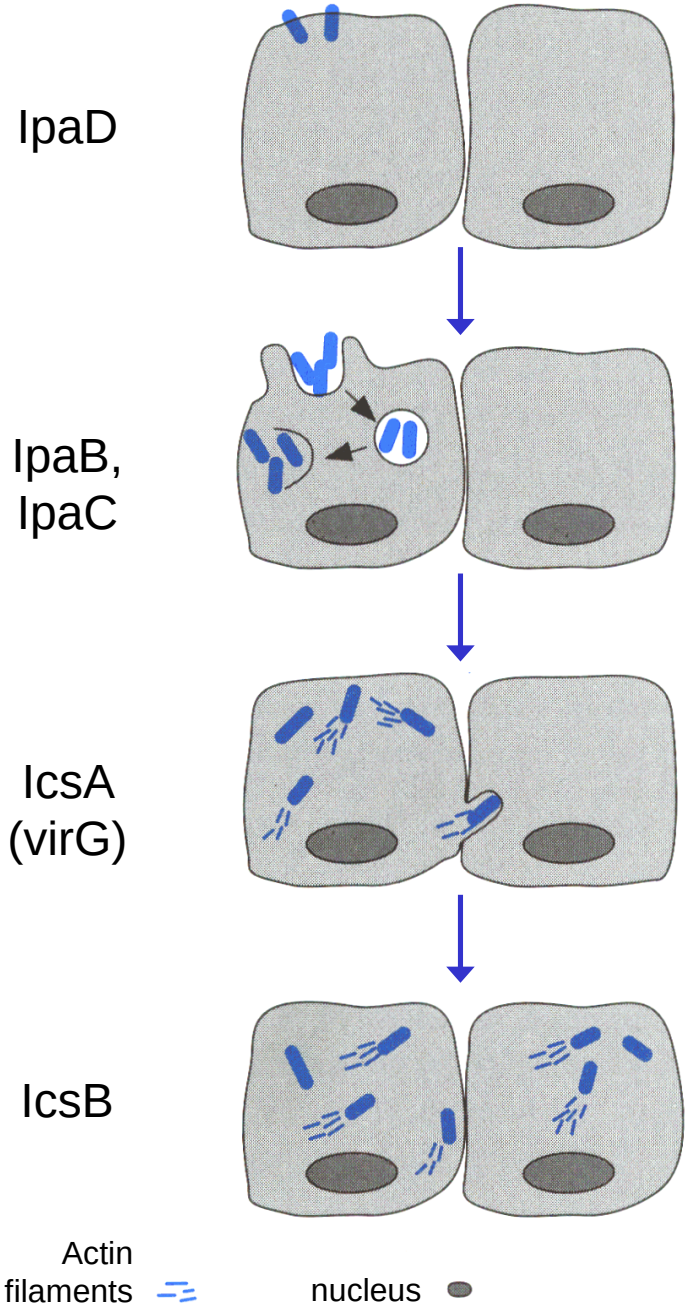
Subgrouped in four "species":

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



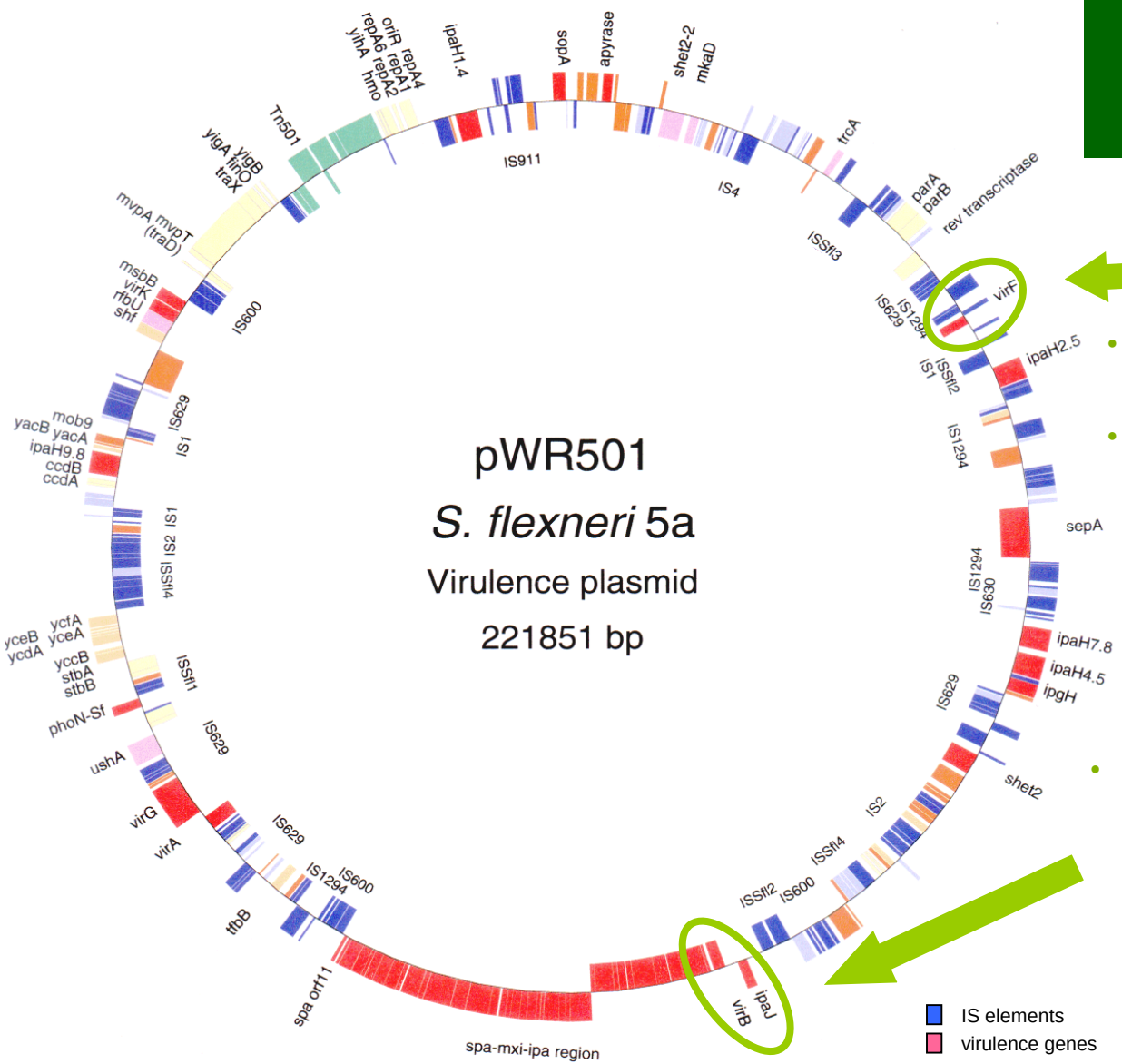
Model for *Shigella* invasion of the colonic mucosa





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

Genetic organization of pINV



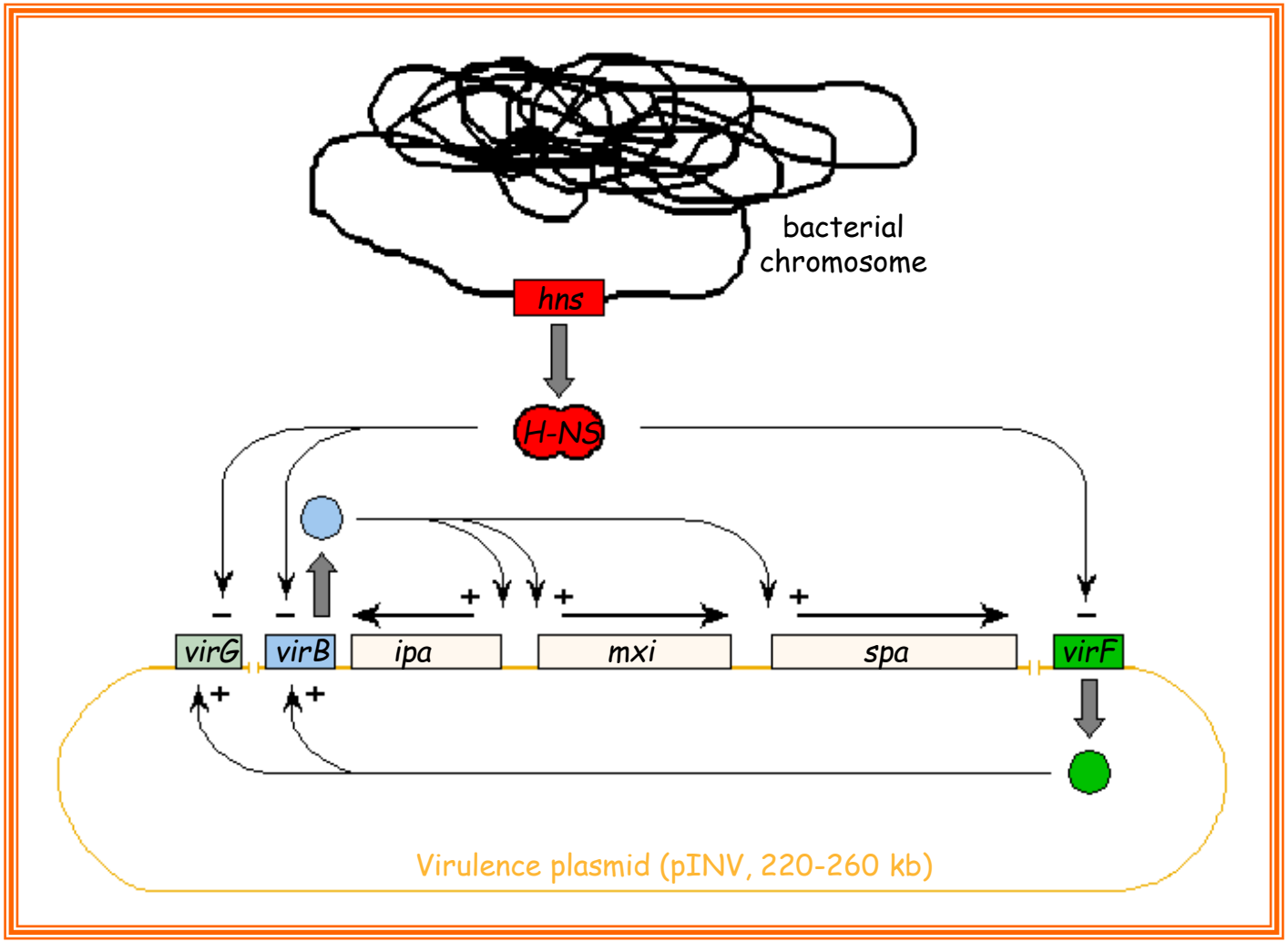
virF is ...

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

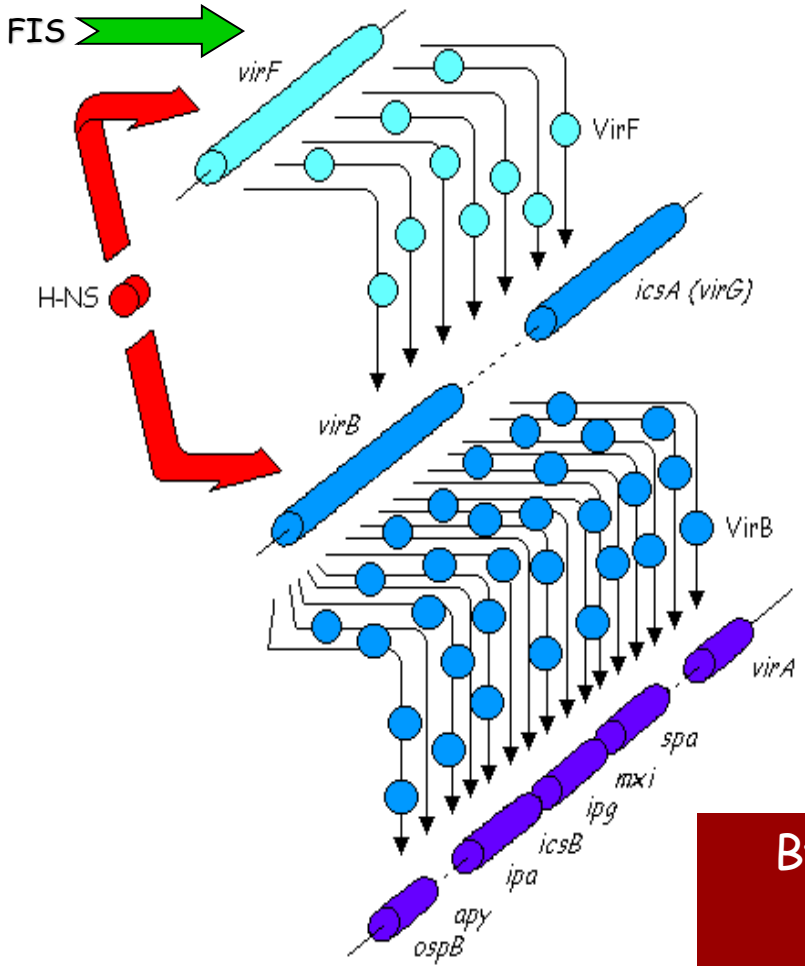
virB is ...

- ... 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

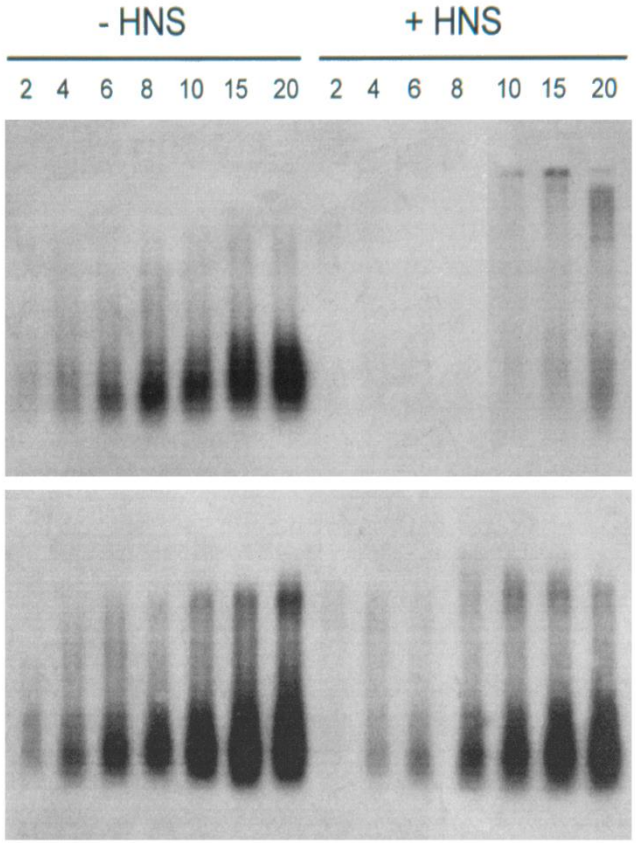


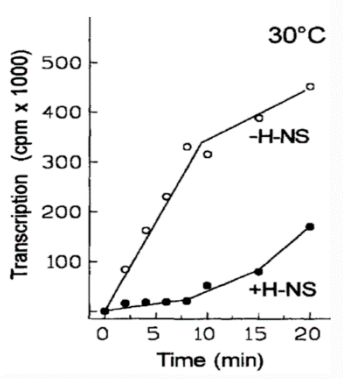
VirF is ...

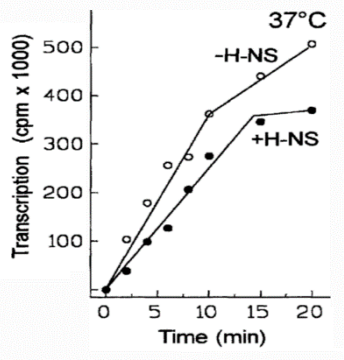
- ... expressed at 37°C
- ... is controlled antagonistically by two nucleoid proteins H-NS (repressor) and FIS (activator)

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

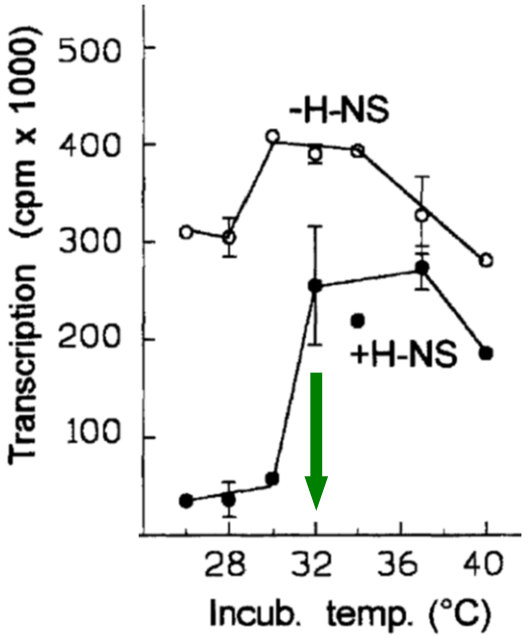
H-NS is able to bind to and repress *virF* only at low temperature



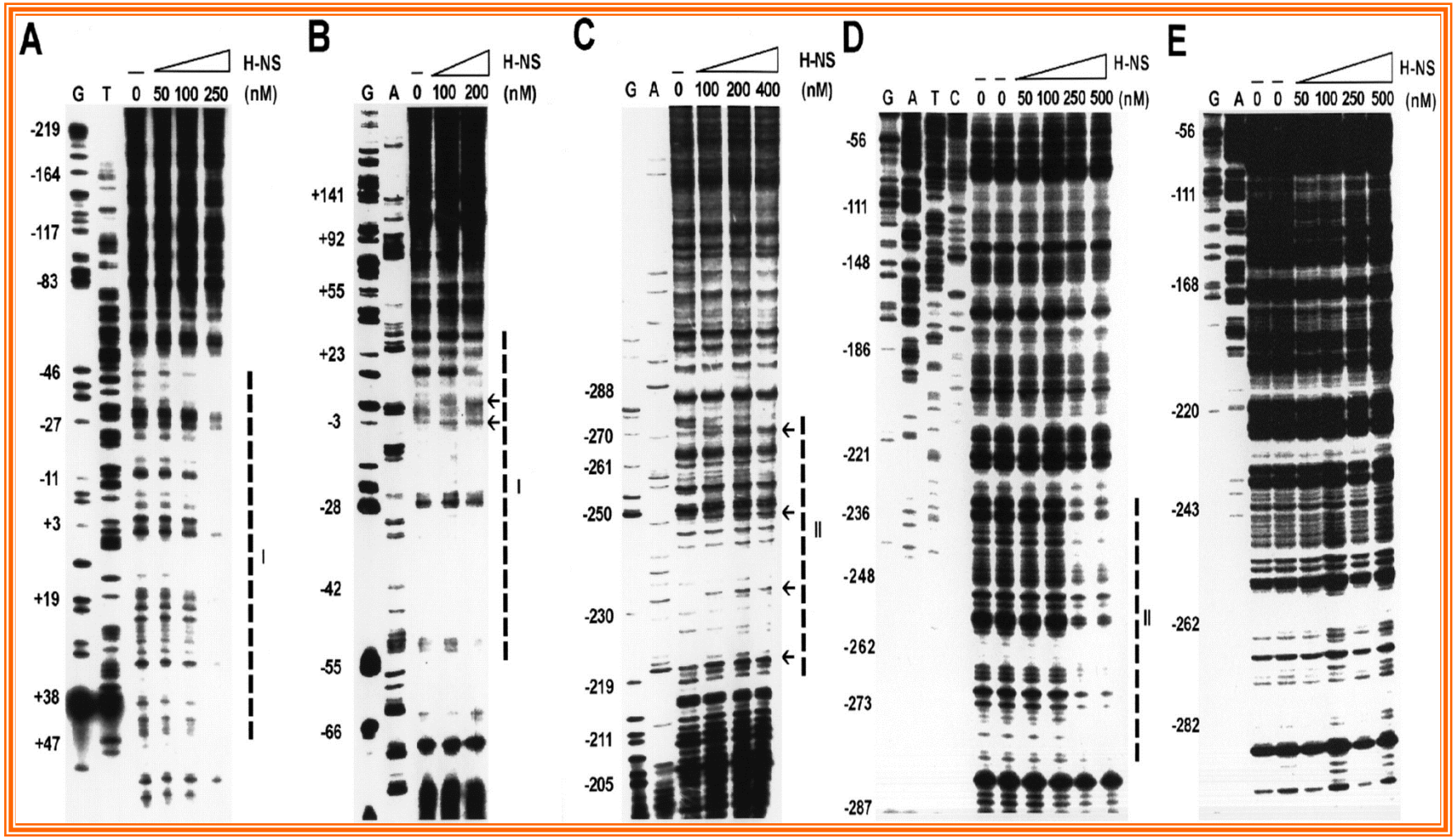




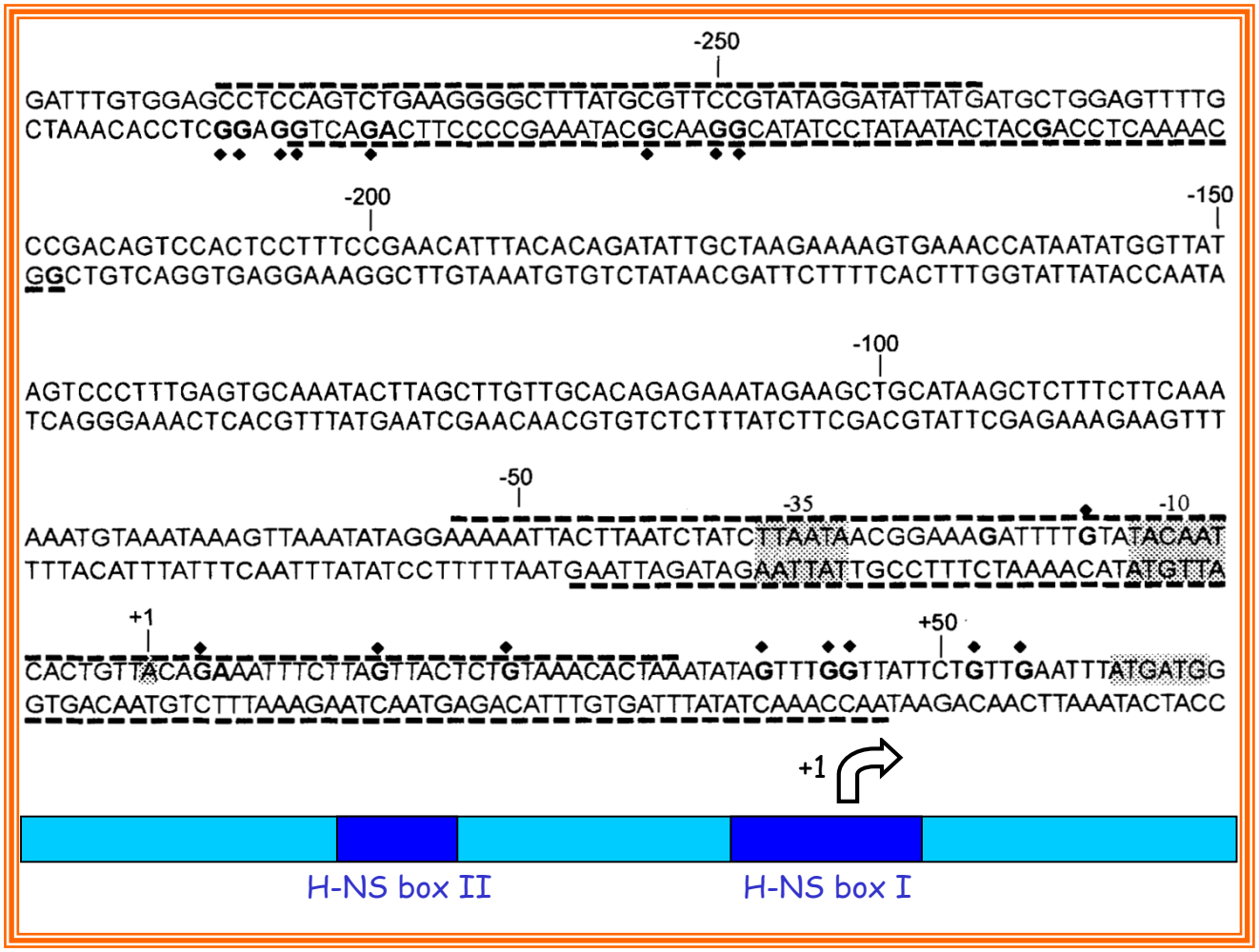
…more precisely:
virF transcription is inhibited by H-NS only below 32°C



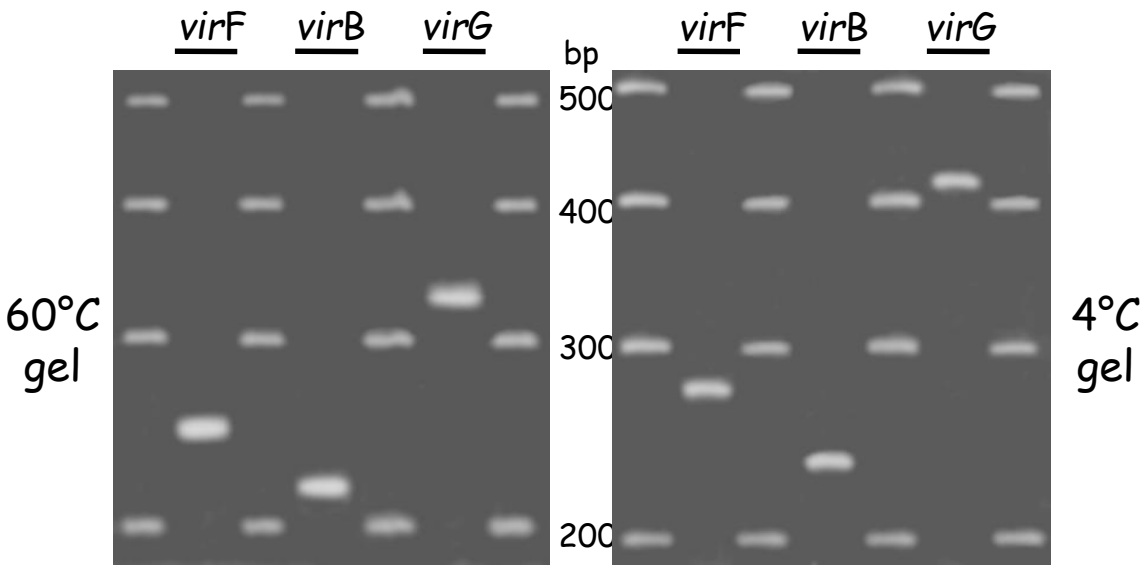
DNaseI footprinting of the *virF* promoter region by H-NS



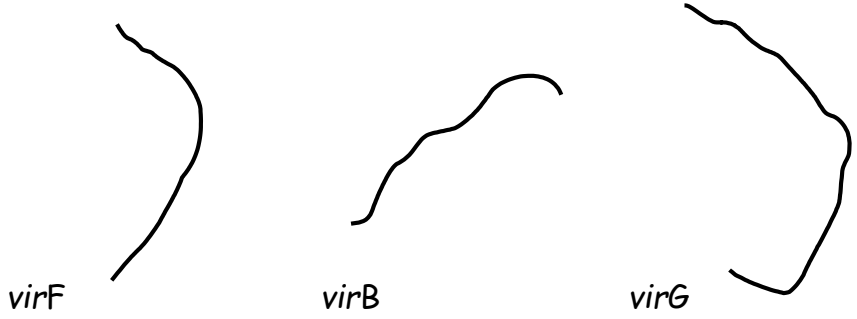
The *virF* promoter region



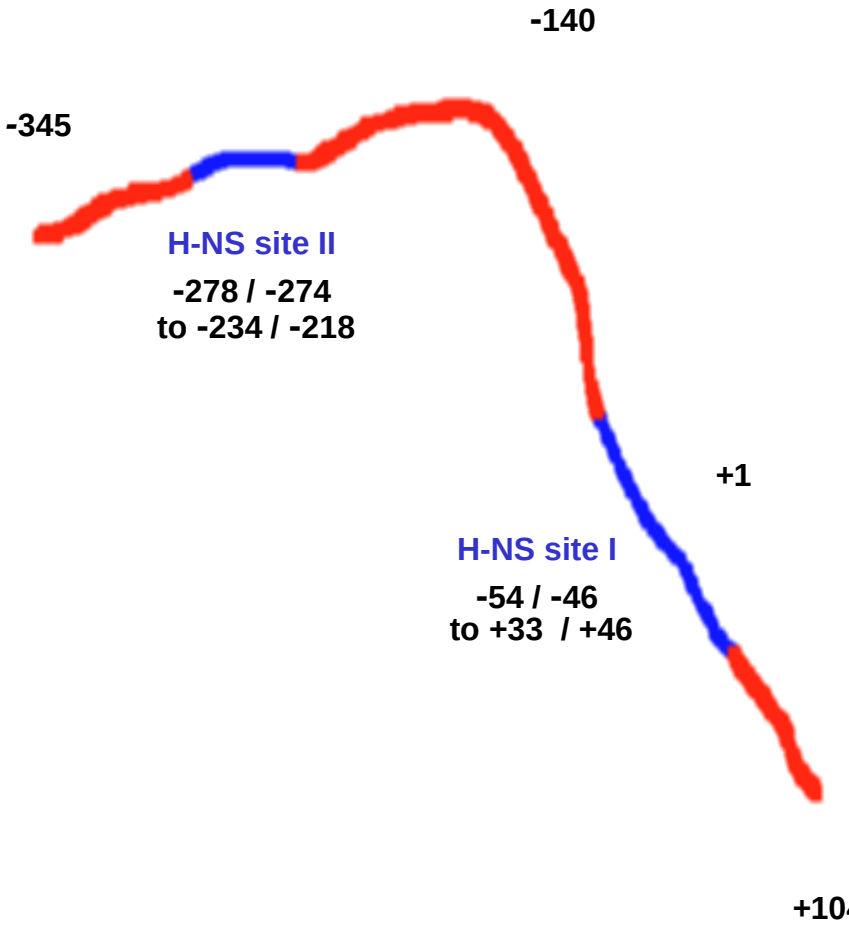
Curvature in H-NS regulated vir promoters



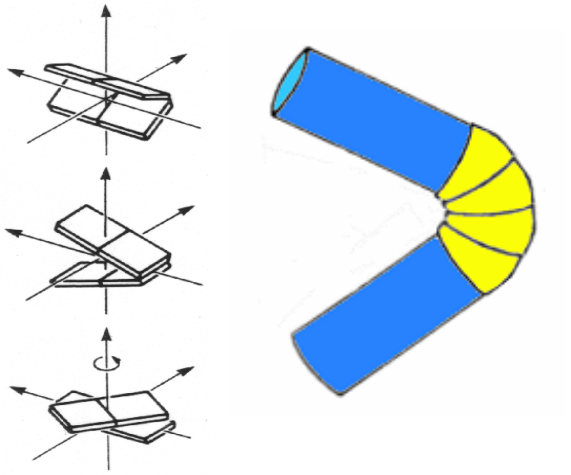
Computer-generated models



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

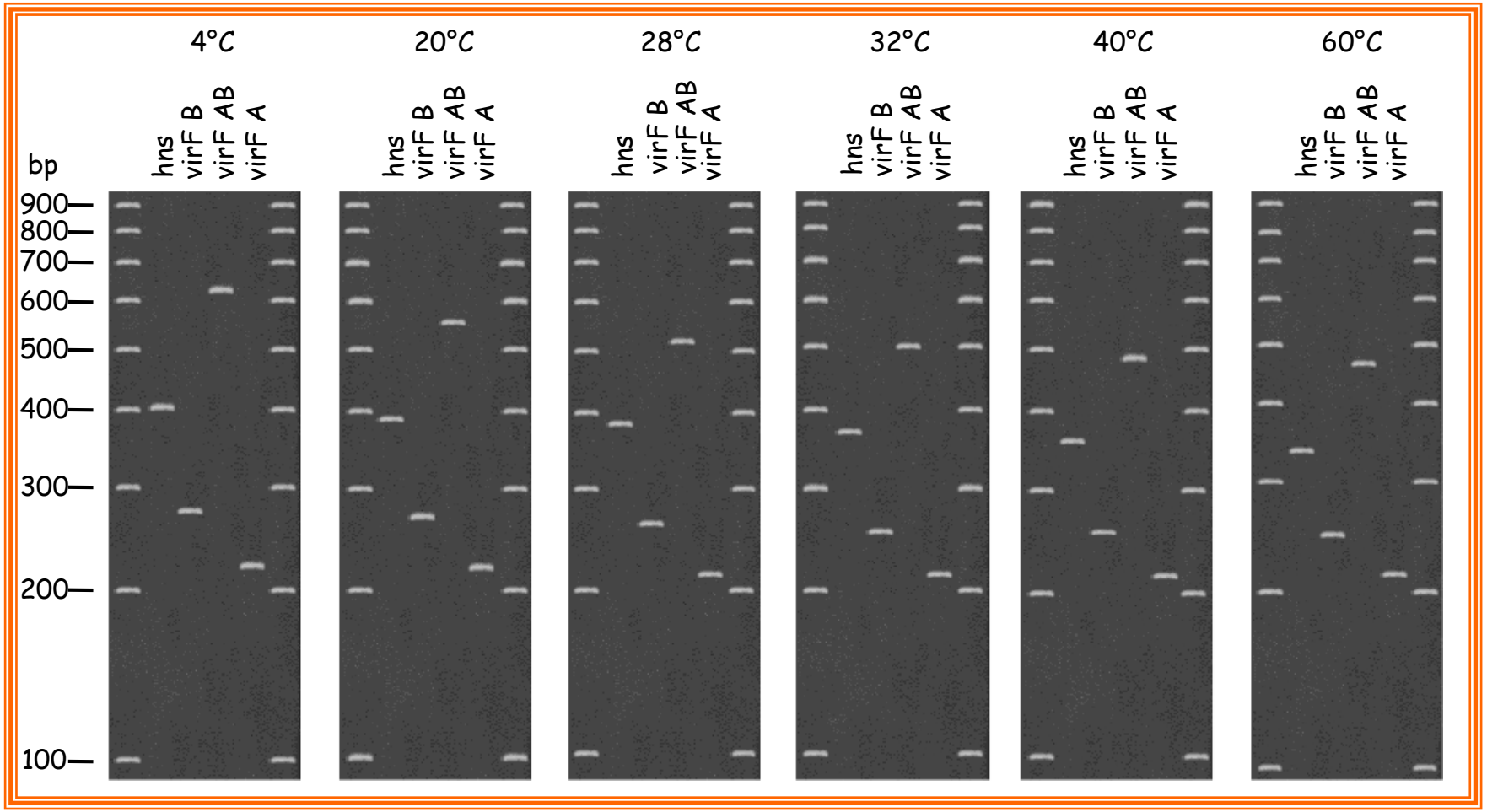


DNA bending : the **wedge model** for sequence mediated curvature

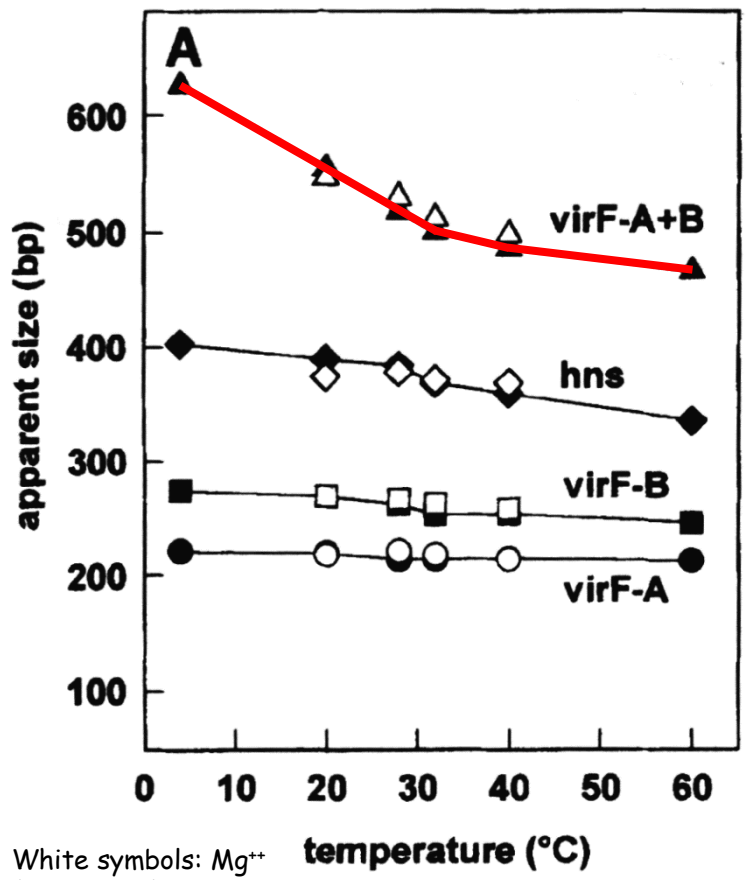


Temperature-dependent curvature of the *virF* promoter

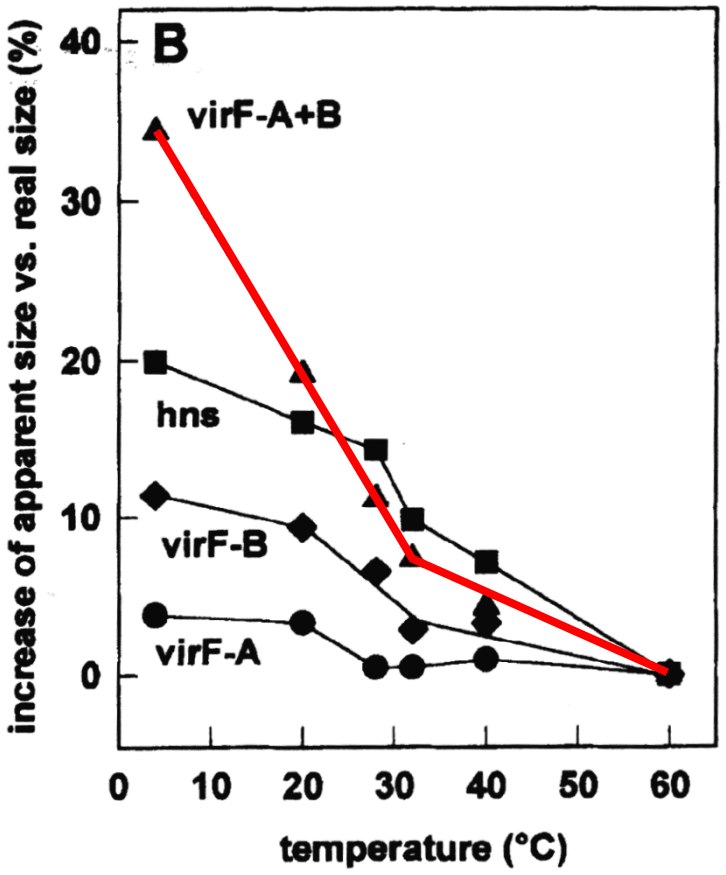
Acrilamide separations



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

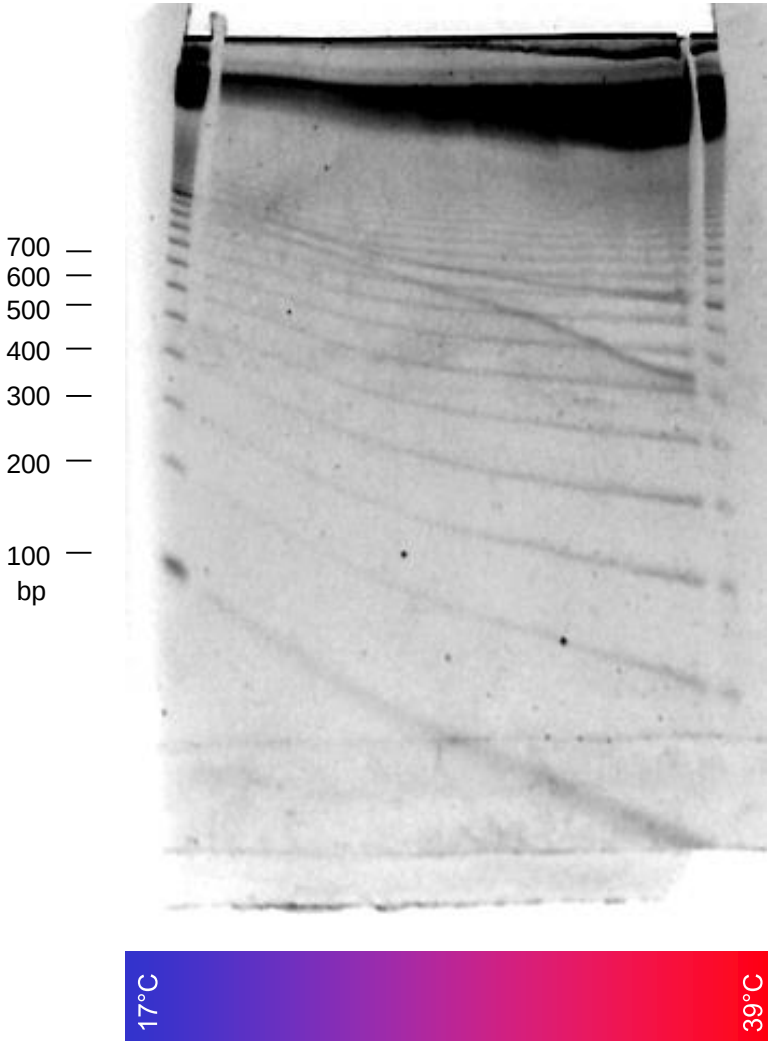


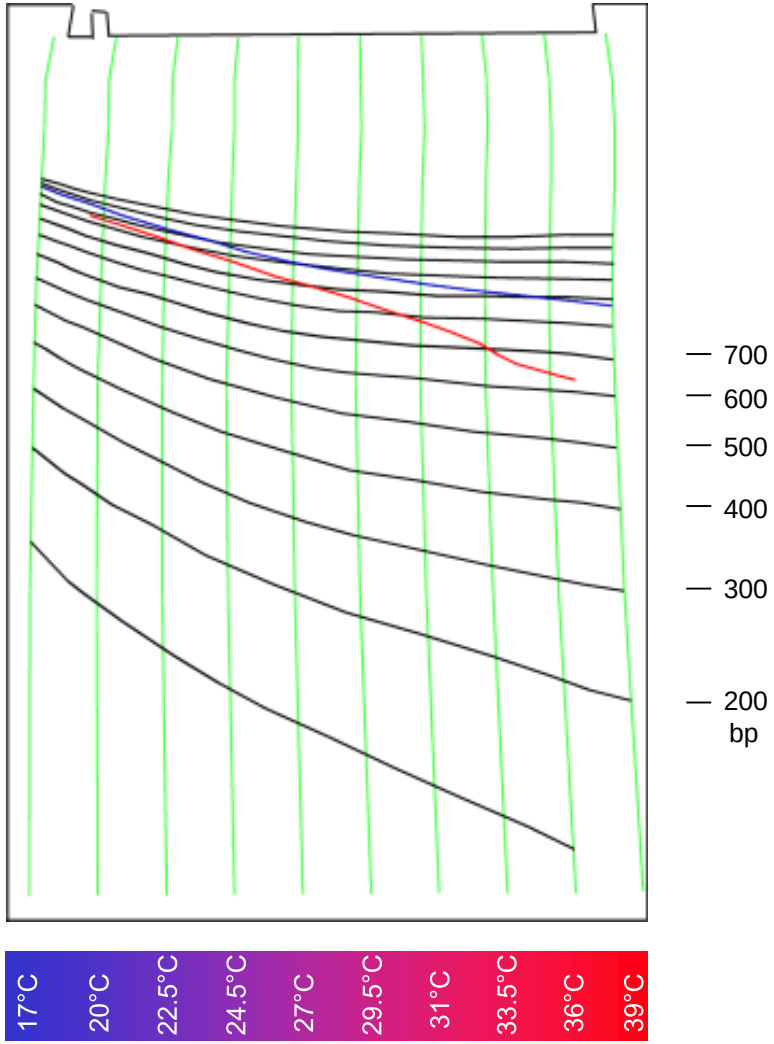
White symbols: Mg^{++}
Black symbols: no Mg^{++}



Temperature-dependent curvature of the *virF* promoter

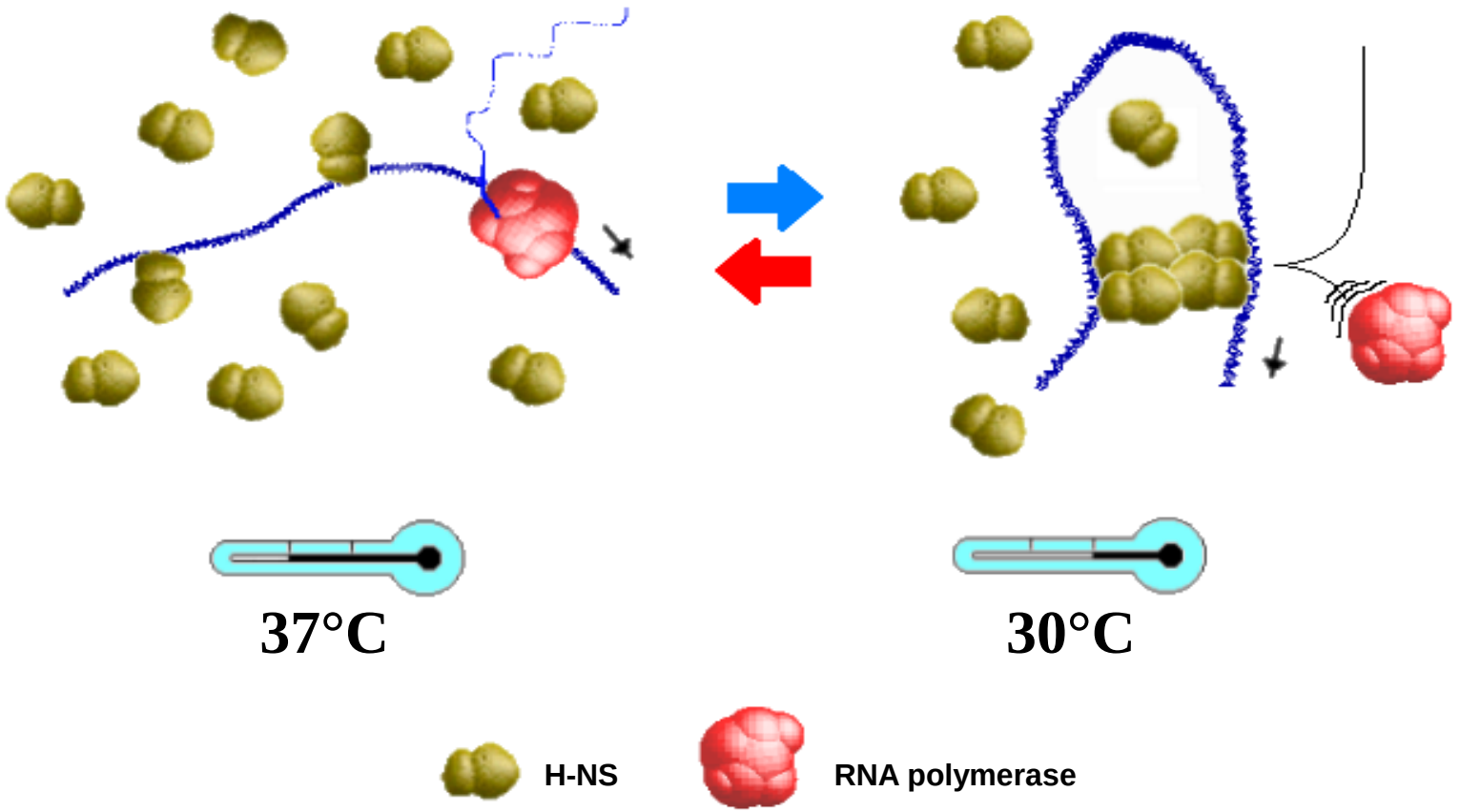
TGGE separation





Temperature-dependent *virF* expression

Working model

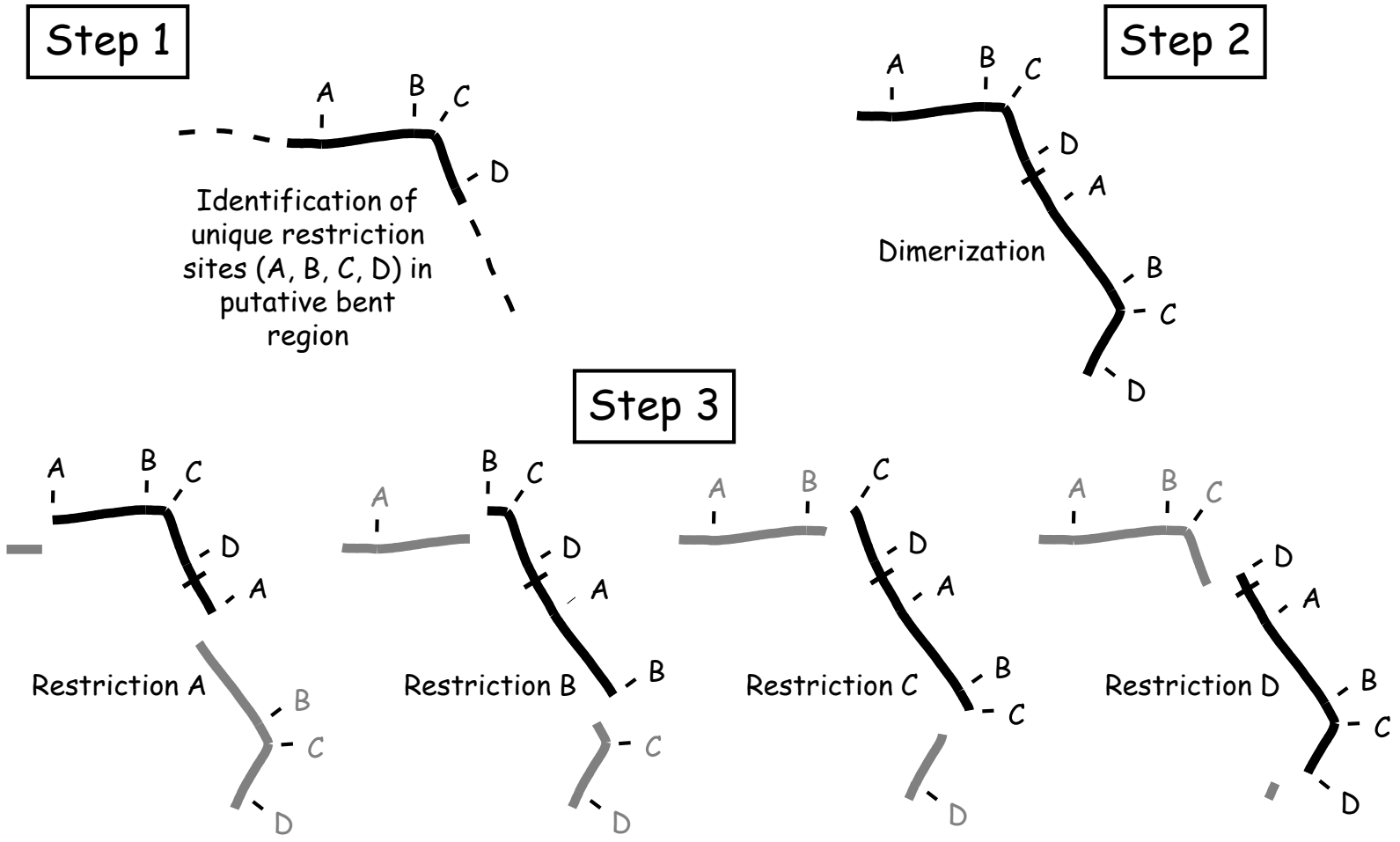


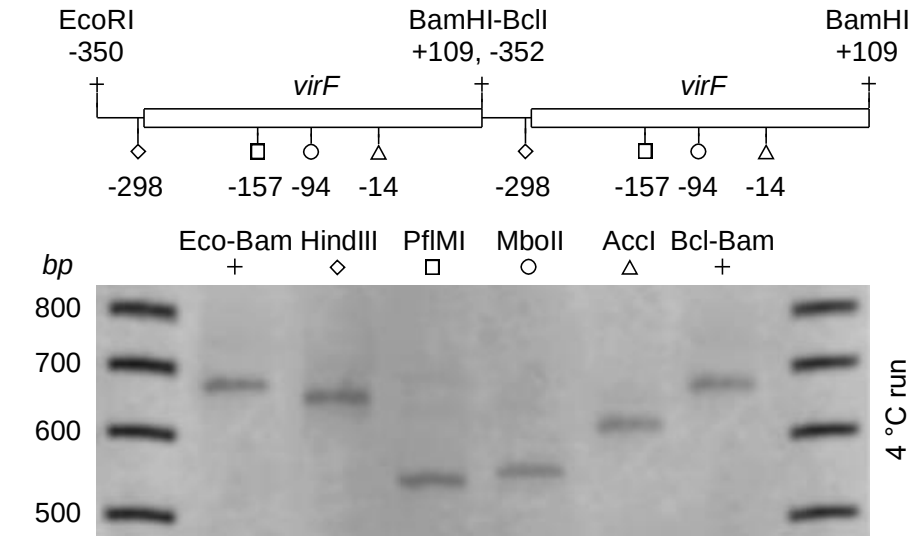
By which mechanism is the *virF* gene
allowed to be expressed
only at the host temperature?

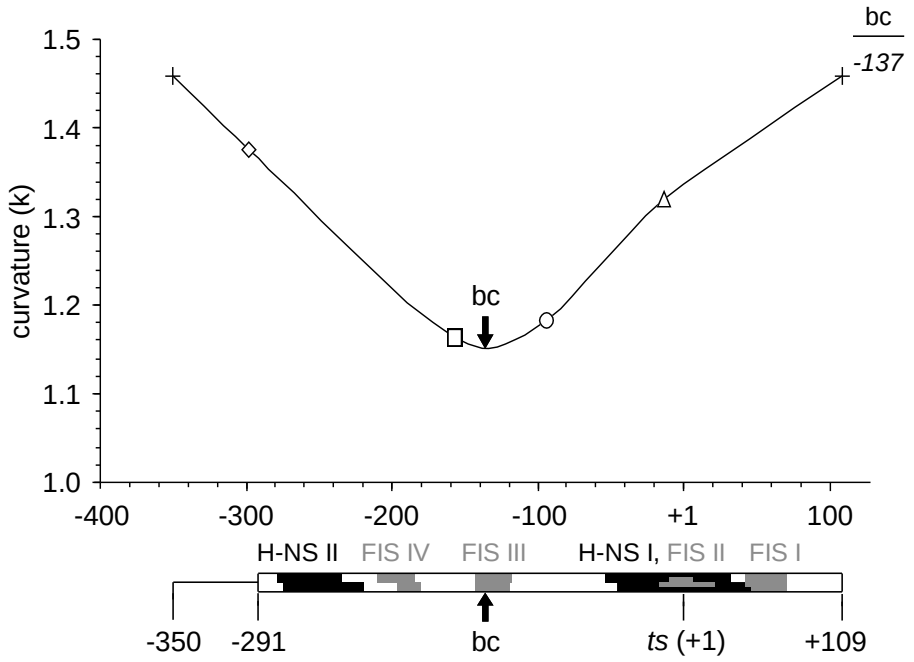
Small RNAs are emerging as key regulators
of virulence gene expression in bacteria.
Is this true also in Shigella?

The circular permutation assay

Rationale



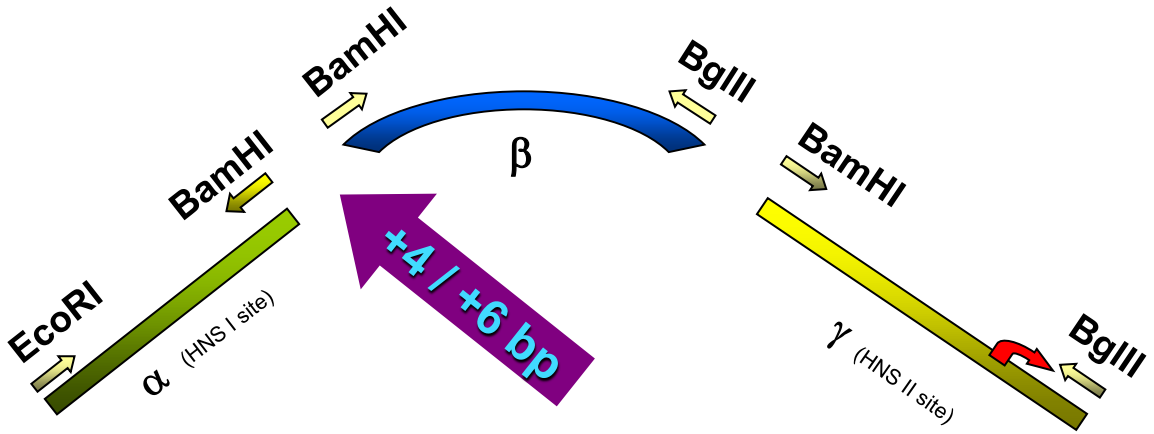


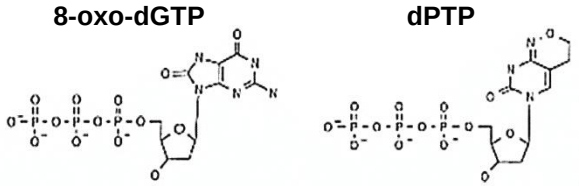


Circular permutation assay on the *virF* promoter region

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

Molecular dissection of the *virF* promoter: mutagenesis of the β-region and shifting the β-region by ~½helix turn





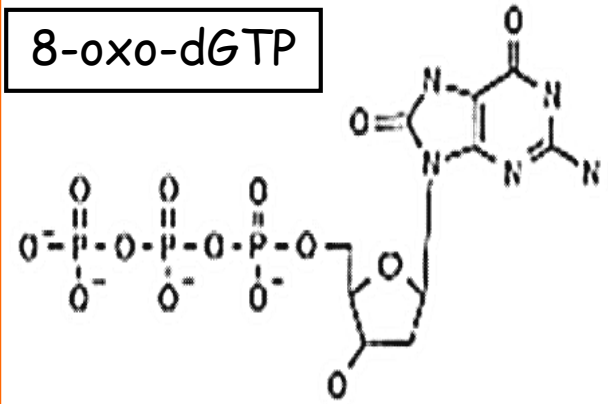
GCGCGCAAAAAGCGCGTAAAAGCGCGATAAAGC

GCGCGCAGAGAGCGCGTAGAAGCGCGACAAAGC

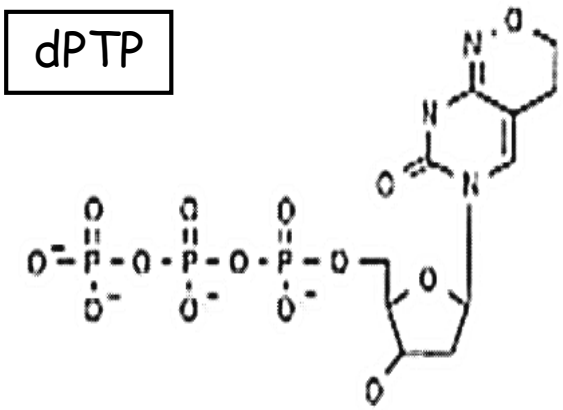
Mutagenesis of the bent region

Mutagens used

8-oxo-dGTP



dPTP



A=T → T=A, C≡G

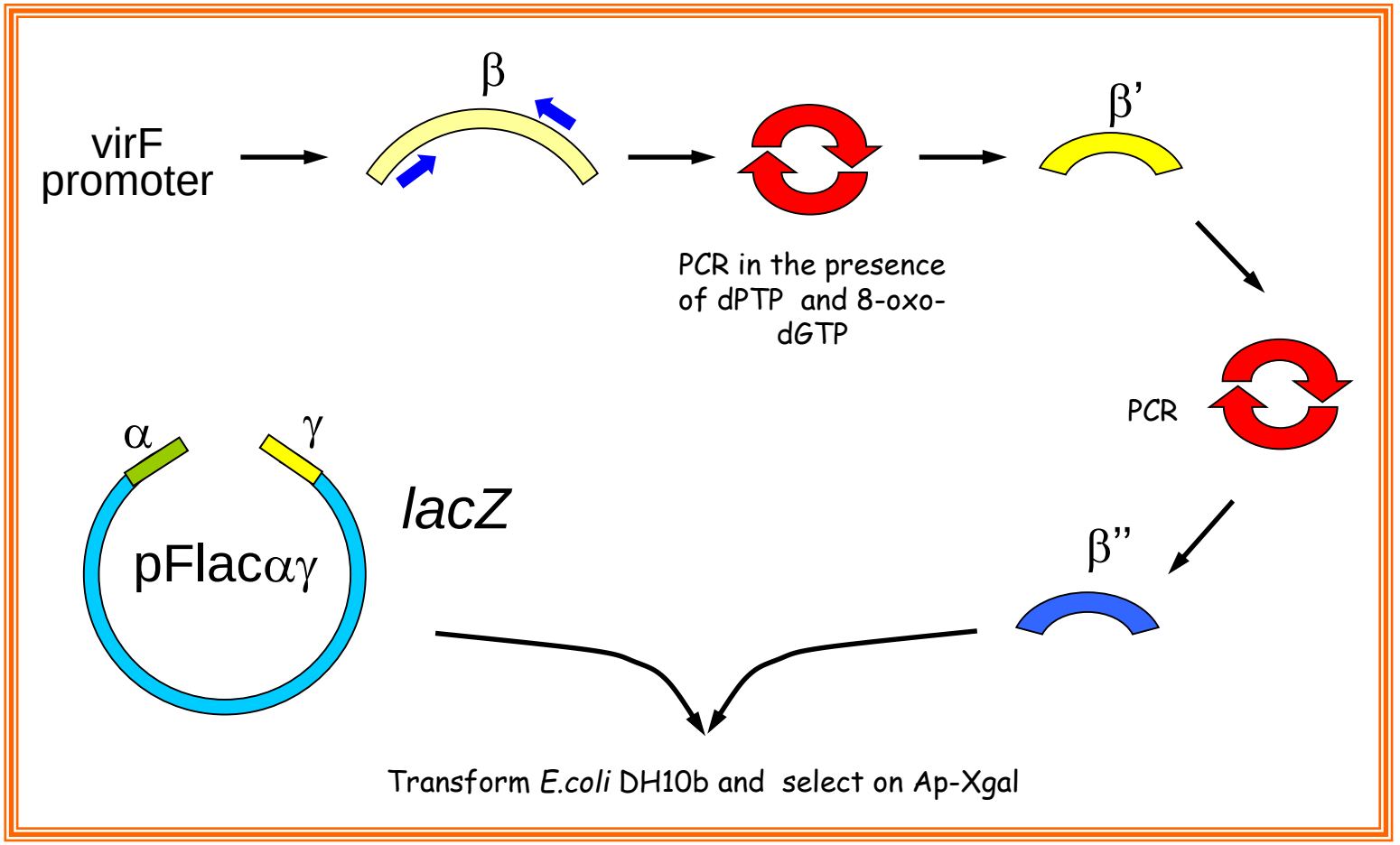
A=T → G≡C

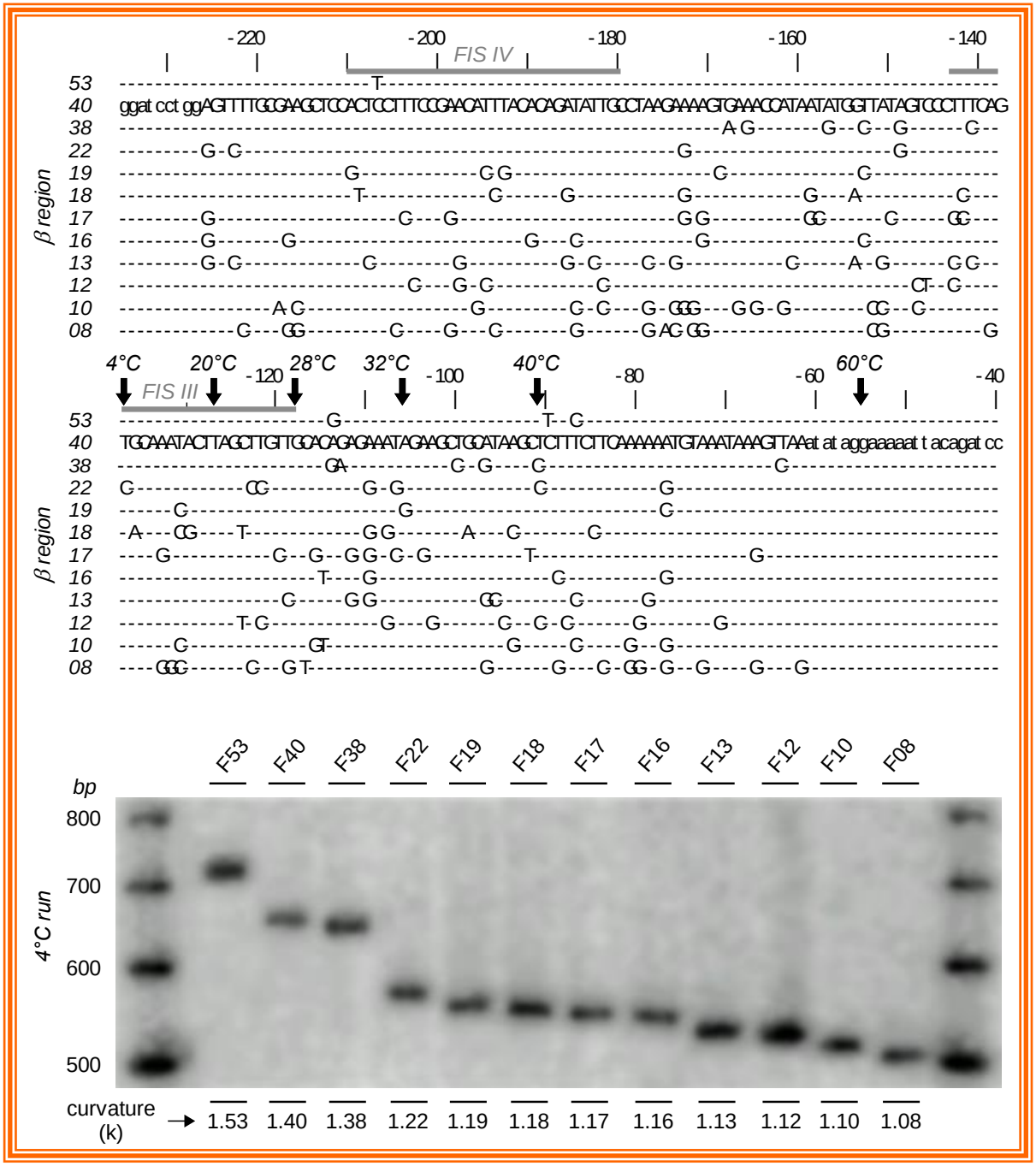
GCGCGCAAAAAGCGCGTAAAAGCGCGATAAAGC

GCGCGCAGAGAGCGCGTAGAAGCGCGACAAAGC

Mutagenesis of the bent region

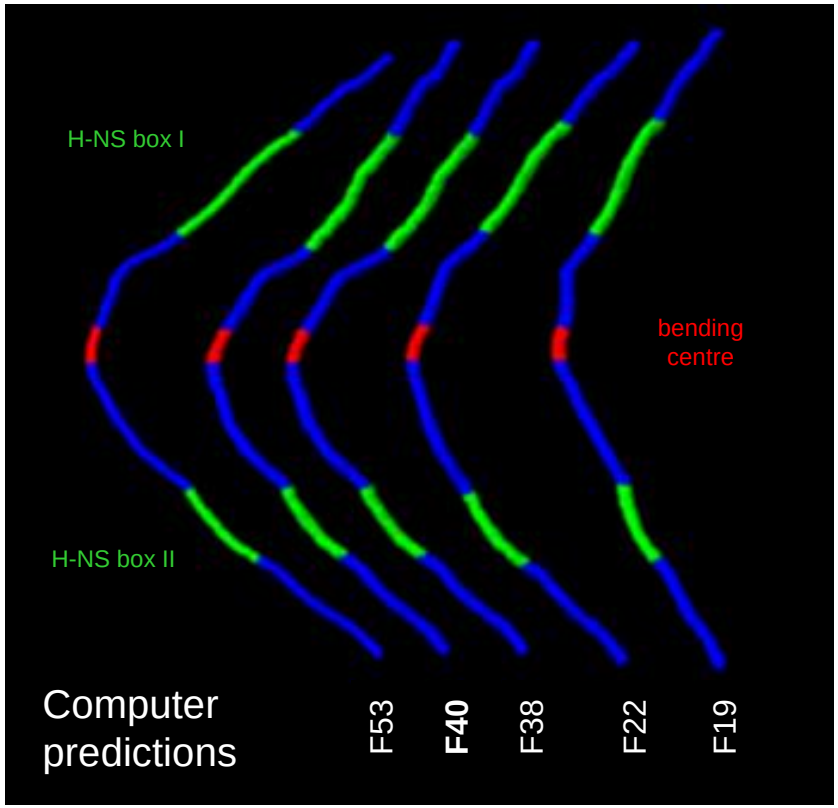
Strategy for the construction of β-region mutants

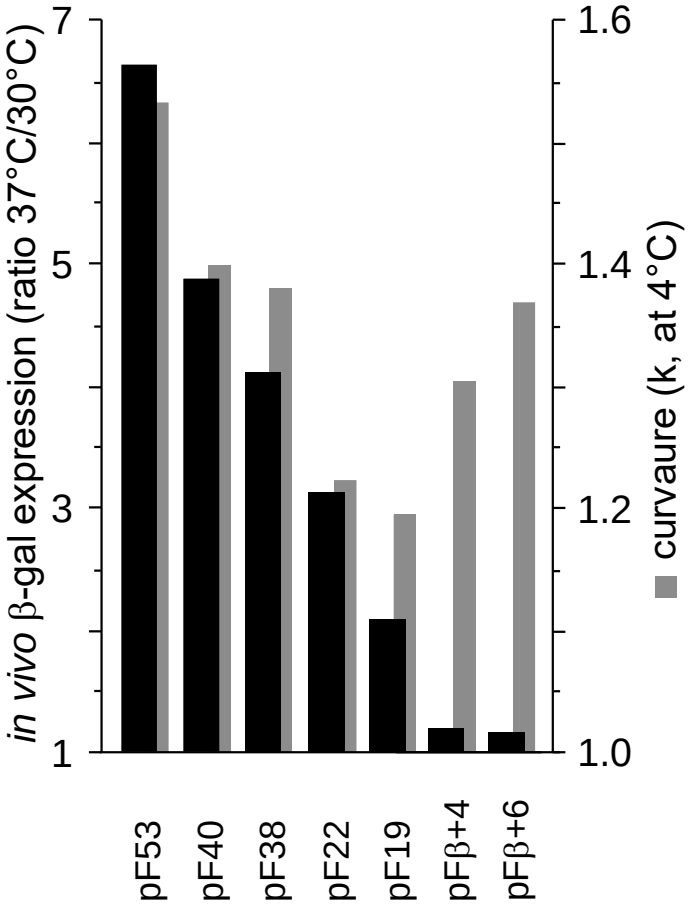




Acrilamide separation (4°C) of β-region mutants

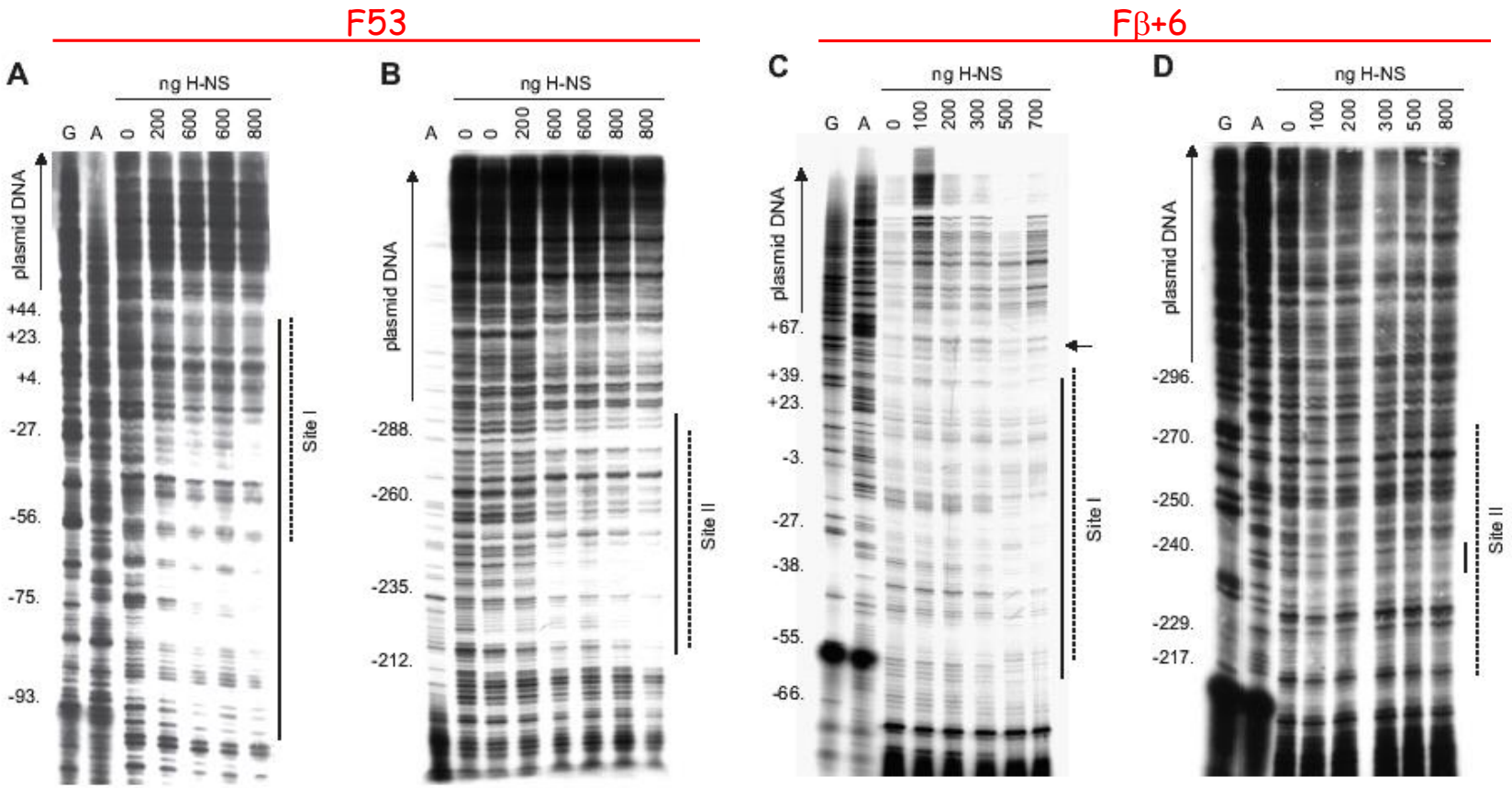
Correlation between intrinsic curvature and thermoregulated expression of the *virF* promoter



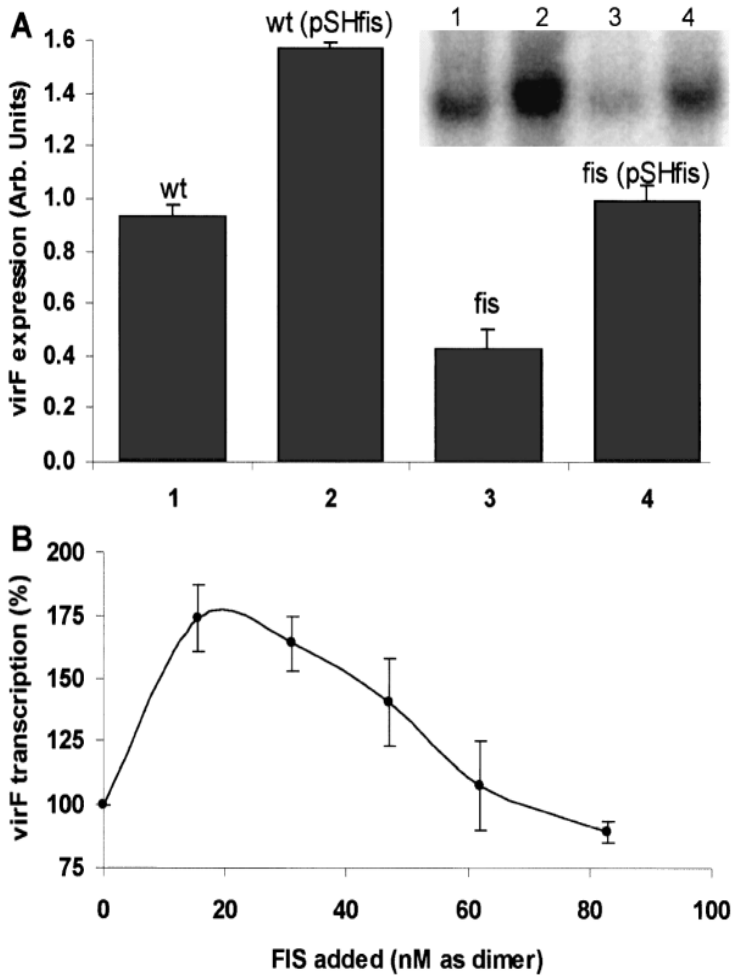


DNAseI footprints reveal that H-NS:

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



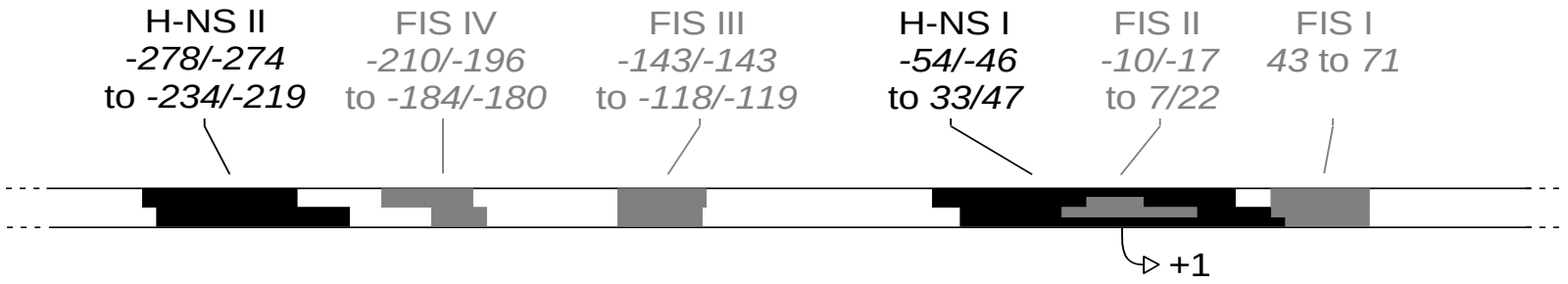
The *virF* gene is positively regulated by **FIS**, another nucleoid protein

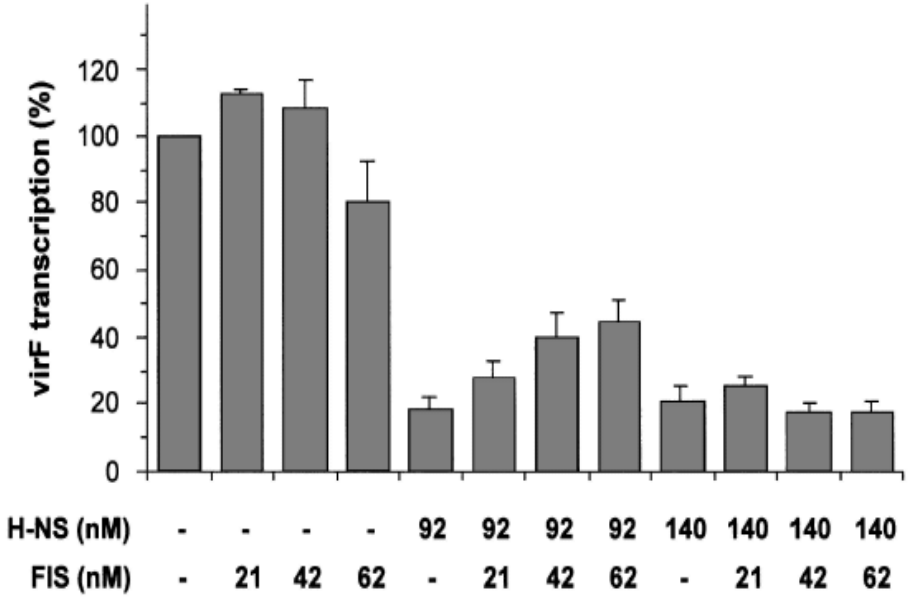


FIS (Factor for Inversion Stimulation)

- 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 …

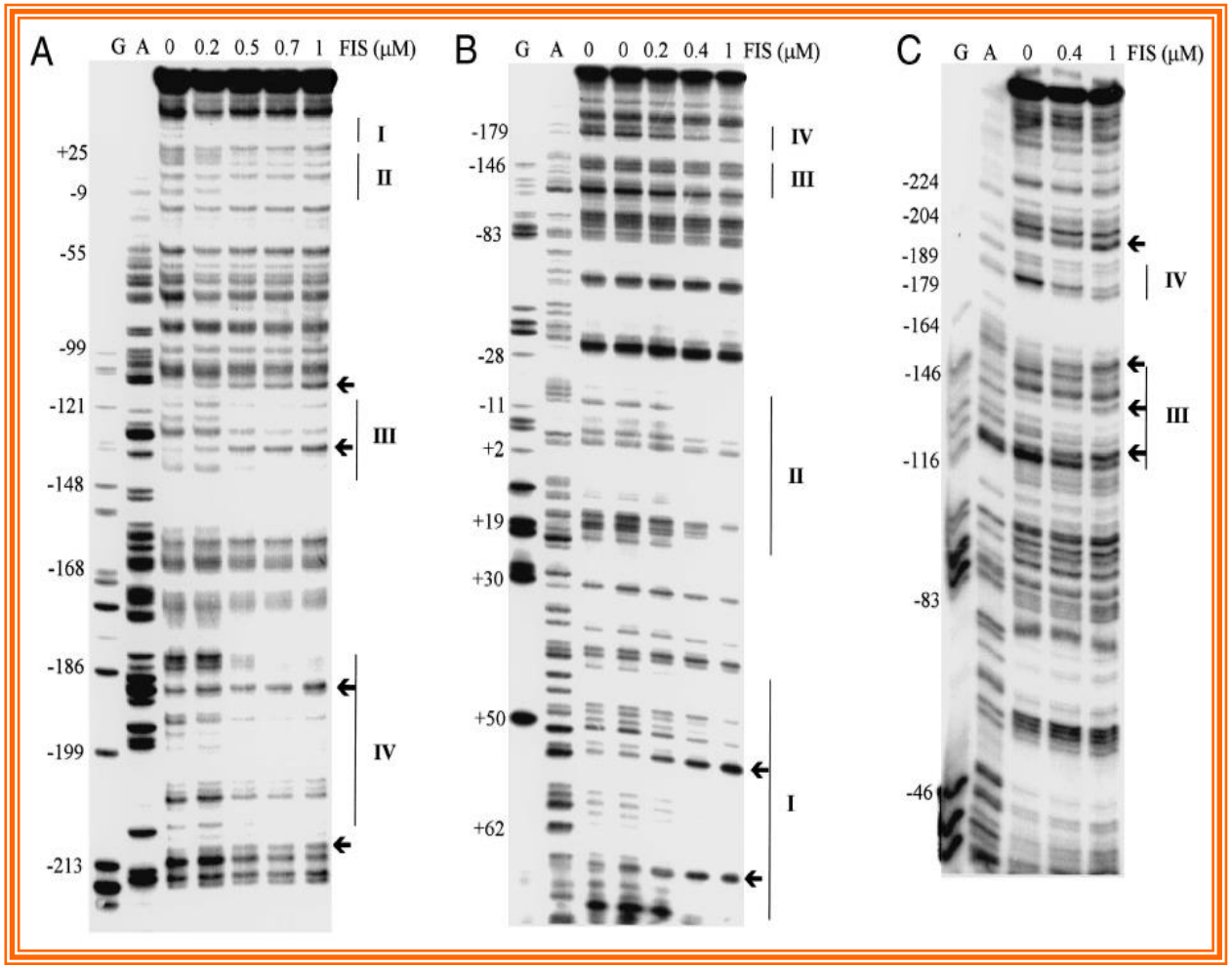




In vitro transcription in the presence of both, H-NS and FIS

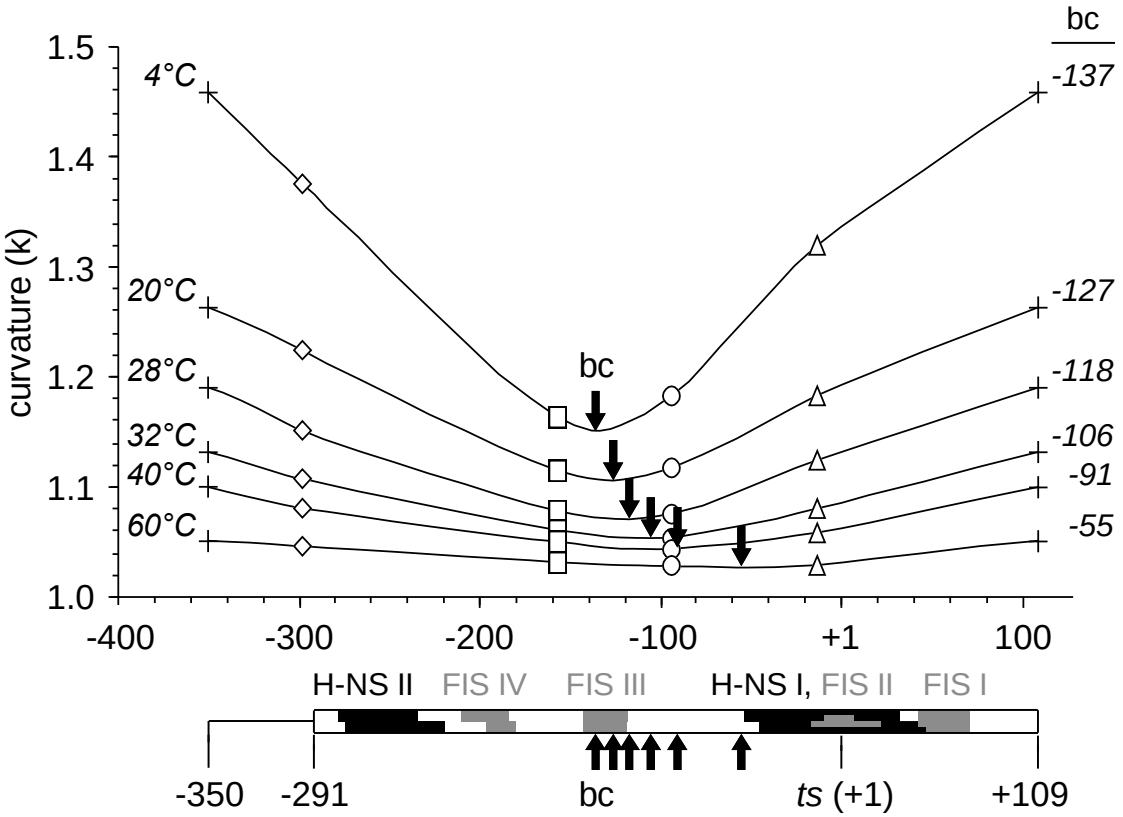
… and alleviates H-NS-mediated repression of the *virF* promoter at 31°C

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



Circular permutation assay on the *virF* promoter region

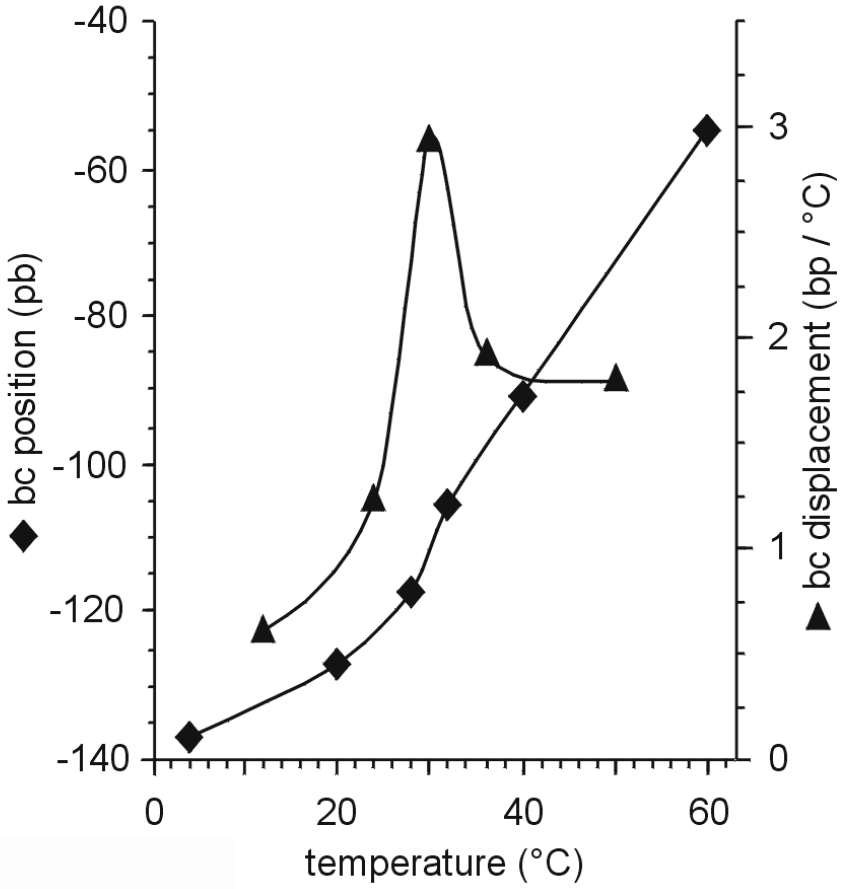
Effect of temperature



Curvature is reduced as temperature increases

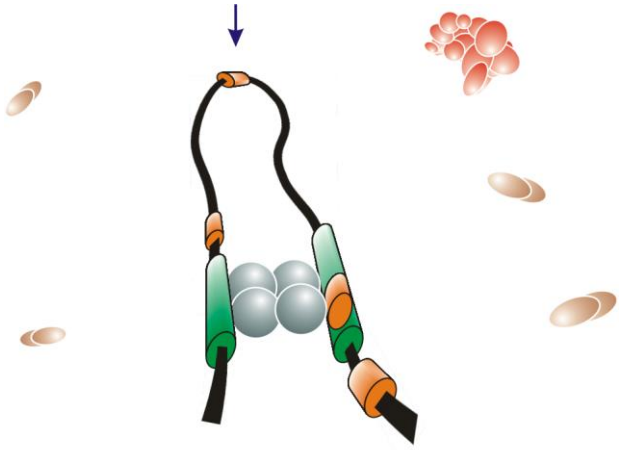
Do temperature changes alter the position of the bending centre?

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

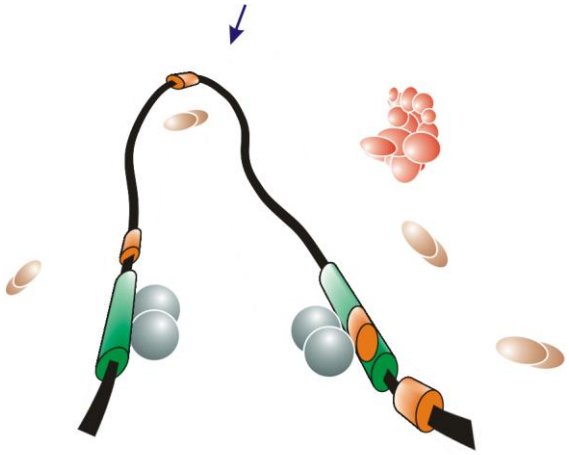


- The displacement of the bending centre is not a linear function of temperature.
- The maximum displacement occurs between 28°C and 32°C.

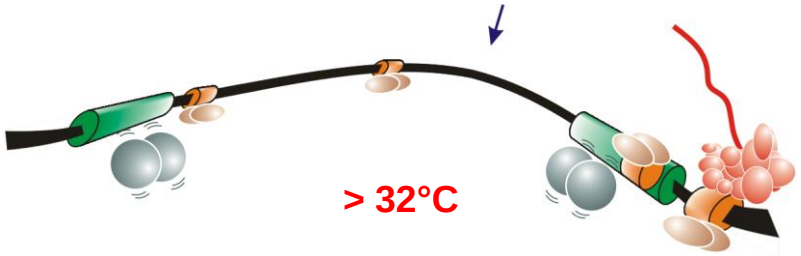
The thermodependent expression of *virF* is mediated by changes in DNA bending of its promoter



< 28°C



28 - 32 °C



> 32°C

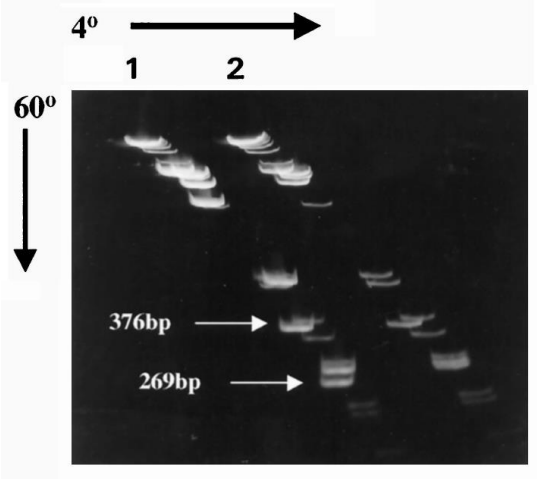
H-NS and its binding sites

FIS and its binding sites

RNA polymerase

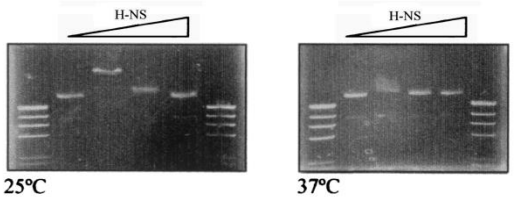
Bending center

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



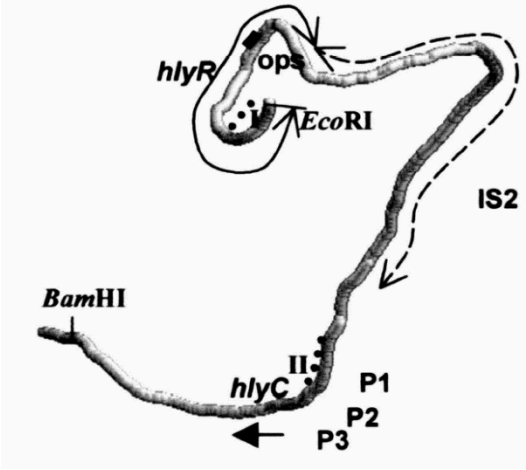
The *Yersinia enterocolitica* virulence plasmid contains DNA bends which melt at 37°C

(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.)

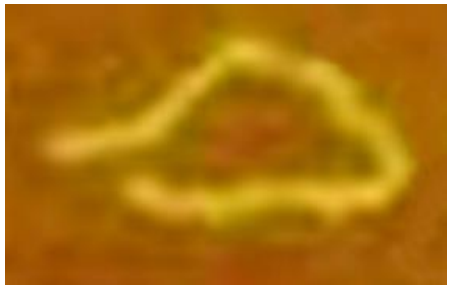


From pathogenic bacteria to *E. coli* :

How far does intrinsic DNA curvature sustain bacterial transcription?

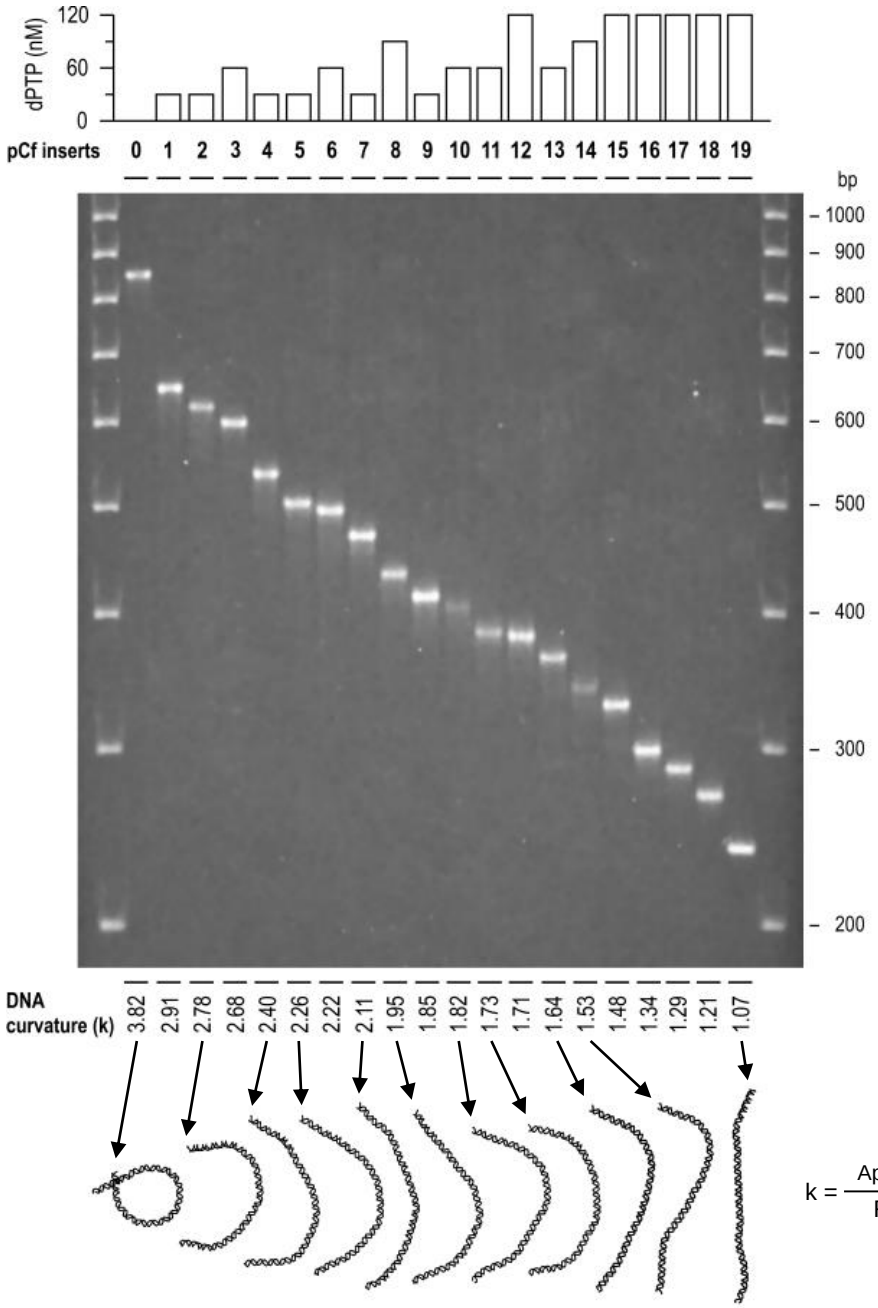
The experimental approach

The 211 bp fragment from the kinetoplast DNA of Crithidia fasciculata, known to be endowed with strong curvature,

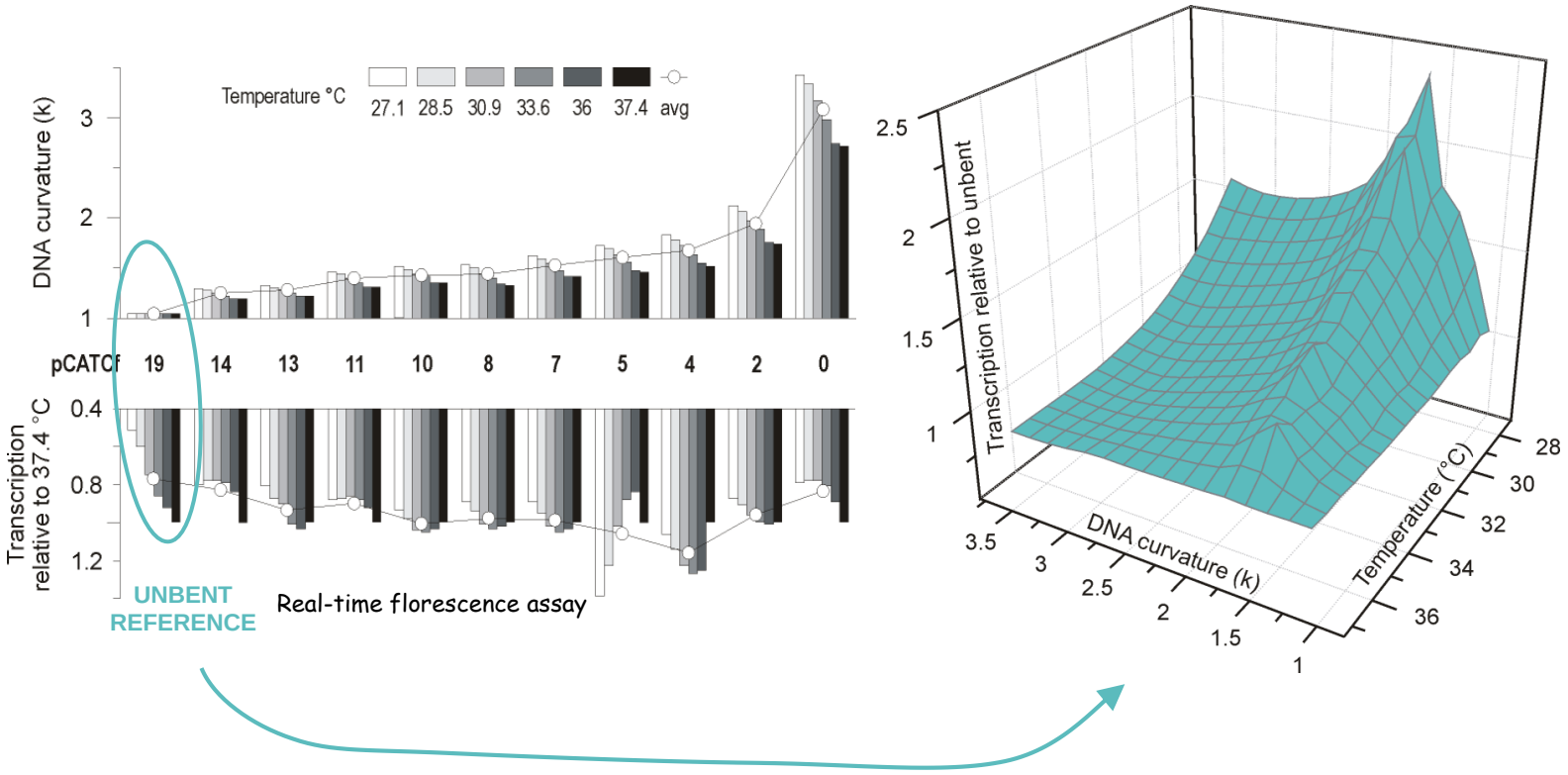


has been randomly mutagenized in order to obtain a spectrum of fragments covering a wide curvature range.

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



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

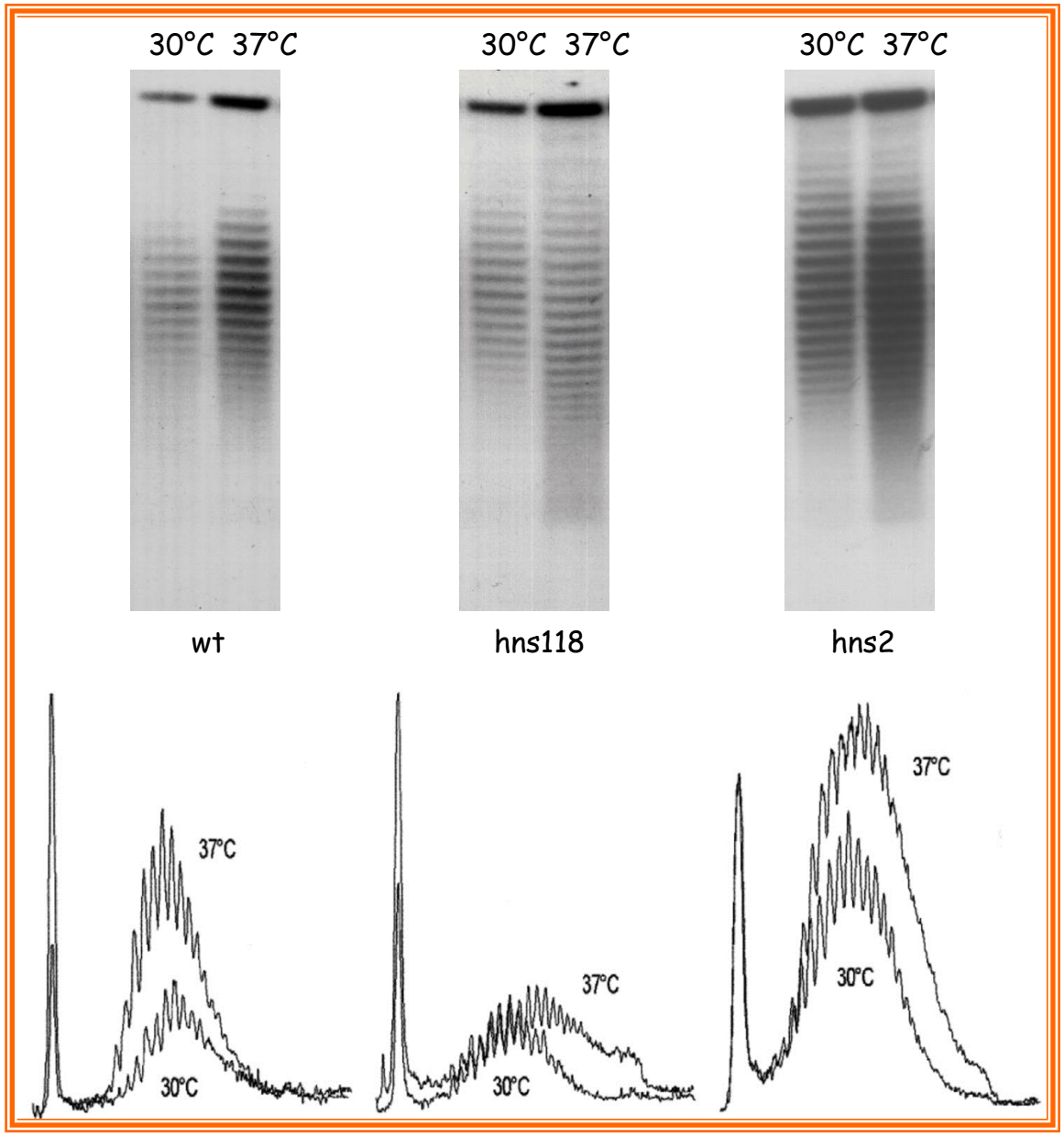


In short:

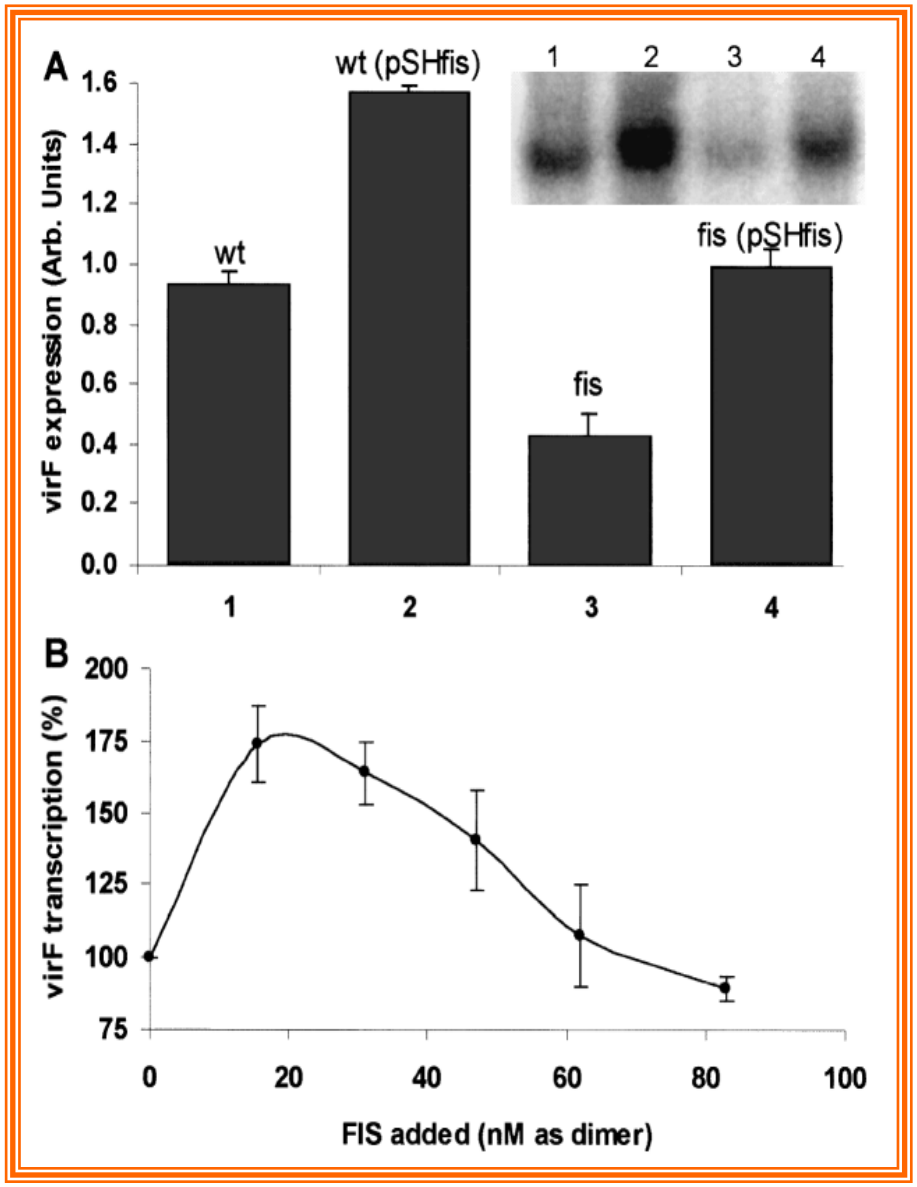
Curved DNA regions are frequently located upstream bacterial promoters.

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.

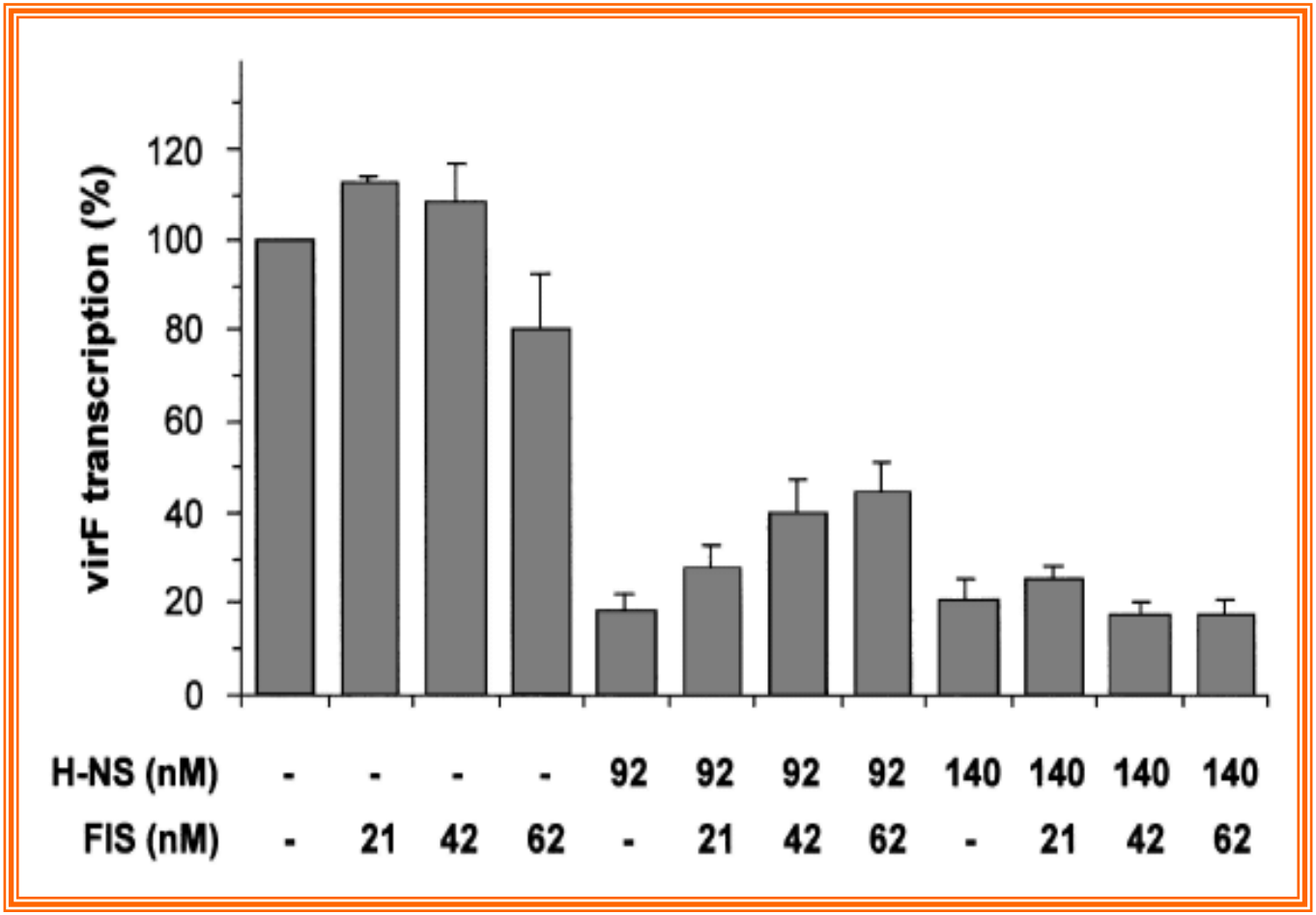


Distribution of pMYSH6504 topoisomers in *hns* $^{+}$ and *hns* $^{-}$ strains at 30°C and at 37°C



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



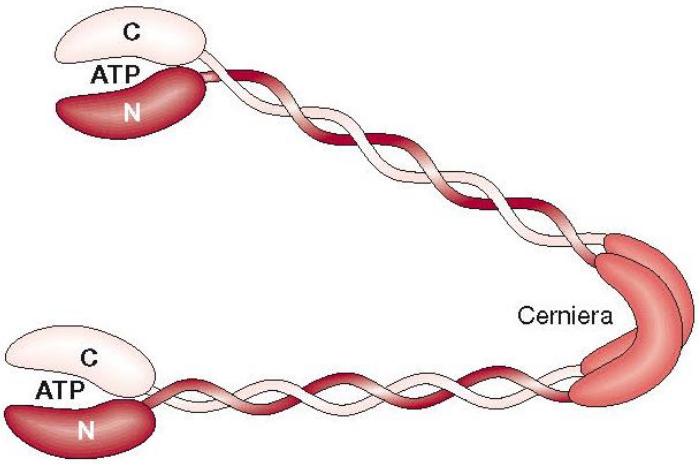
La proteina MukB una proteina SMC like

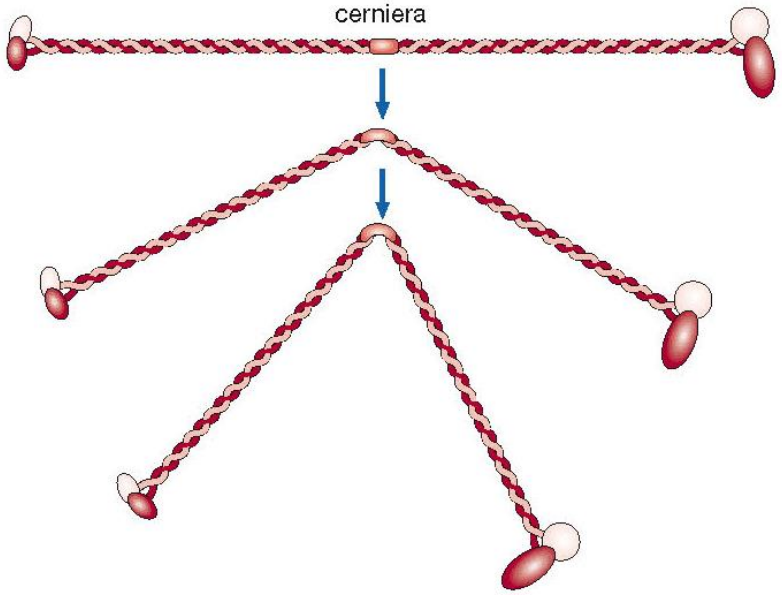
La proteina MukB è una proteina SMC like (Structural Maintanance of Chromosome) presente in molti batteri.

In assenza della proteina MukB i batteri diventano termosensibili e a temperatura permissiva hanno una crescita ridottissima : si osserva una decondensazione del DNA ed una perdita del nucleoide ad alta frequenza.

La proteina MukB svolge un ruolo importante anche nella segregazione dei cromosomi in seguito a divisione cellulare.

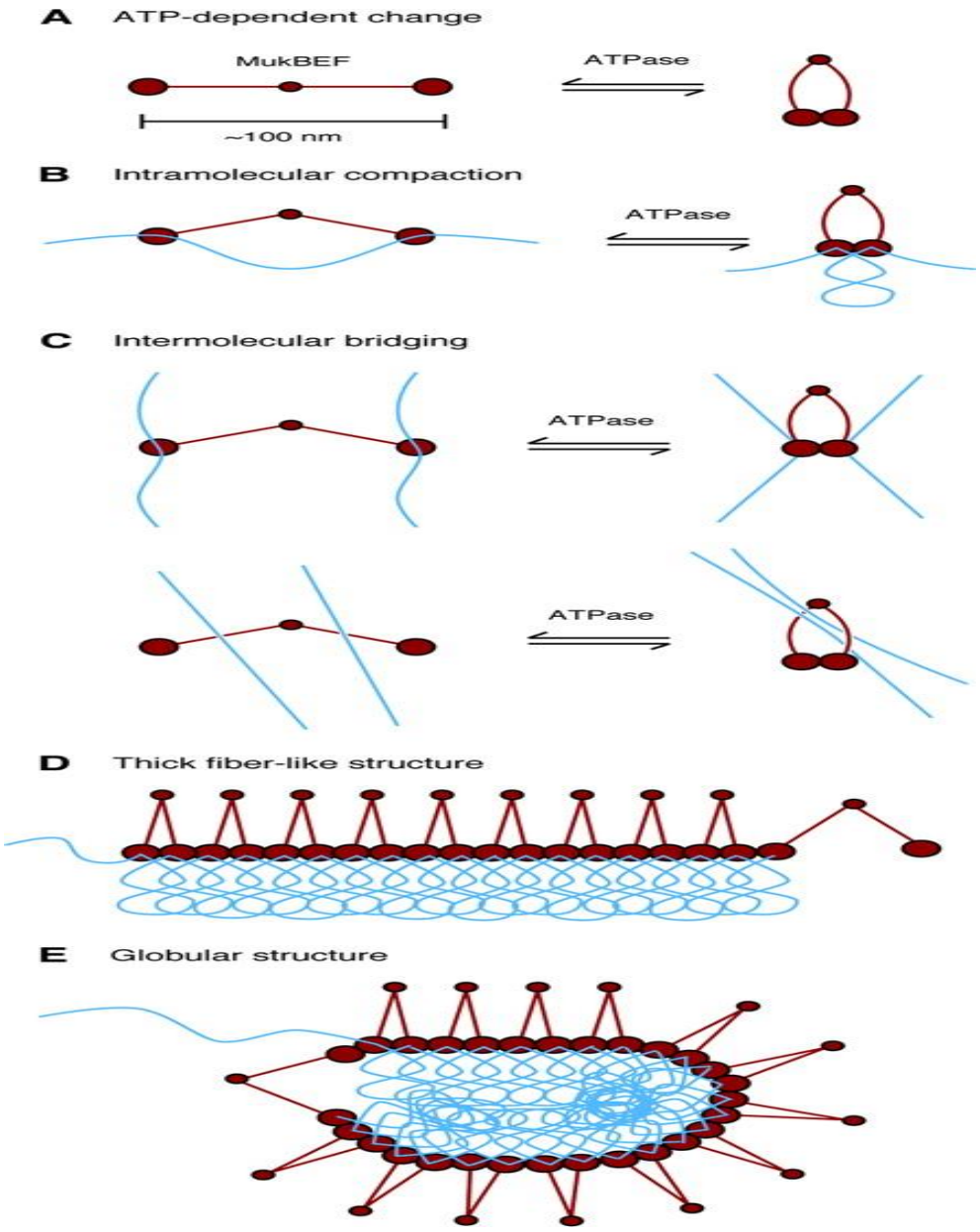
La perdita di MukB e della proteina del nucleoide HU è letale per la cellula.





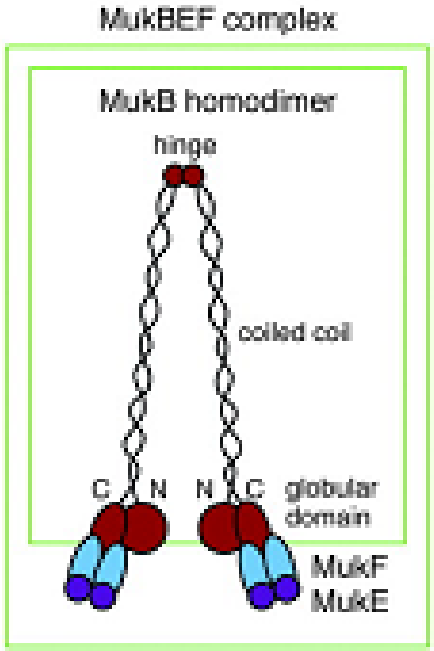
La proteina Muk B come molte proteine della famiglia SMC ,è costituita da due domini globulari N- e C- terminali (teste) separati da 2 regione coiled-coil intervallate da una terza regione globulare che costituisce una cerniera flessibile.

Le proteine MuKB sono omodimeri ed hanno nelle regioni C- e N-terminali dei domini ATPasici conservati.

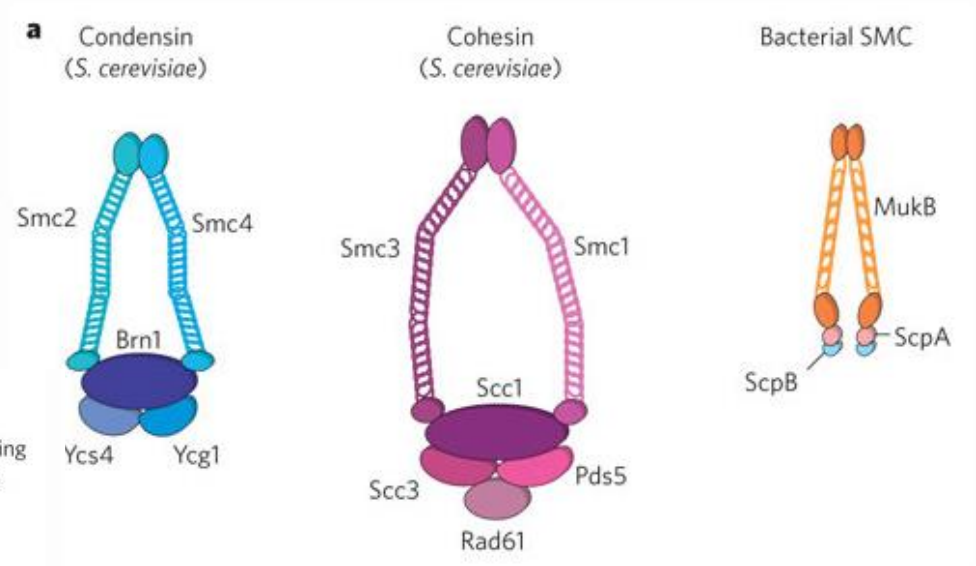


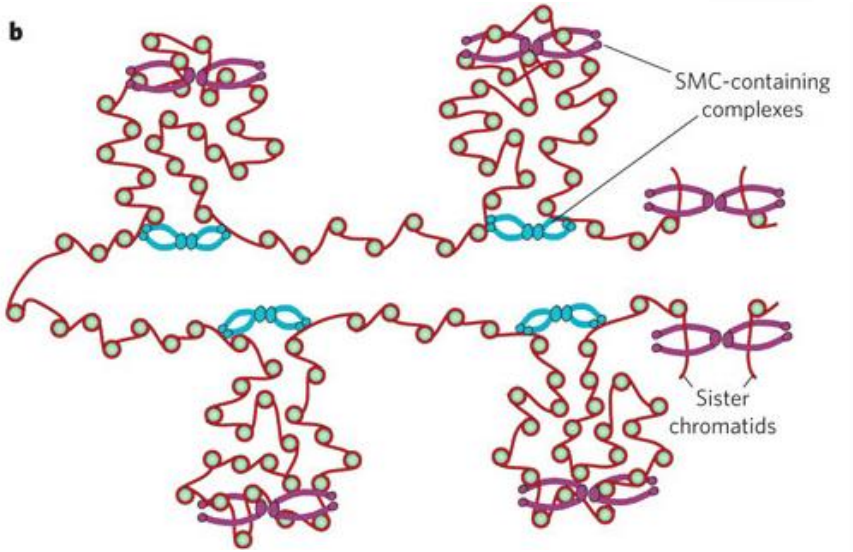
Nella forma chiusa la proteina scorre sul DNA ed è in grado di indurne il ripiegamento.

La proteina MukB potrebbe legare il DNA in due punti interagendo con i domini testa e poi grazie alla flessibilità della regione cerniera provocarne il ripiegamento e la condensazione



La condensina Batterica costituita dalla proteina MukB che appartiene alla famiglia delle SMC proteins (Structural Maintance of Chromosome) e dalle proteine MukFE è in grado di compattare il DNA in presenza di ATP con un meccanismo simile a quello osservato negli euc





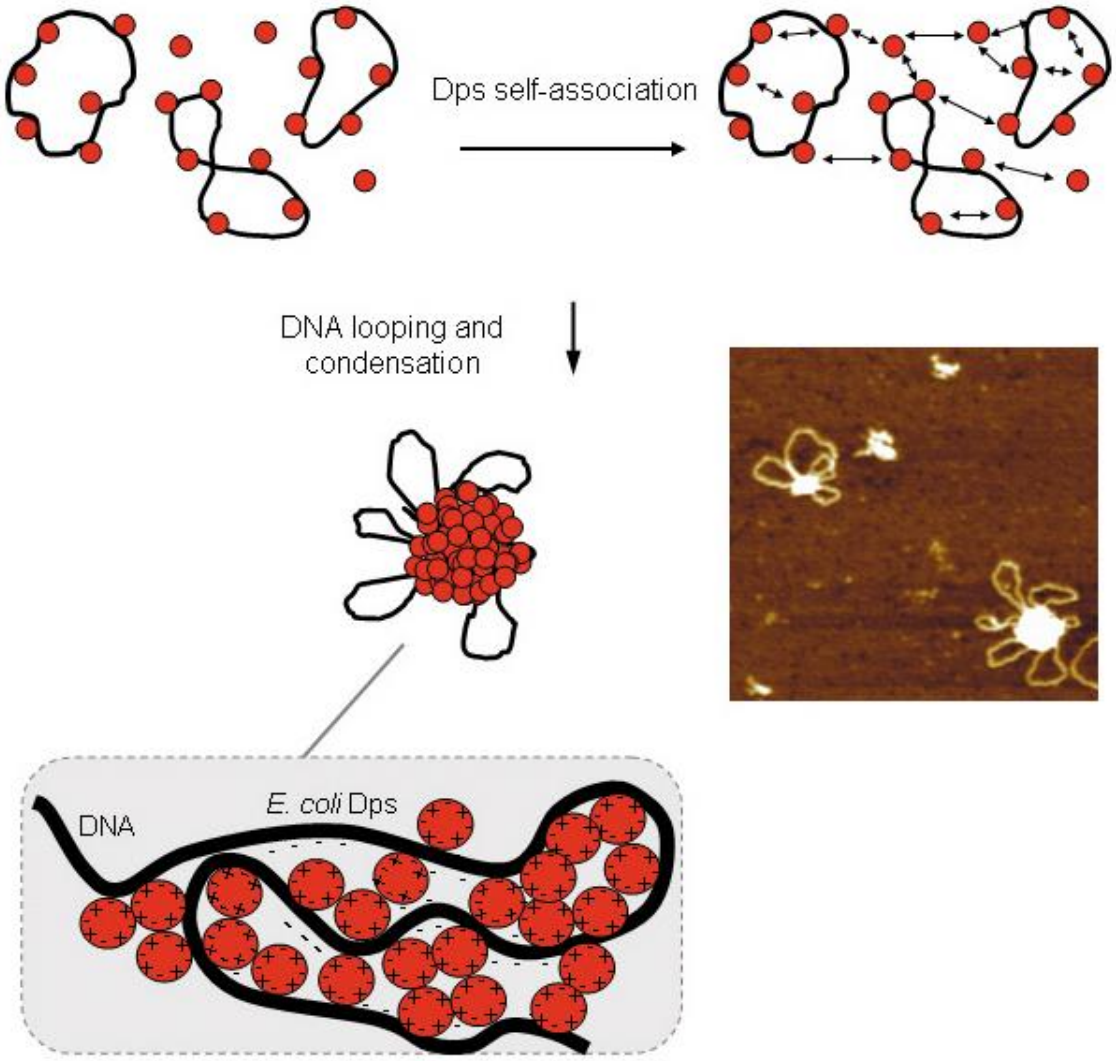
La proteina Dps (Dna Binding protein from starved cells)

forma un complesso costituito da 12 monomeri di 19 KDa

Il complesso Dps contiene uno ione Fe e rende il DNA resistente allo stress ossidativo

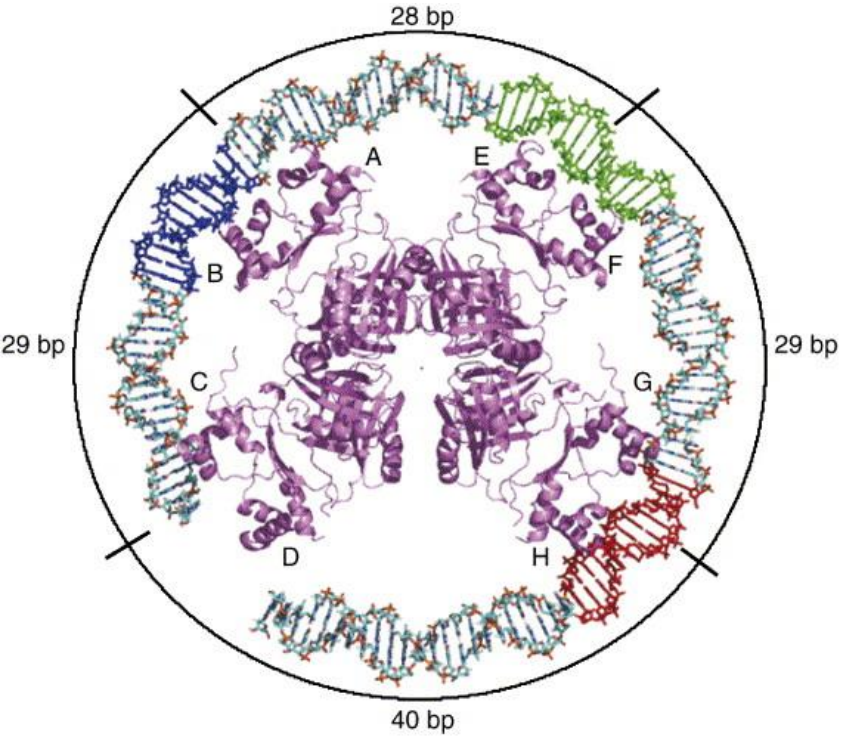
E' presente in alto numero di copie circa 20.000

Si lega al DNA a livello di sequenze non specifiche



I residui di lisina localizzati all'estremità N terminale di Dps carichi positivamente promuovono la condensazione del DNA in quanto interagiscono sia con il DNA che con le regioni cariche negativamente delle molecole adiacenti di Dps

Lrp Leucine responsive regulatory protein



Ottamero di LRP

influenza ala trascrizione del 10% dei geni di E.coli e a seconda del target il suo effetto può essere potenziato o meno dalla presenza di leucina.

I geni regolati comprendono geni coinvolti nell'acquisizione e metablismo degli AA oltre a geni di virulenza quali quelli coinvolti nella sintesi di alcuni pili.

Lrp riconosce una sequenza consenso degenerata sul DNA e modifica la struttura del DNA con il suo legame.

Esiste in diversi stati oligomerici , dimero, ottamero o esadecamero.