

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

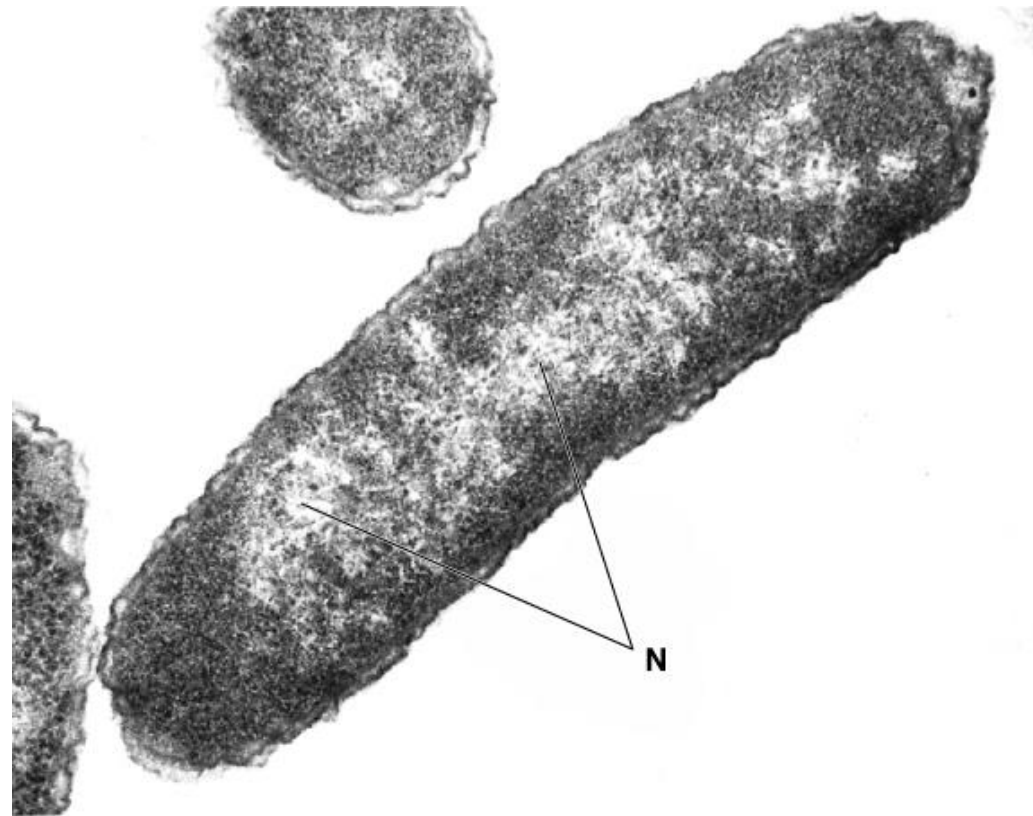
RNA

RNA polimerasi

Topoisomerasi

Proteine basiche

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



IL NUCLEOIDE

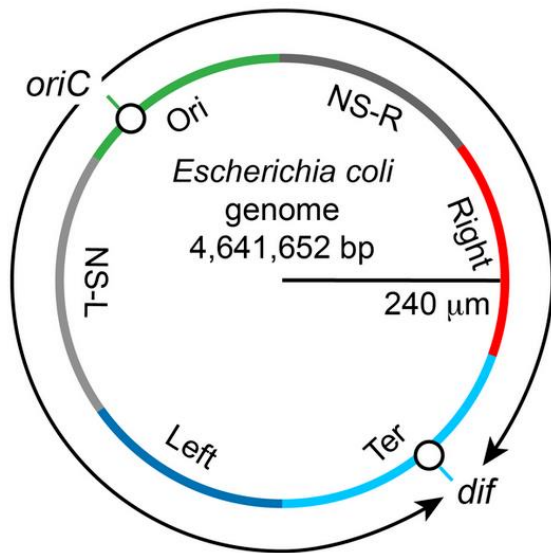
- la molecola di DNA cromosomico di E.coli è lunga circa 1.6mm
- è contenuta in una cellula di 2 μm di lunghezza e 1 μm di larghezza
- Un compattamento casuale della molecola determinerebbe un volume di circa 200mm³ circa 400 volte superiore al volume del nucleotide
- Il volume del nucleotide è di circa 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 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 .

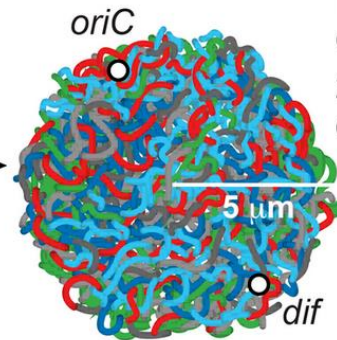
Elemento importante è anche il posizionamento rispetto all'origine: geni posizionati in posizione prossimale all'origine sono espressi di più in modo statisticamente significativo rispetto a quelli in posizione distale

A. Circular *E. coli* genome



B. Random coil of the DNA

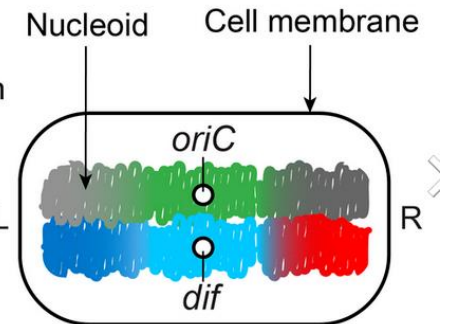
Inherent
polymeric
property



Random coil volume = $\sim 523 \mu\text{m}^3$

- 1) 1000-fold condensation
- 2) Spatial organization

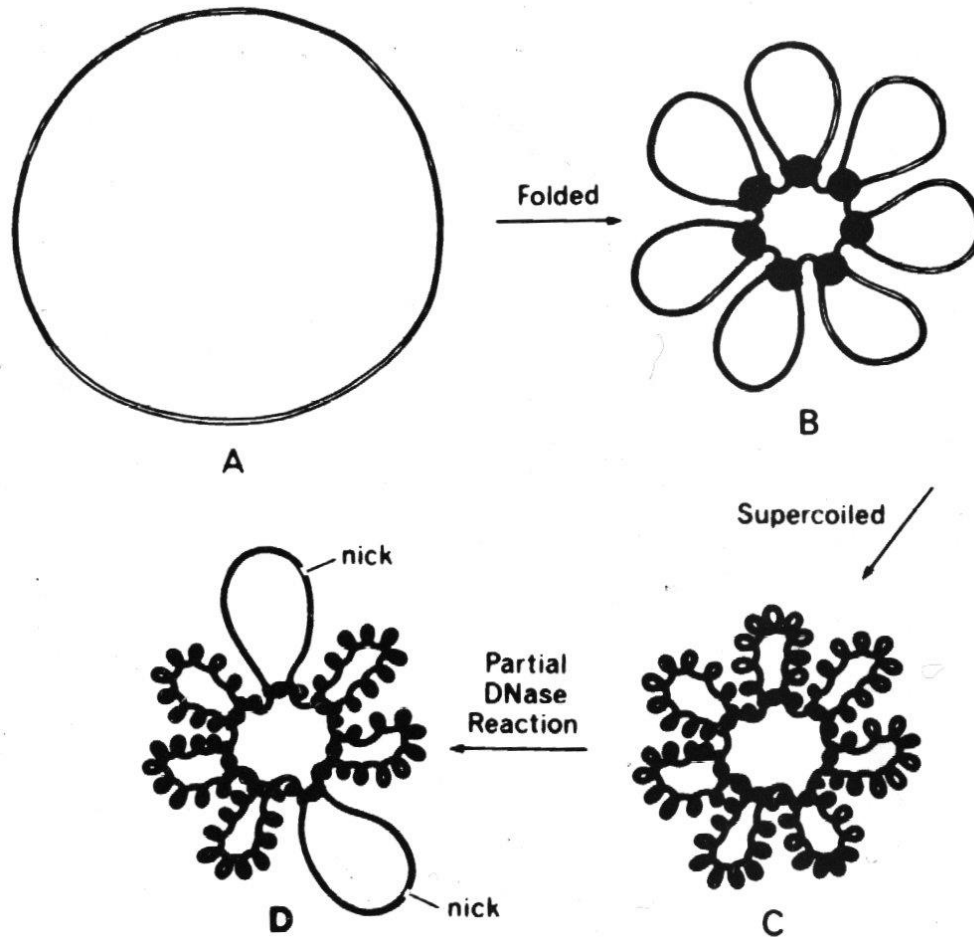
C. Genome organization *in vivo*



0.5 μm

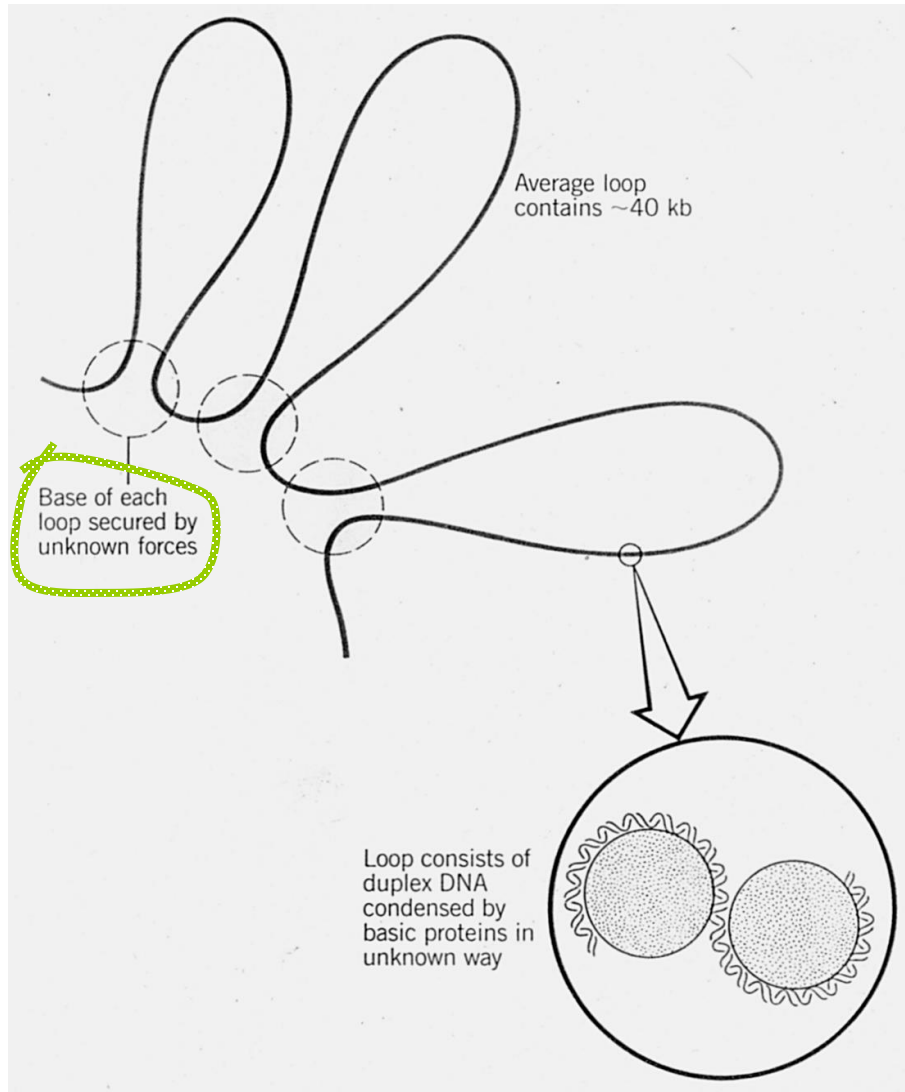
Nucleoid volume $< 1.0 \mu\text{m}^3$

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 sedimentaz.)
- + 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, ...)

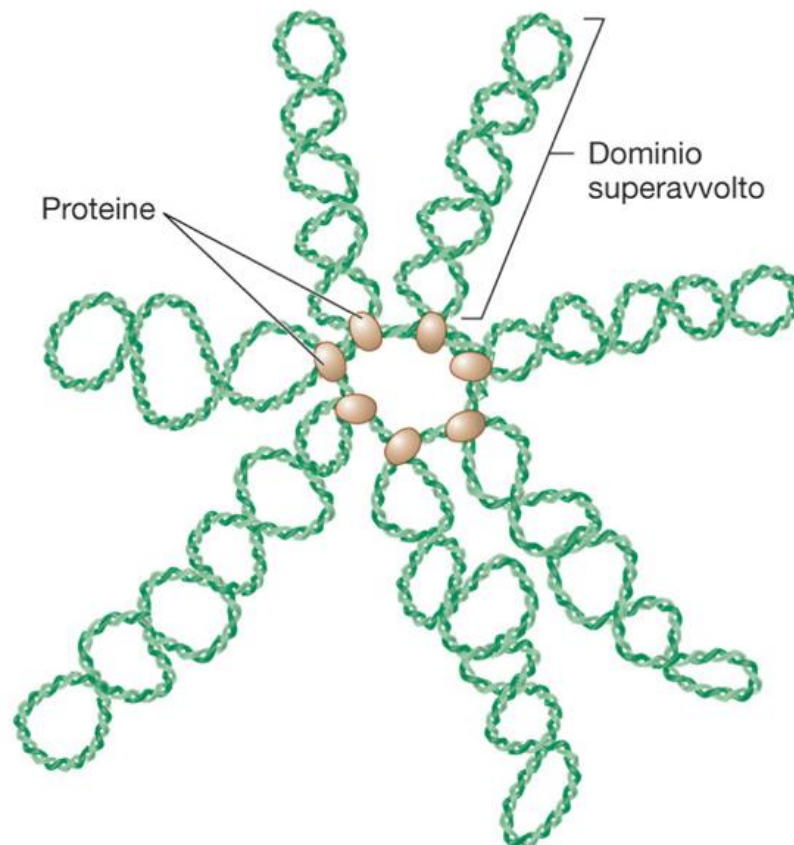
• DNA ancorato?



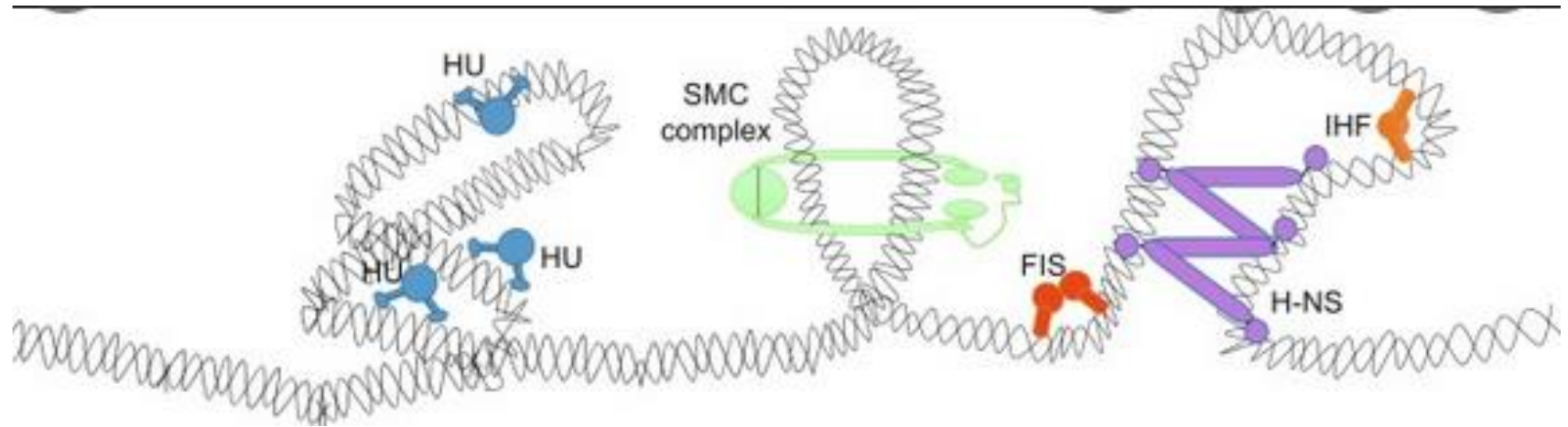
Rilevanza funzionale in vivo?

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

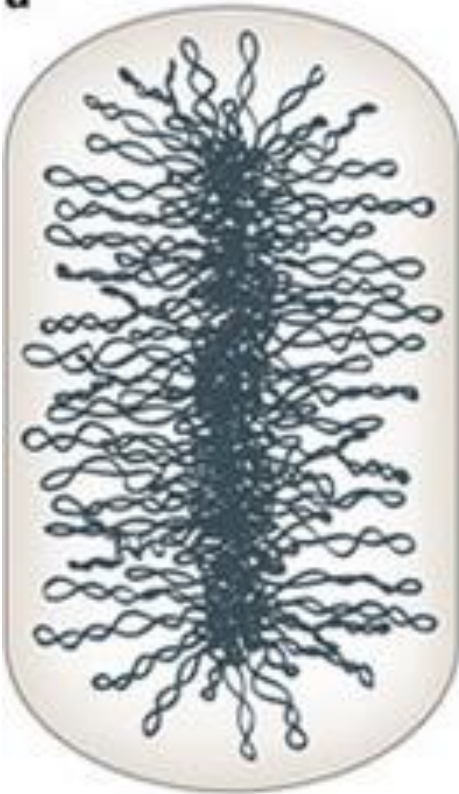


Quali sono le proteine associate al nucleotide?
Come riescono a compattare il cromosoma batterico?

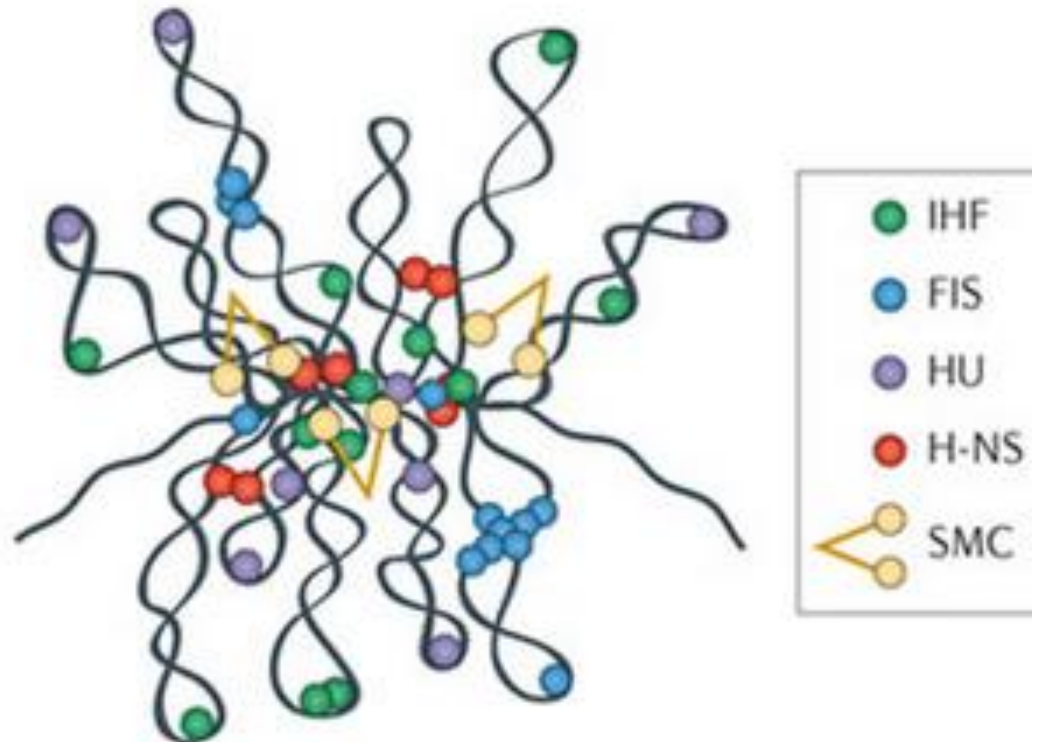
SMC complex corrisponde alle proteine MUK

Organizzazione del nucleotide batterico: le principali proteine associate al nucleotide

a

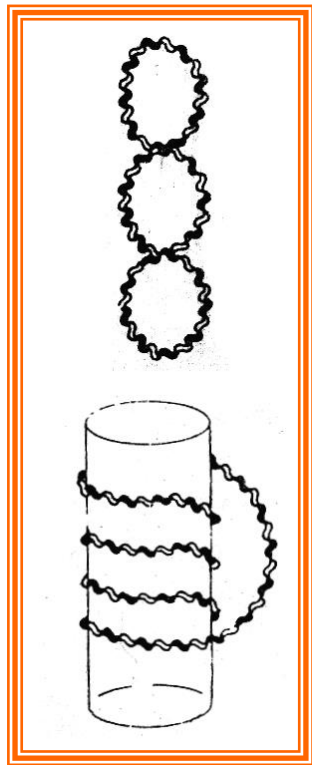


b Nucleoid-associated proteins and SMC complexes

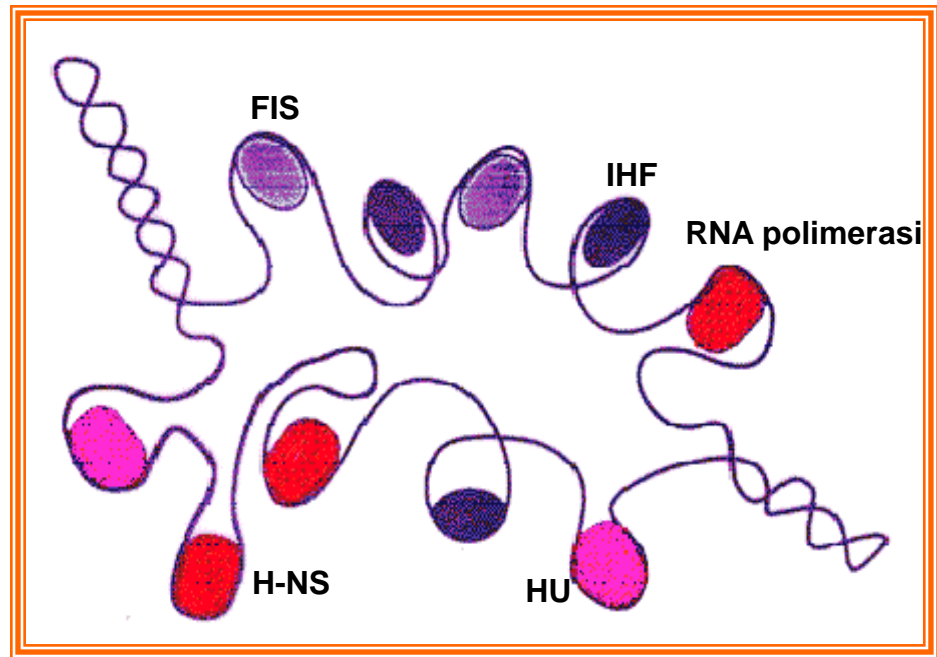


Bacterial chromatin organization

Plectonemic
condition

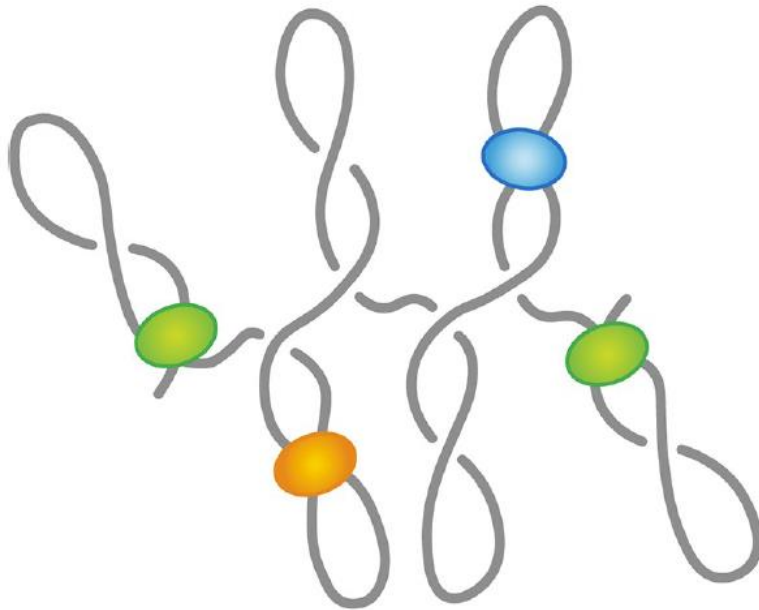


Toroidal
condition



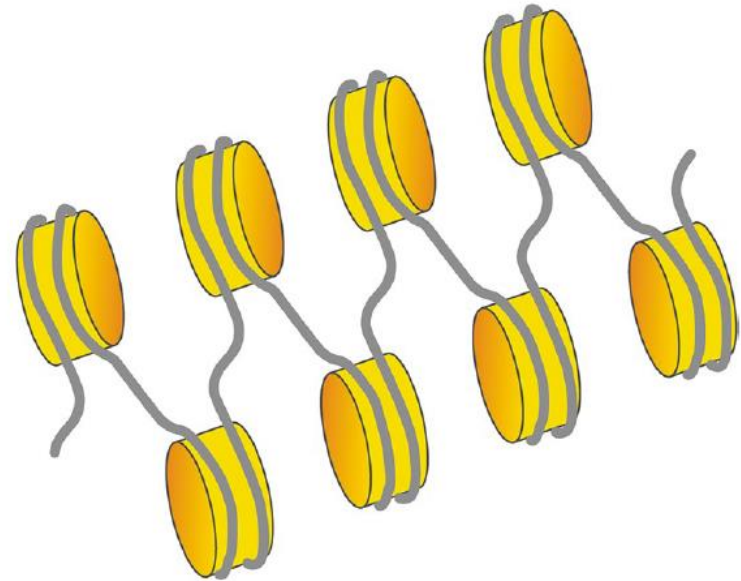
Co-existence of
plectonemic and toroidal conditions

A. Bacteria



Negative plectonemic supercoils

B. Eukaryotes



Negative toroidal supercoils

Fig 5. Basic units of genomic organization in bacteria and eukaryotes. **A.** A bacterial genome organizes as plectonemic supercoils. Half of the supercoils are present in free form, and nucleoid-associated proteins (NAPs), shown as colored spheres, restrain the remaining half. **B.** In contrast, a eukaryotic genome organizes as toroidal supercoils, induced by the wrapping of DNA around histone proteins (orange color). An octamer of histones with 146 wrapped DNA refers to as nucleosome, and the genome organizes into a repeating array of nucleosomes.

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 del nucleotide (definite precedentemente istone simili) è risultato complesso

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

HU

IHF

FIS

H-NS

Dps

Lrp

Principali proteine associate
al nucleotide

Le principali proteine associate al nucleotide batterico

(NAP nucleoid associated proteins)

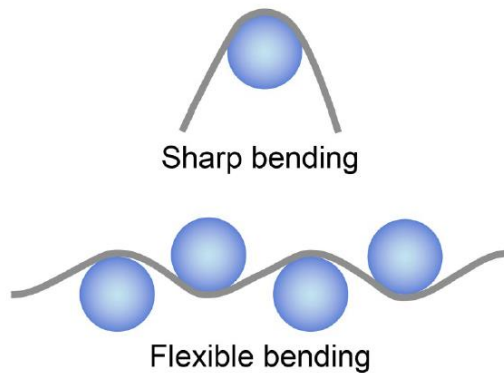
NAP	Properties and abundance	Structure	Genes	Main functional activities
HU (Heat Unstable nucleoid protein)	<ul style="list-style-type: none"> • basic • abundant in exponential phase (15000-30000 dimers/cell) 	heterodimer; HU α , 9.2 kDa HU β , 9.5 kDa	<i>hnpA</i> (90.4 min) <i>hnpB</i> (9.9 min)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • basic • abundant in stationary phase (25000-3000 dimers/cell) • high amino acid identity between IHF and HU subunits • DNA binding preference: WATCAANNNTTR 	heterodimer; IHF α , 11.2 kDa IHF β , 10.7 kDa	<i>himA</i> (38.6 min) <i>himD</i> (25 min)	<ul style="list-style-type: none"> • induces very strong DNA curvature (up to 140°) • participates in site-specific recombination, transposition, and DNA replication
FIS (Factor for Inversion Stimulation)	<ul style="list-style-type: none"> • basic • abundant in exponential phase (20000-40000 dimers/cell) • DNA binding preference: GNYAWWWTRNC 	homodimer, 2x11.2 kDa	<i>fis</i> (73.4 min)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • non basic • 20000-40000 dimers/cell • binding form may be tetramer or higher oligomer • induced during cold-shock 	homodimer, 2 x 15.4 kDa	<i>hns</i> (27.8 min)	<ul style="list-style-type: none"> • recognizes curved DNA • alters DNA topology • induces DNA curvature • influences recombination

Table 1. Properties and the abundance of major nucleoid-associated proteins of *E. coli*.

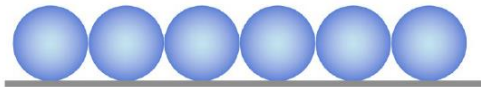
Protein	Molecular mass (kDa)	Native functional unit	Abundance ¹ in growth phase	Abundance ¹ in stationary phase
HU α and HU β	~ 9	Homo- and hetero-dimer	55,000 (23)	30,000 (12.5)
IHF α and IHF β	~ 11	Heterodimer	12,000 (5)	55,000 (23)
H-NS	~ 15	Homodimer	20,000 (8)	15,000 (6)
Fis	~ 11	Homodimer	60,000 (25)	Undetectable
Dps	~ 19	Dodecamer	6,000 (0.4)	180,000(12.5)

¹Abundance (molecules/cell) data were taken from [16]. The number in the parenthesis is micromolar concentration calculated using the following formula: (number of native functional units/Avogadro number) x (1/cell volume in liter) x 10³. Cell volume in liter (2 x 10⁻¹⁵) was determined by assuming volume of the *E. coli* cell to be 2 μm^3 .

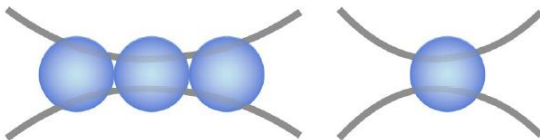
A. DNA bending



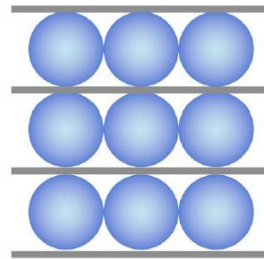
B. DNA stiffening (coating)



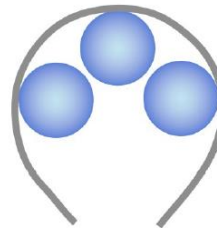
C. DNA bridging



D. DNA bunching



E. DNA wrapping



Non solo curvatura... le diverse funzioni delle proteine associate al nucleoside sul DNA

- A. Bend curvare
- B. Stiffen irrigidire,
Coat rivestire
- C. Bridge creare ponti
- B. Bunch raggruppare
- E. Wrap avvolgere

Le proteine associate al nucleotide (NAP proteins)

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

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- 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"> • basic • abundant in exponential phase (15000–30000 dimers/cell) 	heterodimer; HU α , 9.2 kDa HU β , 9.5 kDa	<i>hupA</i> (90.4 min) <i>hupB</i> (9.9 min)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • basic • abundant in stationary phase (25000–3000 dimers/cell) • high amino acid identity between IHF and HU subunits • DNA binding preference: WATCAANNNTTR 	heterodimer; IHF α , 11.2 kDa IHF β , 10.7 kDa	<i>htmA</i> (38.6 min) <i>htmD</i> (25 min)	<ul style="list-style-type: none"> • induces very strong DNA curvature (up to 140°) • participates in site-specific recombination, transposition, and DNA replication
FIS (factor for inversion stimulation)	<ul style="list-style-type: none"> • basic • abundant in exponential phase (20000–40000 dimers/cell) • DNA binding preference: GNYAWWWTRNC 	homodimer, 2 × 11.2 kDa	<i>fis</i> (73.4 min)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • non basic • 20000–40000 dimers/cell • binding form may be tetramer or higher oligomer • induced during cold-shock 	homodimer, 2 × 15.4 kDa	<i>hns</i> (27.8 min)	<ul style="list-style-type: none"> • recognizes curved DNA • alters DNA topology • induces DNA curvature • influences recombination
StpA (suppressor of <i>td</i> mutant phenotype A)	<ul style="list-style-type: none"> • basic • 10000–15000 copies/cell • high amino acid identity with H-NS • able to form heterodimers with H-NS 	dimer?, n × 15.3 kDa	<i>stpA</i> (60.2 min)	<ul style="list-style-type: none"> • 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 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 nucleotide 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

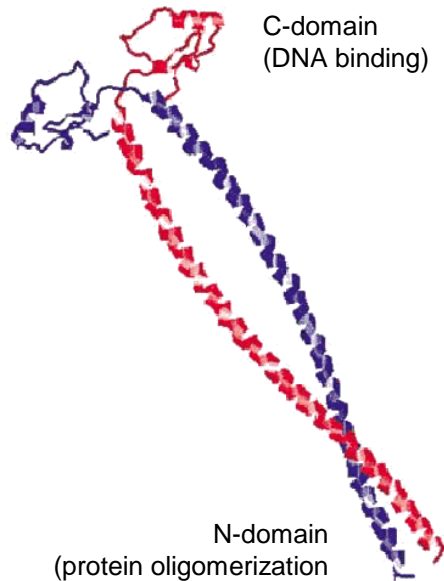
IHF



HU



H-NS

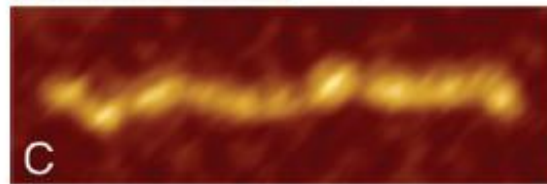
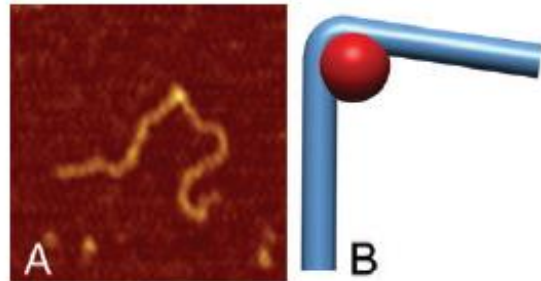


FIS



Nucleoid
proteins are
homo- or
heterodimers

HU lega il DNA e lo ripiega



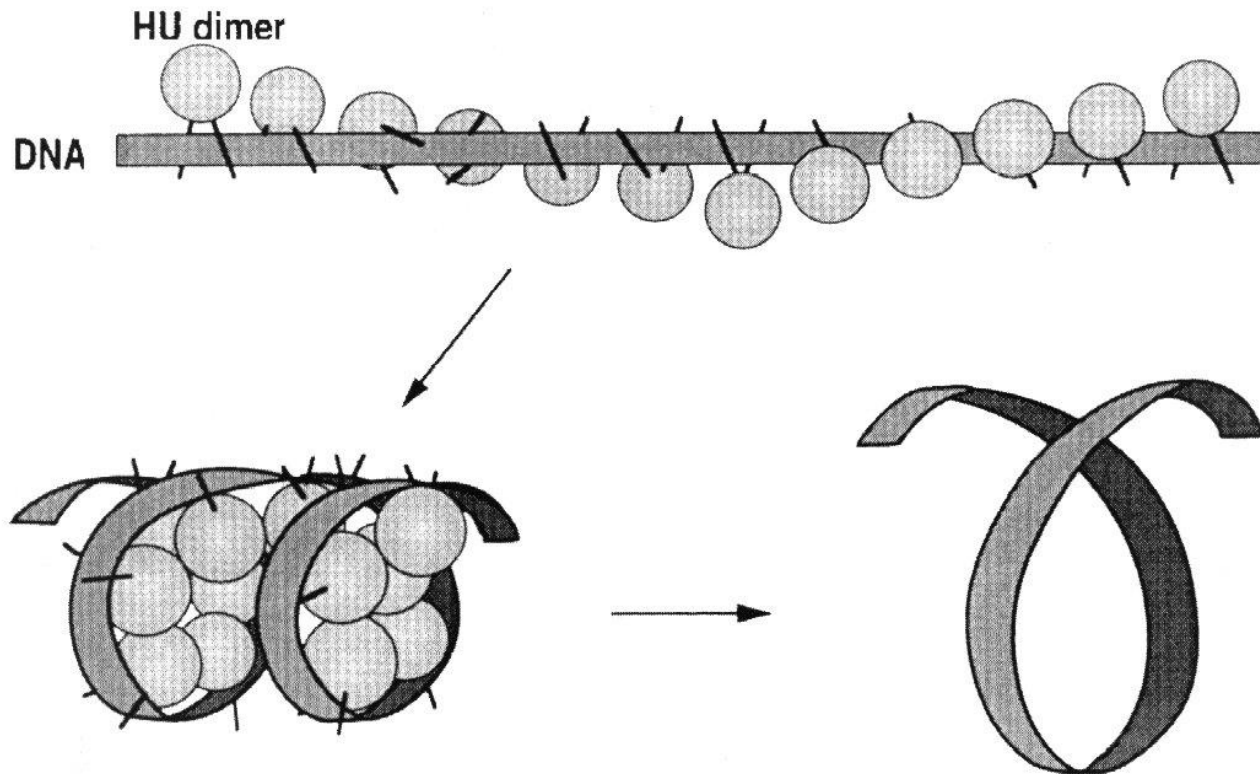
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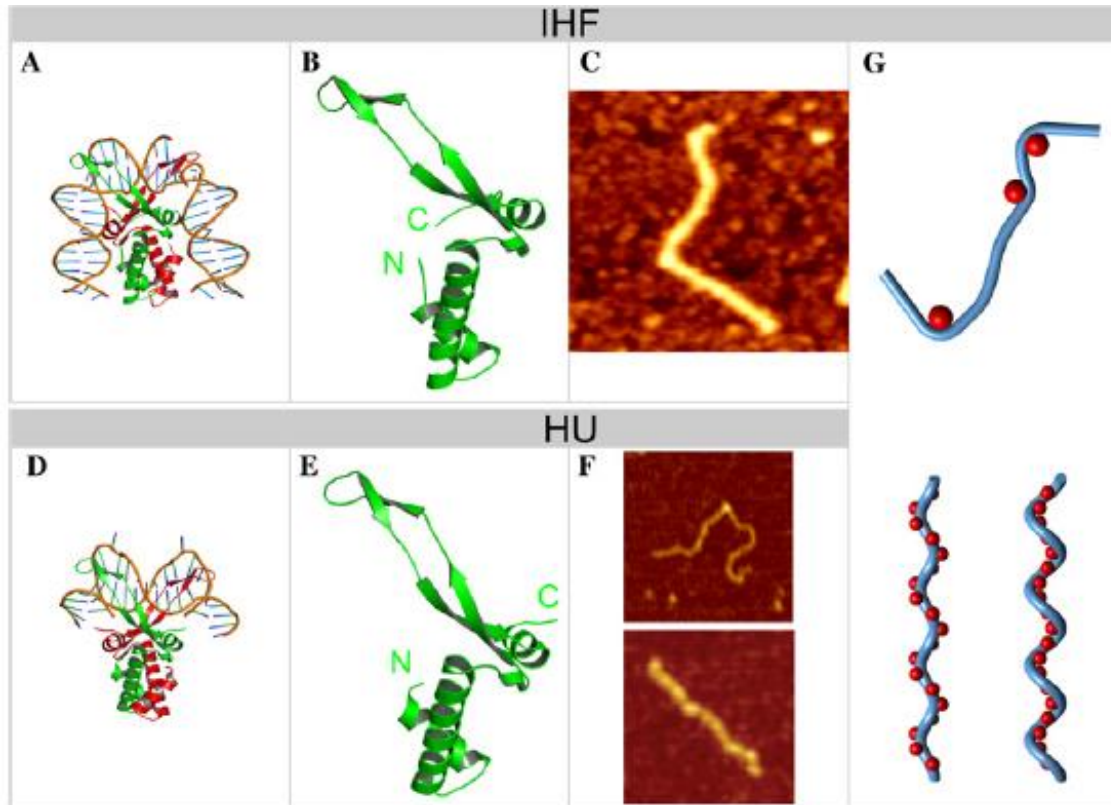
E

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	pM	geni
Eterodimero		
IHF α	11.2 kDa	himA (38.6 min)
IHF β	10.5 kDa	himD (25 min)

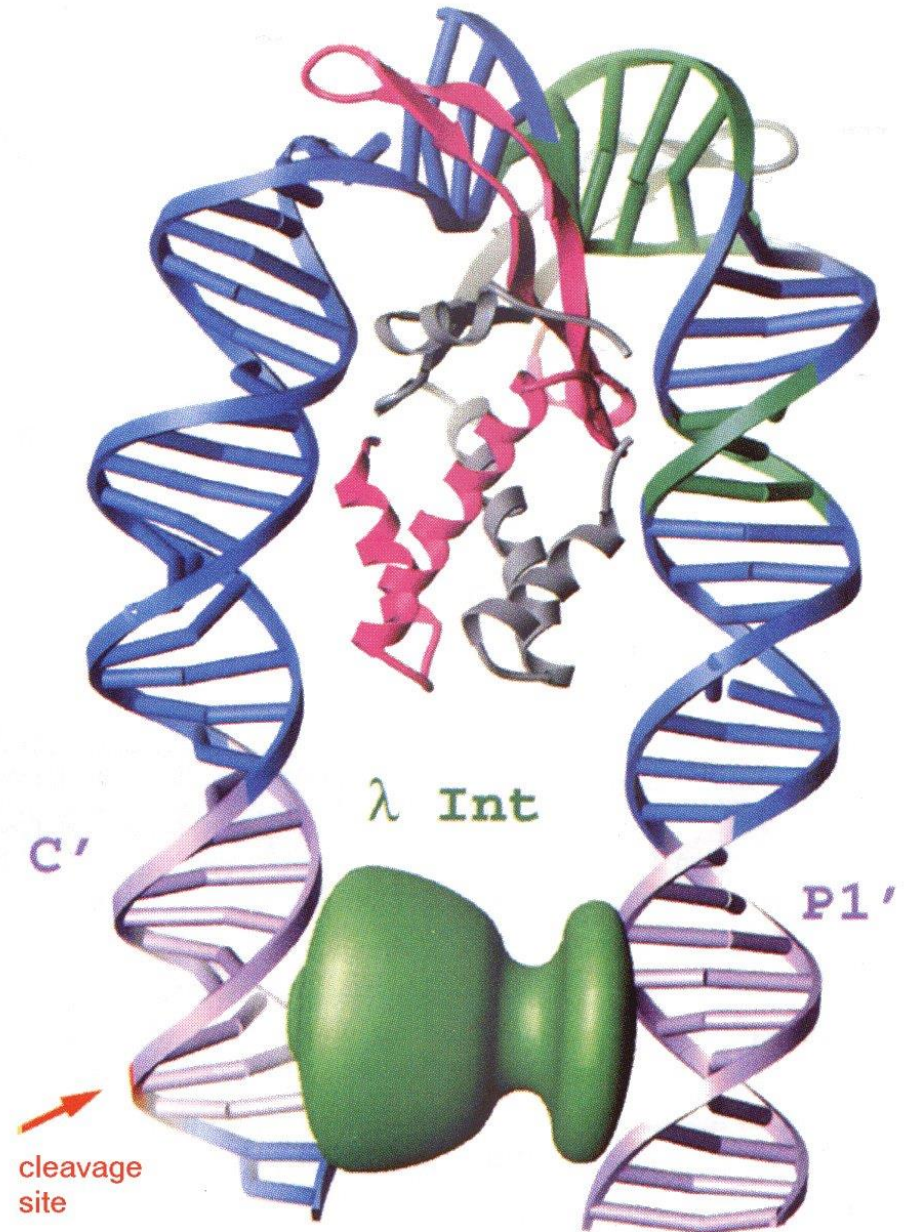
IHF ; Caratteristiche funzionali

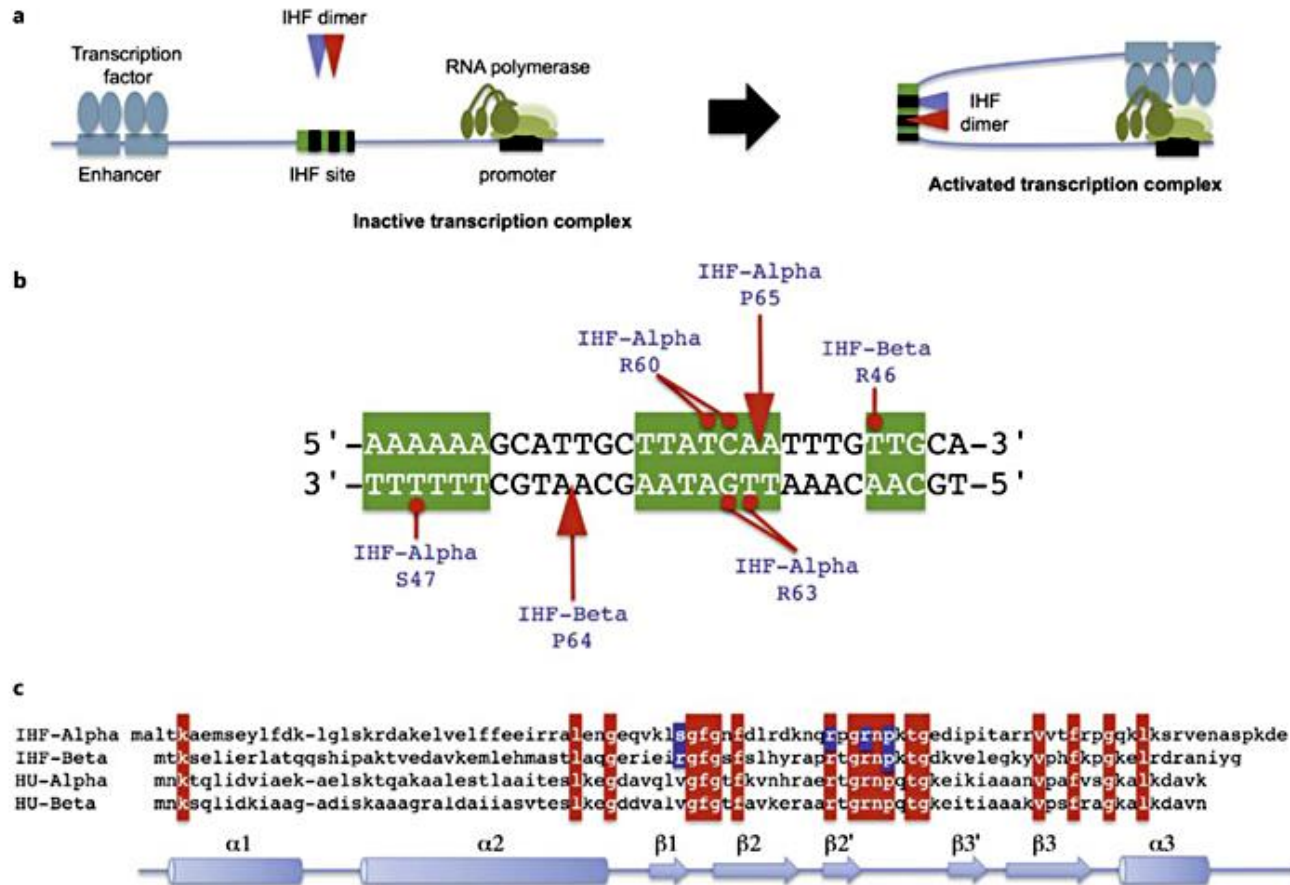
- Induce forte curvatura nel DNA (fino a 140°)
- Interviene nella ricombinazione sito specifica
- Interviene nella trasposizione

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° permette a siti di DNA distanti di trovarsi ravvicinati favorendo così sia processi di trascrizione che di regolazione

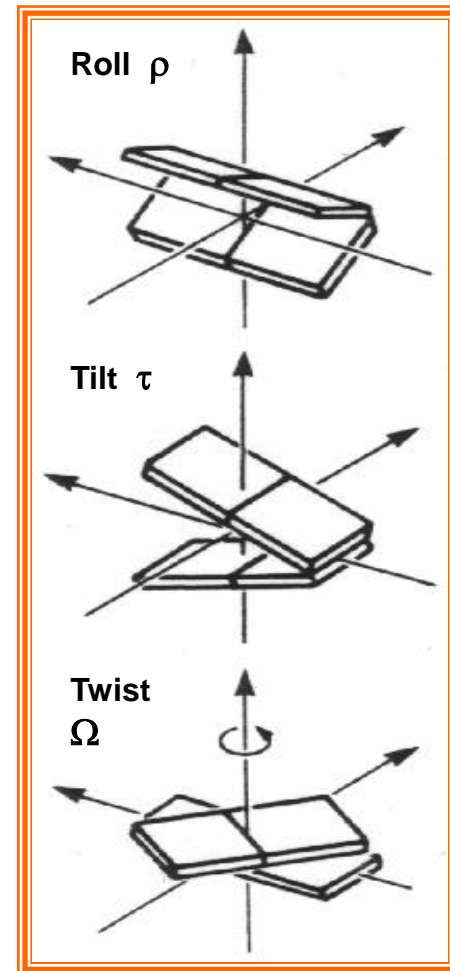
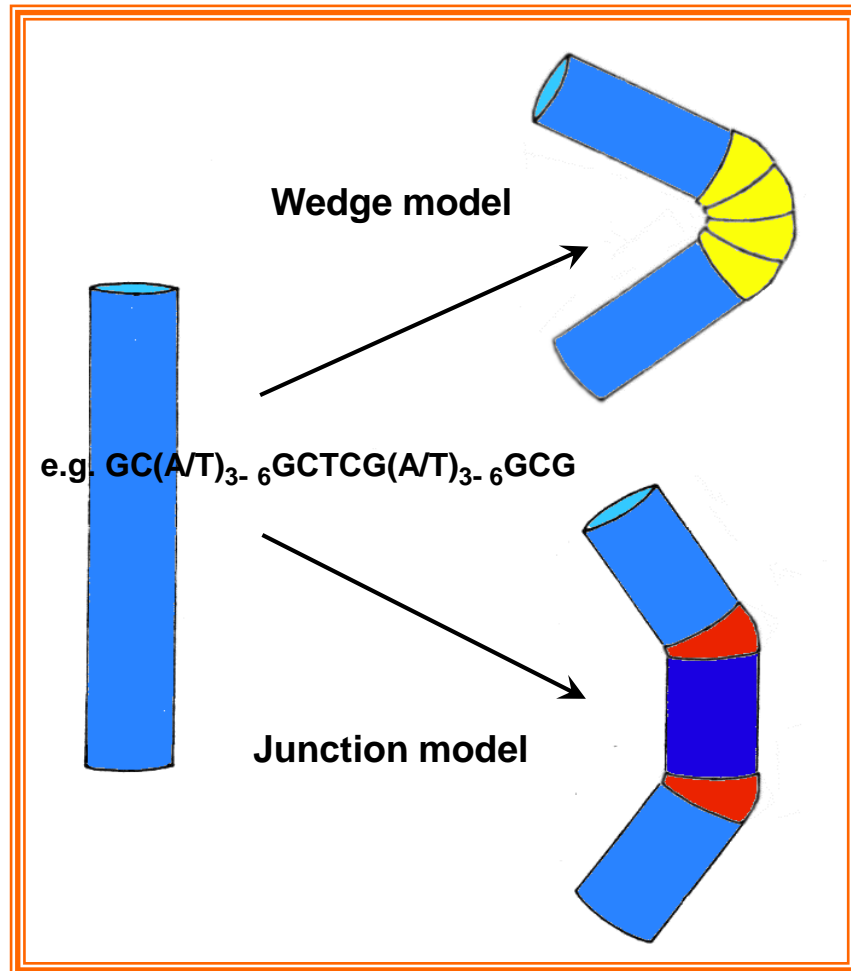
La curvatura
mediata da IHF può
facilitare il legame
della Proteina Int
con siti di DNA
distanti tra loro.

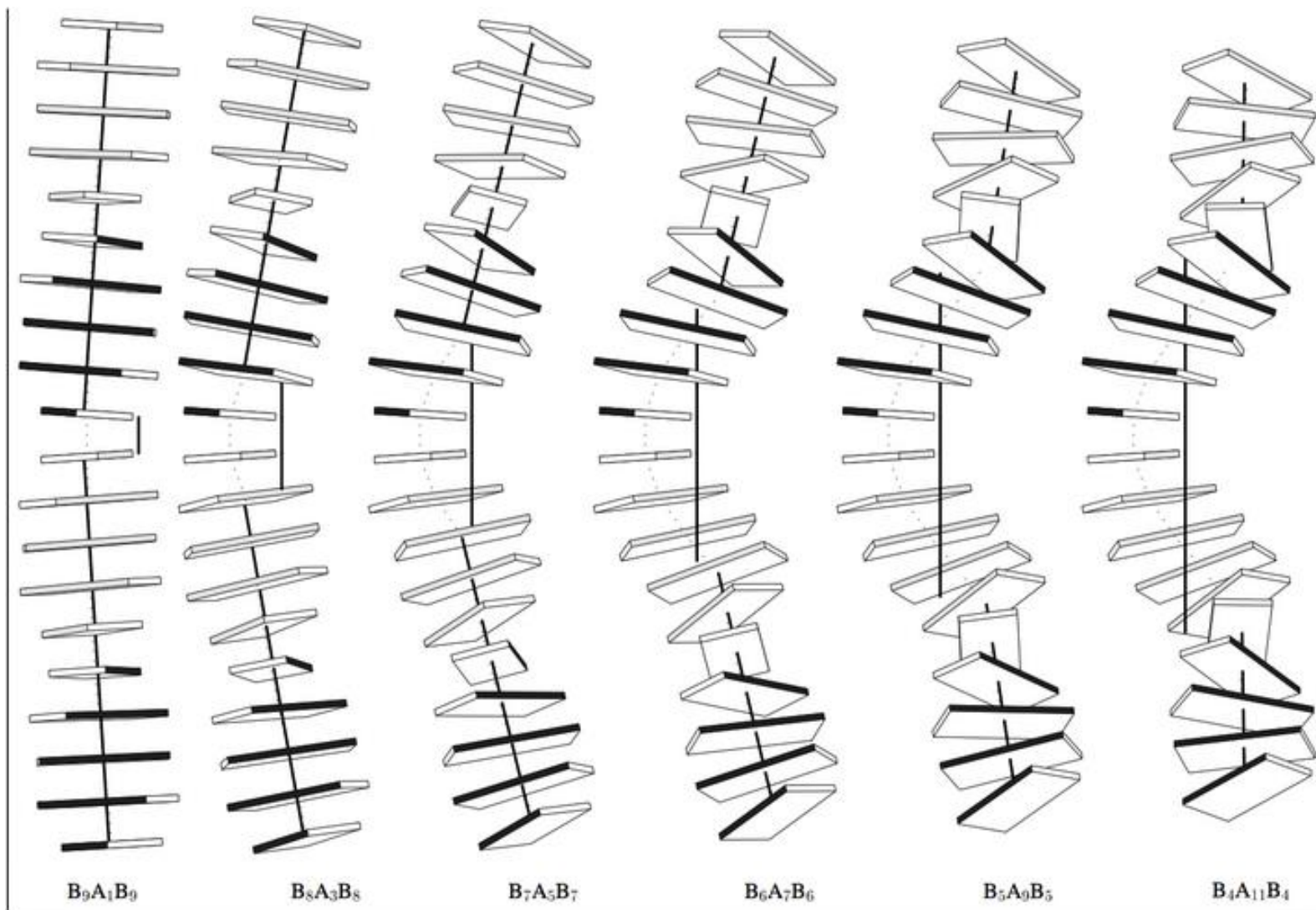




IHF and its paralogue HU. **a** The interaction of IHF with its target site and the consequences for the pathway of the DNA are shown. Here, RNA polymerase (containing $\sigma 54$) has formed an inactive complex with a promoter, and the bending of the DNA by IHF causes a transcription factor, bound as two dimers to two copies of the upstream-located enhancer sequence, to make physical contact with RNA polymerase, activating transcription. Not to scale. **b** The details of the IHF site sequence are shown, with the highlighted residues being the conserved members of the IHF binding site consensus. The amino acids in the α - and the β -subunits of IHF that interact with the DNA sequence are shown. In the cases of proline residues P65 (α -subunit) and P64 (β -subunit), the protein makes an insertion into the minor groove of the DNA duplex, bending it by up to 180° . **c** An alignment of the α - and β -subunits of the paralogous IHF and HU proteins from *E. coli* strain W3110 is shown together with a summary of the main structural features of each monomer. Amino acids that are completely conserved in all four proteins are highlighted..

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

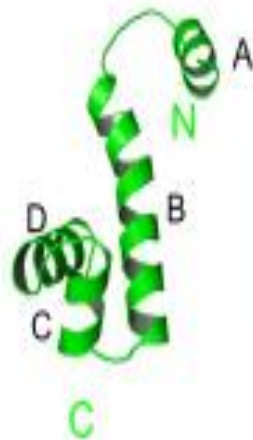
fis (27.4 min)

FIS

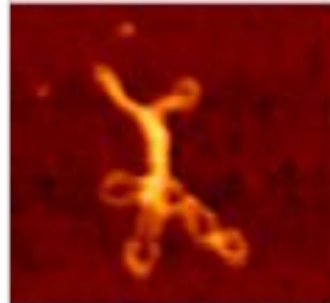
H



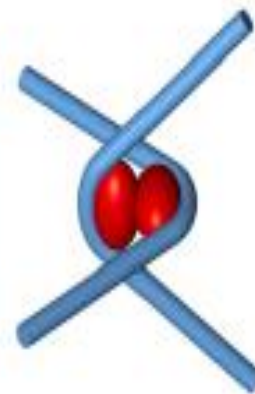
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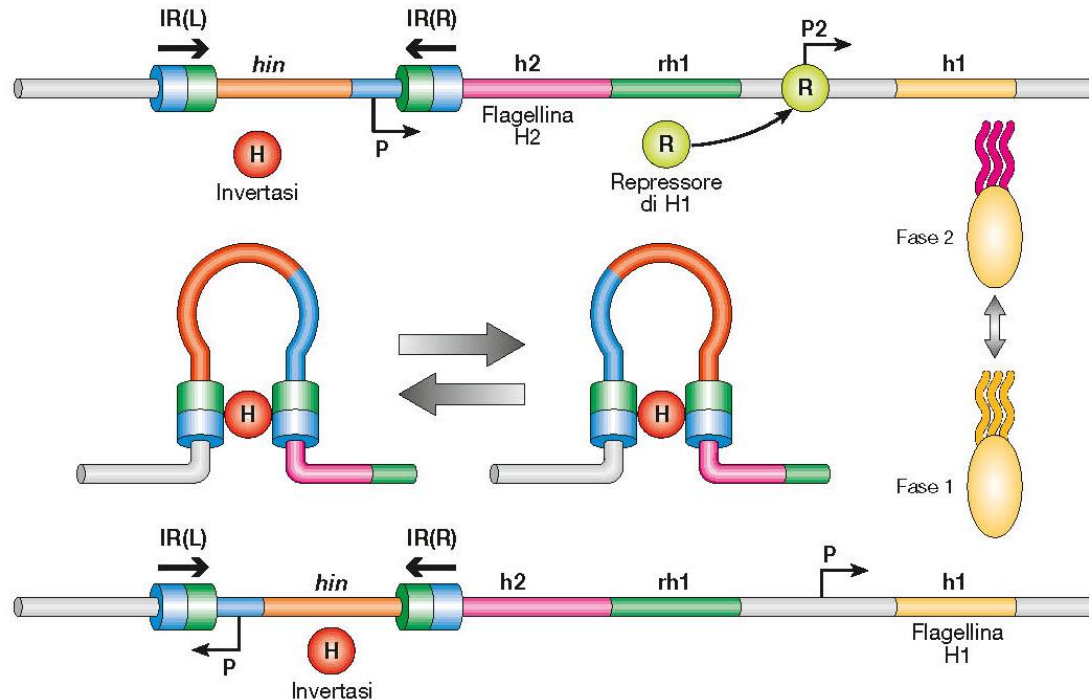
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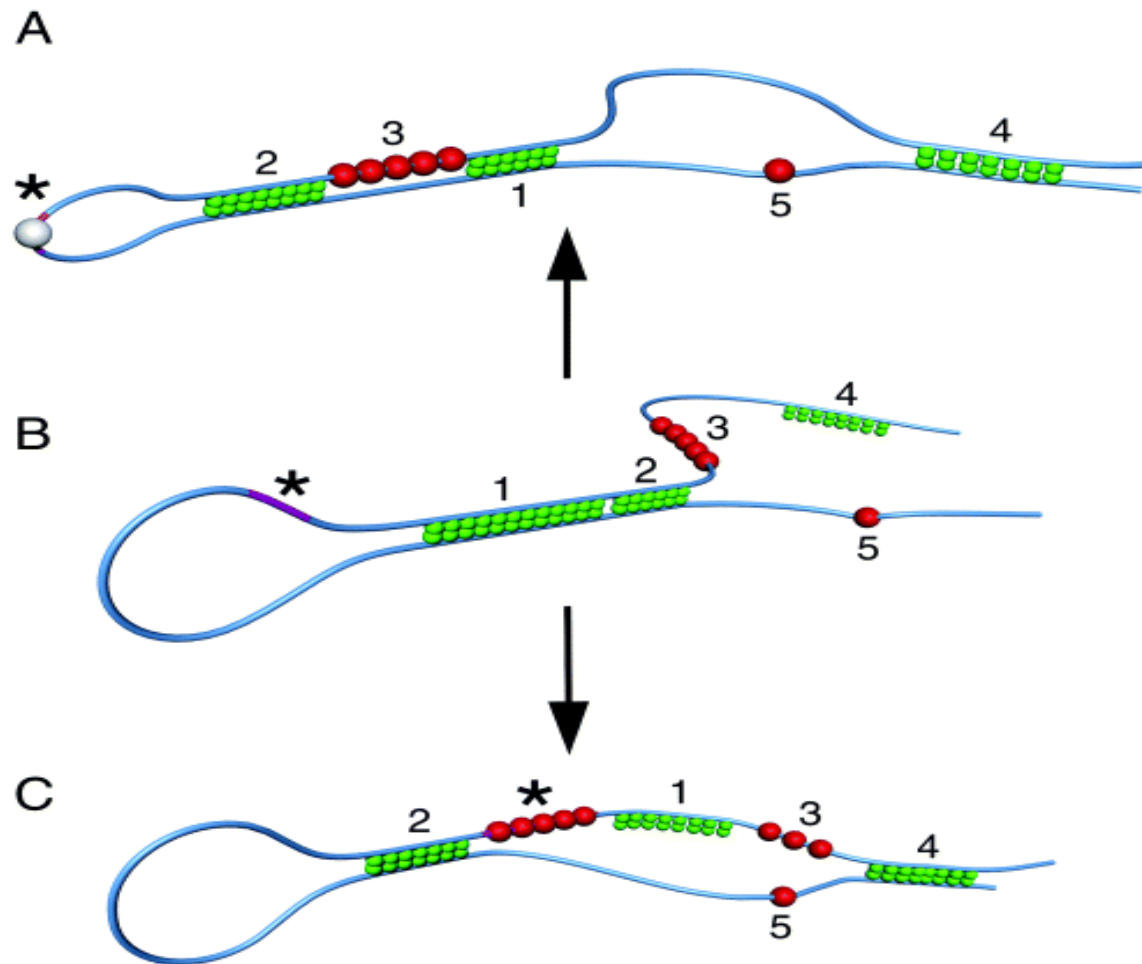


K



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

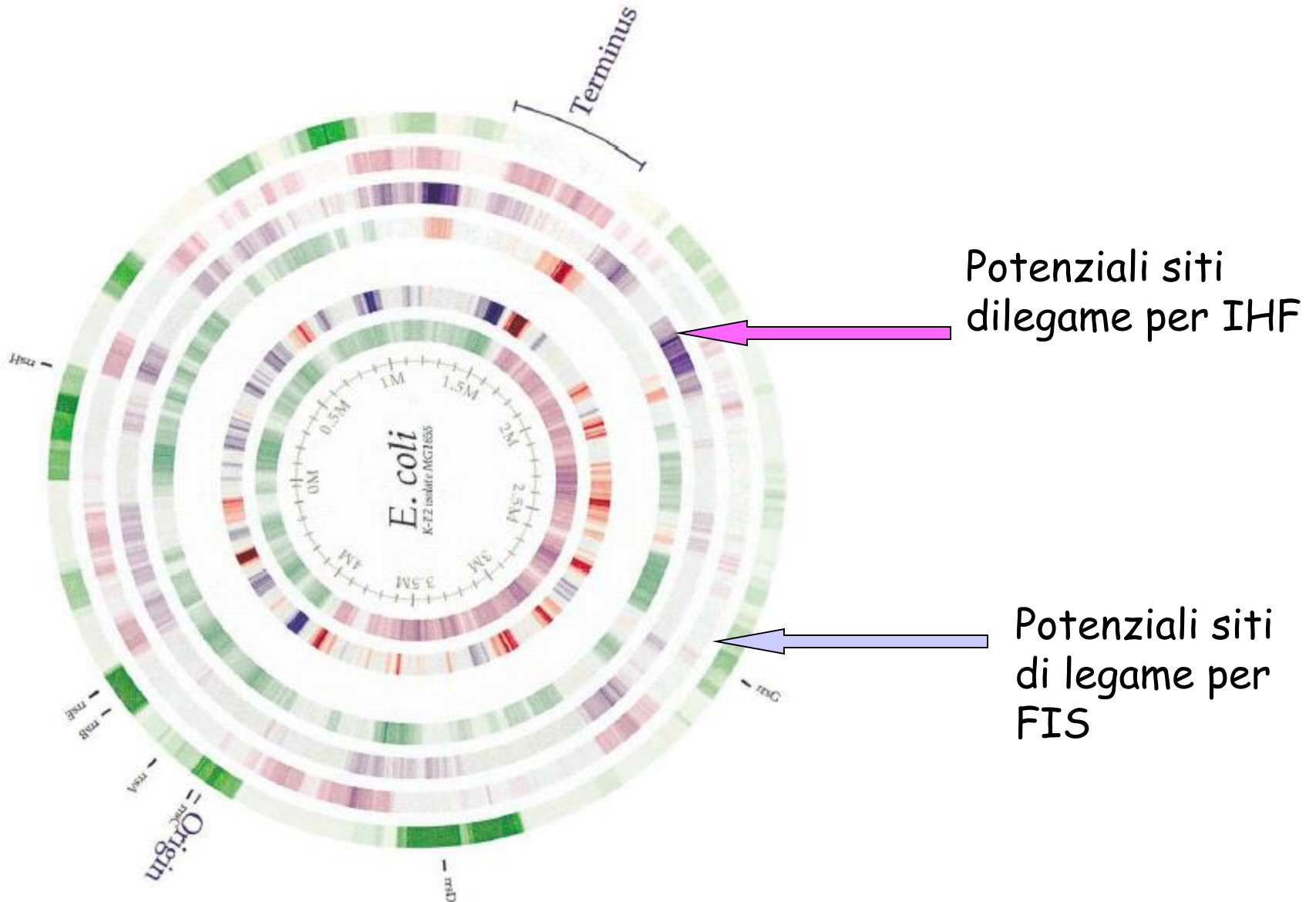




The nucleoid is very dynamic in nature. The organization of the supercoiled loops and the relative orientation of sites within are affected by the binding of nucleoid-associated proteins such as HU, H-NS, IHF and Fis. Each of these proteins has different functional interactions with DNA and the proteins of this family can be roughly fitted into two categories: DNA-bridgers and DNA-benders (see main text). Within the context of a supercoiled loop, the configuration is determined by the proteins that are simultaneously bound within the loop. The organization within loops is important for DNA compaction and can also play a role in the modulation of transcription. DNA is depicted in cyan. The green spheres correspond to H-NS (following the mechanism of binding as shown in [Fig. 1B](#)). The red spheres correspond to HU (either in its bending or its rigidification mode). The grey sphere corresponds to a DNA bending protein, such as IHF, Fis or HU. Although the described type of reorganization can take place only within a supercoiled loop, DNA topology has been omitted in the visualization of these loops for the purpose of simplicity.

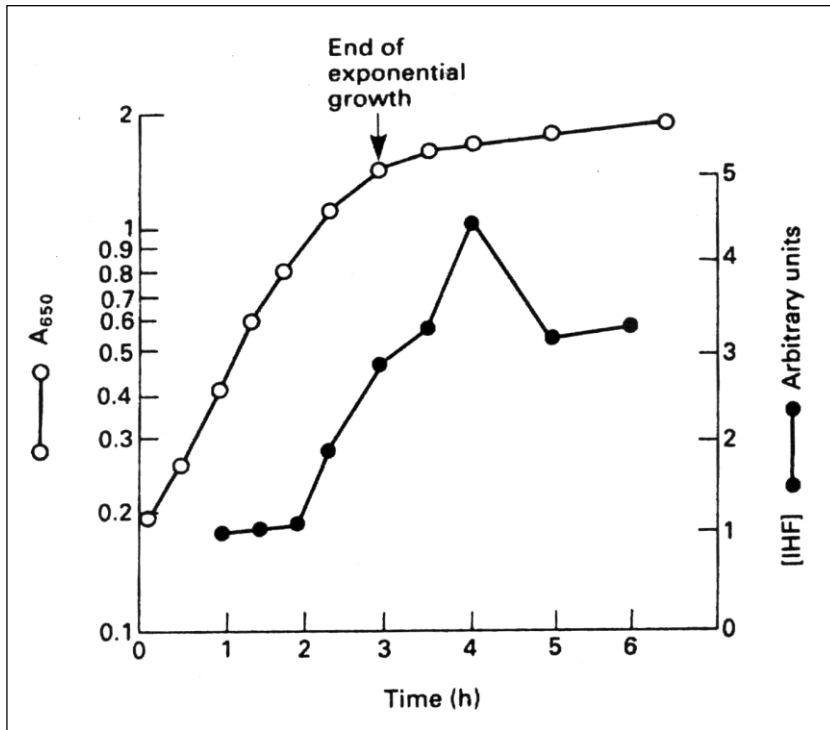
The (arbitrary) starting configuration of a loop is shown in B. H-NS bridges hold together DNA tracts within the loop, some HU molecules are shown rigidifying the DNA, or bending it at another site and one tract of DNA is covered with H-NS but *not* bridged. A binding site is present within the tip of the loop (in purple, and indicated with an asterisk). The binding of proteins at this site results in reconfiguration of the loop. In the first example (A), this site is bound by a protein that bends DNA (e.g. IHF), which imposes relocation of the bound protein to the tip of the loop. In parallel, other bound proteins within the loop will become relocated. In this example, one of the H-NS tracts (2) and the adjacent region with HU bound (3) follow the left-ward directed movement of the tip of the loop. A part of a second H-NS tract (1) is found back bound on the right side of the region with HU. Finally, the spatial vicinity of the loose H-NS tract (4) has changed such that it has become bound to the DNA alongside the DNA bending HU molecule (5). In the second example (C), HU becomes co-operatively bound to the binding site (or rather region). It is energetically unfavourable for a rigid tract of DNA to find itself located within the tip of a loop and therefore it moves away into the stem. The H-NS tract directly aside of the bound HU (1) moves along with it to the right (where it remains loose), whereas the second H-NS tract (2) is found back bridging an area on the left of the region with HU bound (3). Finally, the spatial vicinity of an initially loose H-NS tract (4) has also changed such that it has become bound to the DNA alongside the DNA bending HU molecule (5).

Proteine del nucleoside come regolatori globali

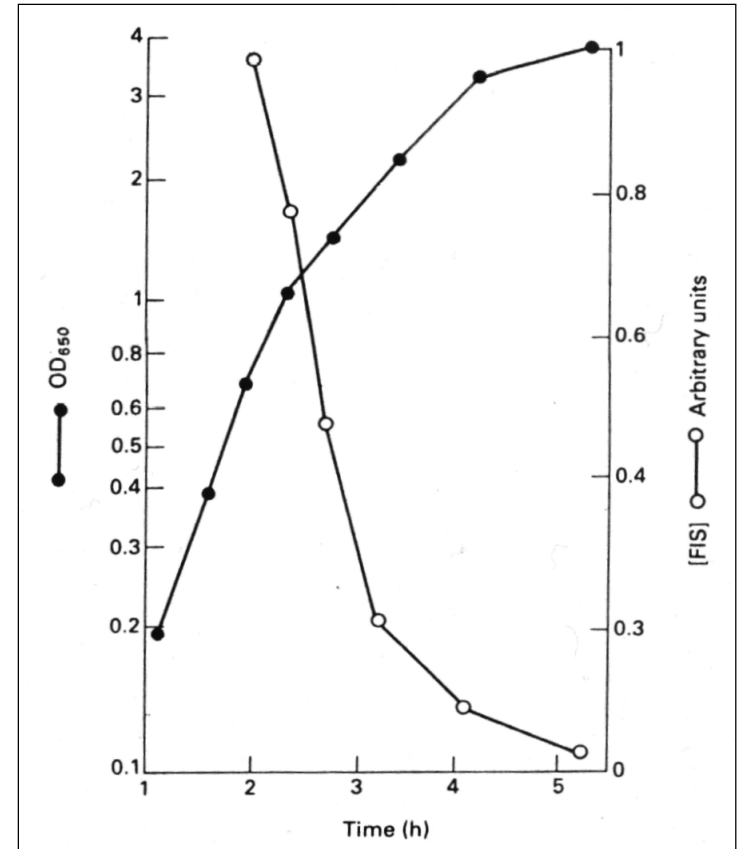


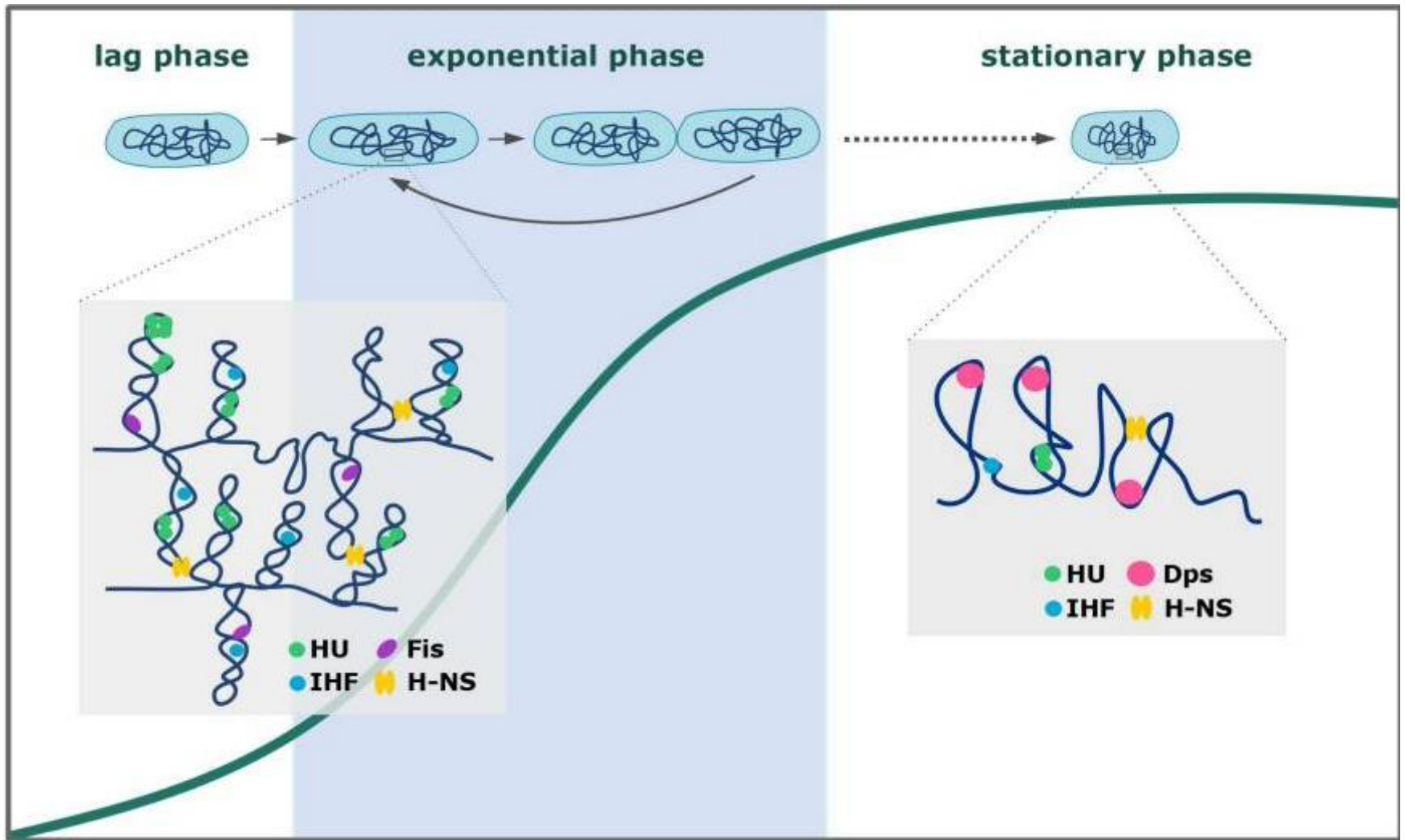
Espressione di IHF e FIS in funzione della curva di crescita

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

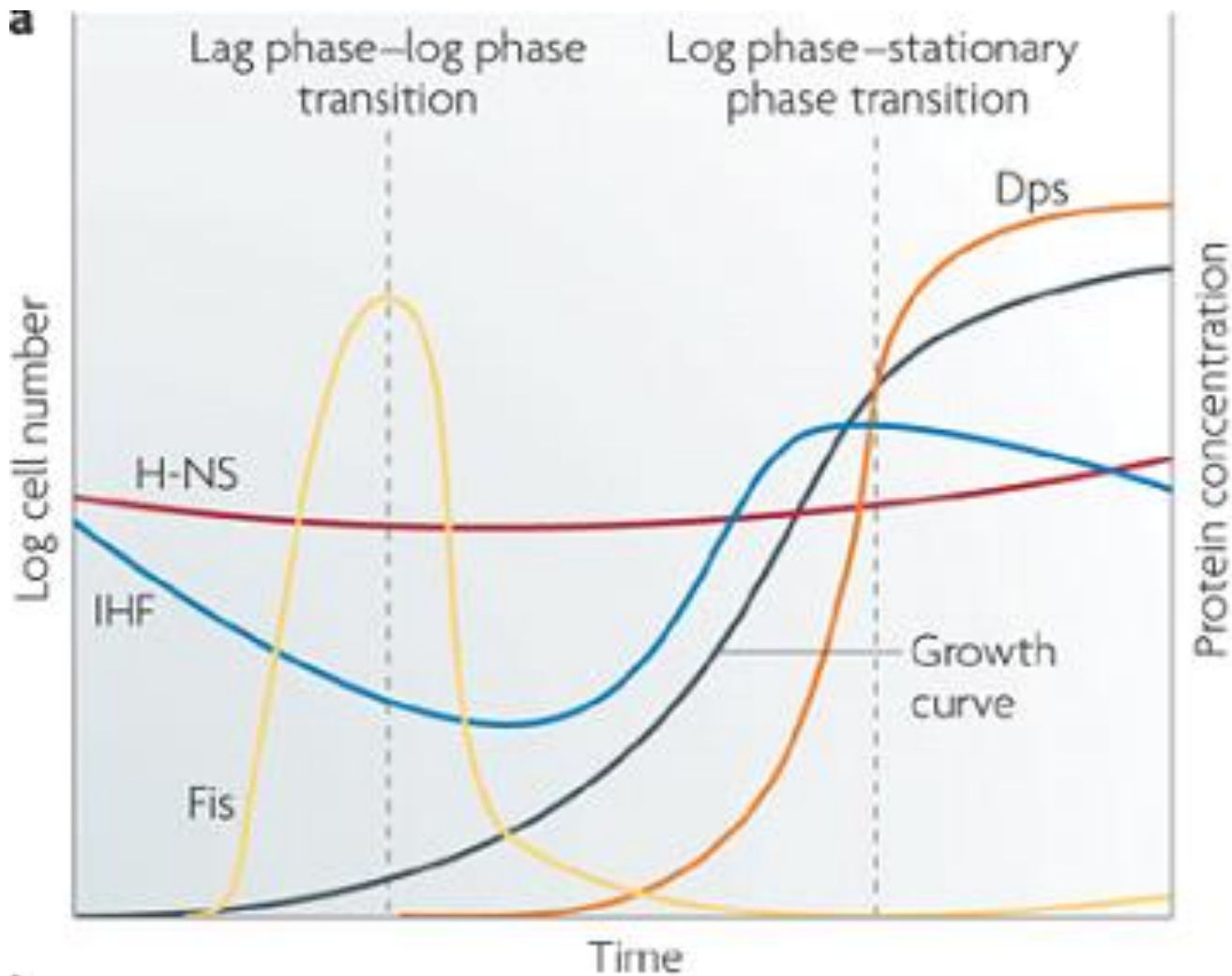


La proteina FIS è invece molto abbondante all'inizio della fase esponenziale





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



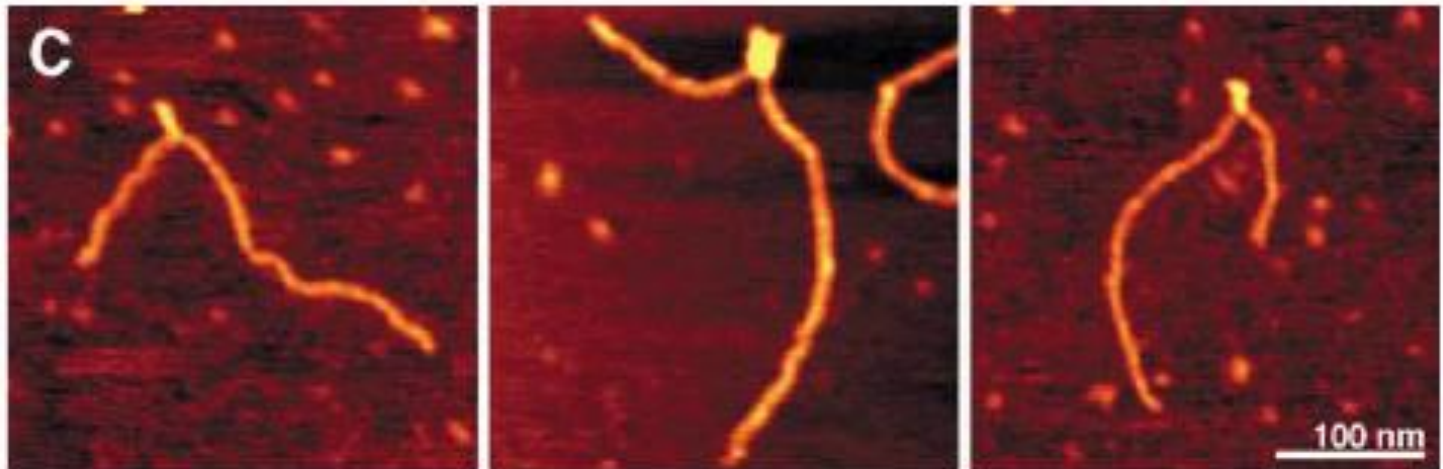
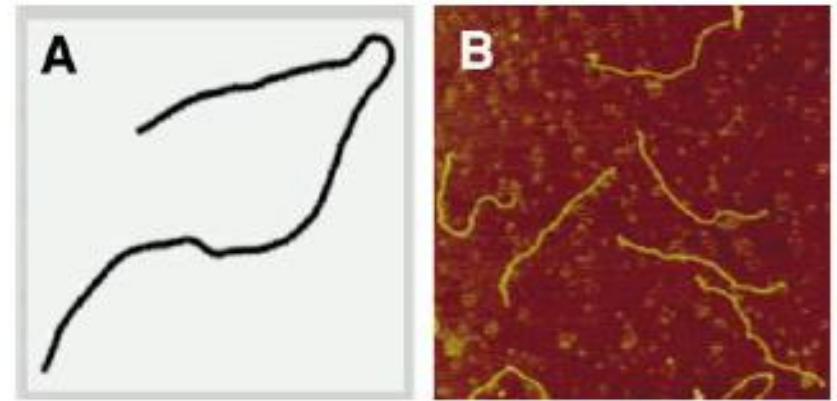
The expression patterns of the four nucleoid-associated proteins (NAPs), DNA protection from starvation protein (Dps), factor for inversion stimulation (Fis), histone-like nucleoid-structuring protein (H-NS) and integration host factor (IHF). A typical bacterial growth curve is shown, with the lag phase-log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

H-NS

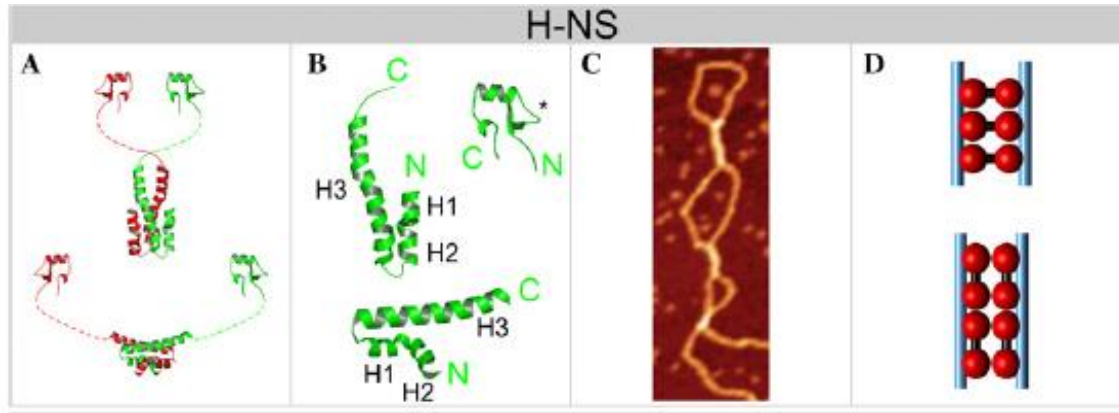
(Histone-like Nucleoid Structuring protein)

- 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



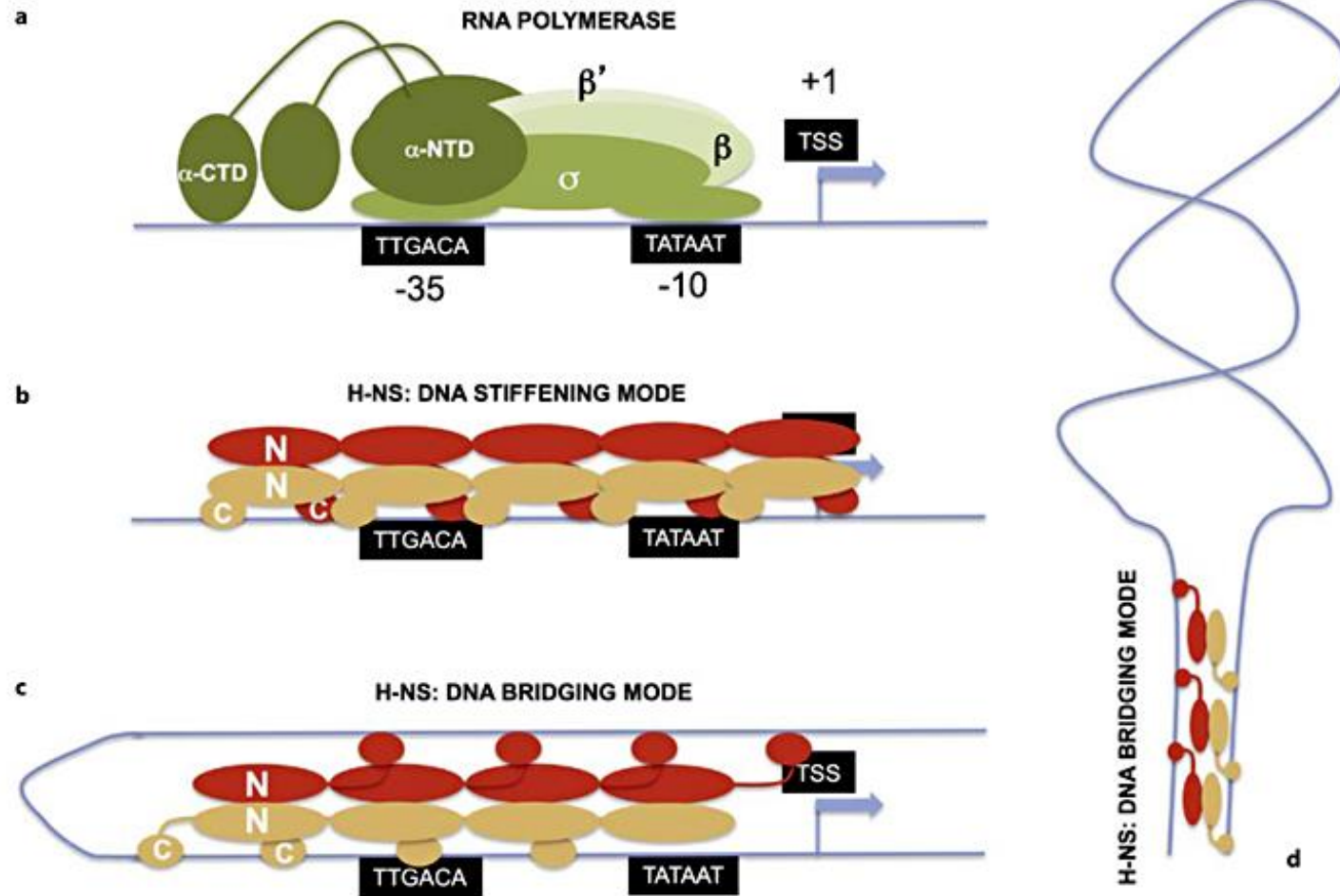
Le proteine NAP che oltre a ripiegare il DNA formano ponti : un esempio H-NS



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

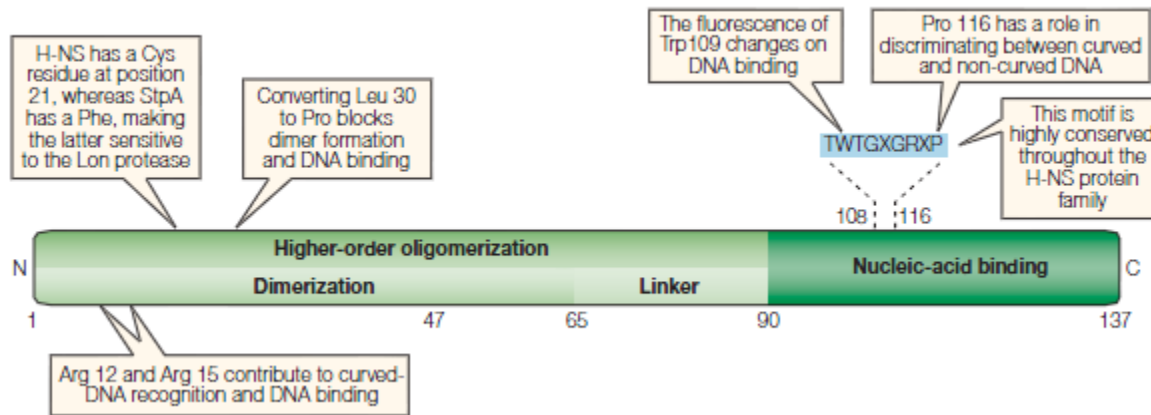
- A) Strutture proposte per il dimero di H-NS
- B) Analisi dei monomeri di H-NS
- C) Formazione delle anse mediate da H-NS
- D) 2 diversi modelli di interazione nella formazione complessi

Altre proteine NAP che formano ponti su LRP e MuKB



H-NS, transcription silencing and chromosome microdomain formation. **a** A standard representation of RNA polymerase bound to a transcription promoter is shown consisting of the principal components of the holoenzyme: the α -subunit, in two copies with their carboxyl-terminal domains (CTD) and amino terminal domains (NTD) shown connected by flexible linkers. The β -, β' - and σ -subunits are also illustrated. The locations of the transcription start site (TSS, +1), the -10 and -35 elements are also shown together with the consensus DNA sequences for the -10 and -35 motifs of promoters that are bound by the RpoD sigma factor of RNA polymerase. **b** The same promoter sequence is shown decorated by the H-NS protein in its DNA stiffening mode, excluding RNA polymerase and silencing transcription. Here H-NS polymerizes along the DNA duplex, and the two DNA-binding motifs of each H-NS dimer bind to the same DNA molecule in *cis*. The H-NS monomers are arranged in an antiparallel orientation within each dimer. **c** H-NS is shown bound to the same promoter element in its bridging mode. Here, the DNA-binding domains of each H-NS dimer (shown in antiparallel configuration) bind to spatially widely separated segments of the same DNA molecule, creating a DNA-protein-DNA bridge that excludes RNA polymerase from the promoter. **d** The bridging function of H-NS can also form loops in DNA, including the 10- to 15-kb microdomain loops that contribute to the higher-order structure of the bacterial nucleoid. The drawings are not to scale.

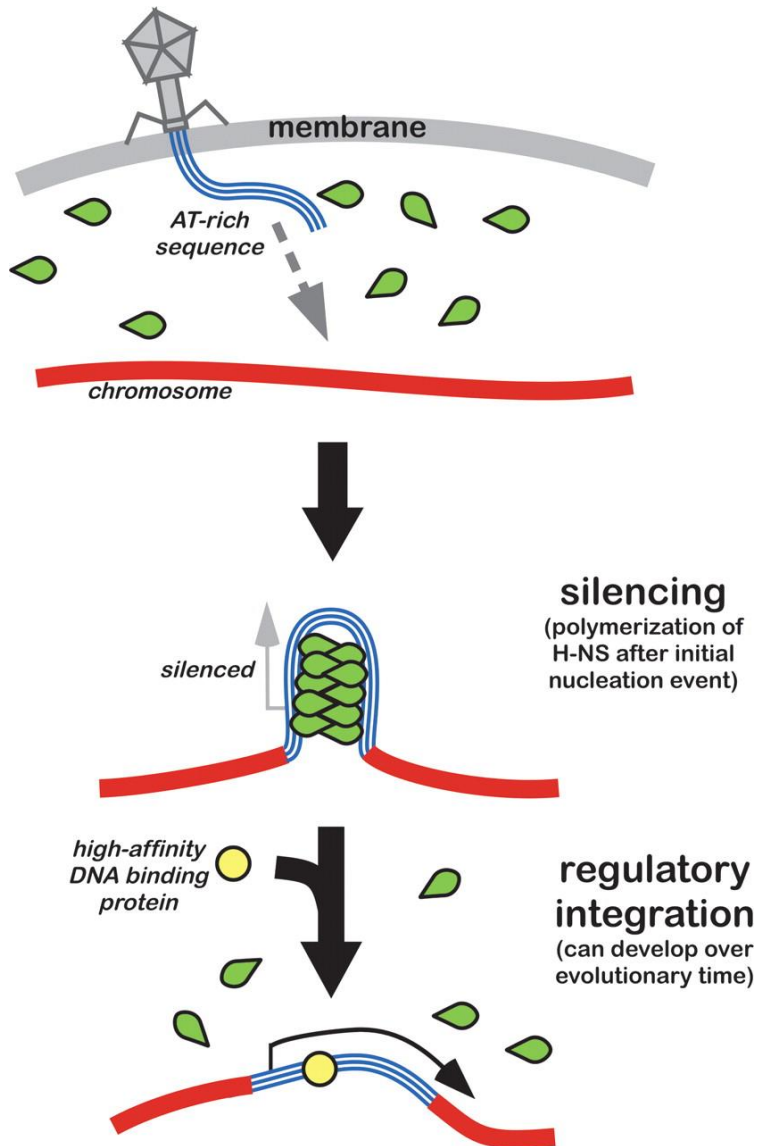
Struttura della proteina H-NS



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

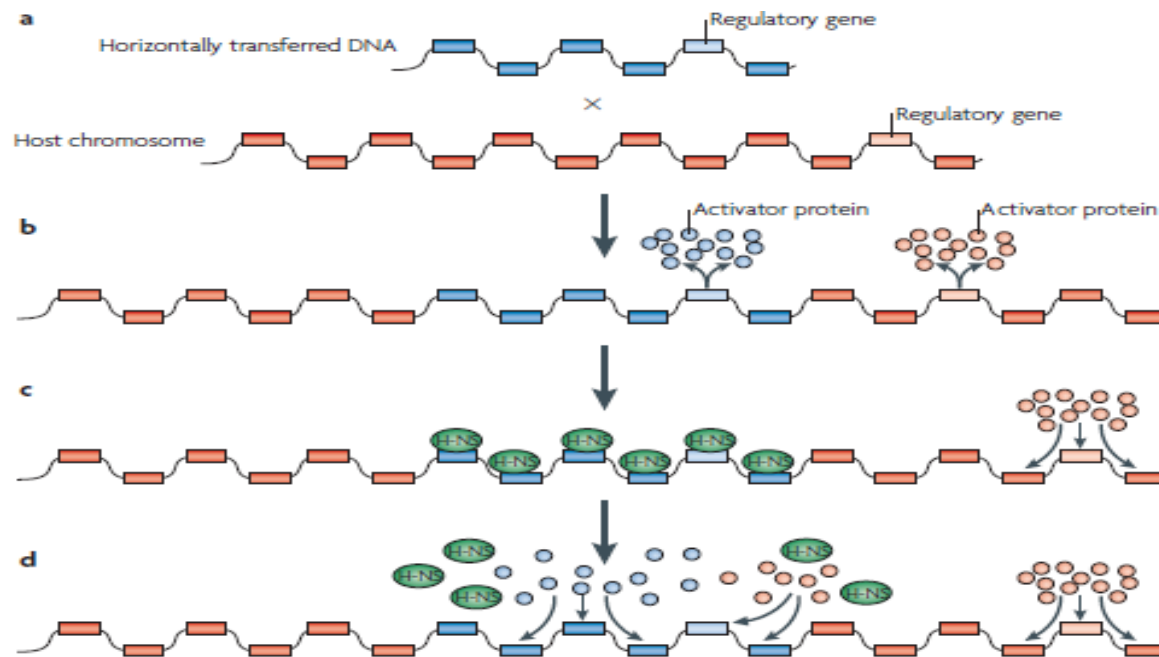
H-NS ed il silenziamento di regione geniche acquisite per HGT



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

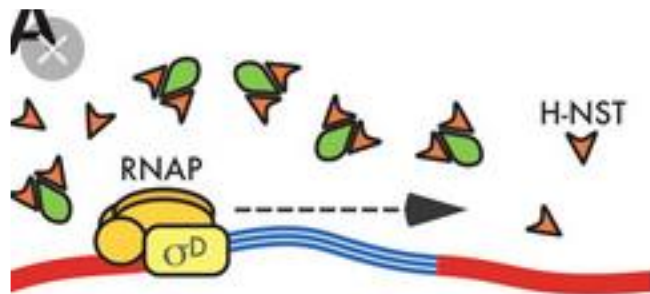
L'eventuale presenza di una proteina regolatrice sequenza specifica con un elevata affinità per il DNA può competere con H-NS per eliminare il silenziamento e permettere nuovamente l'espressione genica in condizioni specifiche.

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

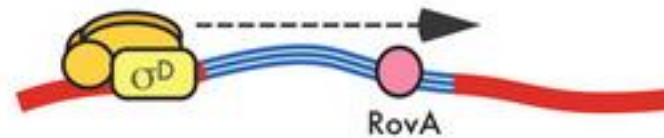


H-NS e il silenziamento dei geni acquisiti tramite HGT

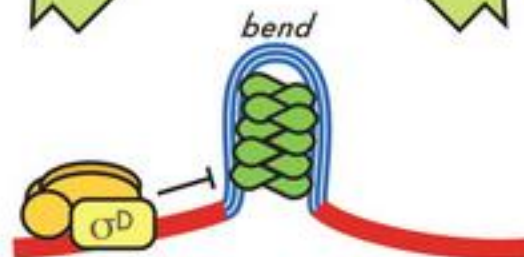
Figure 1 | H-NS as a gene silencer. The figure shows a model for the involvement of H-NS in the repression of horizontally transferred genes, and how this repression can be relieved. **a** | A segment of DNA carrying six genes enters the cell as a result of horizontal transfer. One of these is a regulatory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



antagonism of
multimerization
(e.g. H-NST)



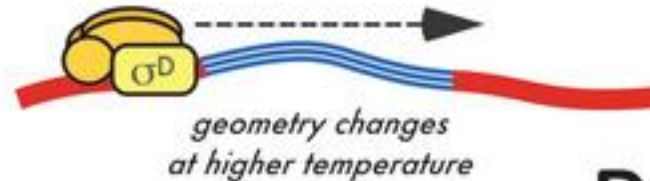
promoter competition
by sequence-specific
DNA-binding proteins
(e.g. ToxT, RovA)



**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)



geometry changes
at higher temperature

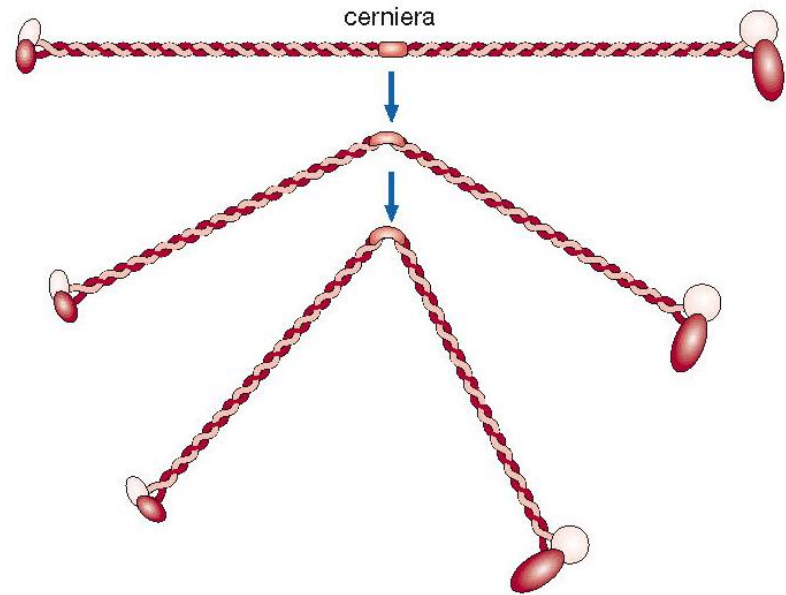
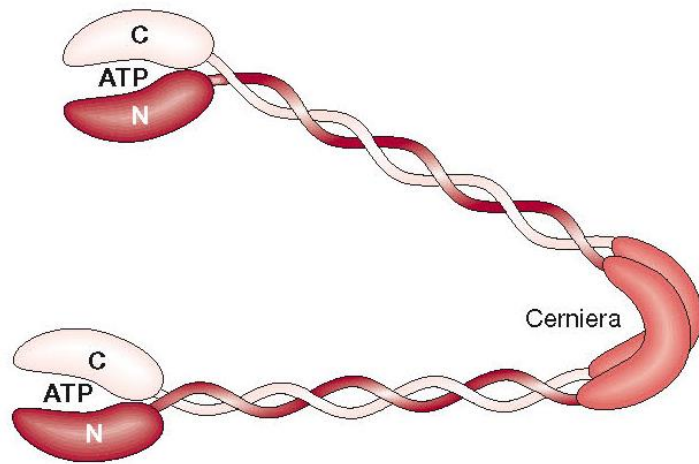
La proteina MukB una proteina SMC like

La proteina MukB è una proteina SMC like (Structural Maintenance 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 nucleotide ad alta frequenza.

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

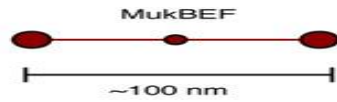
La perdita di MukB e della proteina del nucleotide HU è letale per la cellula.



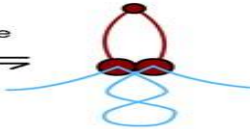
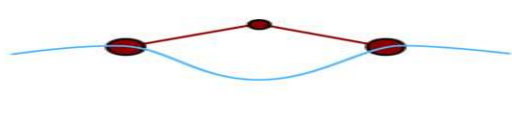
La proteina Muk B come molte proteine della famiglia SMC ,è costituita da due domini globulari N- e C- terminali (teste) separati da 2 regione coiled-coil intervallate da una terza regione globulare che costituisce una cerniera flessibile.

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

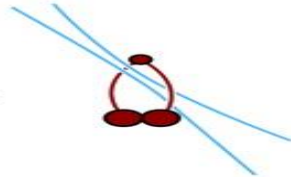
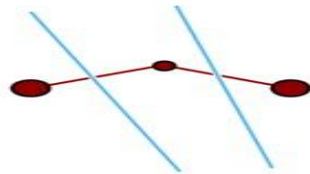
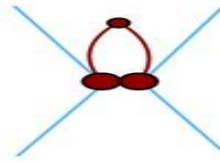
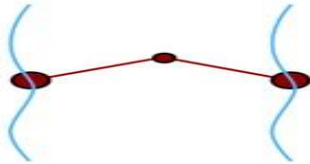
A ATP-dependent change



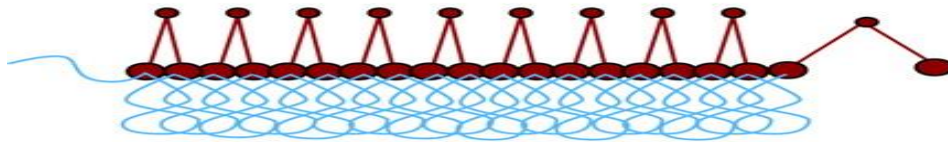
B Intramolecular compaction



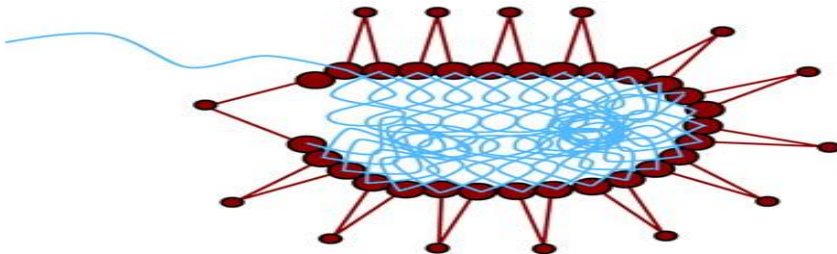
C Intermolecular bridging



D Thick fiber-like structure



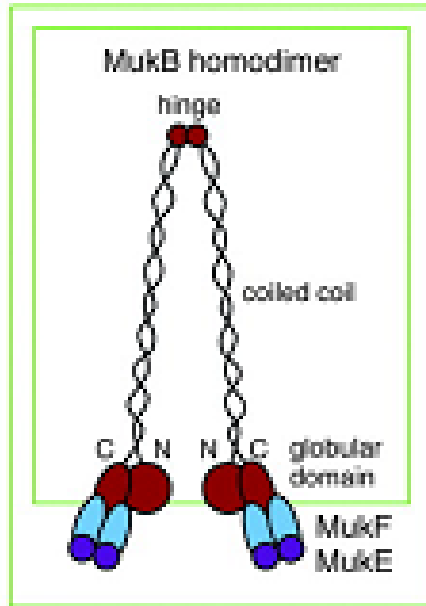
E Globular structure



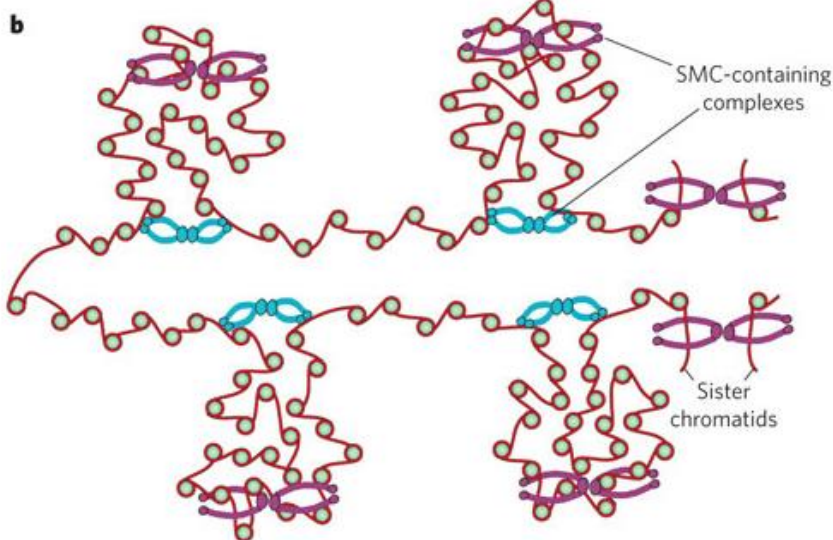
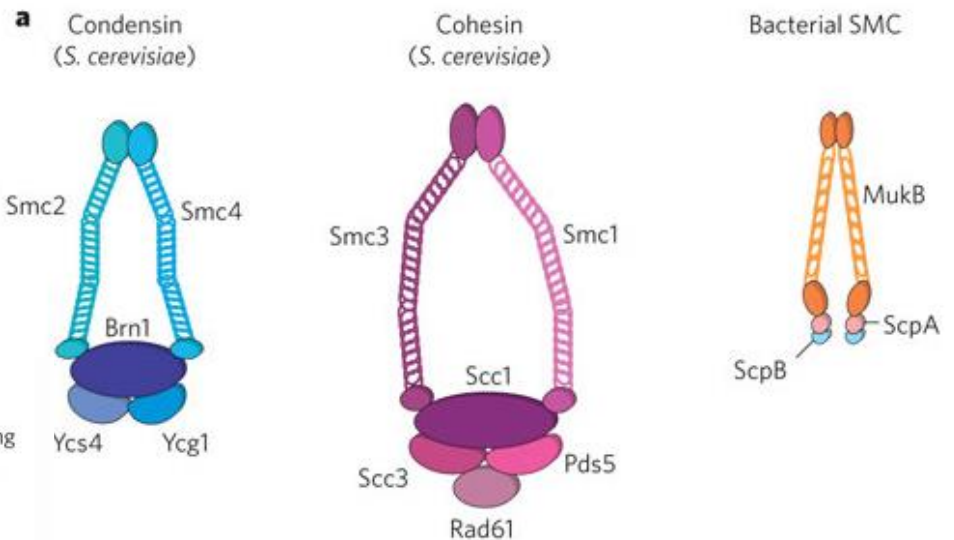
Nella forma chiusa la proteina scorre sul DNA ed è in grado di indurire il ripiegamento.

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

MukBEF complex



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



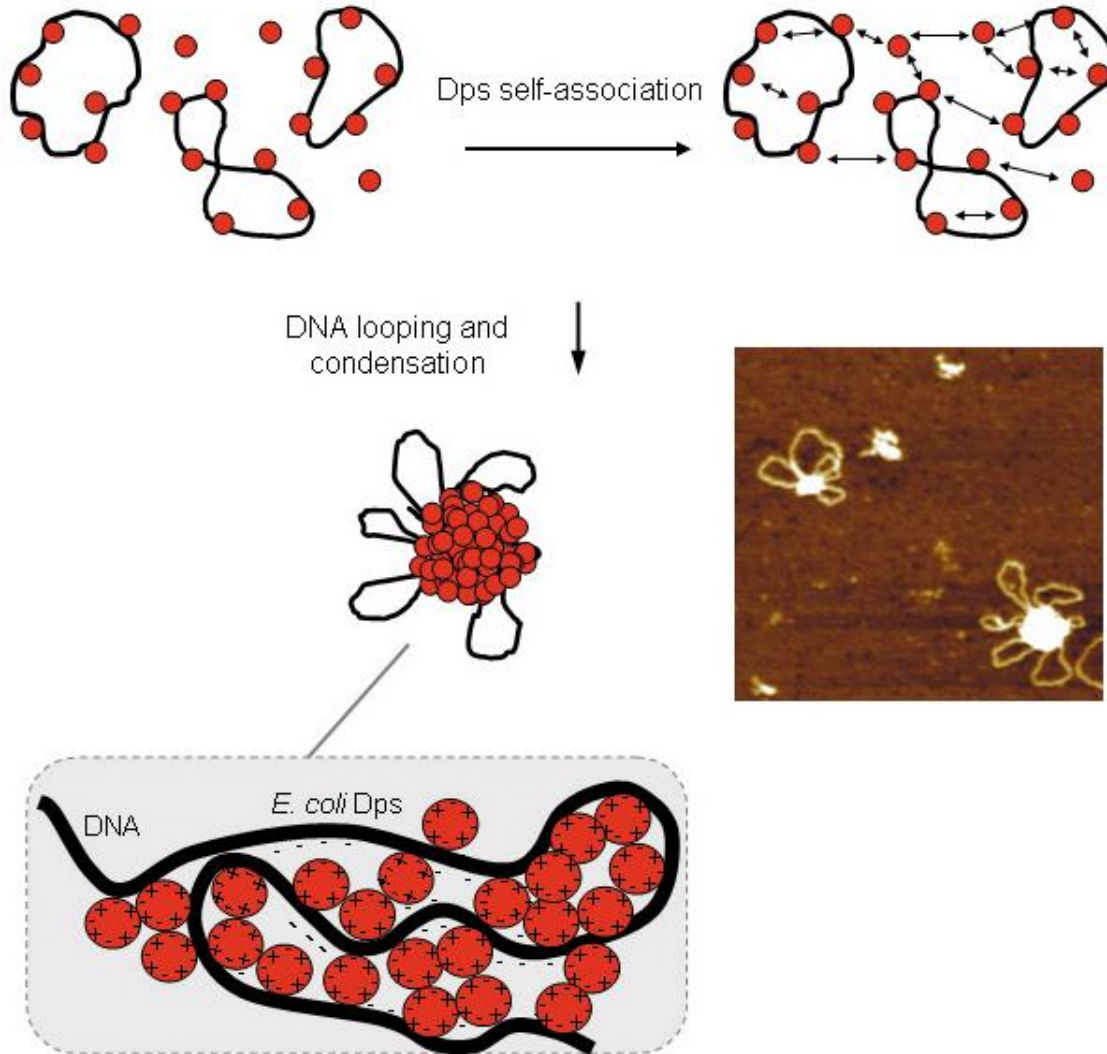
La proteina Dps (Dna Binding protein from starved cells)

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

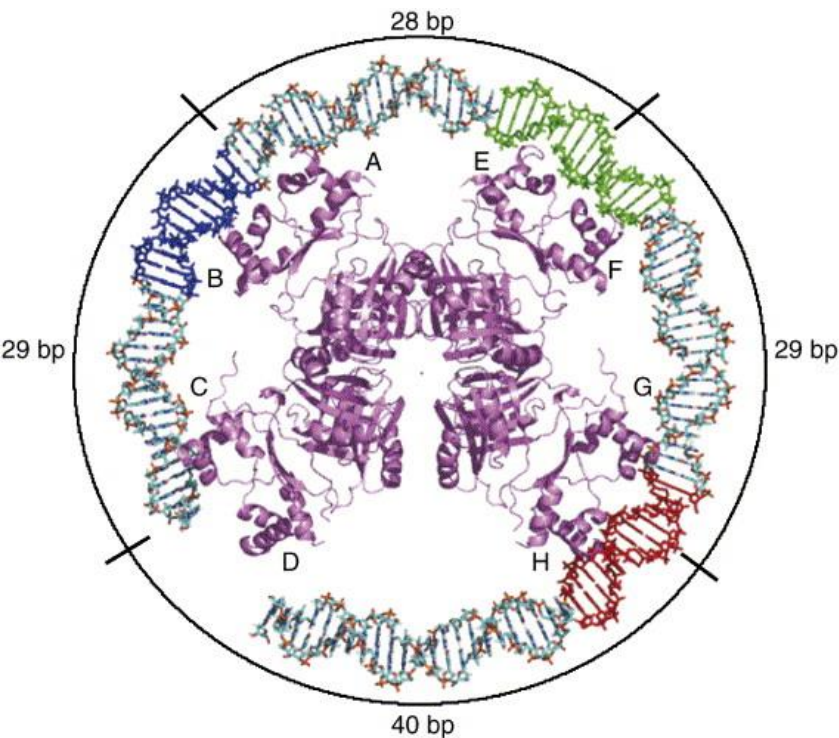
Lrp Leucine responsive regulatory protein

influenza alla 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 metabolismo degli AA oltre a geni di virulenza quali quelli coinvolti nella sintesi di alcuni pili.

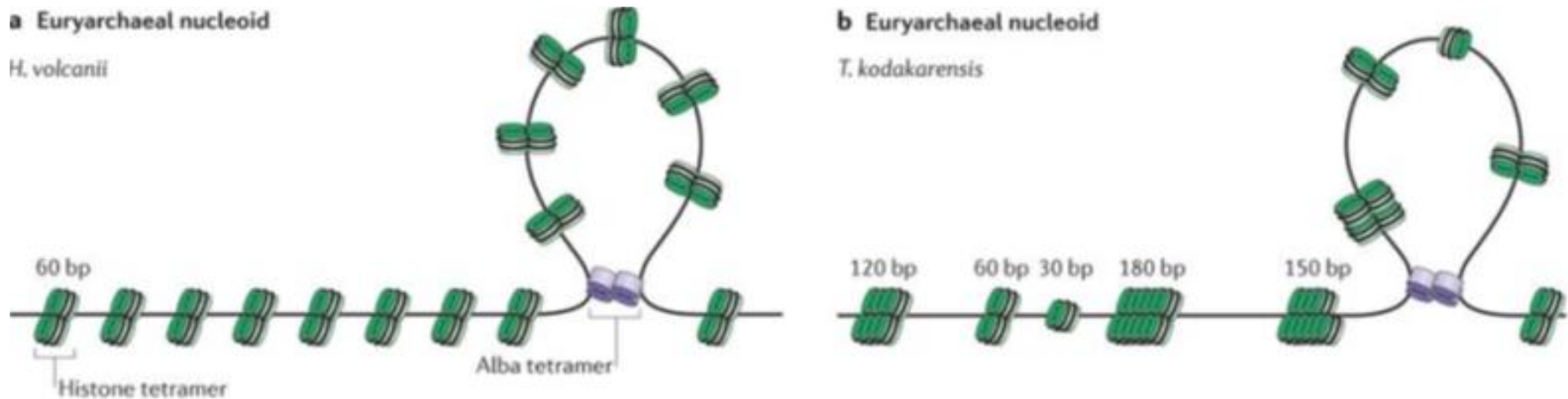
Lrp riconosce una sequenza consenso degenerata sul DNA e modifica la struttura del DNA con il suo legame.

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



Ottamero di LRP

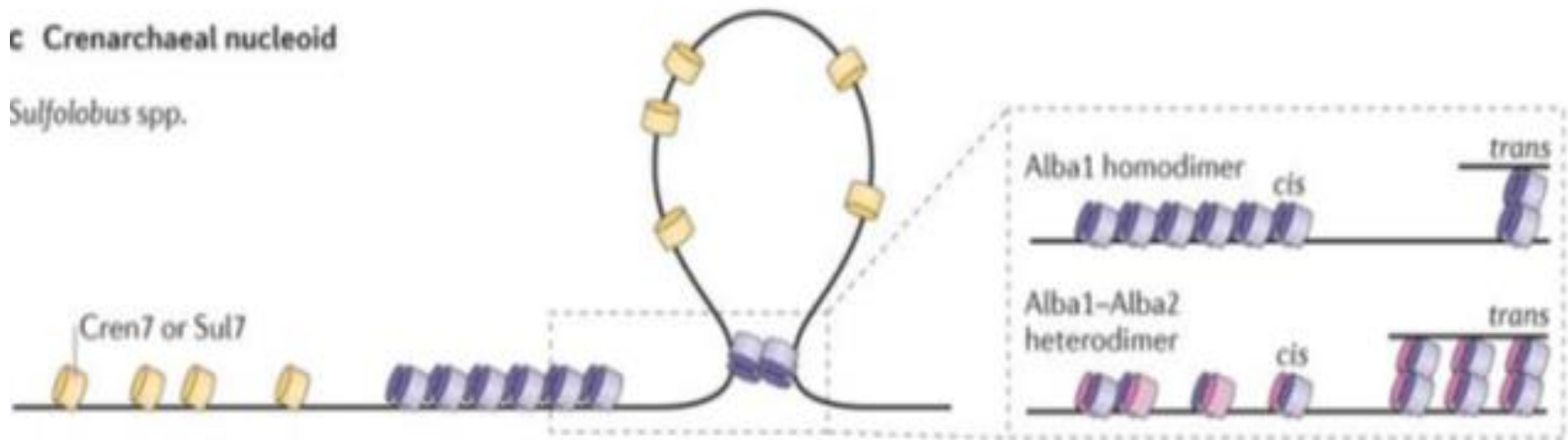
The structure of the archaeal nucleoid varies among different archaeal species depending on the chromatin proteins they express



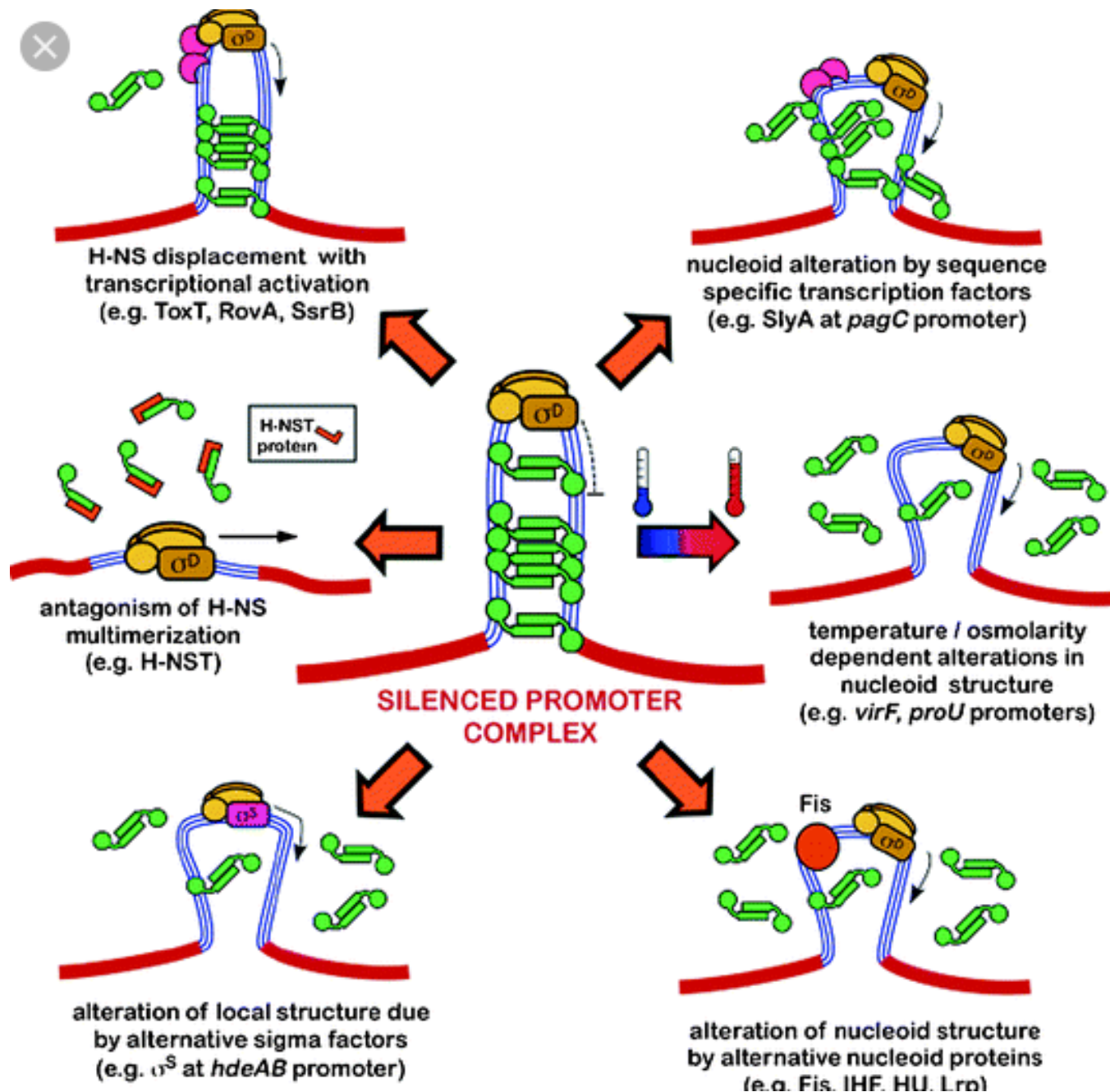
a,b | The euryarchaeal nucleoid is mainly organized by histone proteins that bend or wrap DNA, as well as by Alba that binds to DNA as a homodimer or a heterodimer and that forms looped structures by bridging two DNA duplexes. In *Haloferax volcanii*, histone proteins form tetrameric nucleoprotein structures 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 Crenarchaeal nucleoid

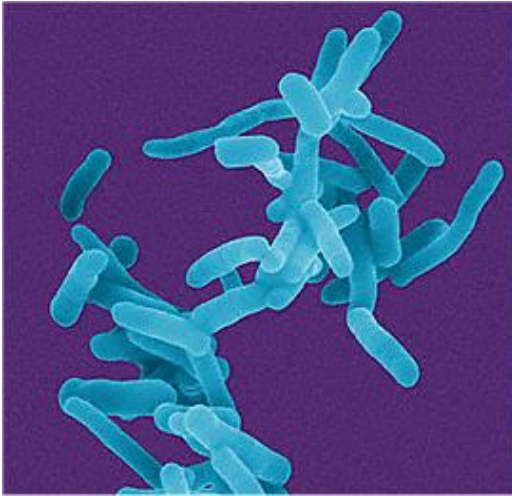
Sulfolobus spp.



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 archaea¹⁵. Alba seems to have an ancient evolutionary history and considerable functional plasticity¹⁶. 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 proteins¹⁹, 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

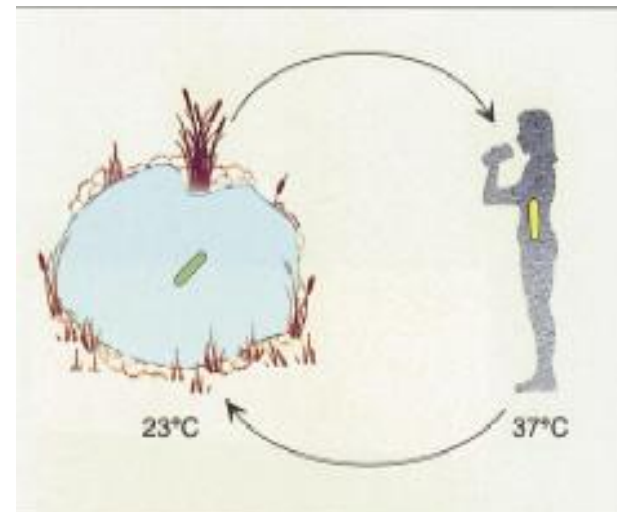


- is a Gram negative, facultative anaerobe
- is an intracellular pathogen
- is the etiological agent of bacillary dysentery, an acute diarrheal disease
- causes 160 million of episodes, determining 1.1 million deaths/year in children and infants in developing countries.

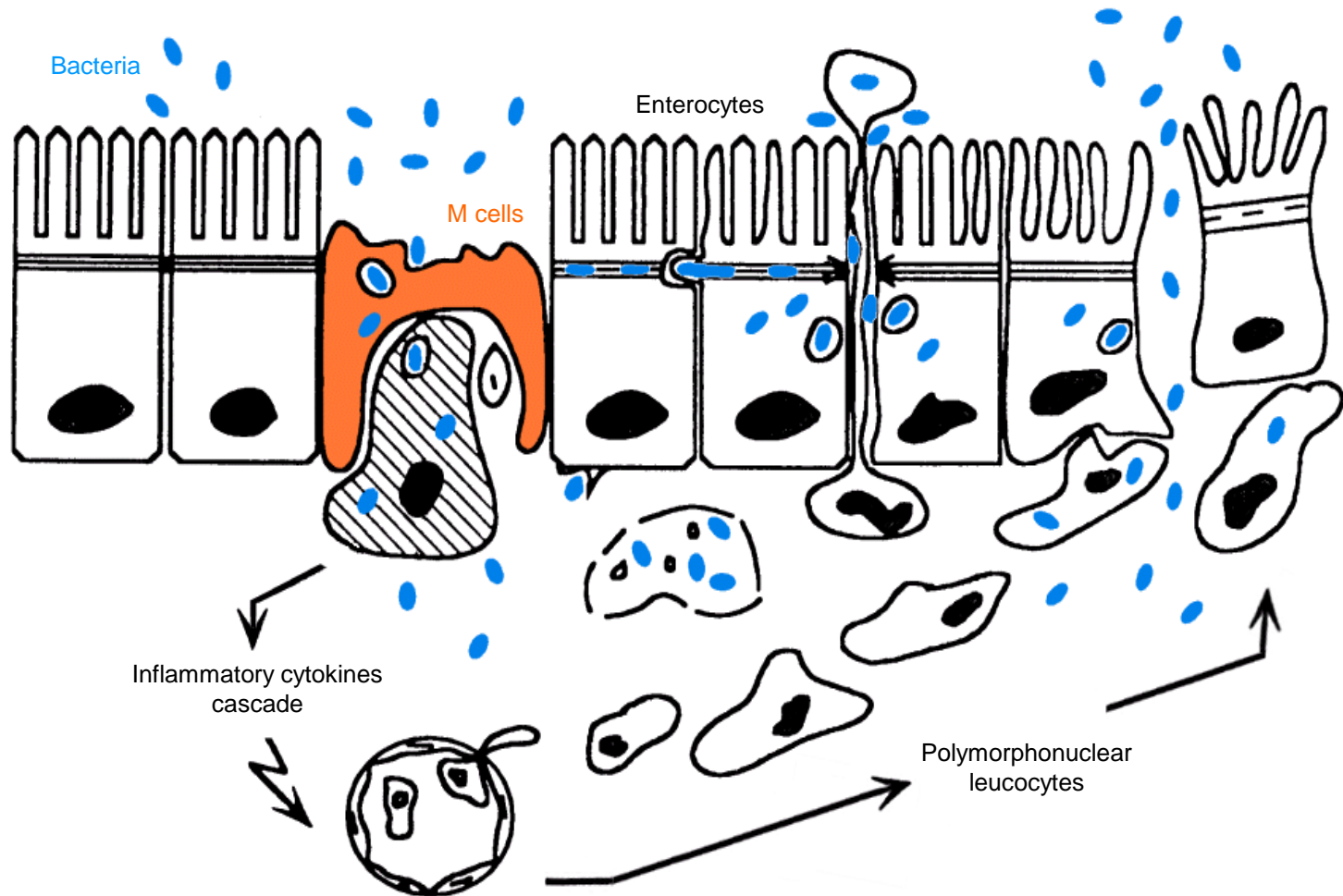
Infection is spread
via fecal-oral route

Subgrouped in four "species":

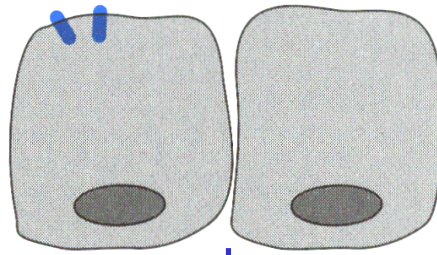
- *Shigella flexneri*
- *Shigella dysenteriae*
- *Shigella boydii*
- *Shigella sonnei*



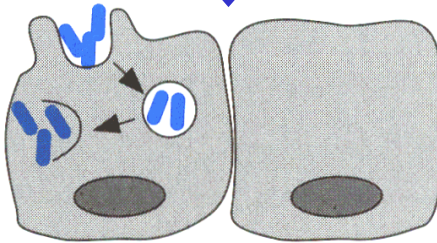
Model for *Shigella* invasion of the colonic mucosa



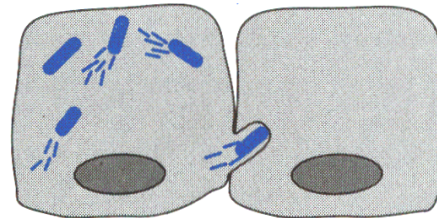
IpaD



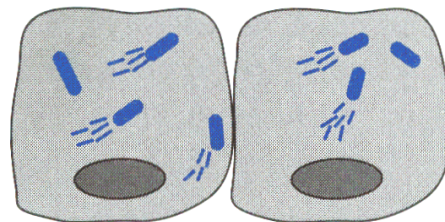
IpaB,
IpaC




IcsA
(virG)



IcsB

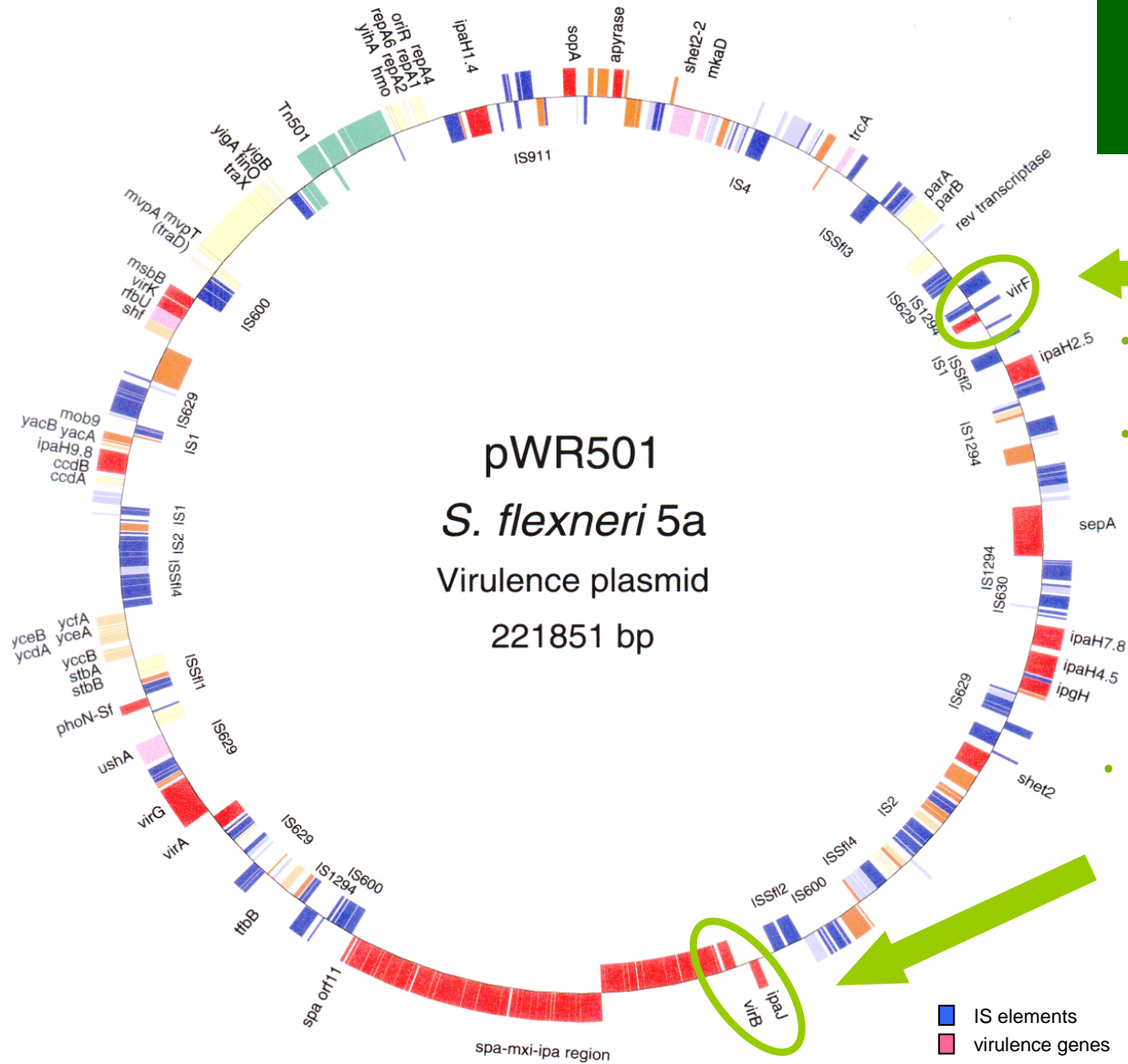


Actin
filaments 

nucleus 

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



Genetic organization of pINV



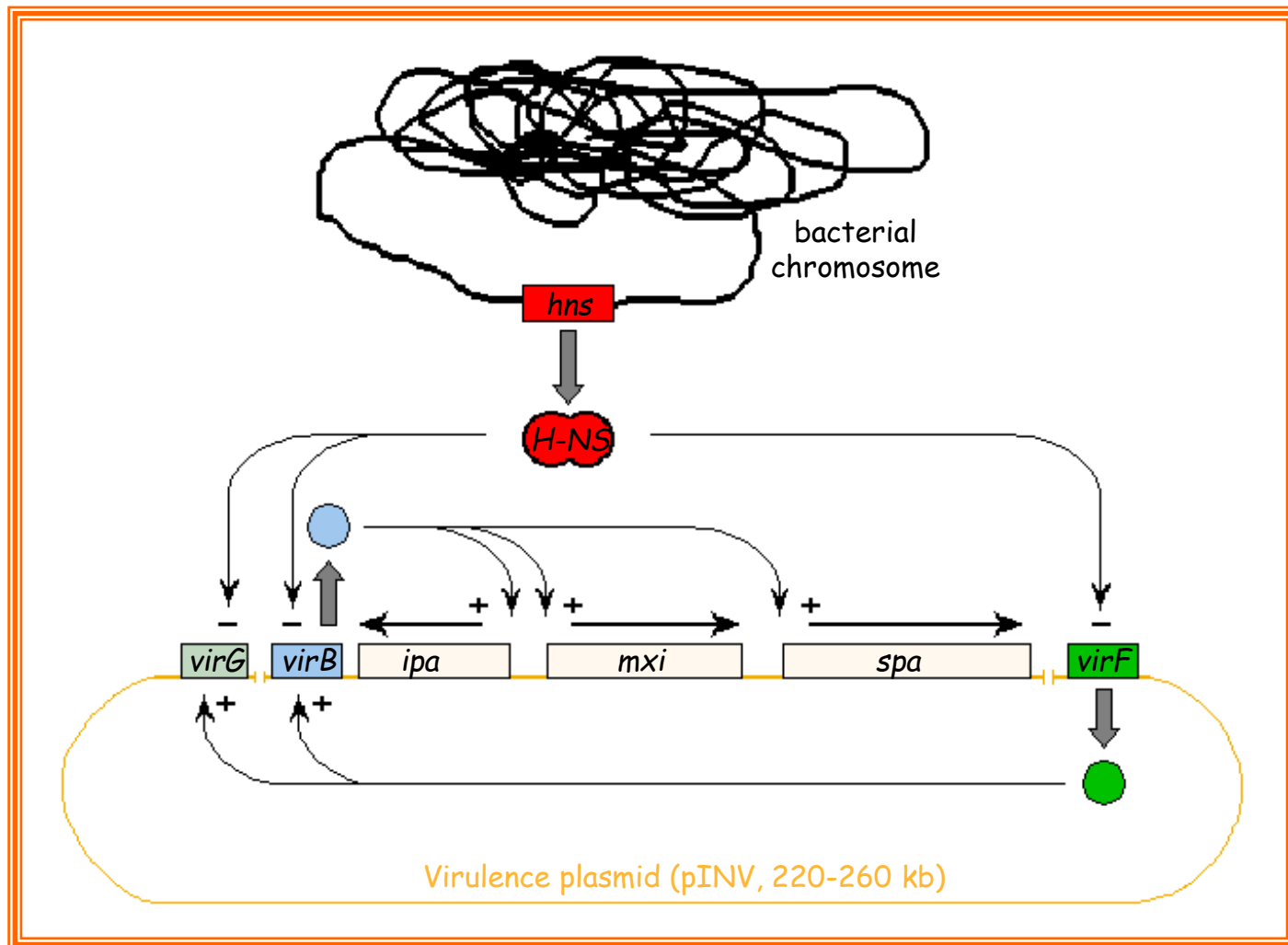
- ... located on a "desert island"
- ... the first positive activator of pINV virulence genes

virB is ...

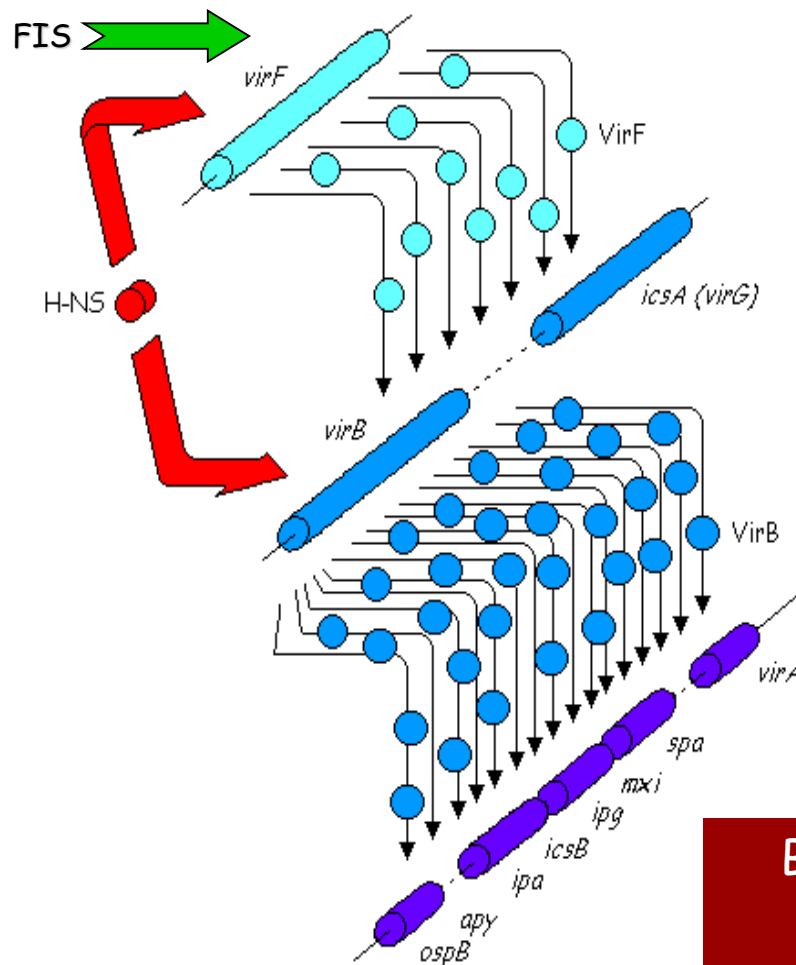
- ... located within the main Pathogenicity Island
- ... the second positive regulator of the plasmid virulence regulons

 IS elements
 virulence genes

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



The expression "cascade" of virulence genes

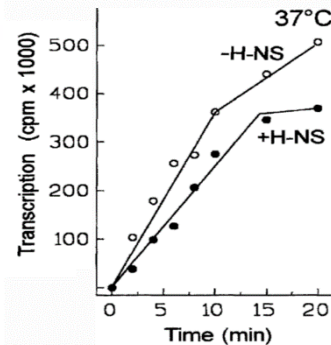
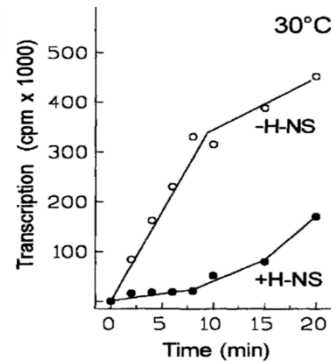
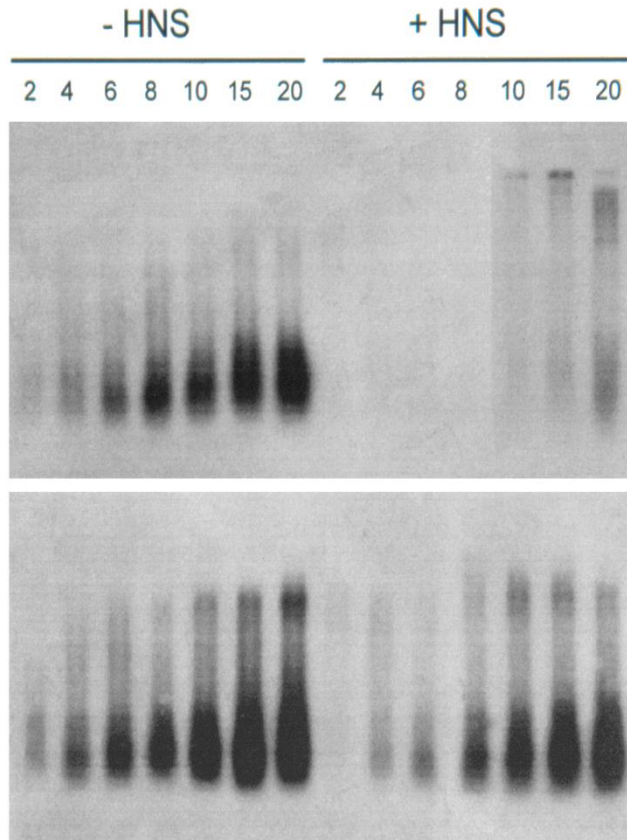


VirF is ...

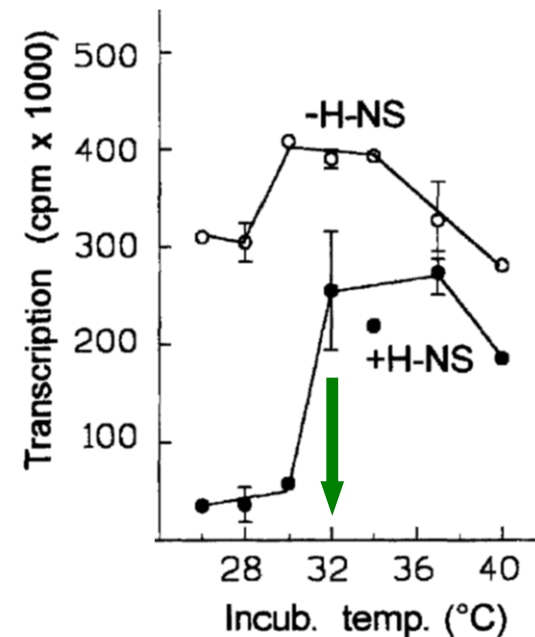
- ... expressed at 37°C
- ... is controlled antagonistically by two nucleoid proteins H-NS (repressor) and FIS (activator)

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

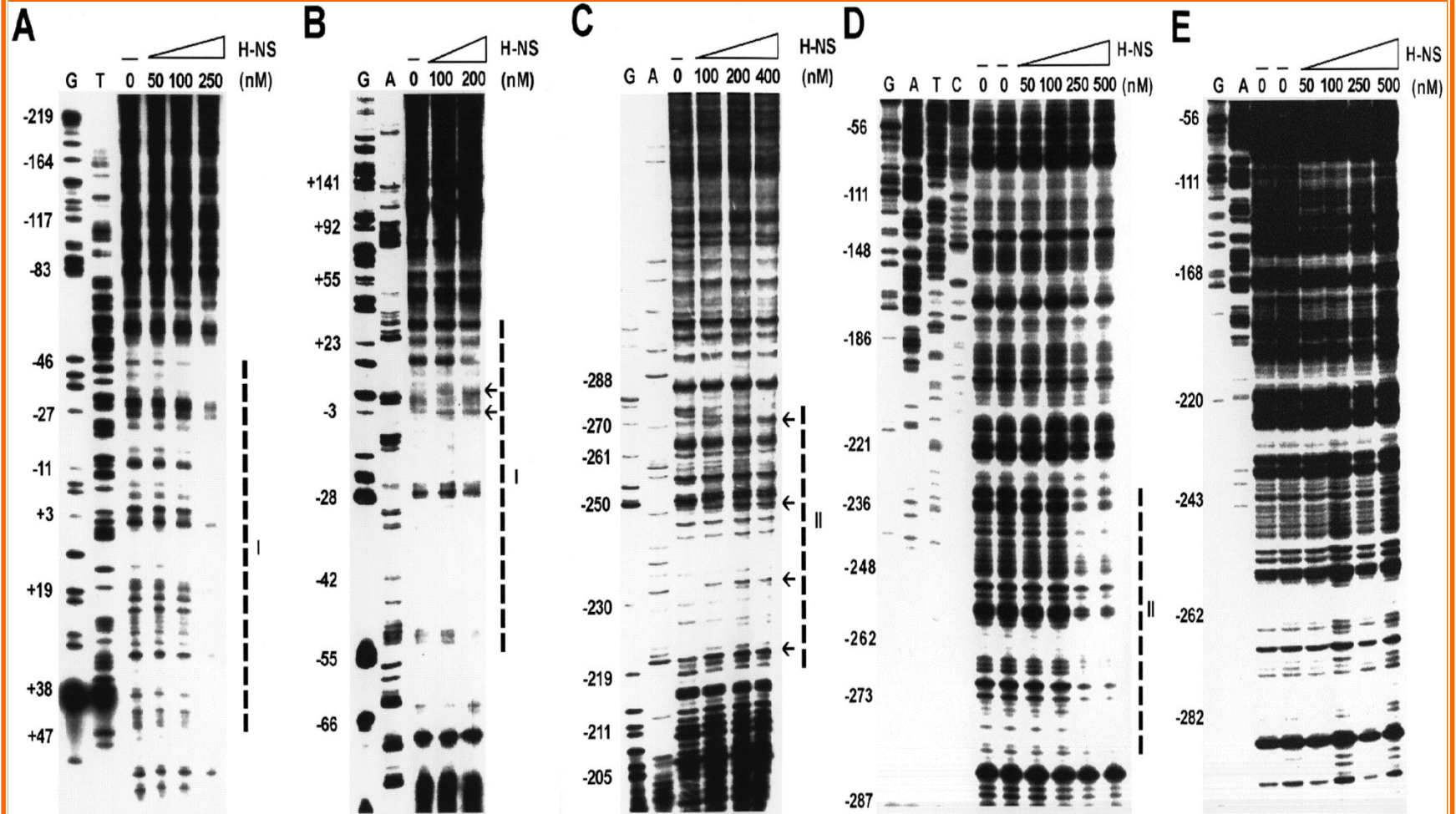
H-NS is able to bind to and repress *virF* only at low temperature



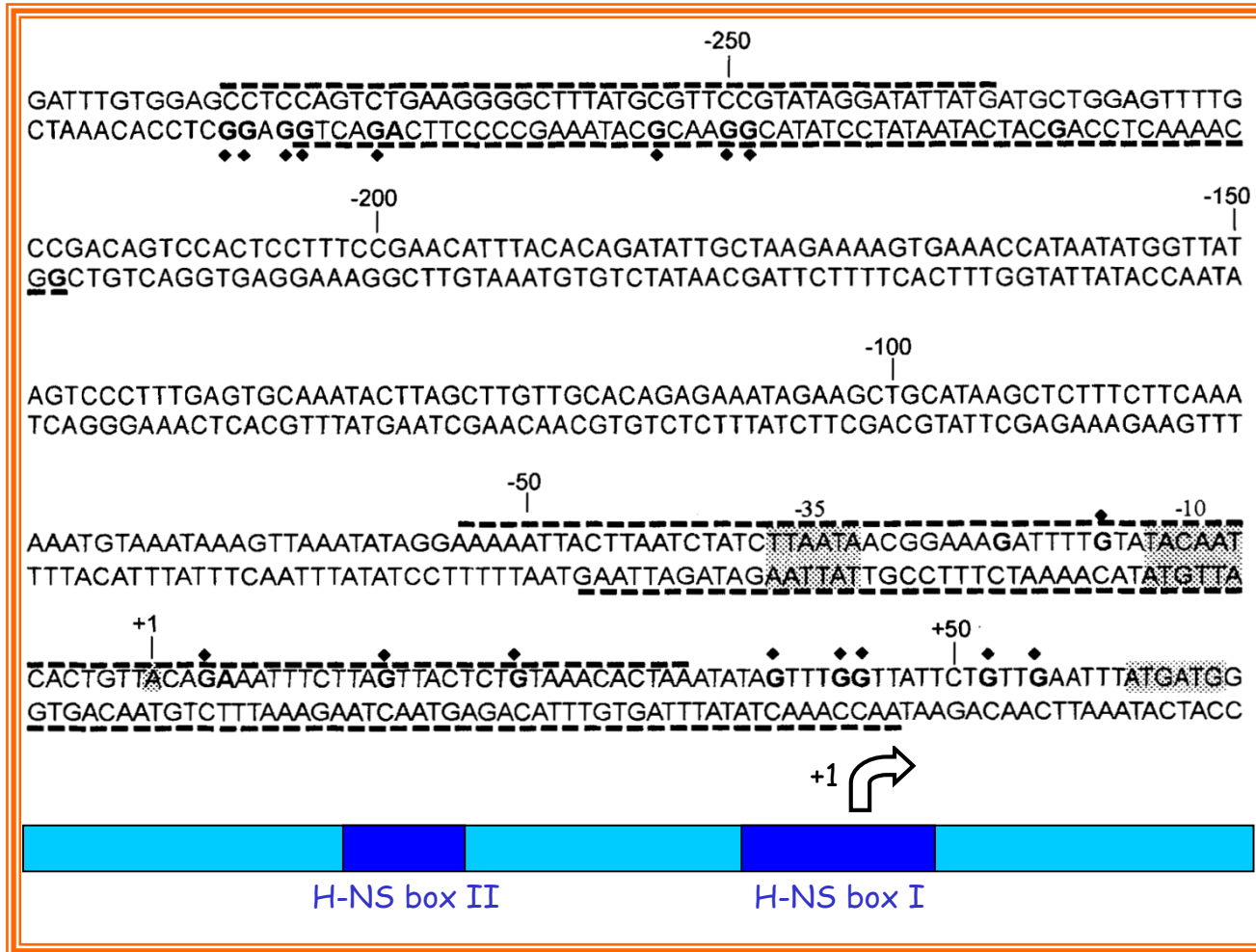
...more precisely:
virF transcription is inhibited
by H-NS only below 32°C



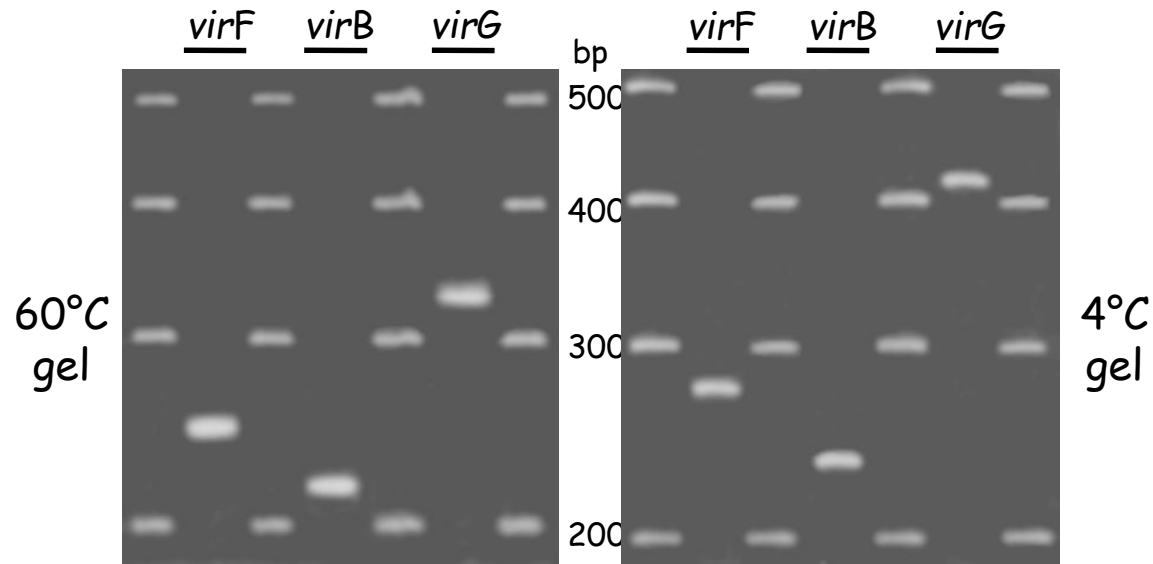
DNaseI footprinting of the *virF* promoter region by H-NS



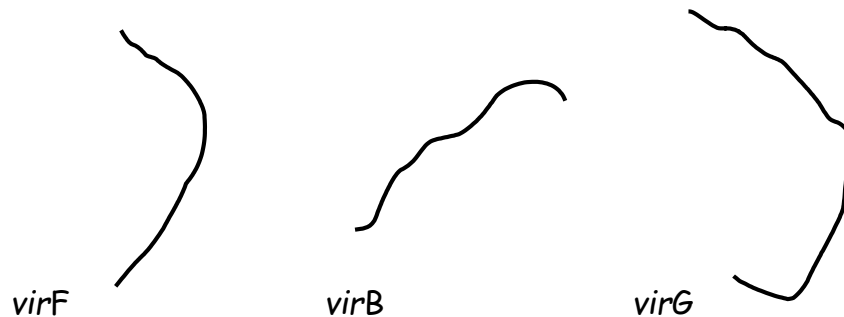
The *virF* promoter region



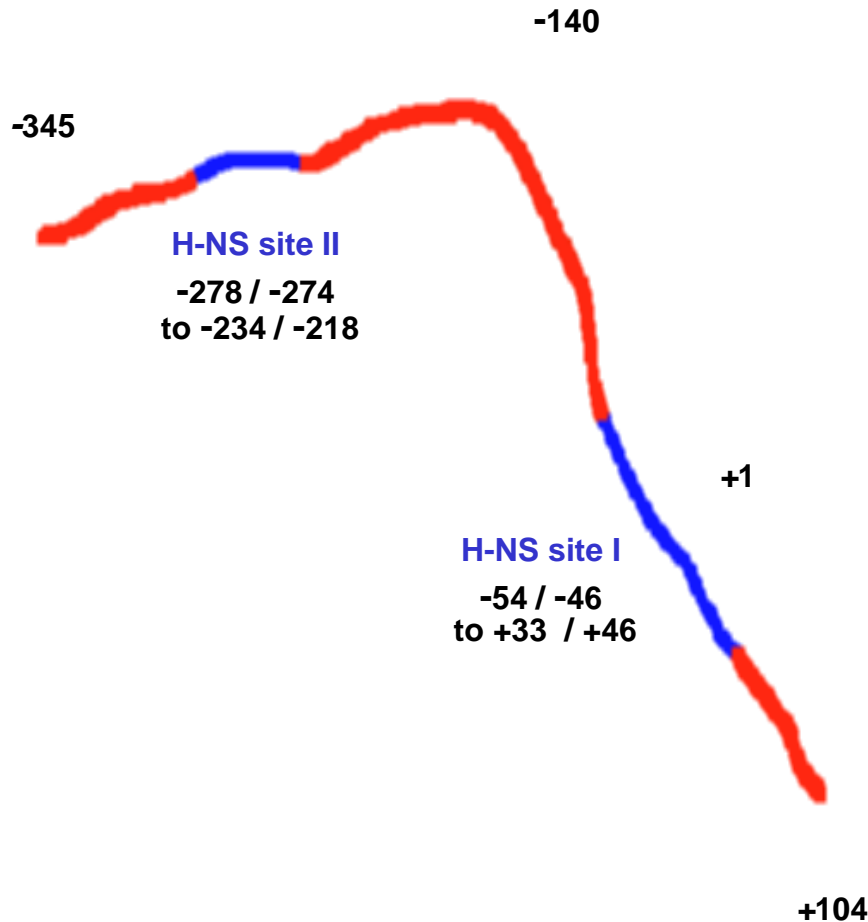
Curvature in *H*-NS regulated *vir* promoters



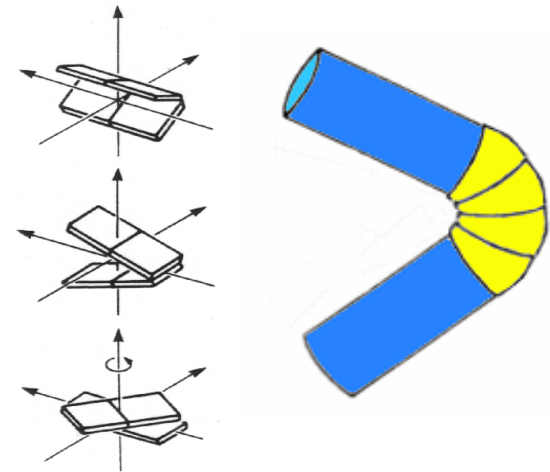
Computer-generated models



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

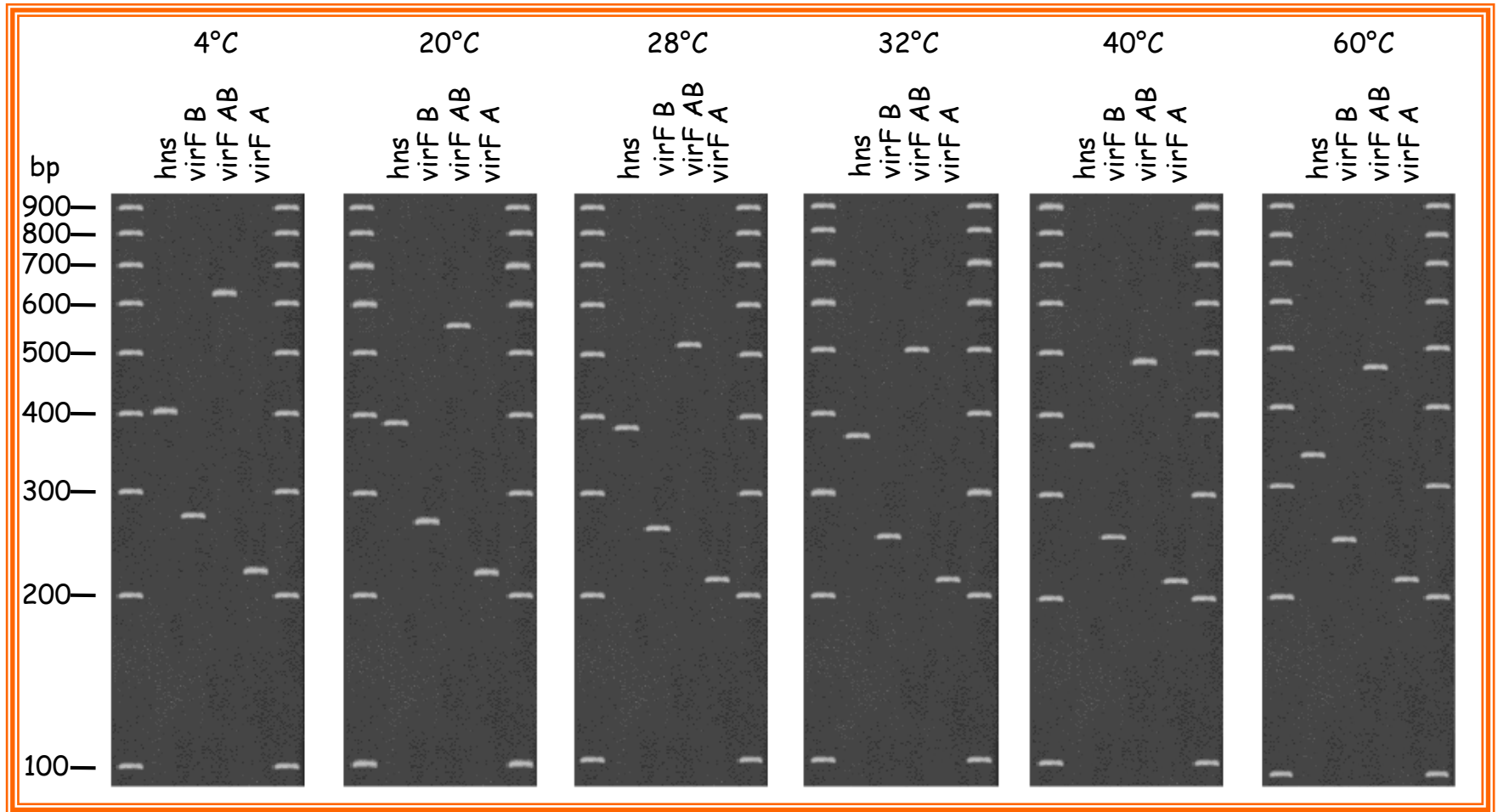


DNA bending : the **wedge model** for sequence mediated curvature

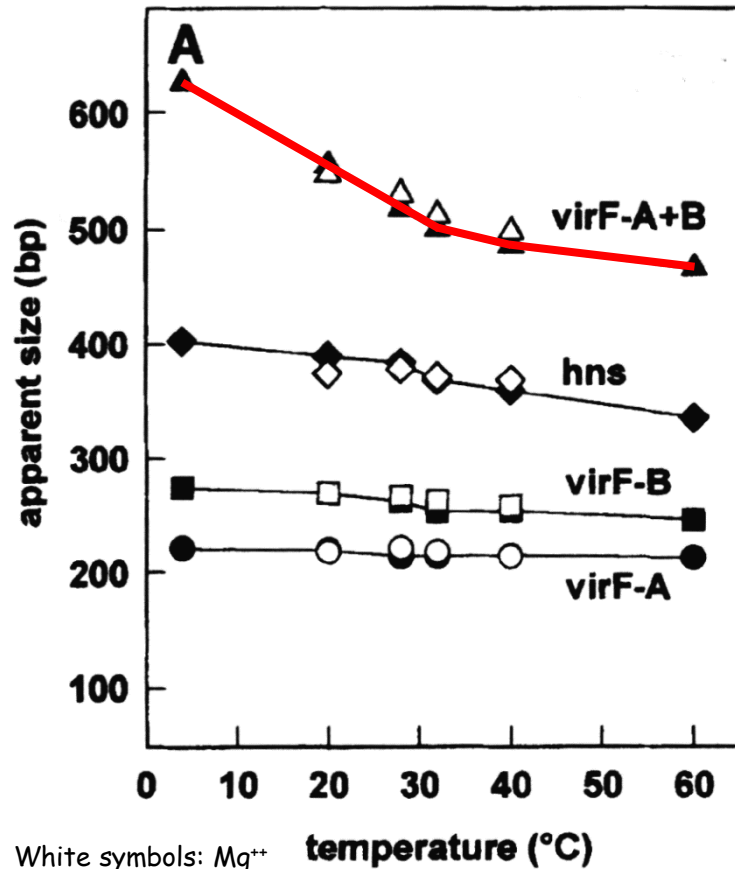


Temperature-dependent curvature of the *virF* promoter

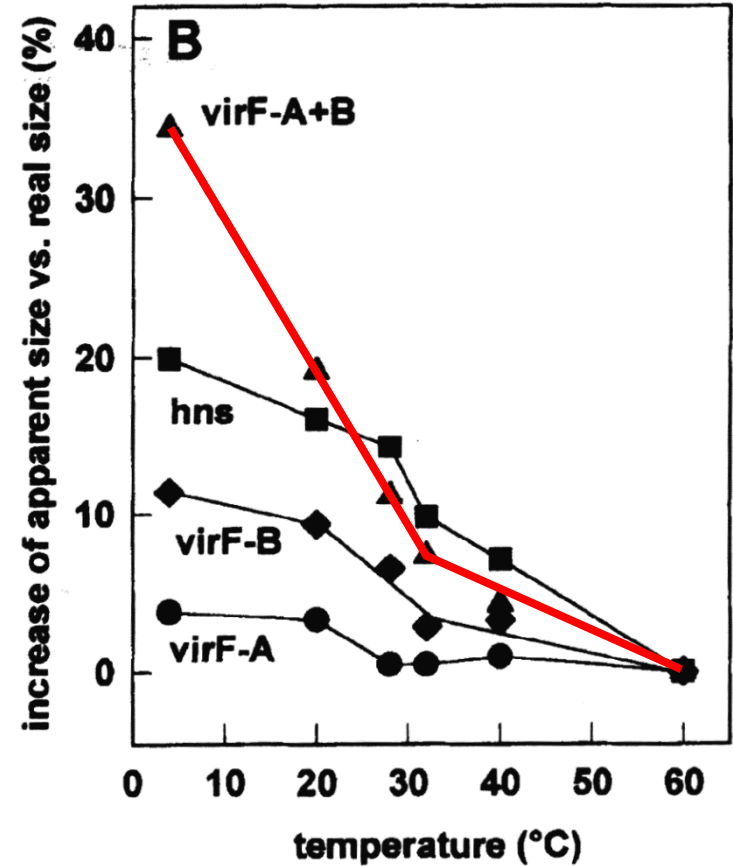
Acrilamide separations



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

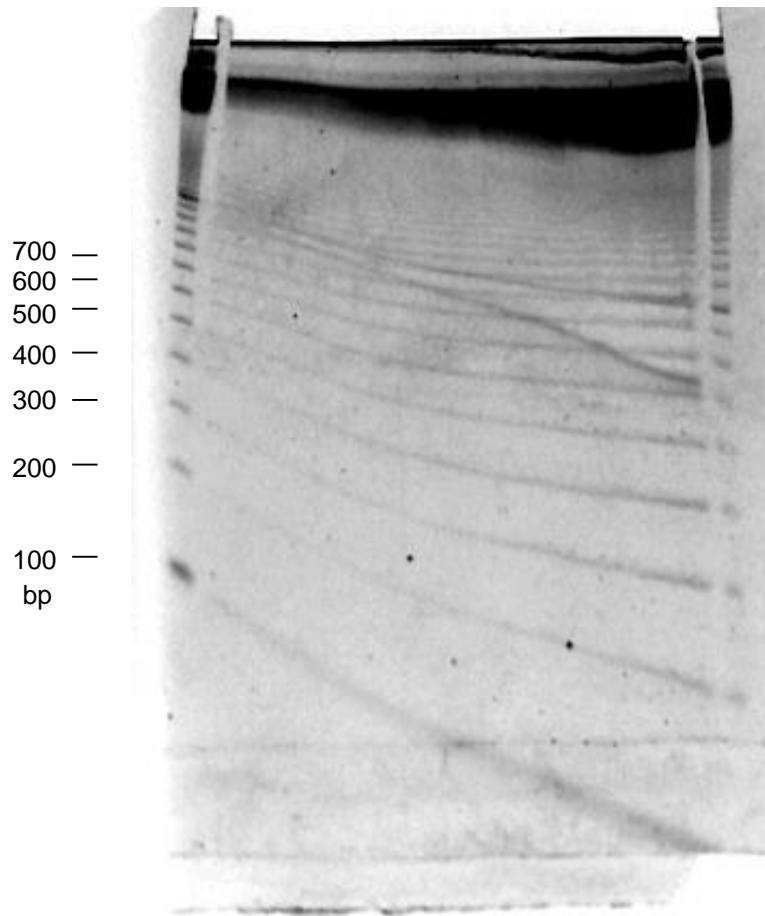


White symbols: Mg^{++}
Black symbols: no Mg^{++}



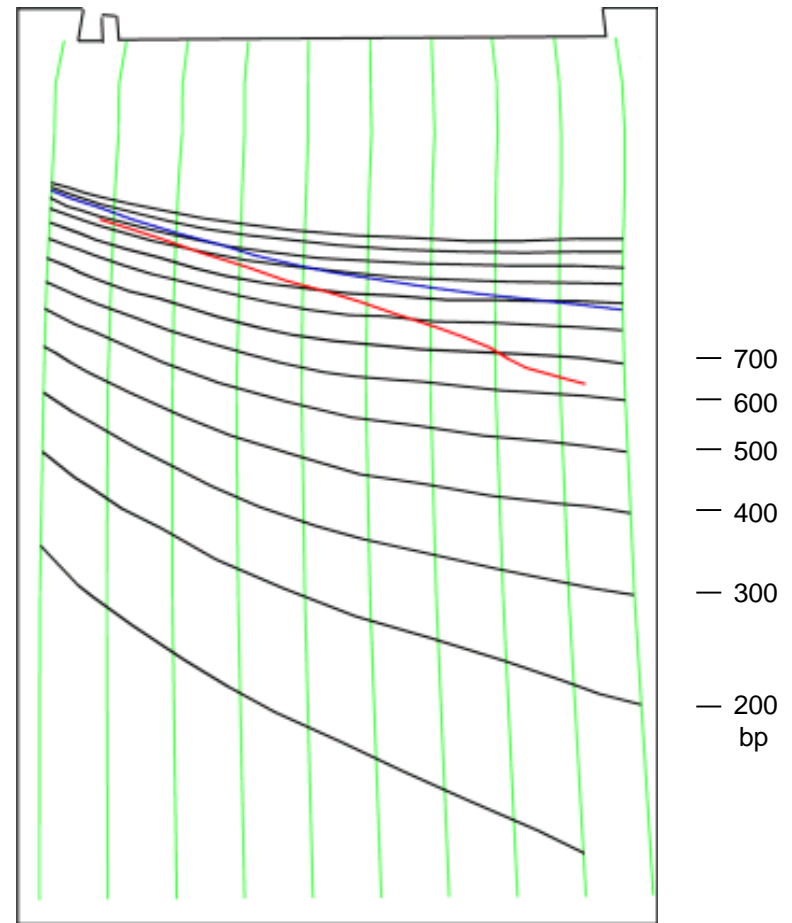
Temperature-dependent curvature of the *virF* promoter

TGGE separation



17°C

39°C



17°C

20°C

22.5°C

24.5°C

27°C

29.5°C

31°C

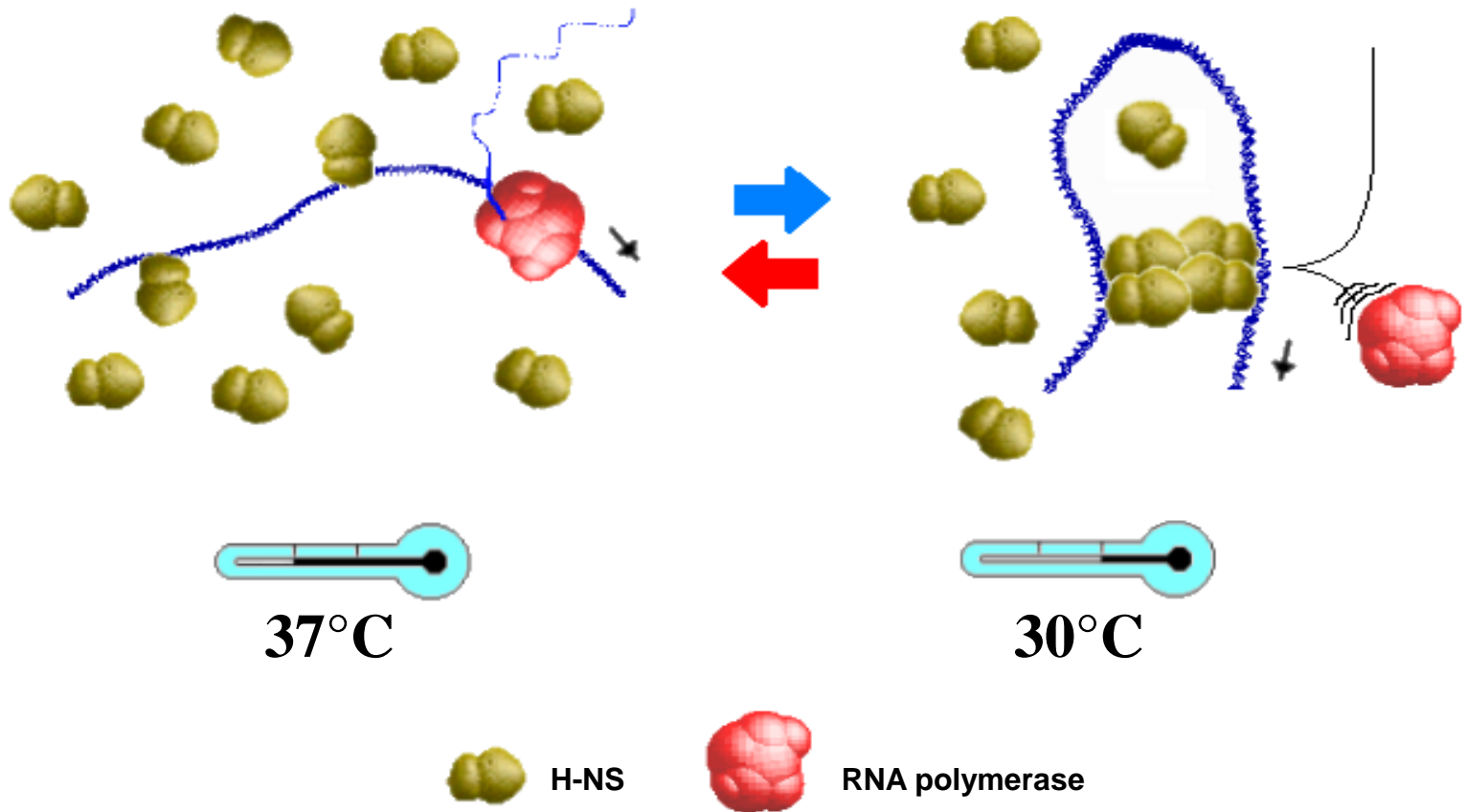
33.5°C

36°C

39°C

Temperature-dependent *virF* expression

Working model



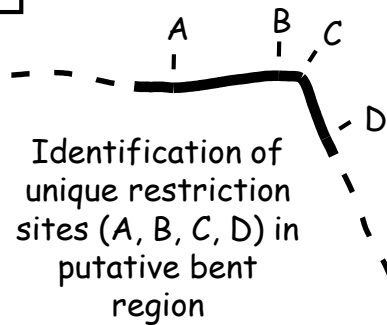
By which mechanism is the *virF* gene
allowed to be expressed
only at the host temperature?

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

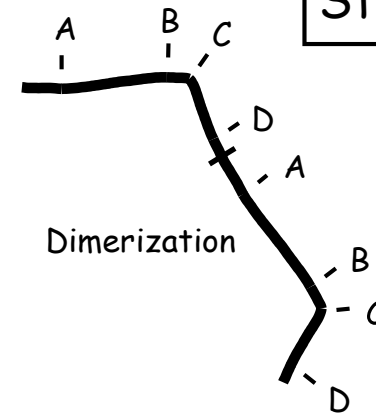
The circular permutation assay

Rationale

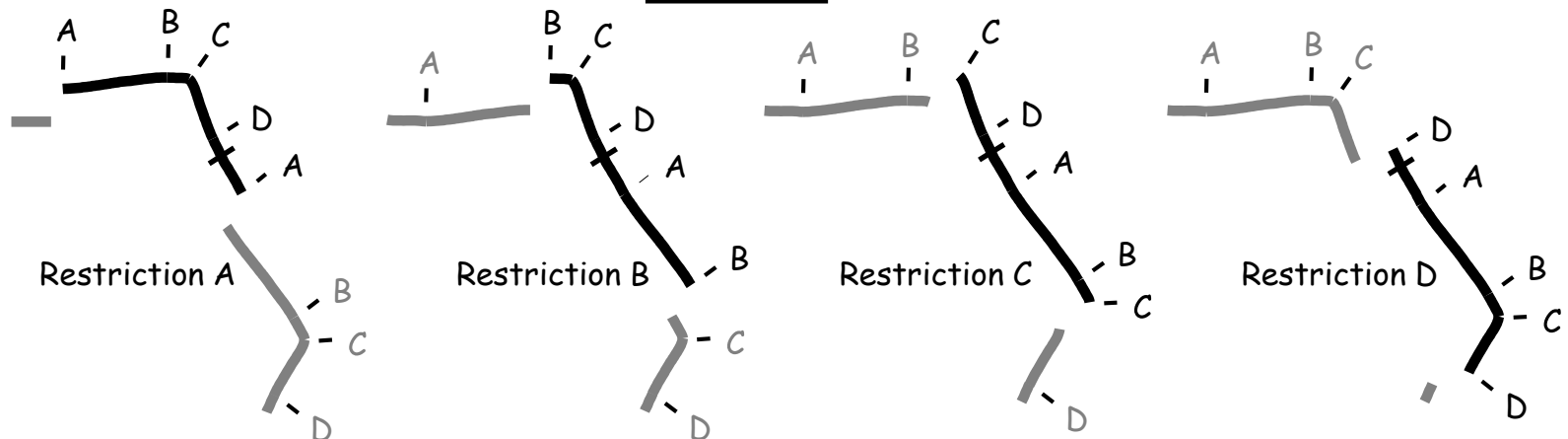
Step 1

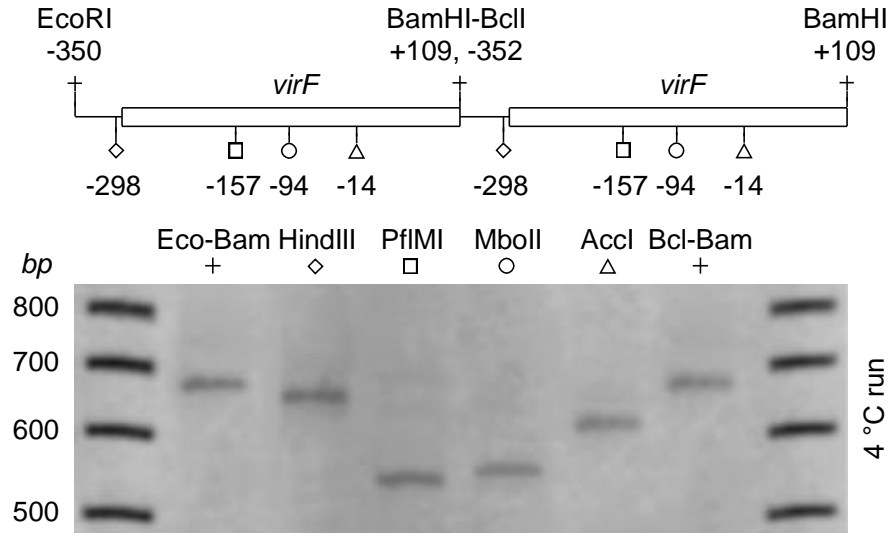


Step 2

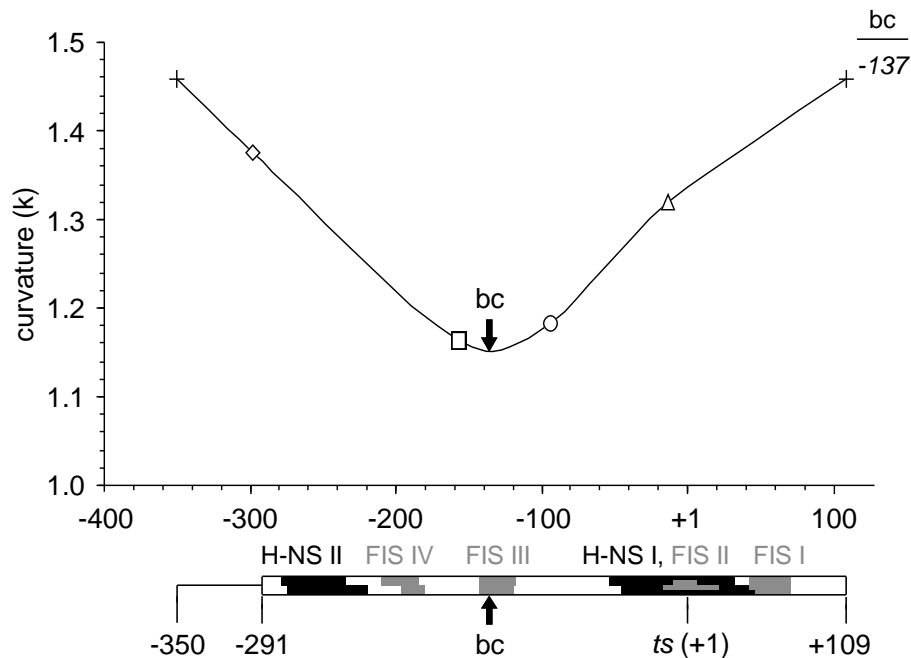


Step 3



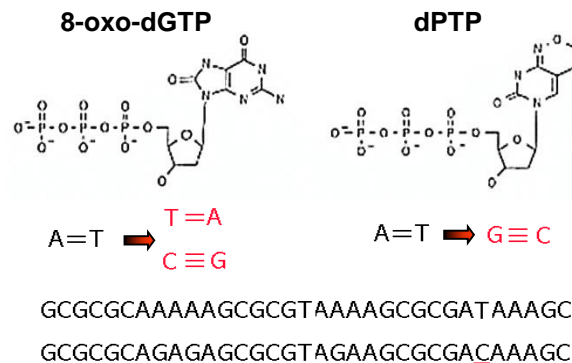
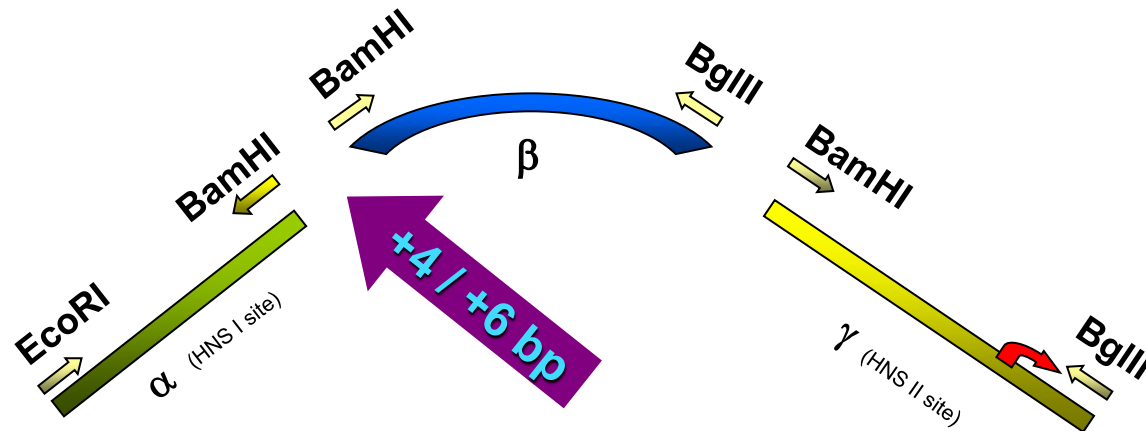


Circular
permutation assay
on the *virF*
promoter region



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

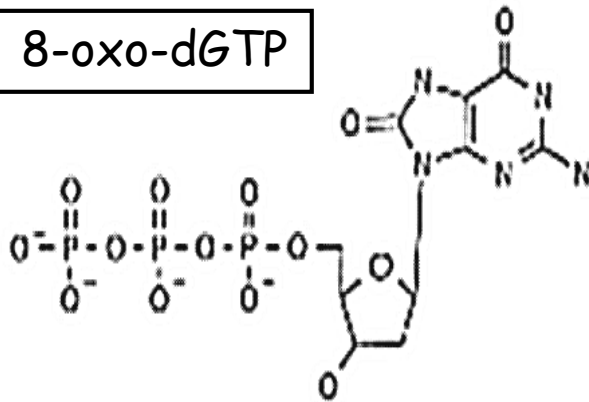
Molecular dissection of the *virF* promoter: mutagenesis of the β -region and shifting the β -region by $\sim\frac{1}{2}$ helix turn



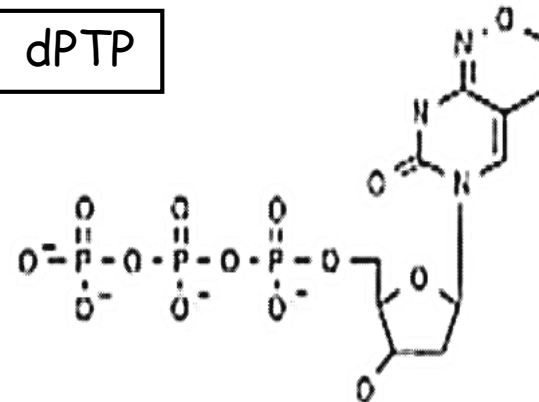
Mutagenesis of the bent region

Mutagens used

8-oxo-dGTP



dPTP



A=T \Rightarrow $\begin{matrix} T \equiv A \\ C \equiv G \end{matrix}$

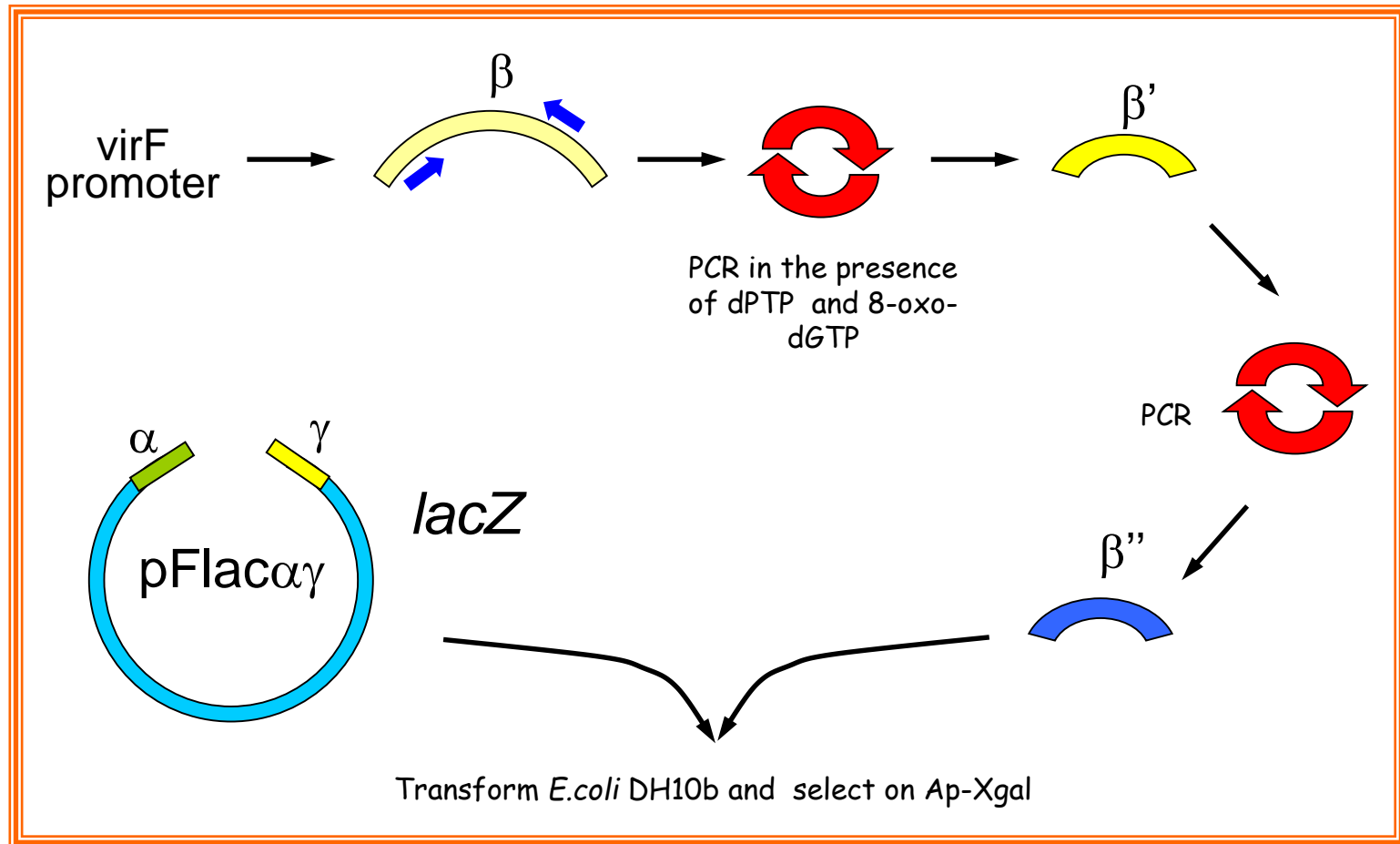
A=T \Rightarrow G \equiv C

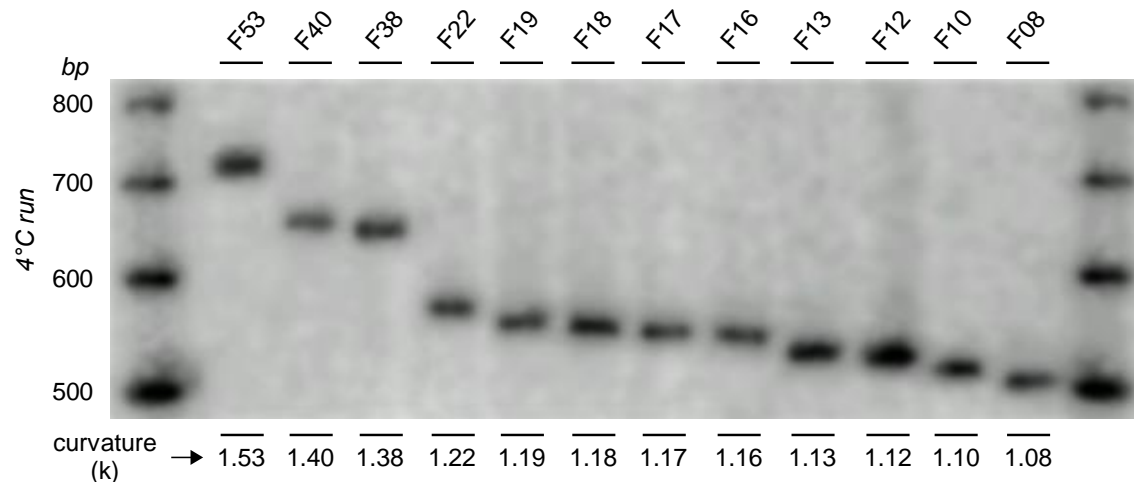
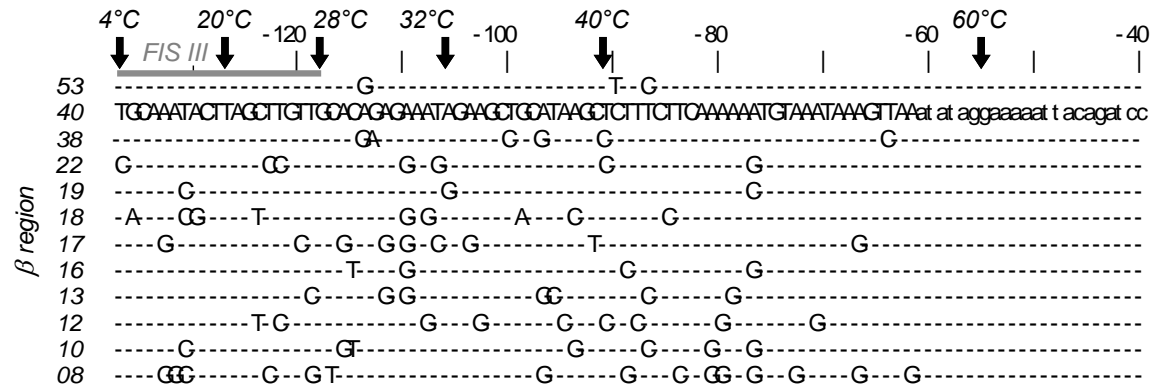
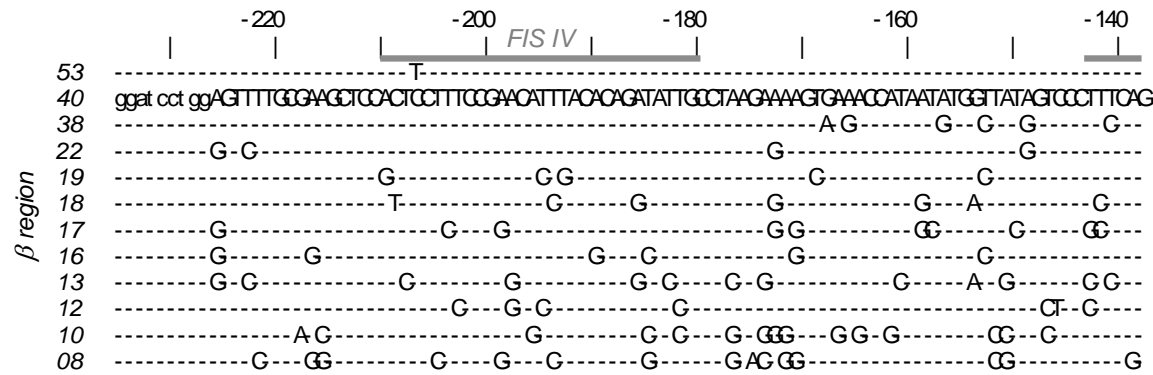
GCGCGCAAAAAGCGCGTAAAAGCGCGATAAAGC

GCGCGCAGAGAGCGCGTAGAAGCGCGACAAAGC

Mutagenesis of the bent region

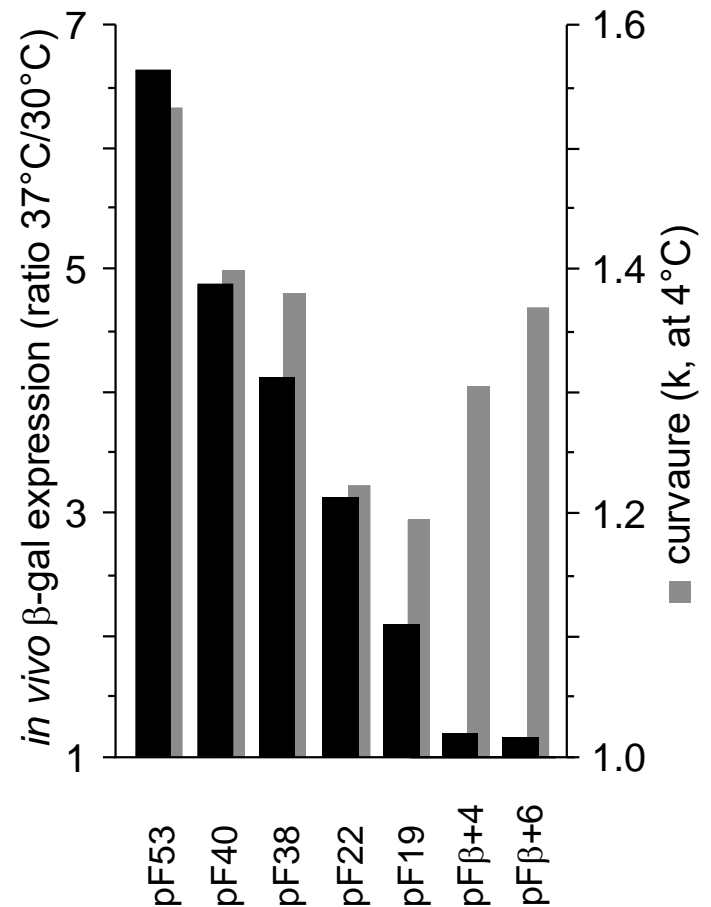
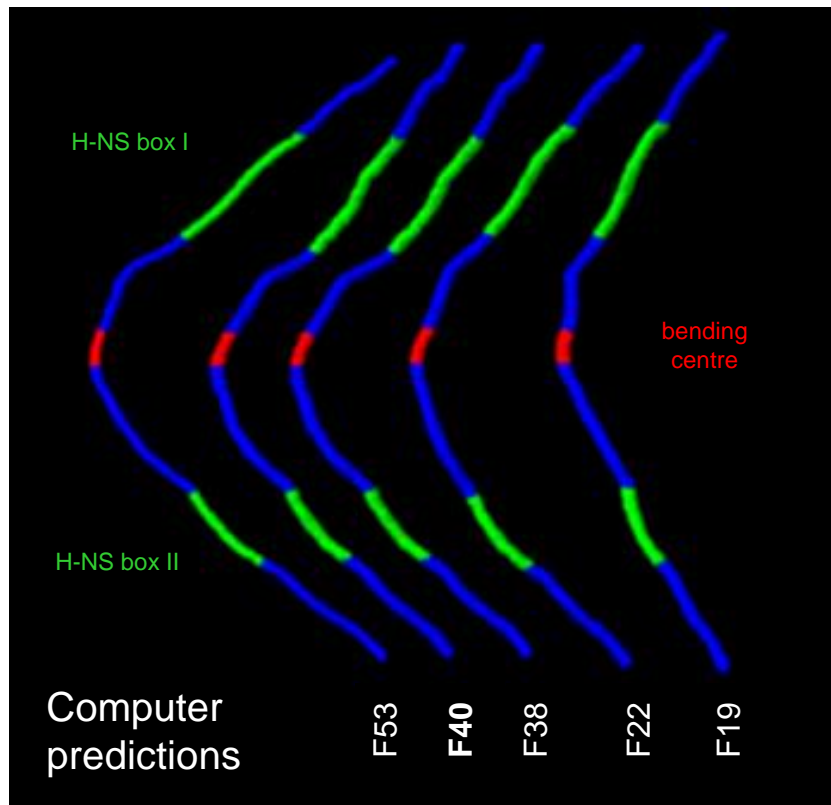
Strategy for the construction of β -region mutants





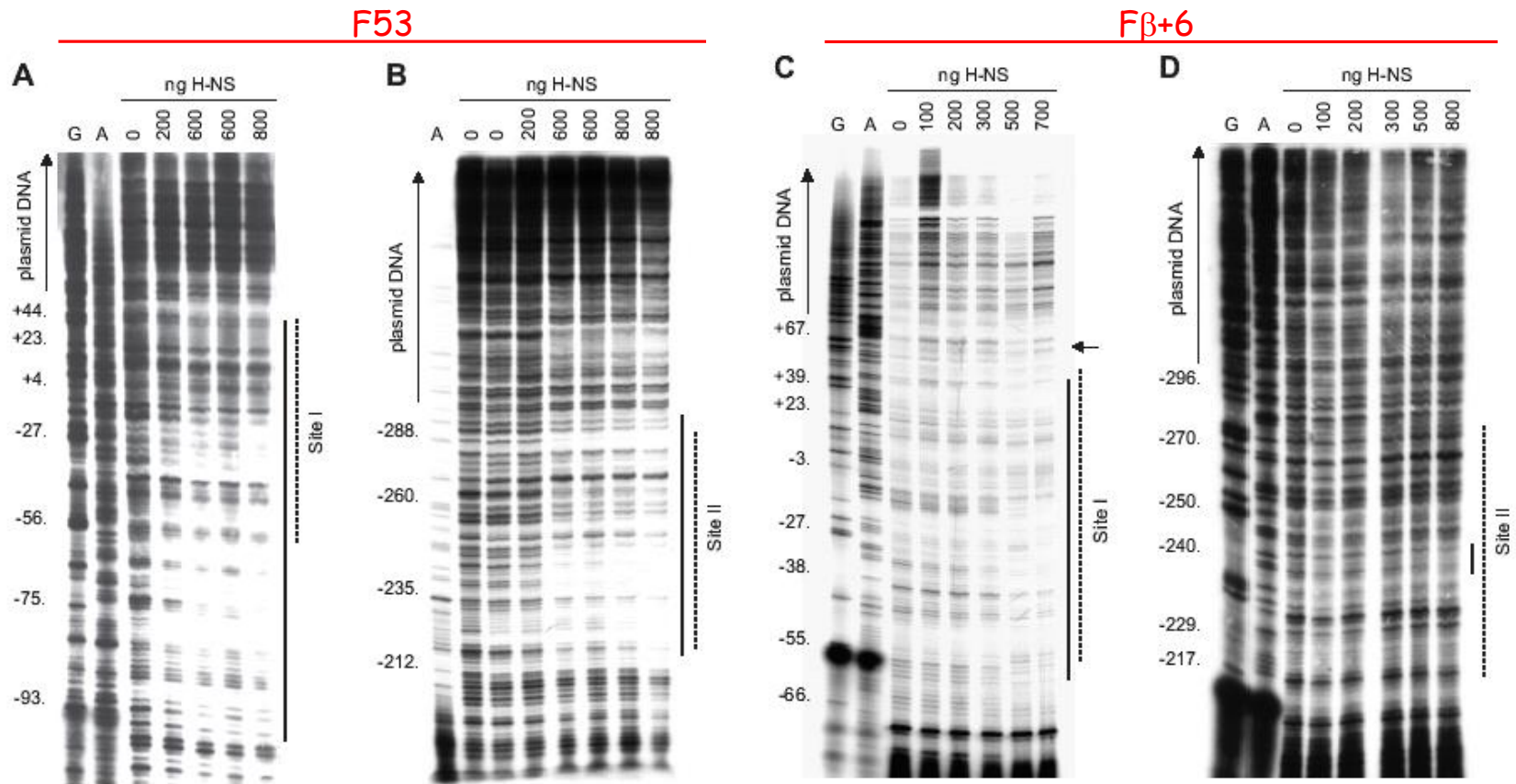
Acrilamide
separation
(4°C) of
 β -region
mutants

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

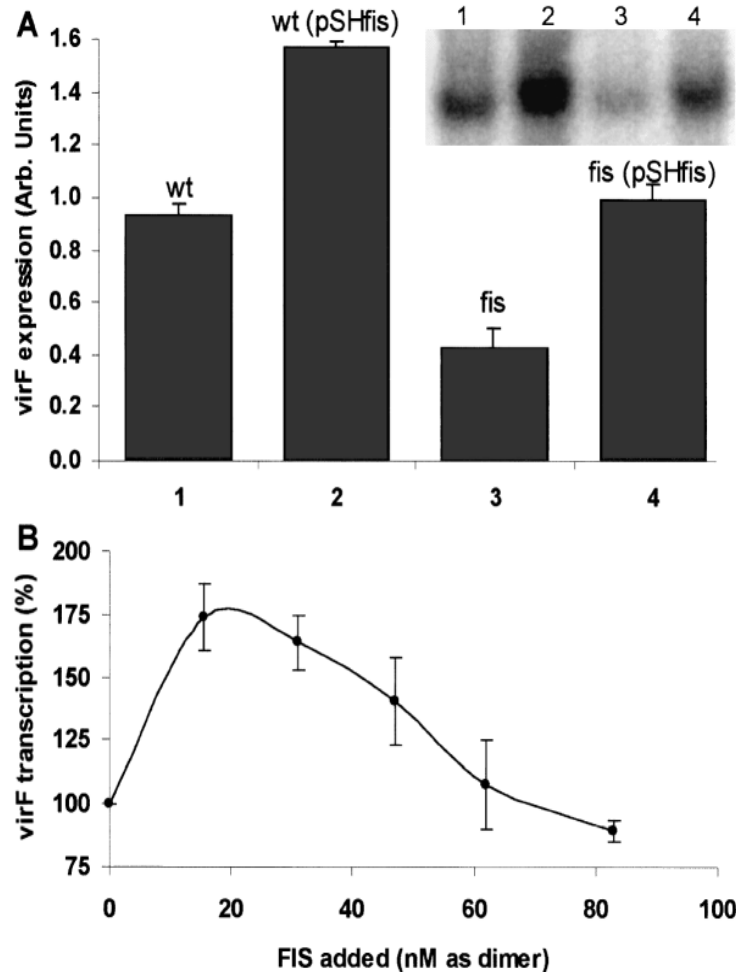


DNaseI footprints reveal that H-NS:

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



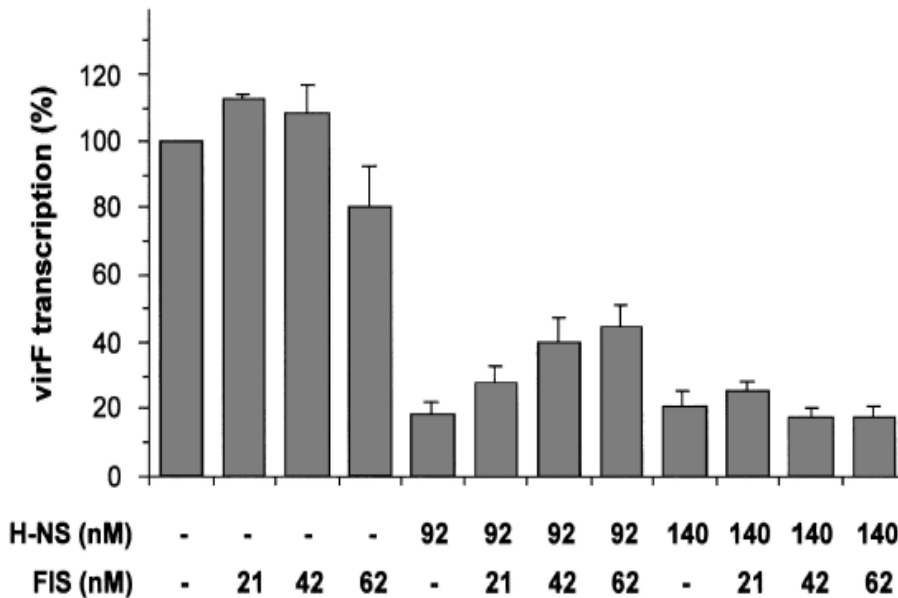
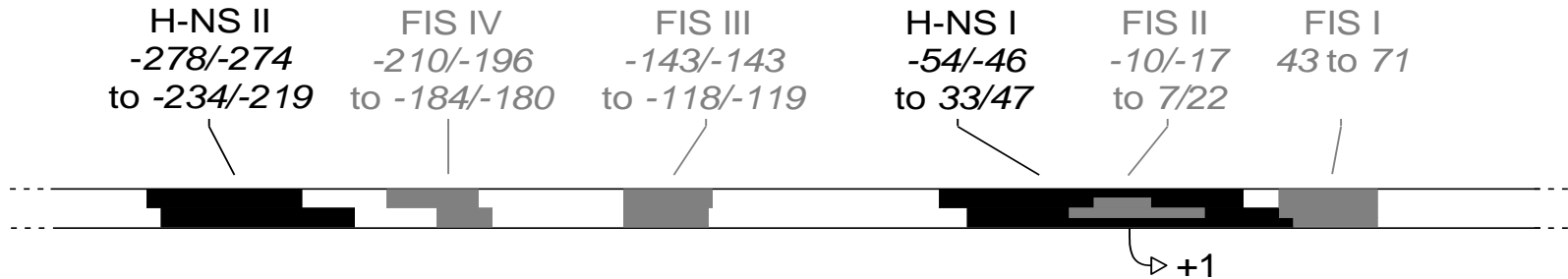
The *virF* gene is positively regulated by FIS, another nucleoid protein



FIS (Factor for Inversion Stimulation)

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

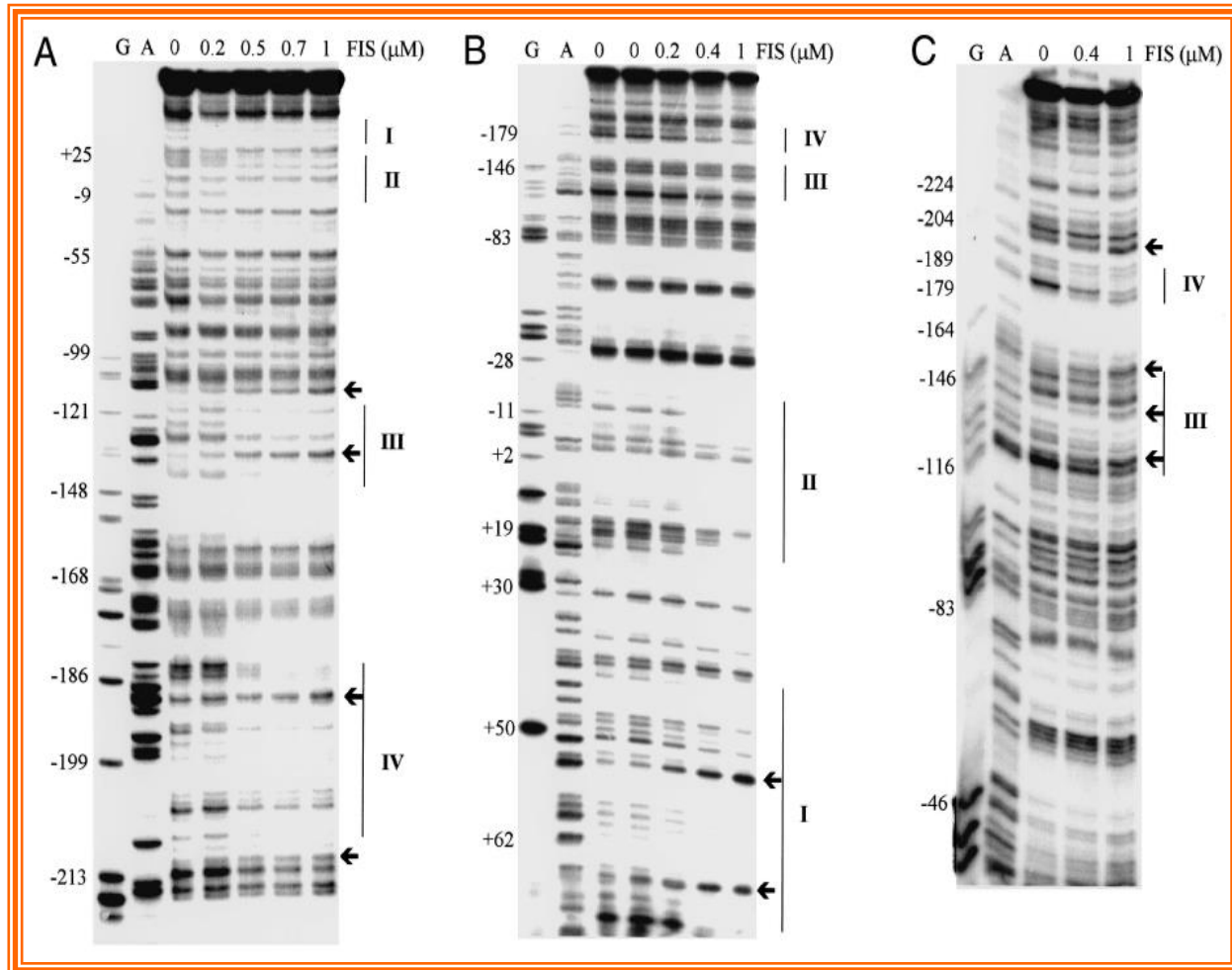
FIS has four binding sites within the *virF* promoter ...



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

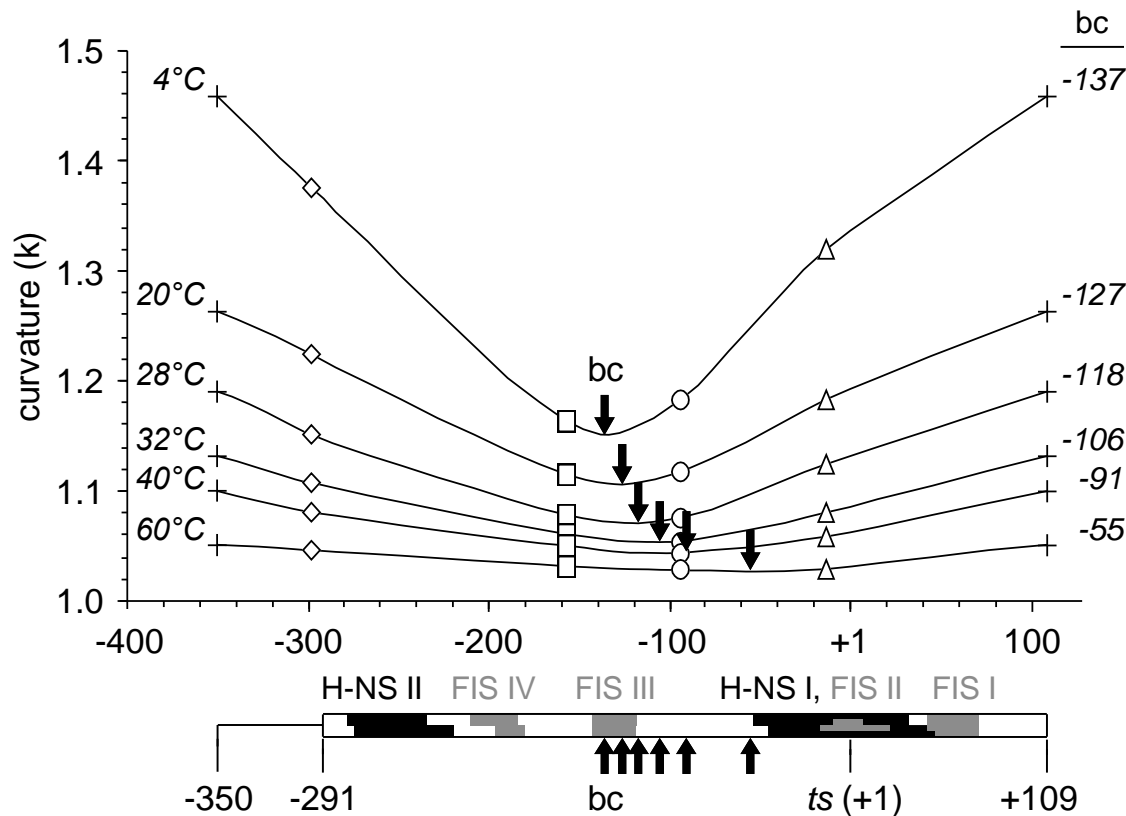
... and alleviates H-NS-mediated repression of the *virF* promoter at 31°C

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



Circular permutation assay on the *virF* promoter region

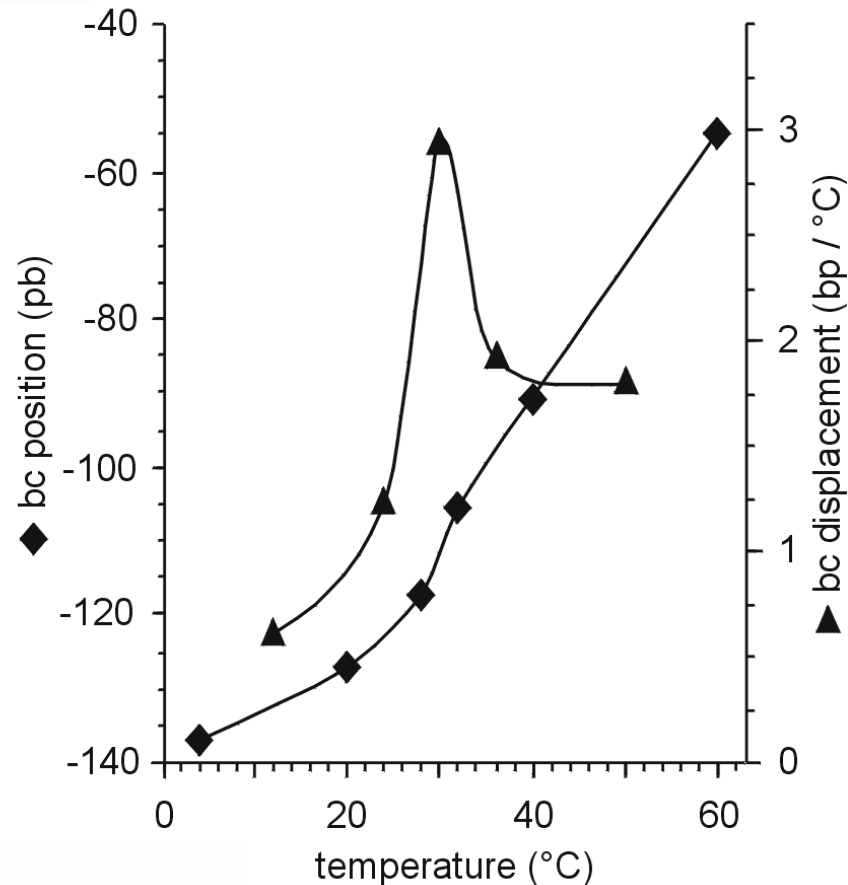
Effect of temperature



Curvature is reduced as temperature increases

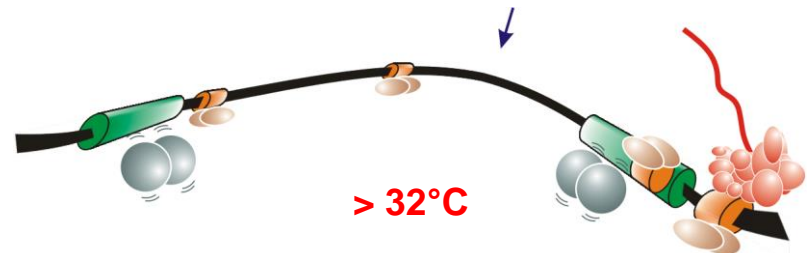
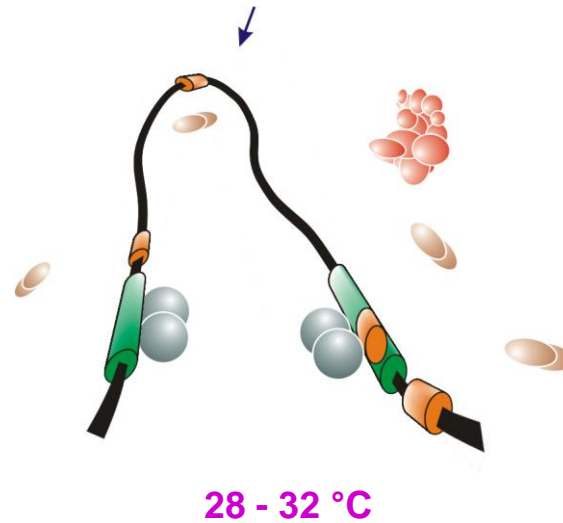
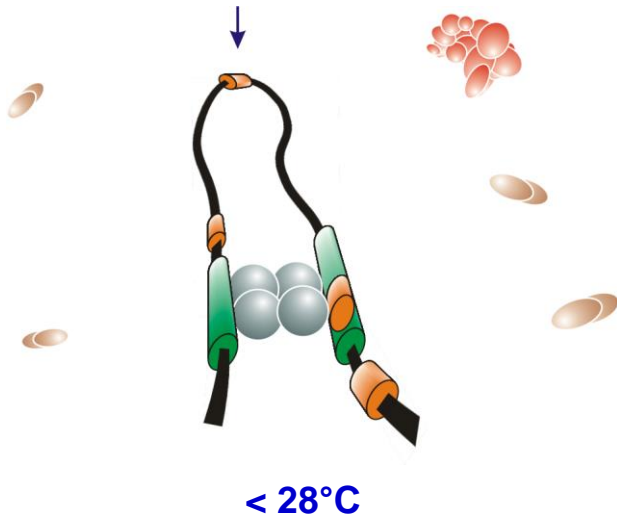
Do temperature changes alter the position of the bending centre?

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



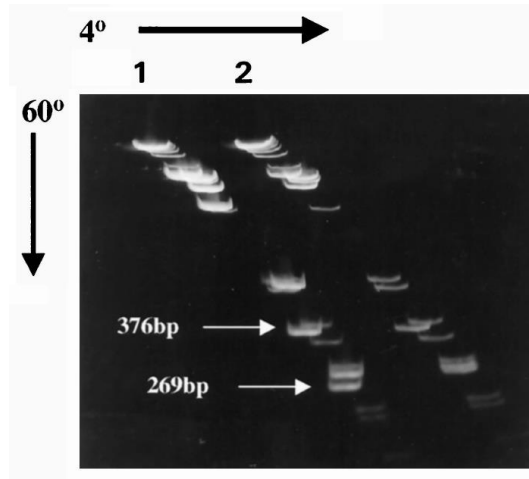
- The displacement of the bending centre is not a linear function of temperature.
- The maximum displacement occurs between 28°C and 32°C.

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



- 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?

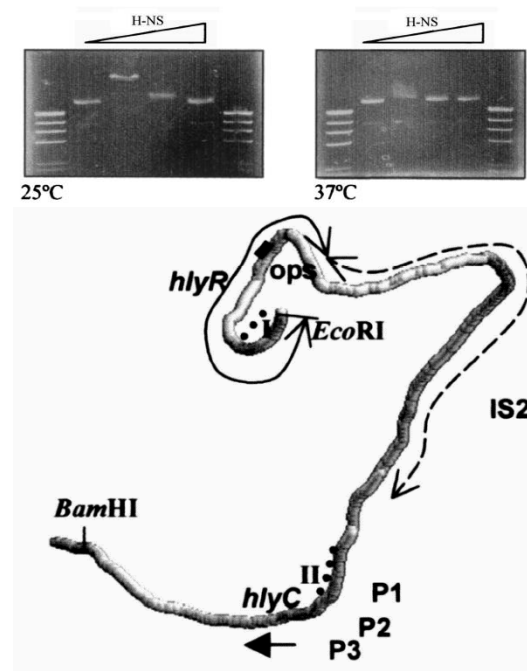


The *Yersinia enterocolitica* virulence plasmid contains DNA bends which melt at 37°C

(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?

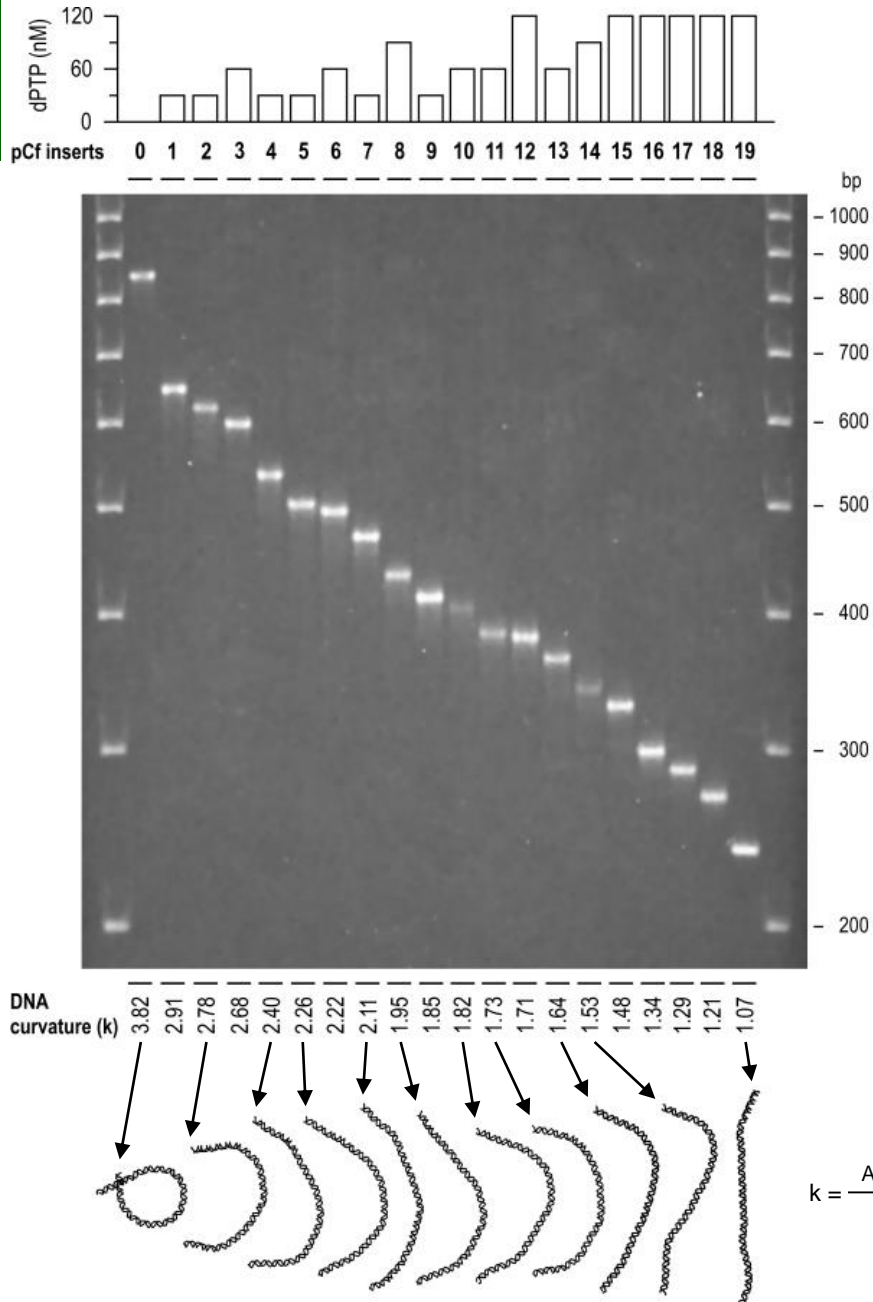
The experimental approach

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

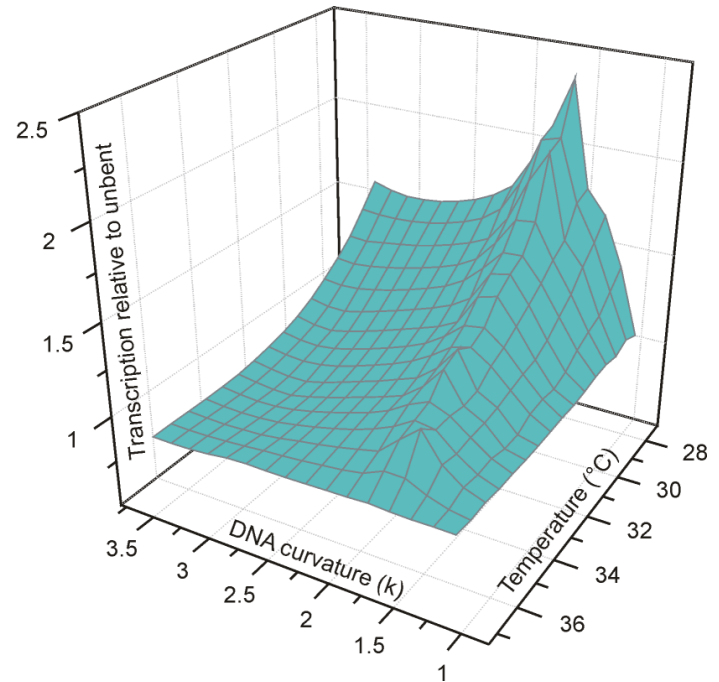
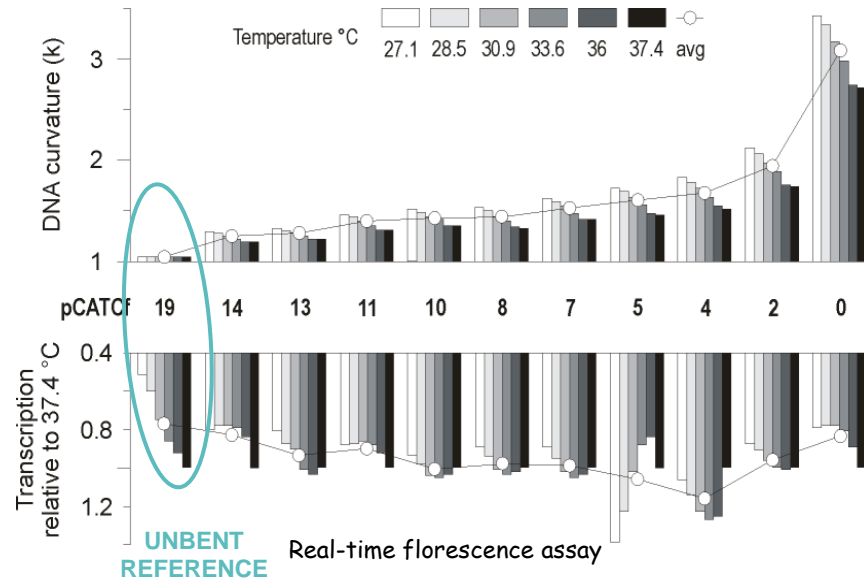


has been randomly mutagenized in order to obtain a spectrum of fragments covering a wide curvature range.

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



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



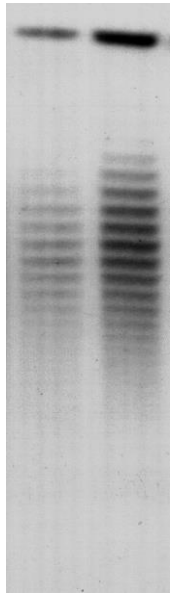
In short:

Curved DNA regions are frequently located upstream bacterial promoters.

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

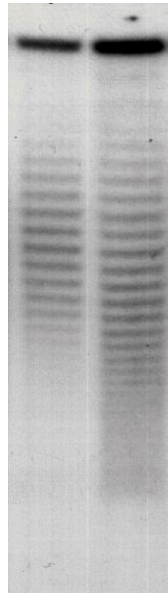
A narrow range of curvature is able to sustain bacterial transcription in vitro.

30°C 37°C



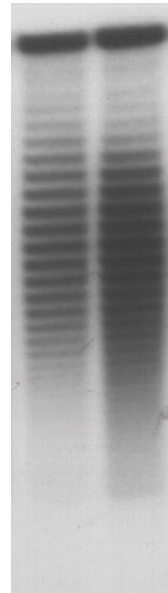
wt

30°C 37°C

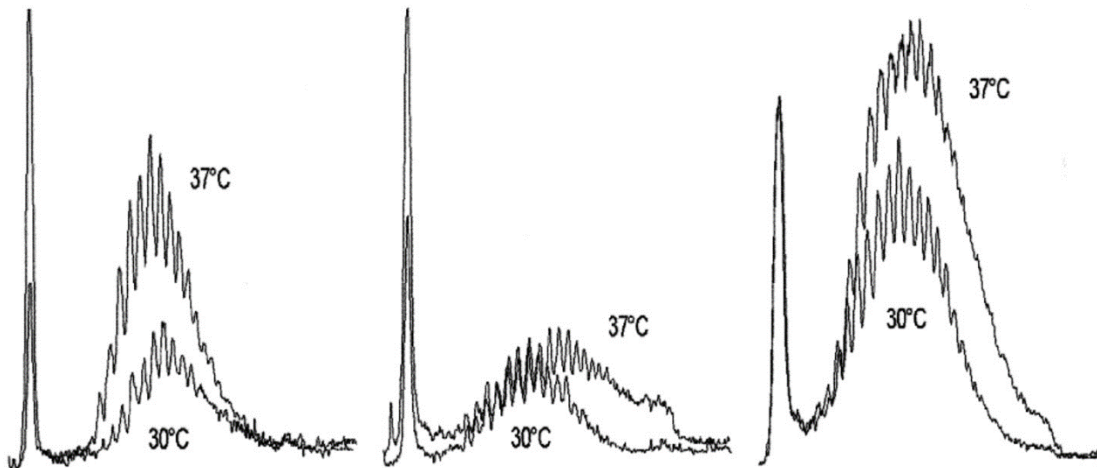


hns118

30°C 37°C

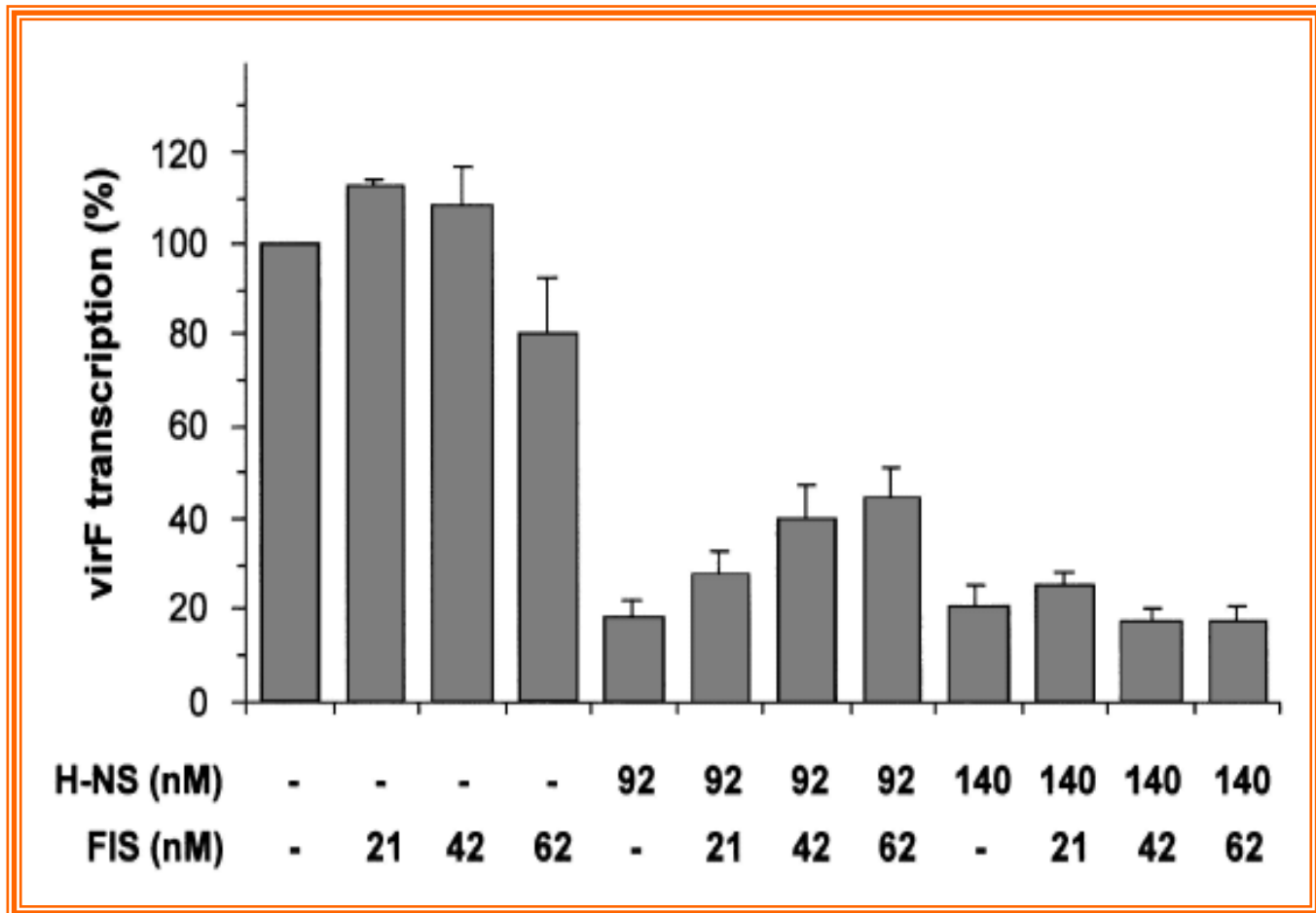


hns2



Distribution of
pMYSH6504
topoisomers in
hns⁺ and *hns*⁻
strains at 30°C
and at 37°C

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



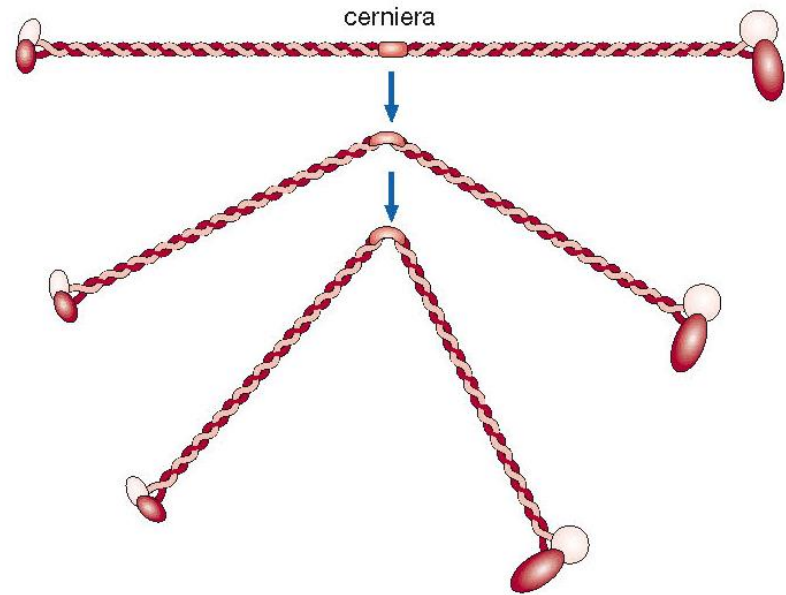
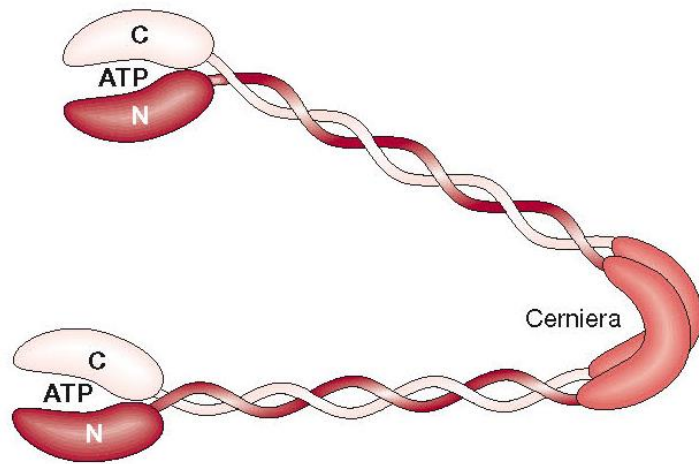
La proteina MukB una proteina SMC like

La proteina MukB è una proteina SMC like (Structural Maintenance 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 nucleotide 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 nucleotide 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.

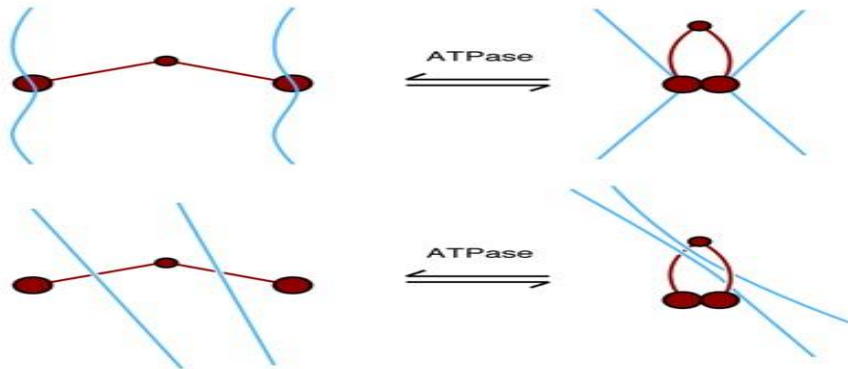
A ATP-dependent change



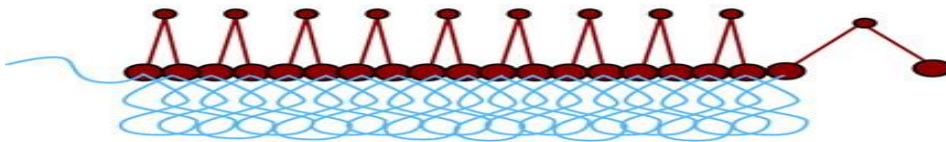
B Intramolecular compaction



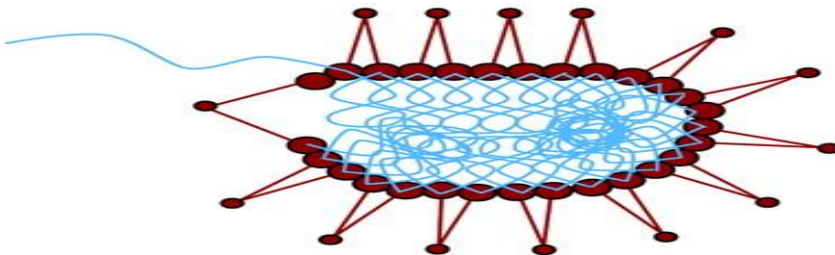
C Intermolecular bridging



D Thick fiber-like structure



E Globular structure



Nella forma chiusa la proteina scorre sul DNA ed è in grado di indurire 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

MukB homodimer

hinge

coiled coil

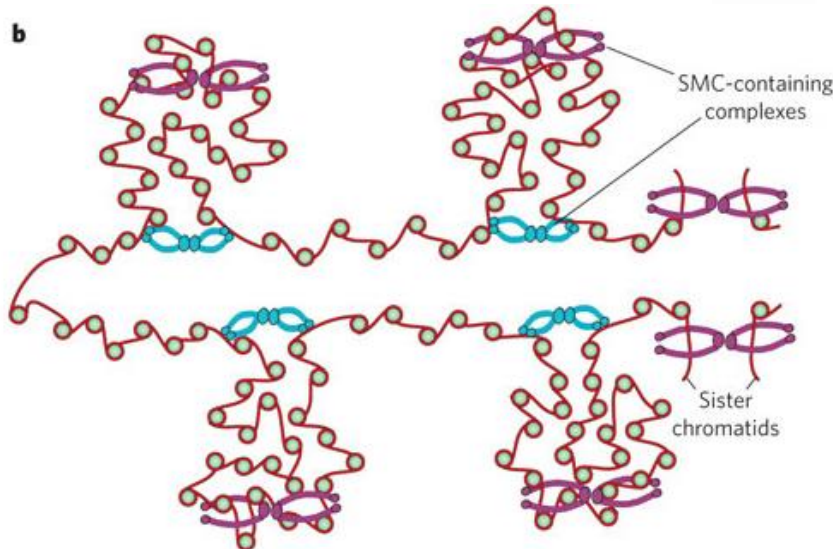
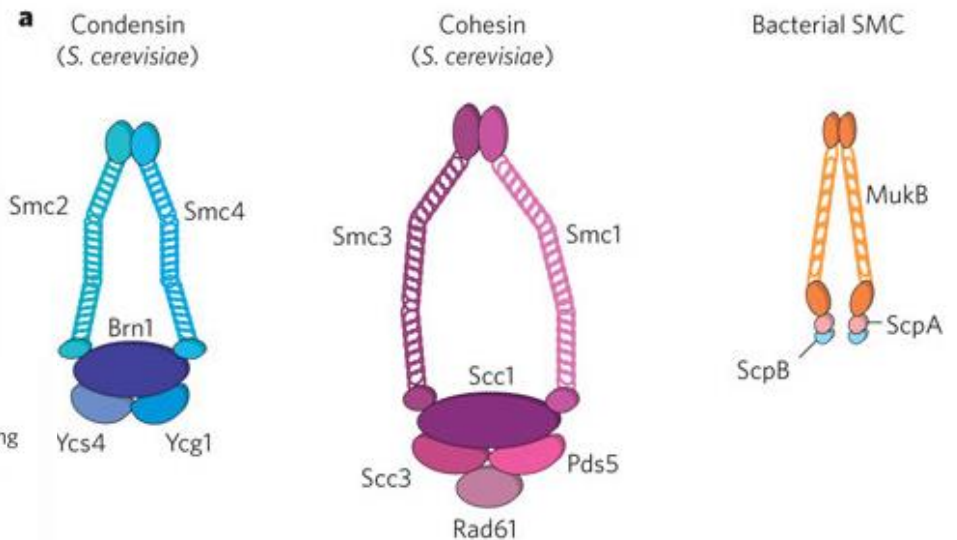
globular domain

MukF

MukE

C N N C

euclidean



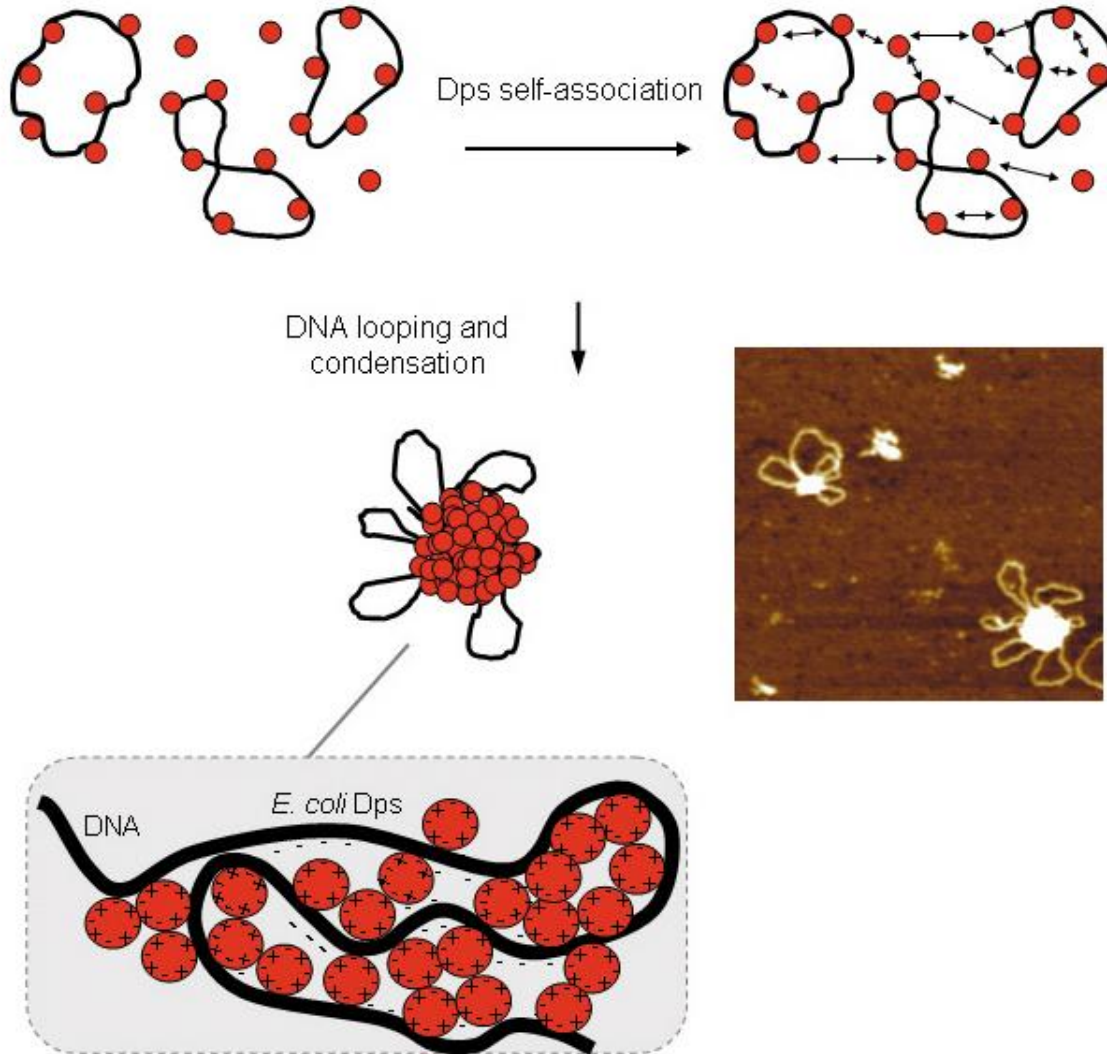
La proteina Dps (Dna Binding protein from starved cells)

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

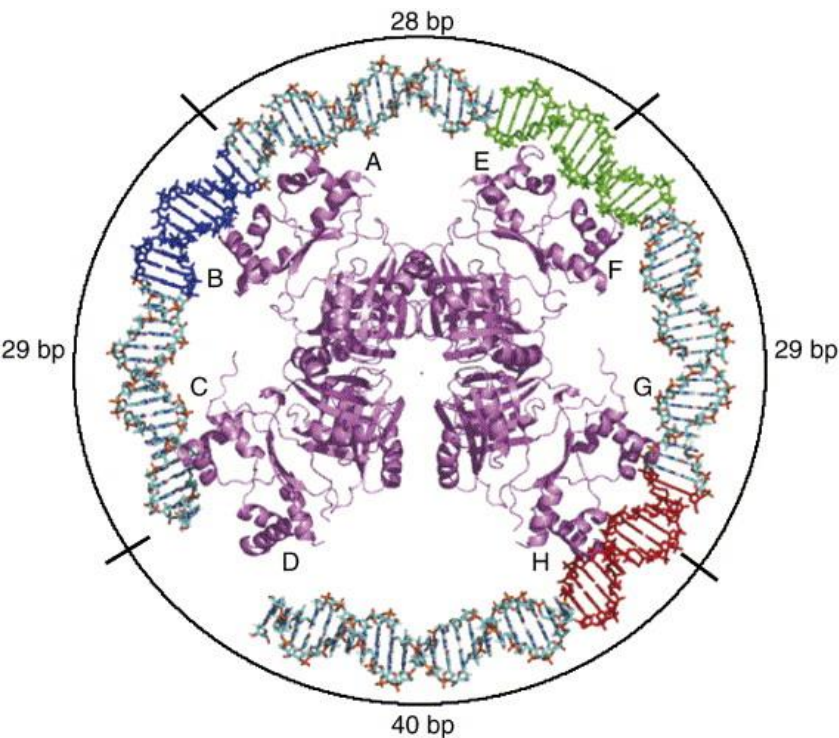
Lrp Leucine responsive regulatory protein

influenza alla 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 metabolismo 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 oligomericici , dimeri, ottamero o esadecamero.



Ottamero di LRP