

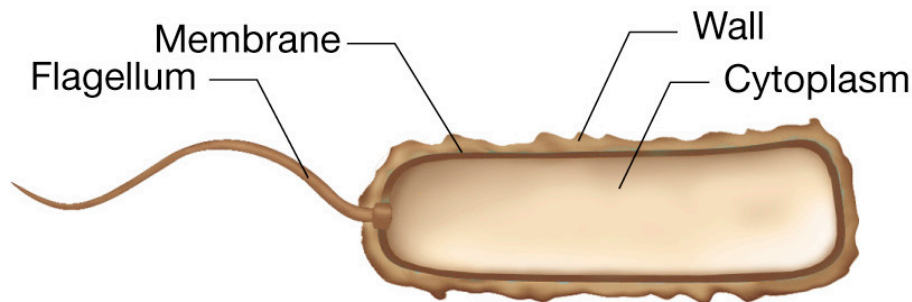
# Localizzazione delle macromolecole nella cellula

Le proteine sono presenti ovunque sia come componenti cellulari che come enzimi

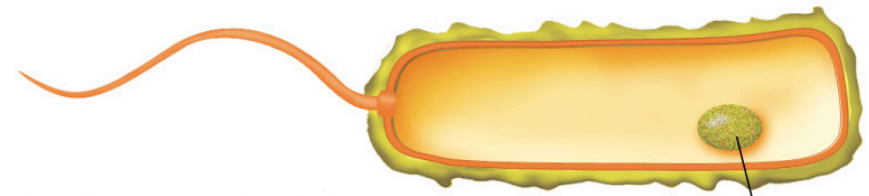
Gli acidi nucleici sono localizzati nel nucleotide, RNA nel citoplasma e nei ribosomi

I polisaccaridi nella parete e nei granuli d'accumulo

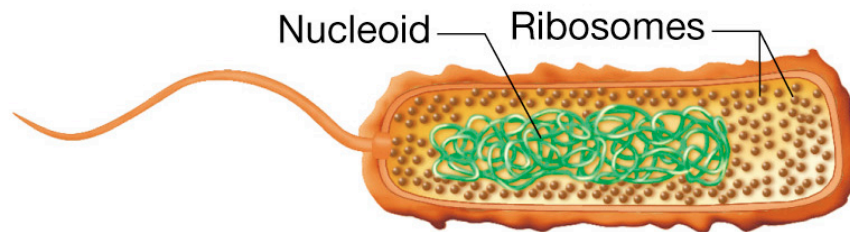
I lipidi nella membrane citoplasmatica, nella parete e nei granuli d'accumulo



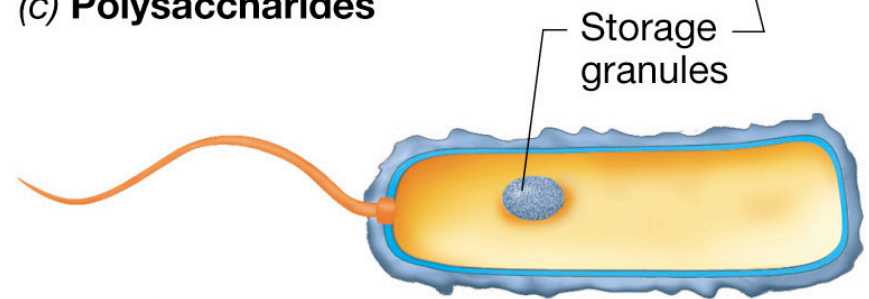
(a) **Proteins**



(c) **Polysaccharides**



(b) **Nucleic Acids**



(d) **Lipids**

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**

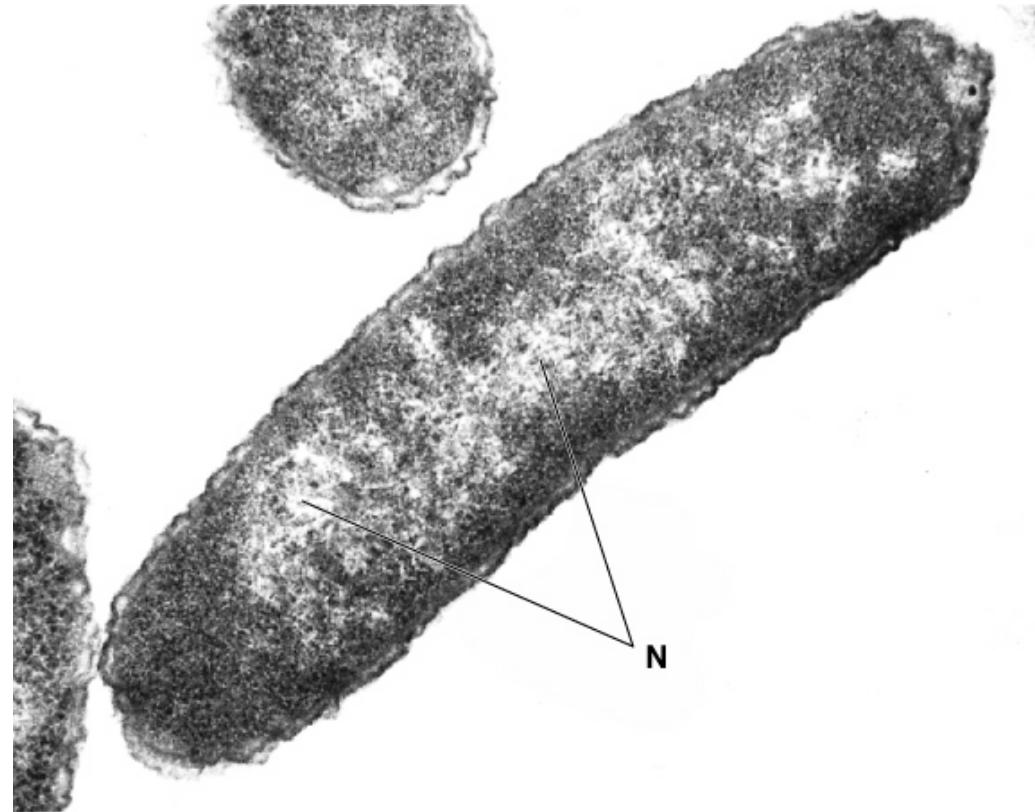
**RNA**

**RNA polimerasi**

**Topoisomerasi**

**Proteine basiche**

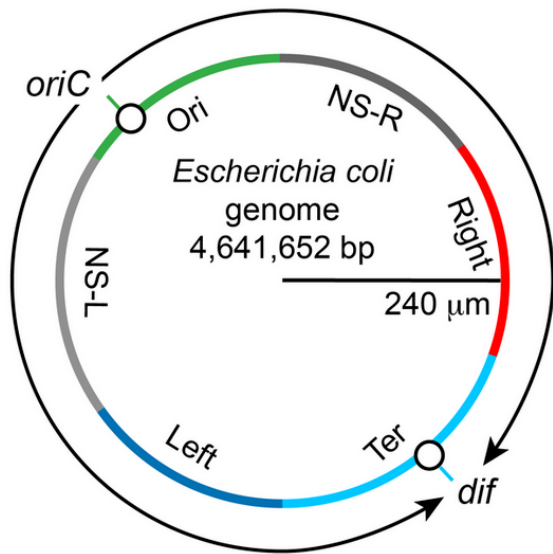
**(proteine associate  
al nucleoide NAP)**



# IL NUCLEOIDE

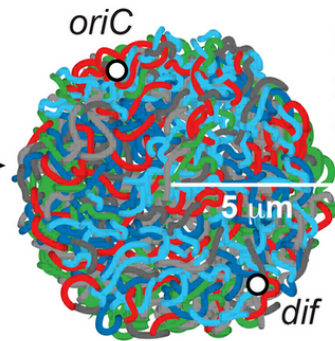
- la molecola di DNA cromosomico di E.coli è lunga circa 1.6mm
- è contenuta in una cellula di 2  $\mu\text{m}$  di lunghezza e 1  $\mu\text{m}$  di larghezza
  - Un compattamento casuale della molecola determinerebbe un volume di circa 200mm<sup>3</sup> circa 400 volte superiore al volume del nucleoide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

### A. Circular *E. coli* genome



### B. Random coil of the DNA

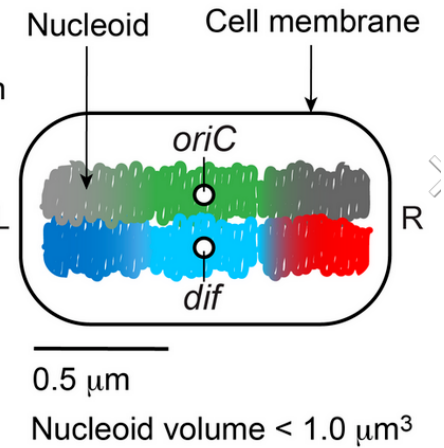
Inherent polymeric property



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

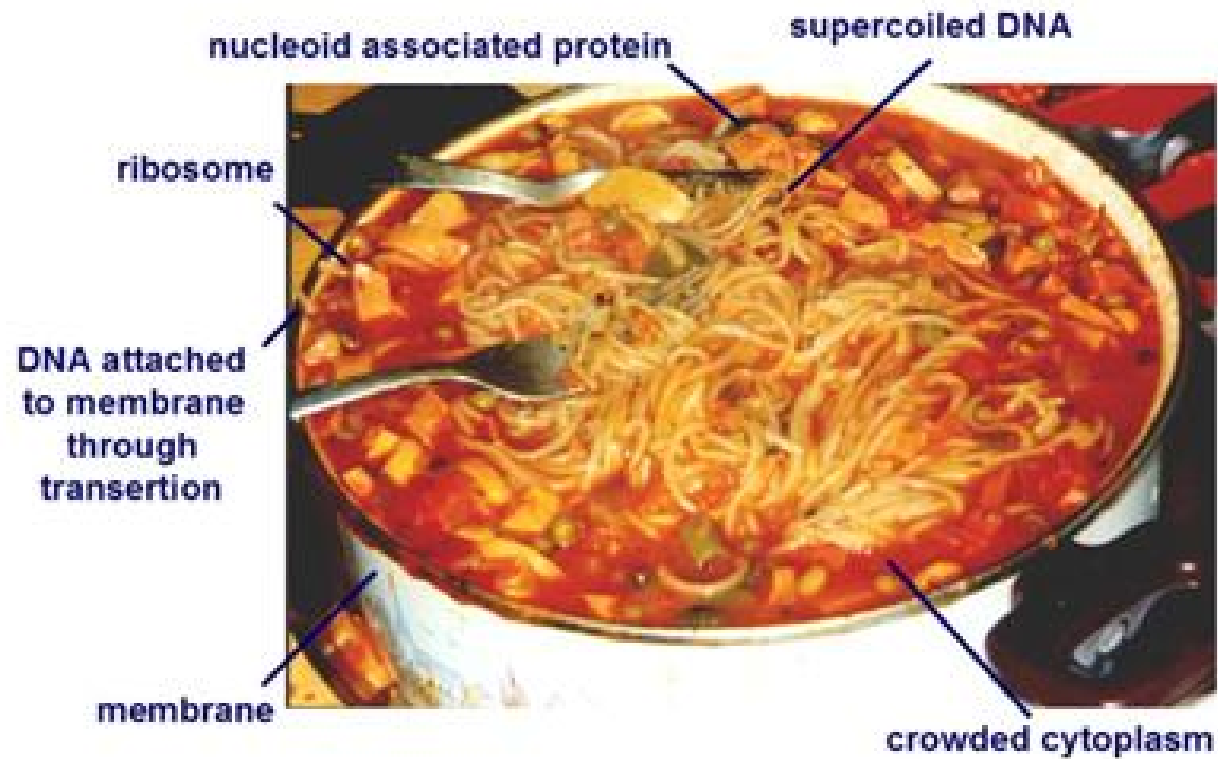
- 1) 1000-fold condensation
- 2) Spatial organization

### C. Genome organization *in vivo*



## nucleoid compaction in *E. coli*

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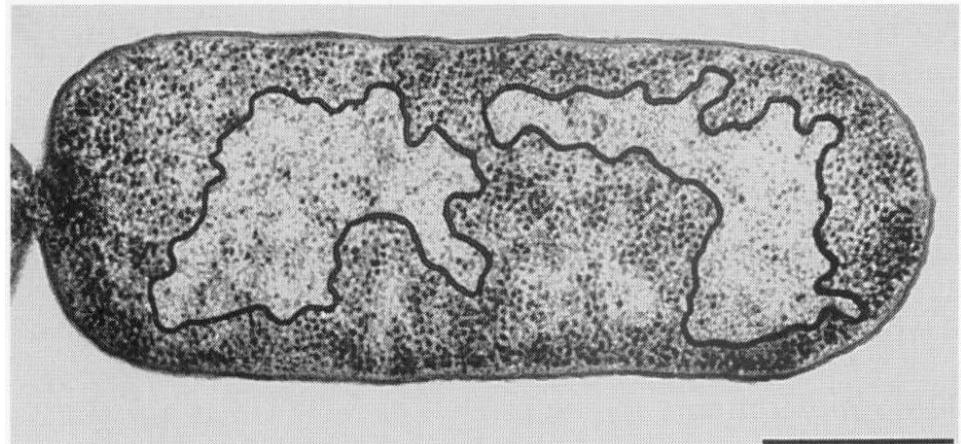
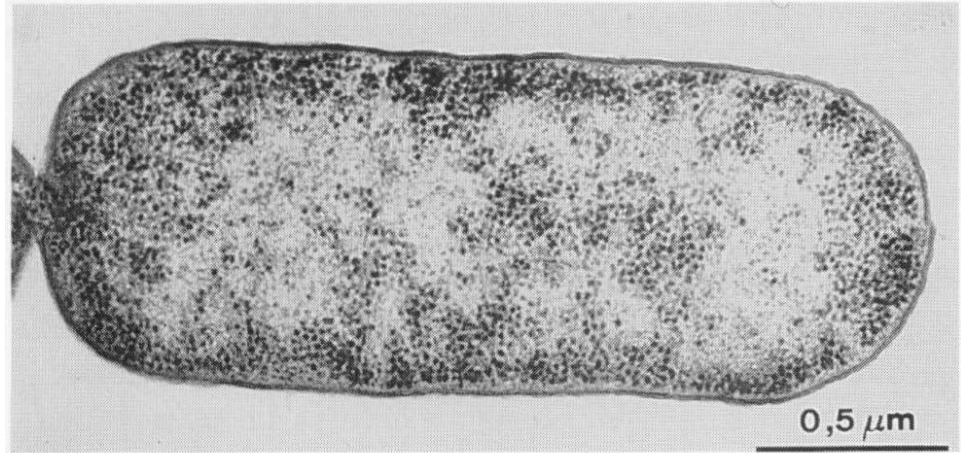


Come gli americani spiegano l'organizzazione del nucleotide....

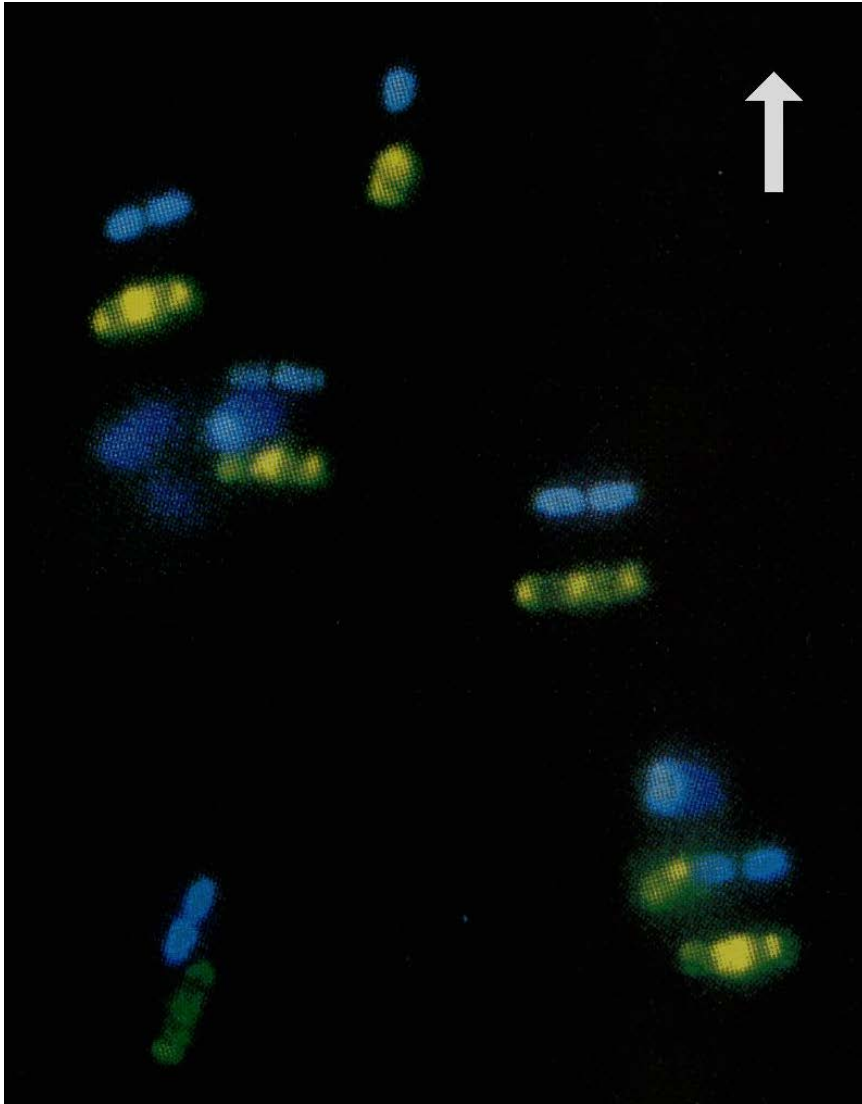
Il nucleoide è una struttura compatta e discreta. Numerose interazioni tra DNA, componenti del citoplasma e della I.M. sono alla base della morfologia compatta e lobulare del nucleoide

Per visualizzare il nucleoide sono stati utilizzati vari approcci:

**la tecnica di criosostituzione** utilizza congelamento della cellule in elio seguita dal trattamento in miscela di tetrossido di osmio in acetone. Le cellule vengono poi colorate con citrato di piombo e acetato di uranile. Il nucleoide appare come una regione libera dai ribosomi localizzata nel mezzo del citoplasma



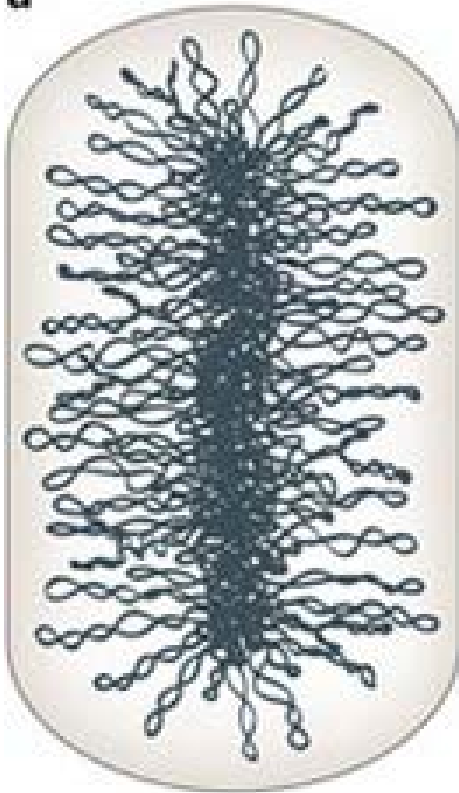
## Analisi per microscopia a fluorescenza dei nucleoidi.



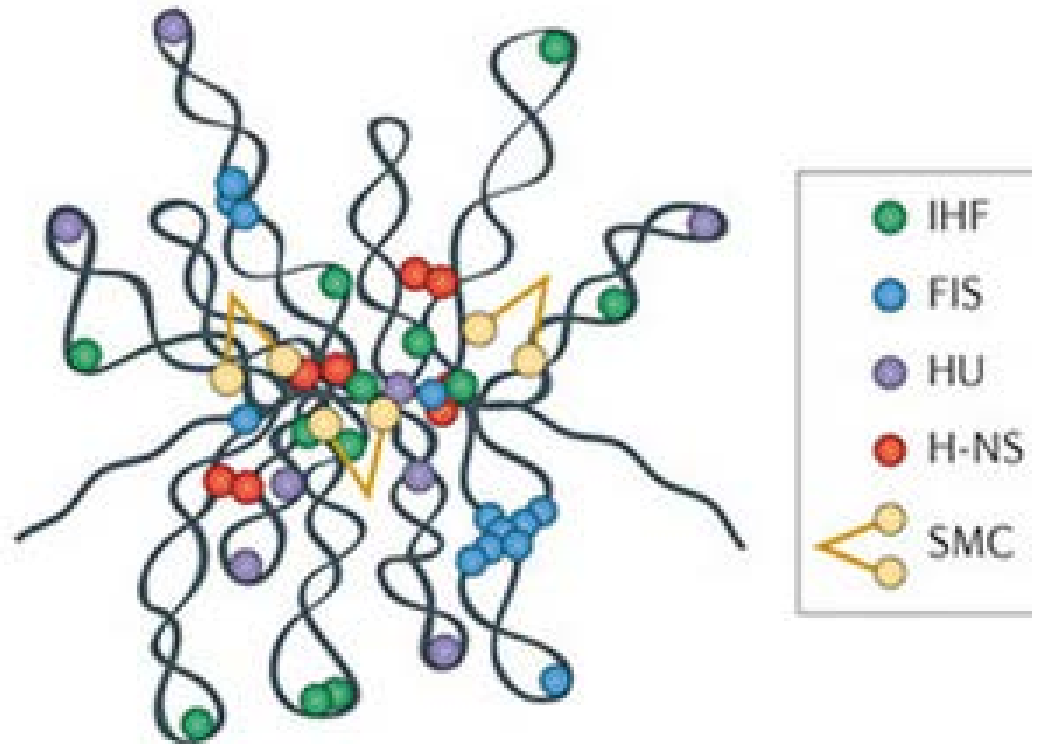
Le cellule sono state colorate con **DAPI** che legandosi preferenzialmente al DNA appare in fluorescenza **BLU** e con **INSULINA** (giallo verde) che non penetra nello spazio occupato dal nucleotide nel citoplasma

# Organizzazione del nucleotide batterico: le principali proteine associate al nucleotide

a



b Nucleoid-associated proteins and SMC complexes:





# Il nucleotide batterico (III)

## Isolamento ...

- 1) Lisozima  
lisi cellulare
- 2) Detergenti non ionici  
solubilizzazione delle membrane e di alcune proteine
- 3) 1M NaCl  
indispensabile per la stabilizzazione della struttura
- 4) Sedimentazione su gradiente di saccarosio

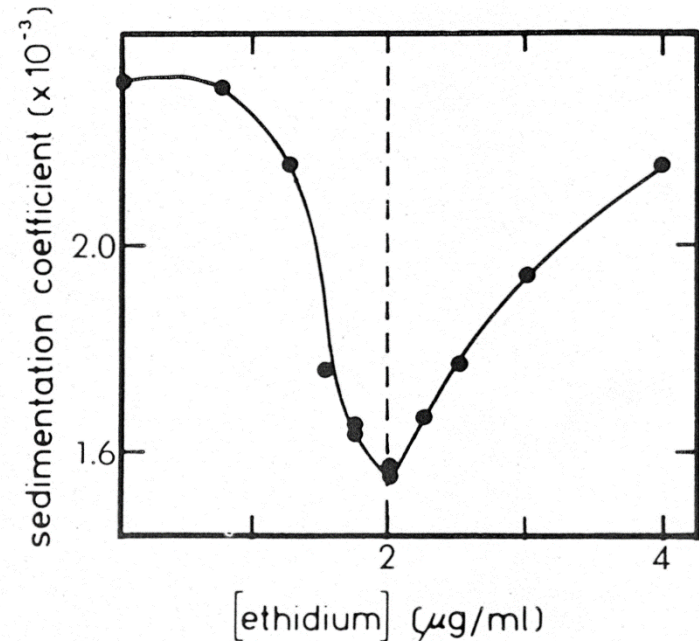
### Composizione (in peso):

- ~ 80% DNA
- ~ 20% proteine

### Nei nuclei eucariotici:

- ~ 45% DNA
- ~ 45% istoni
- ~ 10% proteine non istoniche

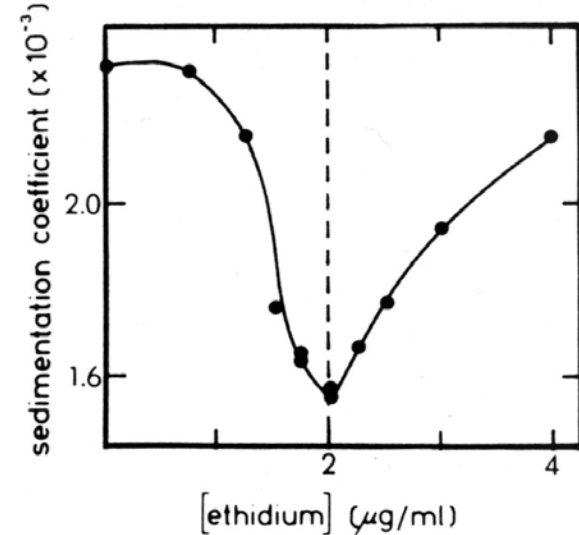
## ... e messa in evidenza dell' organizzazione ad anse



Titration of supercoils in chromosomal DNA. *E. coli* cells were lysed and centrifuged into sucrose density gradients as described in the legend to Fig. 3. Gradients contained various concentrations of ethidium bromide as indicated on the abscissa. The means of the radioactive distribution in the nucleoid and bacteriophage peaks were used to determine relative sedimentation rates.

# Ipotesi dell'organizzazione ad anse del cromosoma batterico.

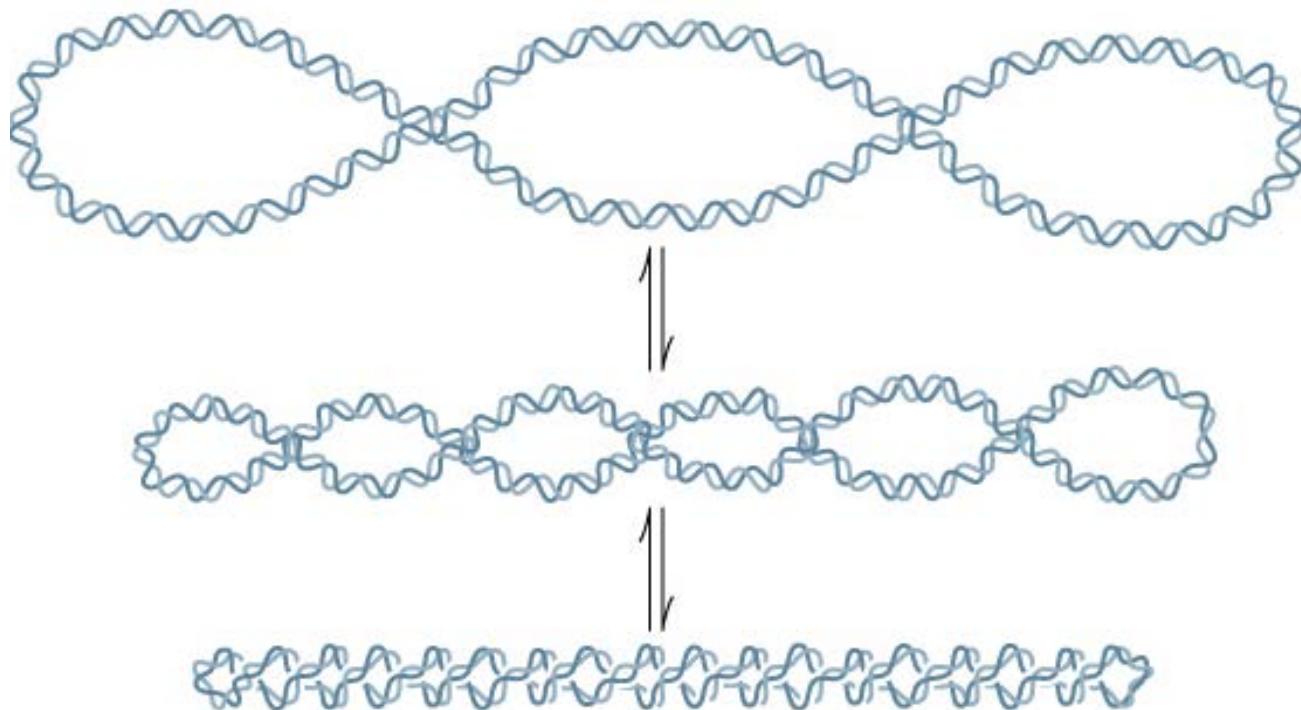
Analisi delle proprietà di sedimentazione dei nucleoidi in presenza di agenti intercalanti fornì le prime prove sperimentali dell'esistenza di domini topologicamente indipendenti. Al crescere della concentrazione di bromuro d'etidio il coefficiente di sedimentazione diminuisce come conseguenza del rilassamento dei superavvolgimenti negativi fino a raggiungere un valore minimo corrispondente al DNA completamente rilassato. L'ingombro sarà massimo e di conseguenza la sua velocità di sedimentazione sarà minima.



Con questo esperimento si riesce a distinguere l'ingombro sterico ovvero si discriminano le forme basandosi sulla frizione che esercitano nel fluido

# Superavvolgimento SUPERCOILING

Il progressivo aumento del grado di superavvolgimento del DNA produce una molecola altamente compattata

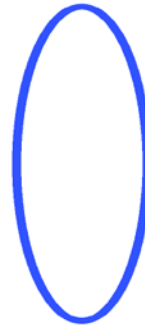


# Risposta agli intercalanti di un dominio di DNA ccc

[EtBr]



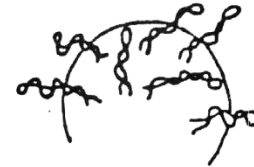
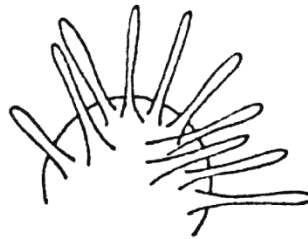
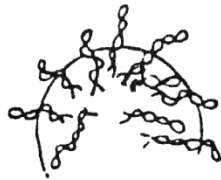
-  
supercoiling  
negativo



0  
rilassamento

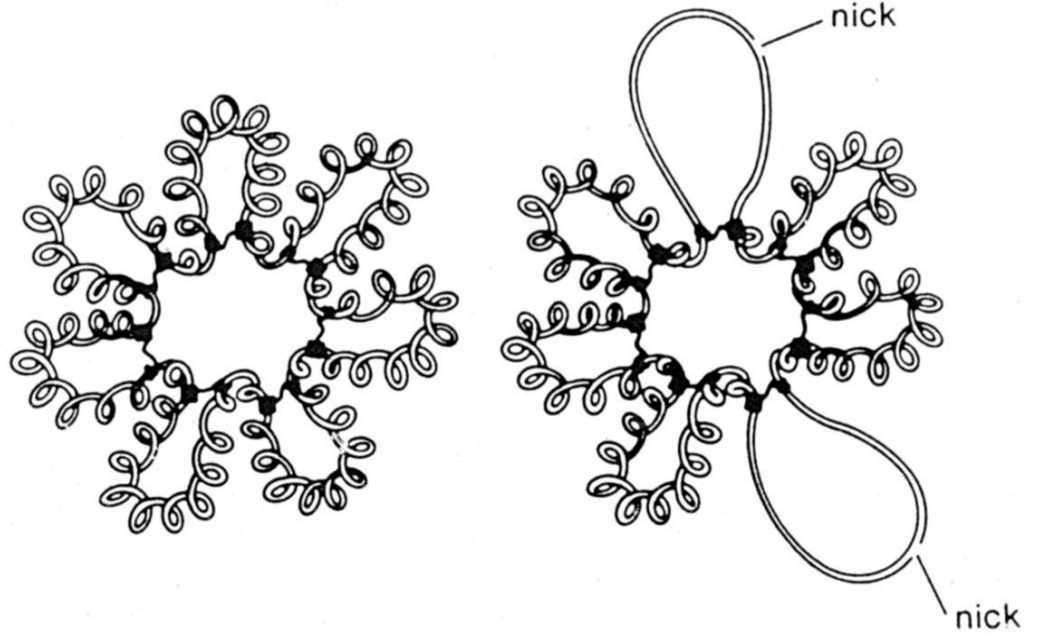
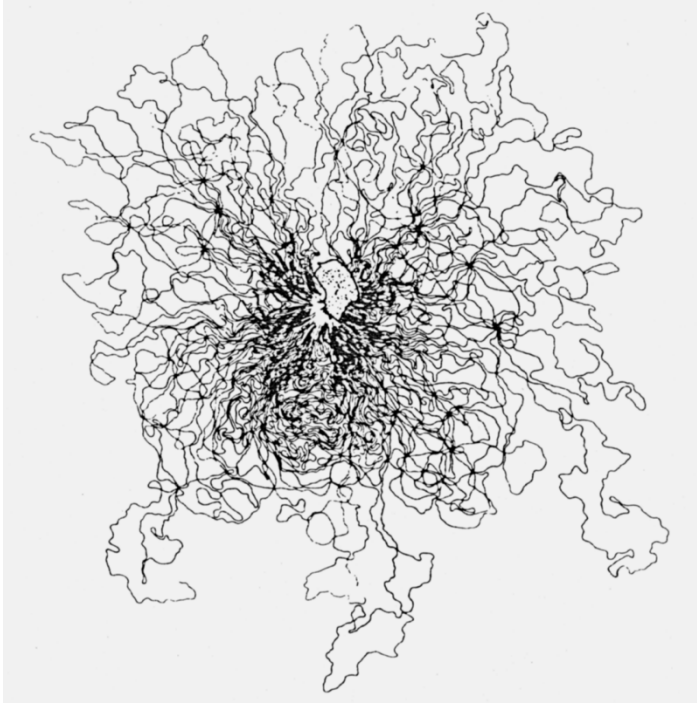


+  
supercoiling  
positivo

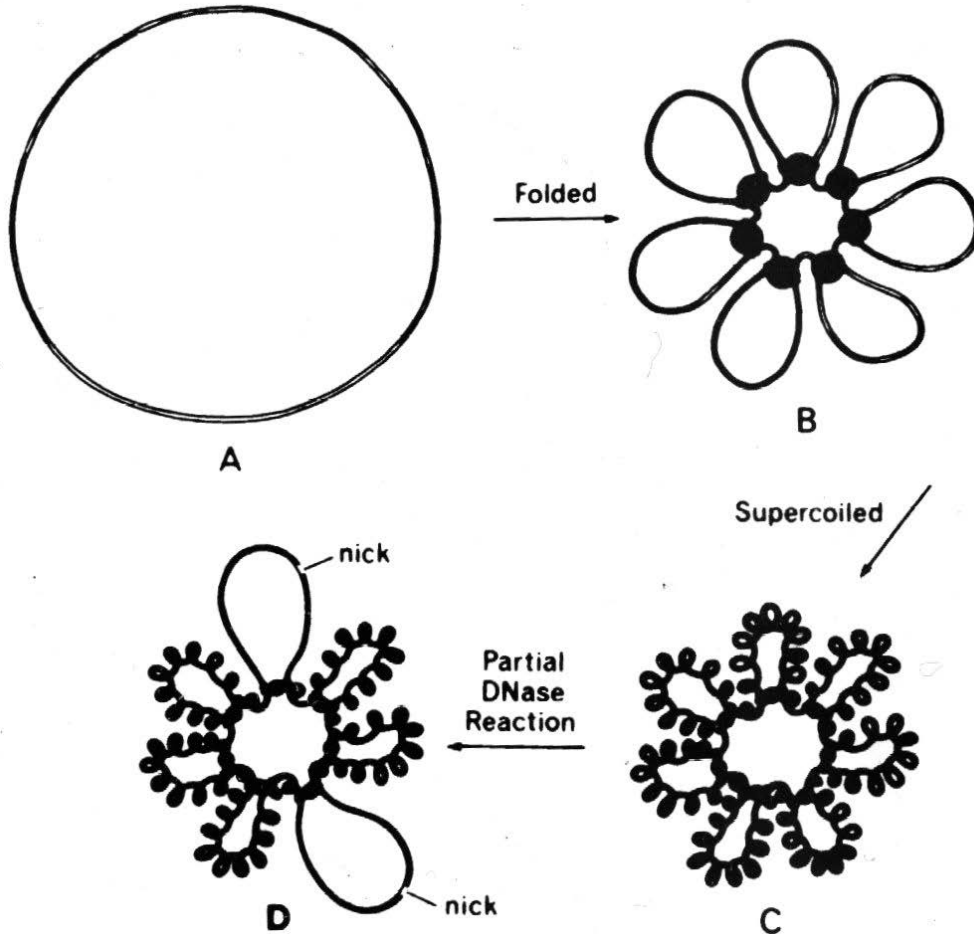


raggio  
dell'alone

# Modello dell'organizzazione ad anse nel nucleotide batterico



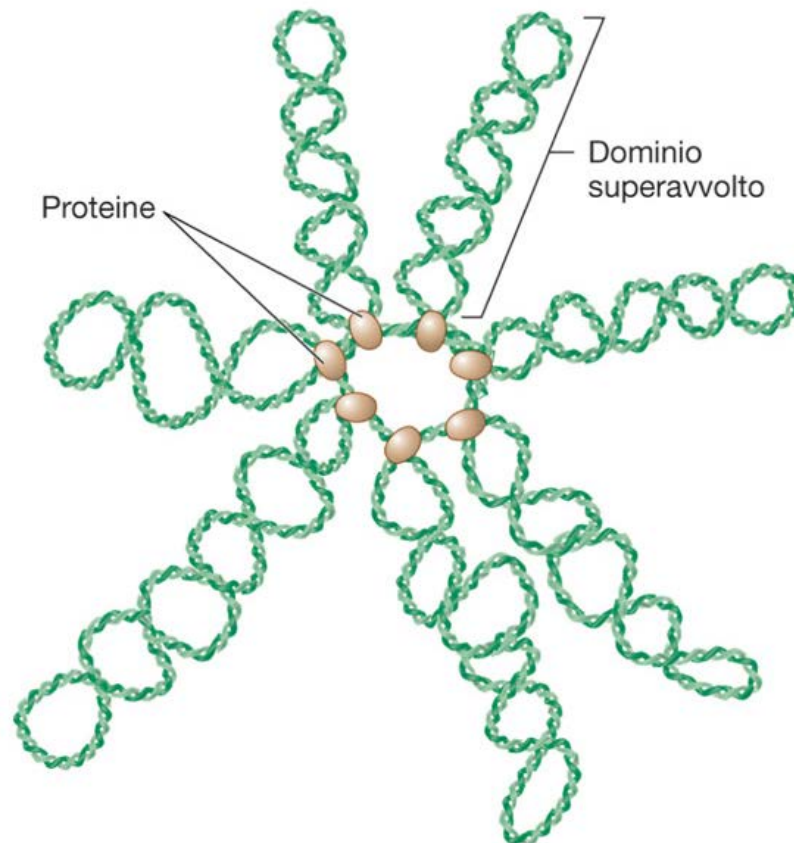
# Organizzazione del cromosoma batterico ad anse



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

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

## La proteina MukB una proteina SMC like

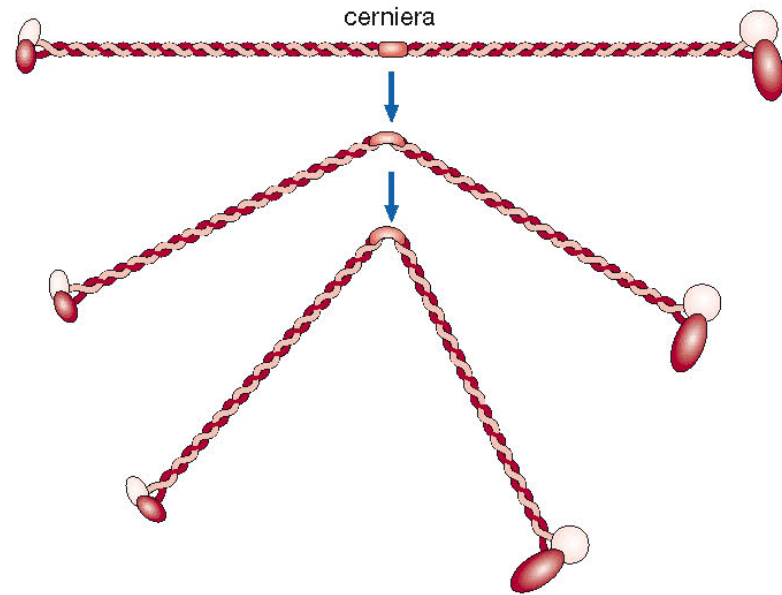
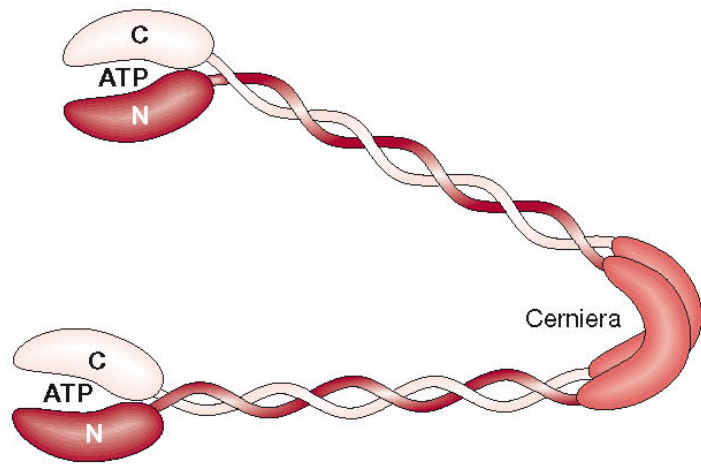
La proteina MukB è una proteina SMC like ( Structural Maintenance of Chromosome) presente in molti batteri.

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.

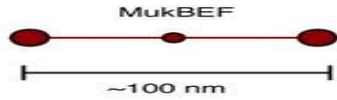




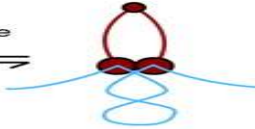
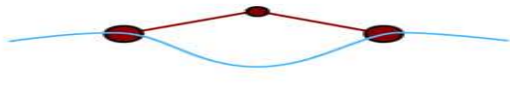
La proteina Muk B come molte proteine della famiglia SMC ,è costituita da due domini globulari N- e C- terminali (teste) separati da 2 regione coiled-coil intervallate da una terza regione globulare che costituisce una cerniera flessibile.

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

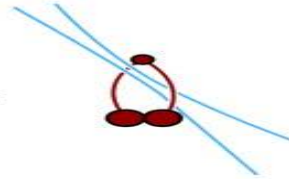
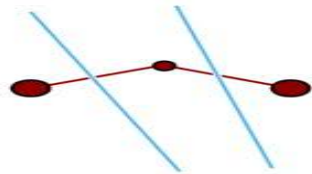
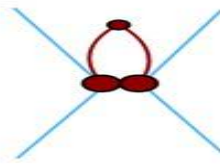
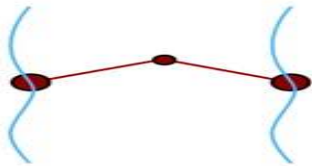
**A** ATP-dependent change



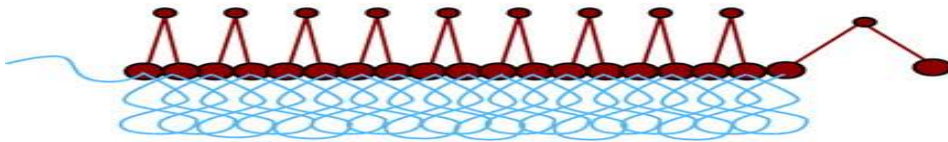
**B** Intramolecular compaction



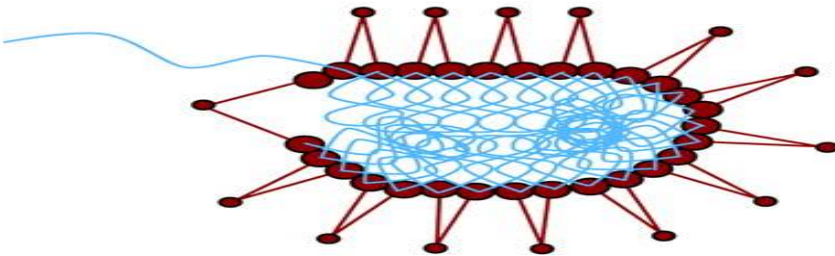
**C** Intermolecular bridging



**D** Thick fiber-like structure

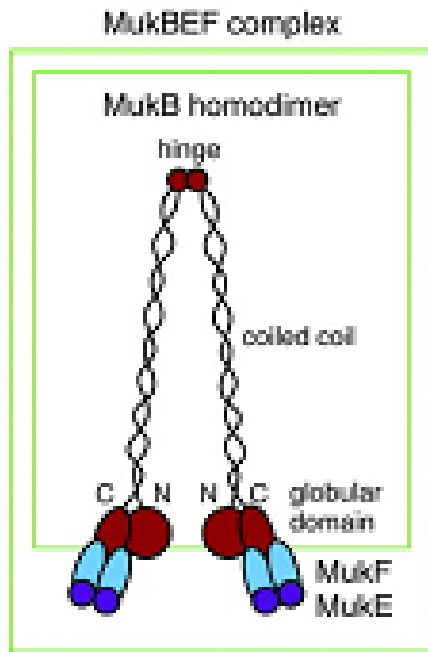


**E** Globular structure

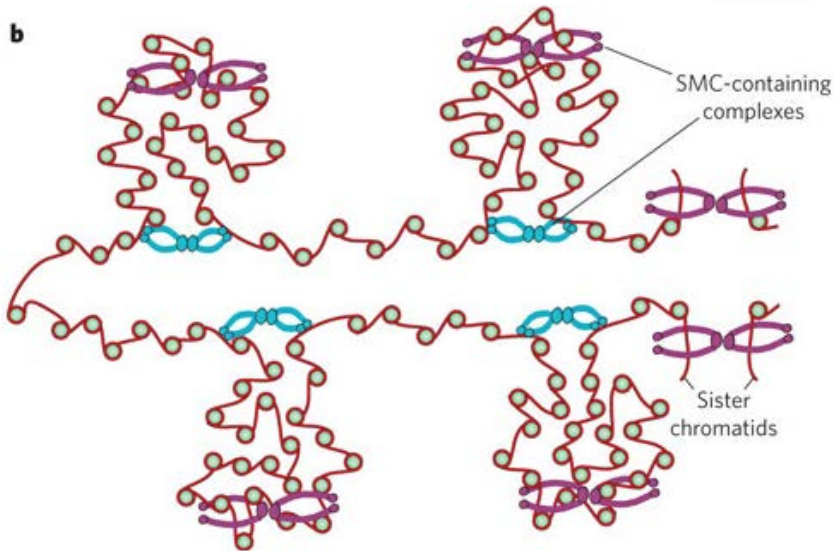
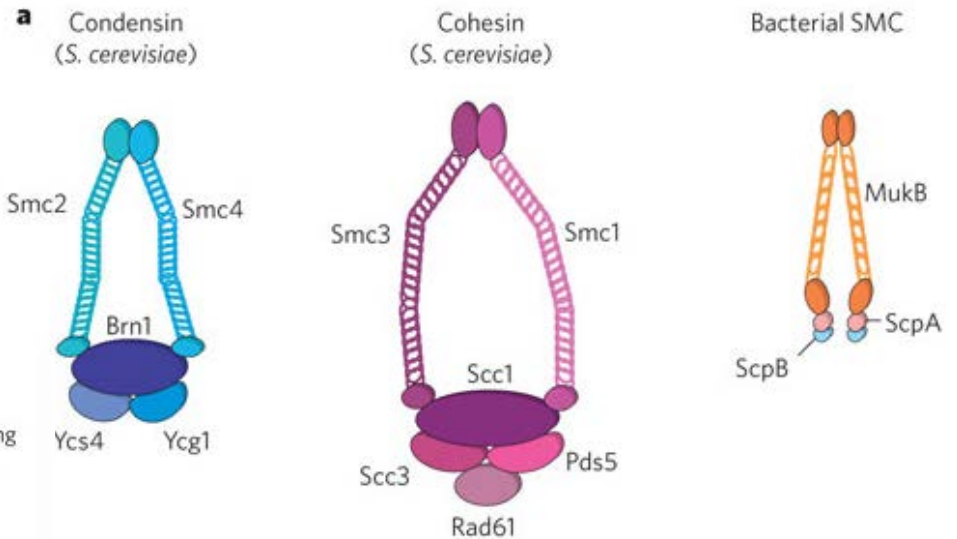


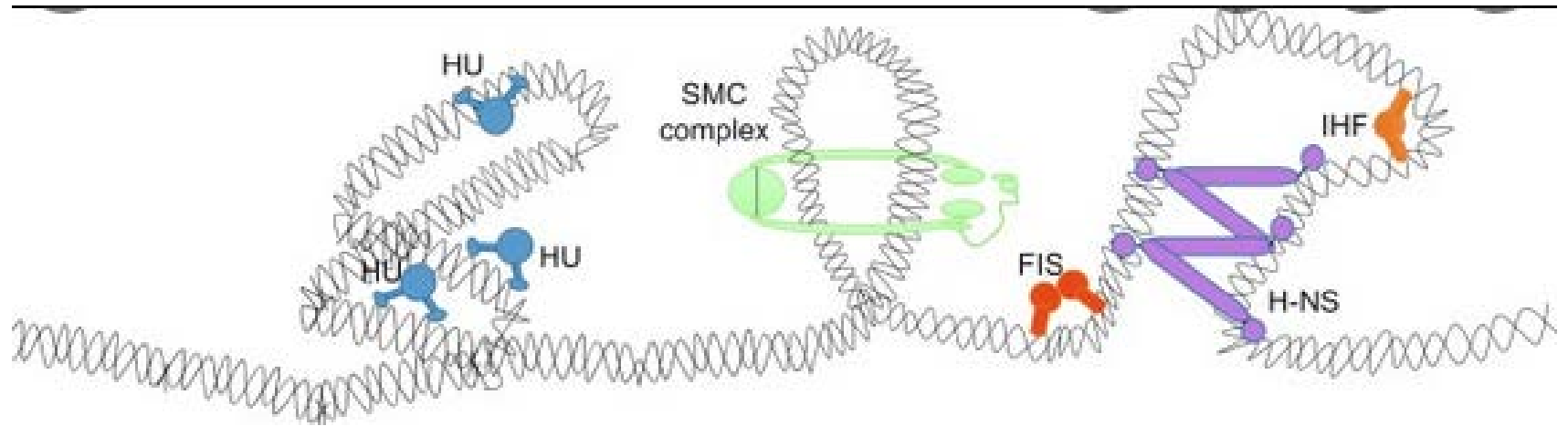
Nella forma chiusa la proteina scorre sul DNA ed è in grado di indurire il ripiegamento.

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



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...





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

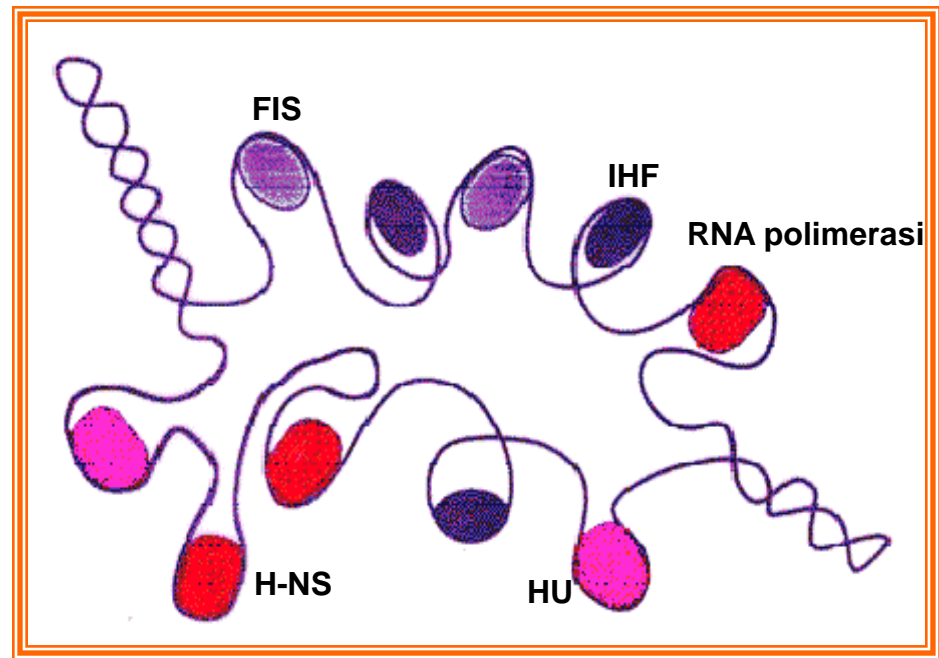
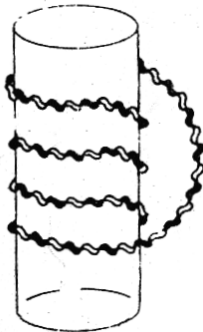
SMC complex corrisponde alle proteine MUK

# Bacterial chromatin organization

Plectonemic  
condition

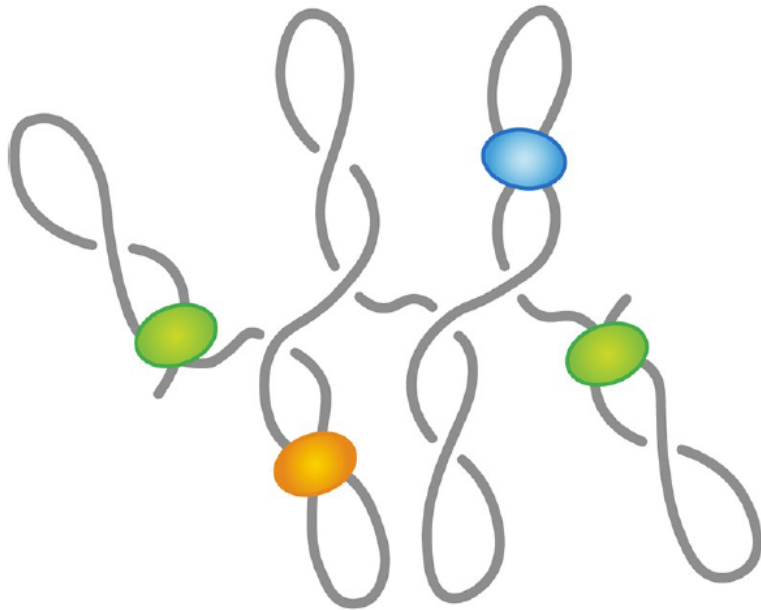


Toroidal  
condition



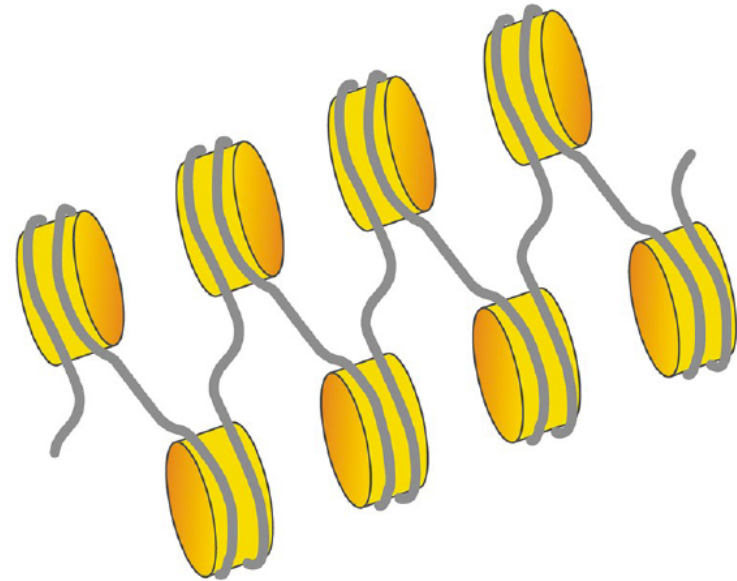
Co-existence of  
plectonemic and toroidal conditions

### 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

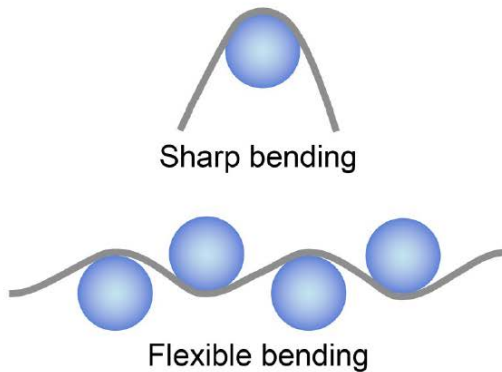
Principali proteine associate  
al nucleotide

# Le principali proteine associate al nucleotide batterico (NAP nucleoid associated proteins)

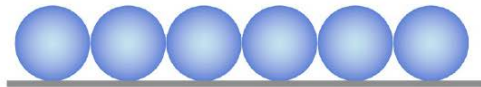
NAP	Properties and abundance	Structure	Genes	Main functional activities
HU (Heat Unstable nucleoid protein)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in exponential phase (15000-30000 dimers/cell)</li> </ul>	heterodimer; HU $\alpha$ , 9.2 kDa HU $\beta$ , 9.5 kDa	<i>hupA</i> (90.4 min) <i>hupB</i> (9.9 min)	<ul style="list-style-type: none"> <li>• compacts DNA into nucleosome-like structures</li> <li>• induces DNA curvature</li> <li>• recognizes curved DNA, gapped regions, and 3-/4way junctions</li> <li>• involved in DNA replication and recombination</li> </ul>
IHF (Integration Host Factor)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in stationary phase (25000-3000 dimers/cell)</li> <li>• high amino acid identity between IHF and HU subunits</li> <li>• DNA binding preference: WATCAANNNTTR</li> </ul>	heterodimer; IHF $\alpha$ , 11.2 kDa IHF $\beta$ , 10.7 kDa	<i>himA</i> (38.6 min) <i>himD</i> (25 min)	<ul style="list-style-type: none"> <li>• induces very strong DNA curvature (up to 140°)</li> <li>• participates in site-specific recombination, transposition, and DNA replication</li> </ul>
FIS (Factor for Inversion Stimulation)	<ul style="list-style-type: none"> <li>• basic</li> <li>• abundant in exponential phase (20000-40000 dimers/cell)</li> <li>• DNA binding preference: GNYAWWWTRNC</li> </ul>	homodimer, 2x11.2 kDa	<i>fis</i> (73.4 min)	<ul style="list-style-type: none"> <li>• induces strong DNA curvature (up to 90°)</li> <li>• alters DNA topology</li> <li>• participates in site-specific recombination, transposition, and DNA replication</li> </ul>
H-NS (Histone-like Nucleoid Structuring protein)	<ul style="list-style-type: none"> <li>• non basic</li> <li>• 20000-40000 dimers/cell</li> <li>• binding form may be tetramer or higher oligomer</li> <li>• induced during cold-shock</li> </ul>	homodimer, 2 x 15.4 kDa	<i>hns</i> (27.8 min)	<ul style="list-style-type: none"> <li>• recognizes curved DNA</li> <li>• alters DNA topology</li> <li>• induces DNA curvature</li> <li>• influences recombination</li> </ul>



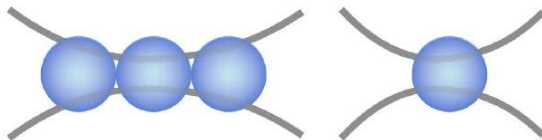
**A. DNA bending**



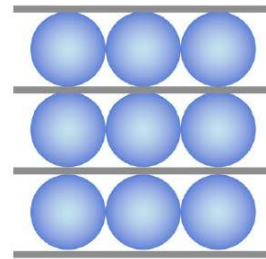
**B. DNA stiffening (coating)**



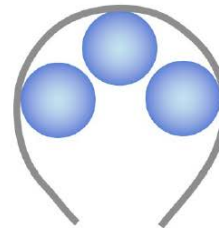
**C. DNA bridging**



**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

NAP	DNA organization
HU	DNA bending, bunching, and stiffening
IHF	DNA bending
H-NS	DNA stiffening and bridging
Fis	DNA bending and bridging

**Fig 2. Nucleoid at  $\geq 1$  kb scale. DNA organization by nucleoid-associated proteins (NAPs).** A straight or curved grey line depicts DNA, and blue sphere depicts a NAP. **A.** NAPs organize DNA by bending it. For example, IHF causes sharp DNA bending (bending angle  $> 160^\circ$ ) upon binding to a specific site, whereas HU introduces flexible bends (bend angles vary between  $10\text{--}180^\circ$ ). IHF also induce flexible bends at non-sequence-specific sites similar to those induced by HU. Fis bends DNA between  $60\text{--}75^\circ$  angle. **B.** In contrast to bending, NAPs can also cause straightening or stiffening of DNA. For example, H-NS spreads along DNA, and as a result, DNA becomes stiff. HU also causes stiffening of DNA at high concentrations ( $\mu\text{m}$  range). **C.** Simultaneous binding of a contiguous tract of NAP molecules (left) or a single NAP molecule (right) to a pair of adjacent or distant DNA sites results in DNA bridging. In an example of DNA bridging, a tract of laterally-bound H-NS molecules bridges two adjacent DNA sites. **D.** DNA bunching or bundling refers to DNA organization in which lateral multimerization of HU triggered by the non-sequence-specific binding brings several parallel DNA segments together, like in a bunch of flowers. **E.** NAP molecules bound adjacent to each other can wrap DNA by coherent bending. Fis molecules bound at tandem sites may organize DNA in this manner.

# 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

Hu $\alpha$

9.2 kDa

hupA (90.4 min)

Hu $\beta$

9.5 kDa

hupB ( 9.9 min)

# La proteina HU

## Funzioni

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

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

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

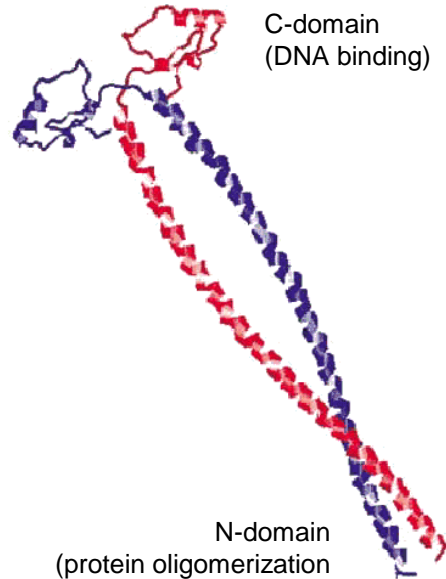
**IHF**



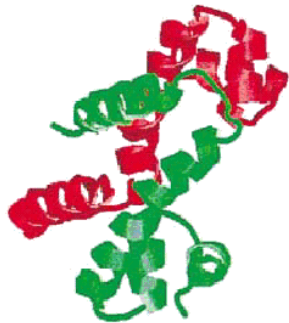
**HU**



**H-NS**



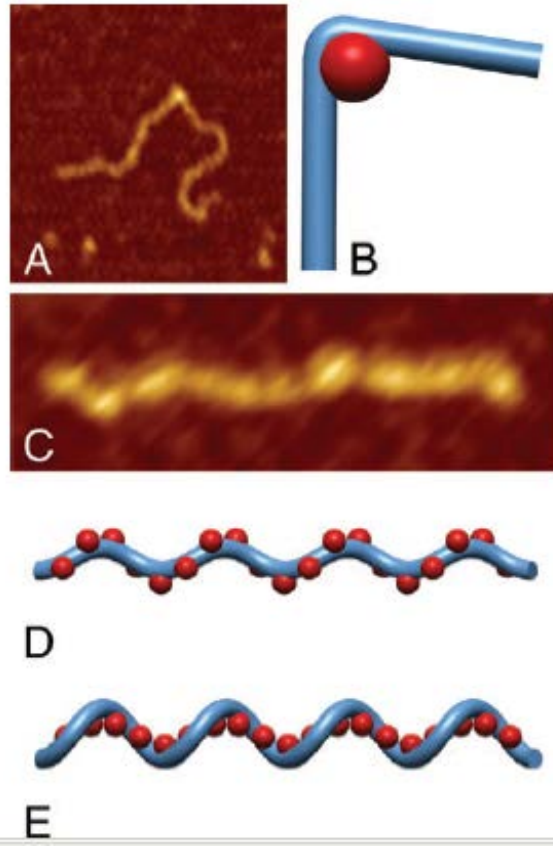
**FIS**



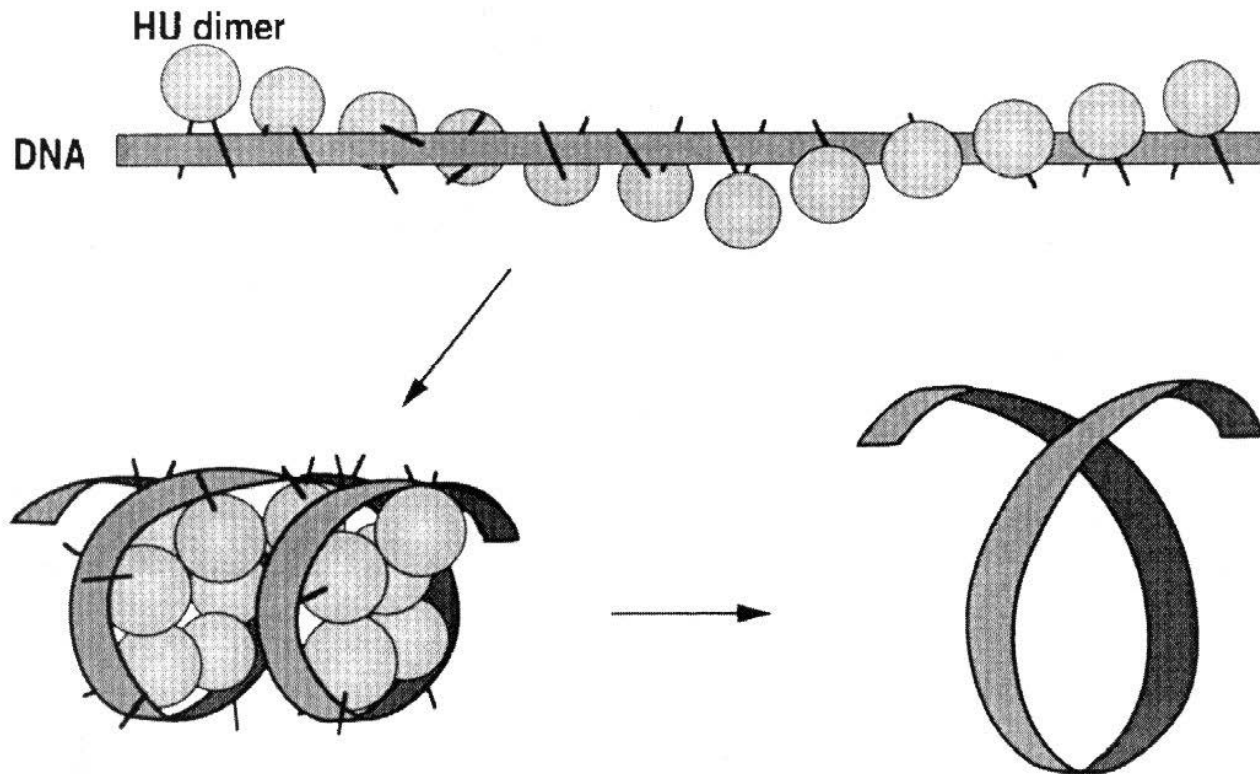
*adapted from  
Ussery et al., 2001*

Nucleoid  
proteins are  
homo- or  
heterodimers

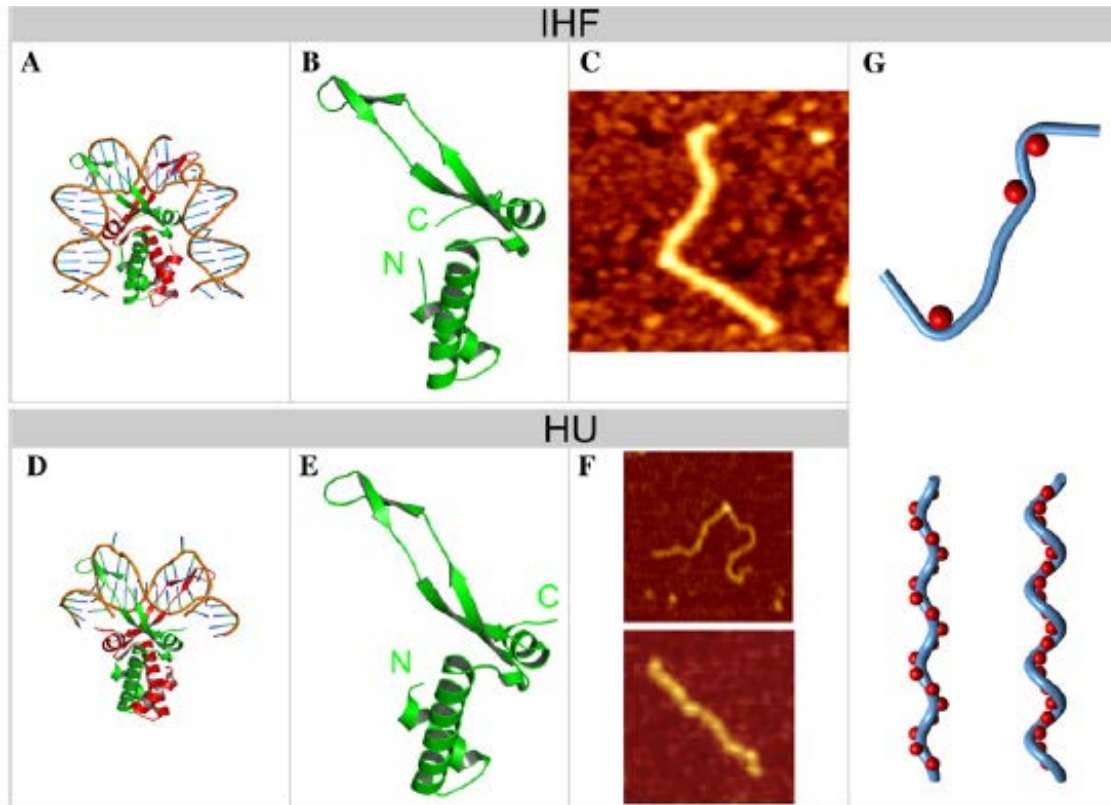
# HU lega il DNA e lo ripiega



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



# Le proteine che ripiegano il DNA





# La proteina IHF Integration Host factor

## Caratteristiche

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

Struttura  
Eterodimero

pM

geni

IHF $\alpha$

11.2 kDa

himA (38.6 min)

IHF $\beta$

10.5 kDa

himD ( 25 min)

# IHF ; Caratteristiche funzionali

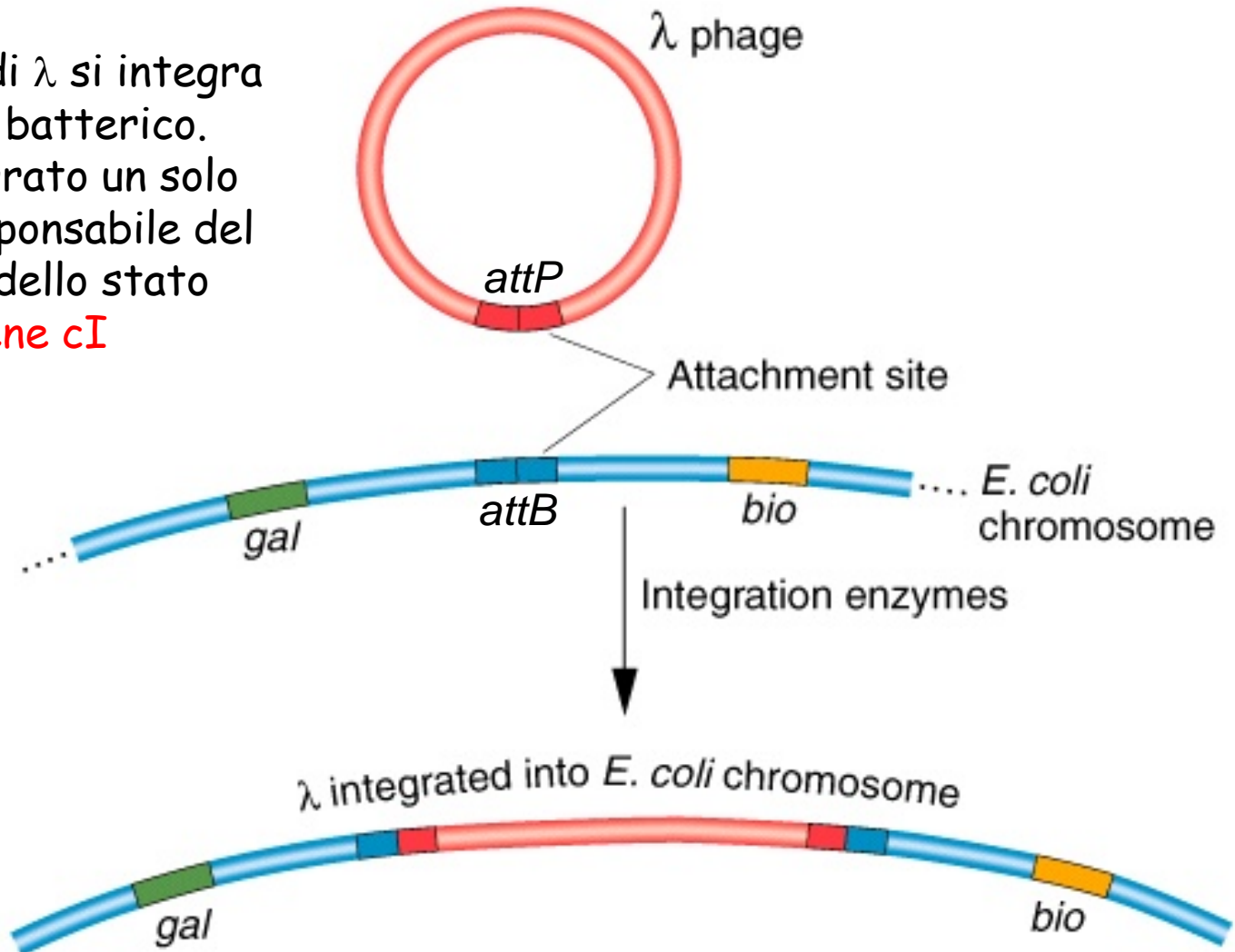
- Induce forte curvatura nel DNA ( fino a  $140^\circ$ )
- Interviene nella ricombinazione sito specifica
- Interviene nella trasposizione

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

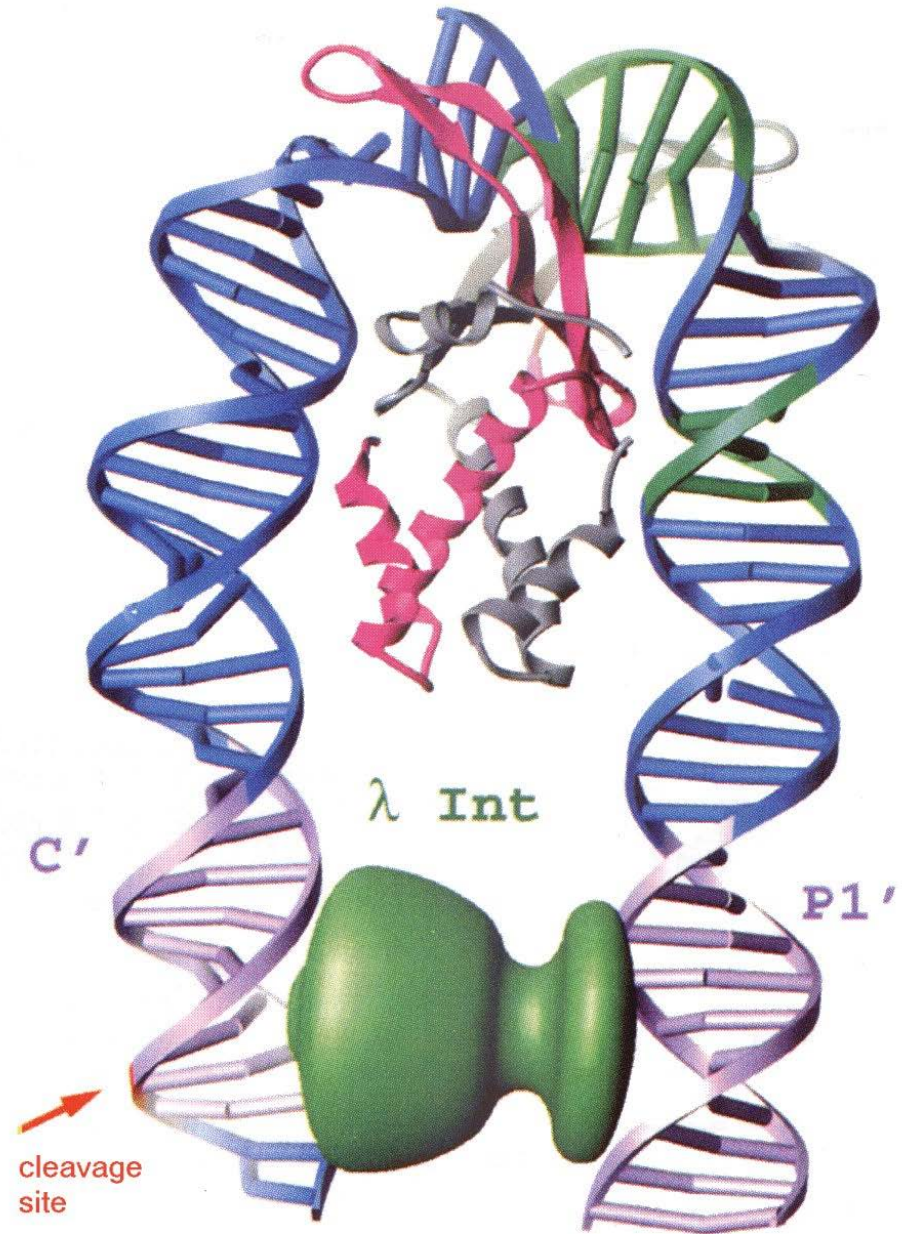
- Isolata come fattore dell'ospite per l'integrazione del fago  $\lambda$  è coinvolta in molte funzioni cellulari.
- Forte ruolo strutturale; curvando il DNA fino a  $140^\circ$  permette a siti di DNA distanti di trovarsi ravvicinati favorendo così sia processi di trascrizione che di regolazione

# La via lisogenica

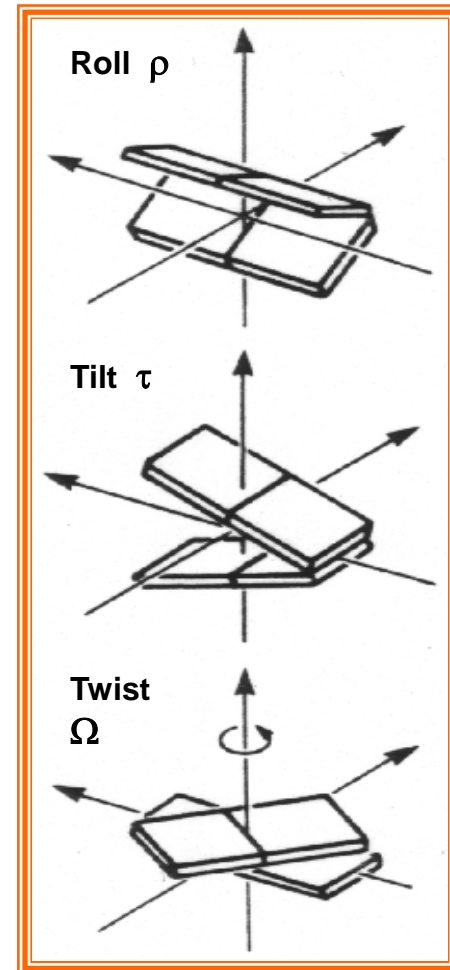
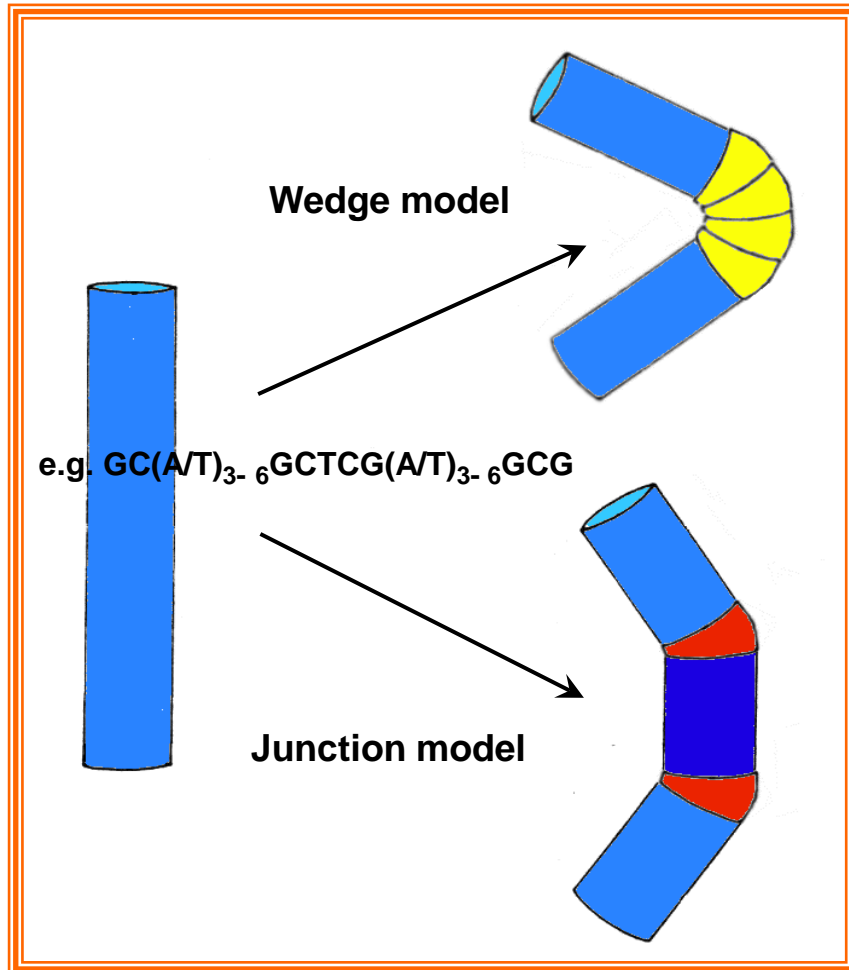
Il cromosoma di  $\lambda$  si integra nel cromosoma batterico. Una volta integrato un solo gene di  $\lambda$  è responsabile del mantenimento dello stato lisogenico: il gene  $cI$

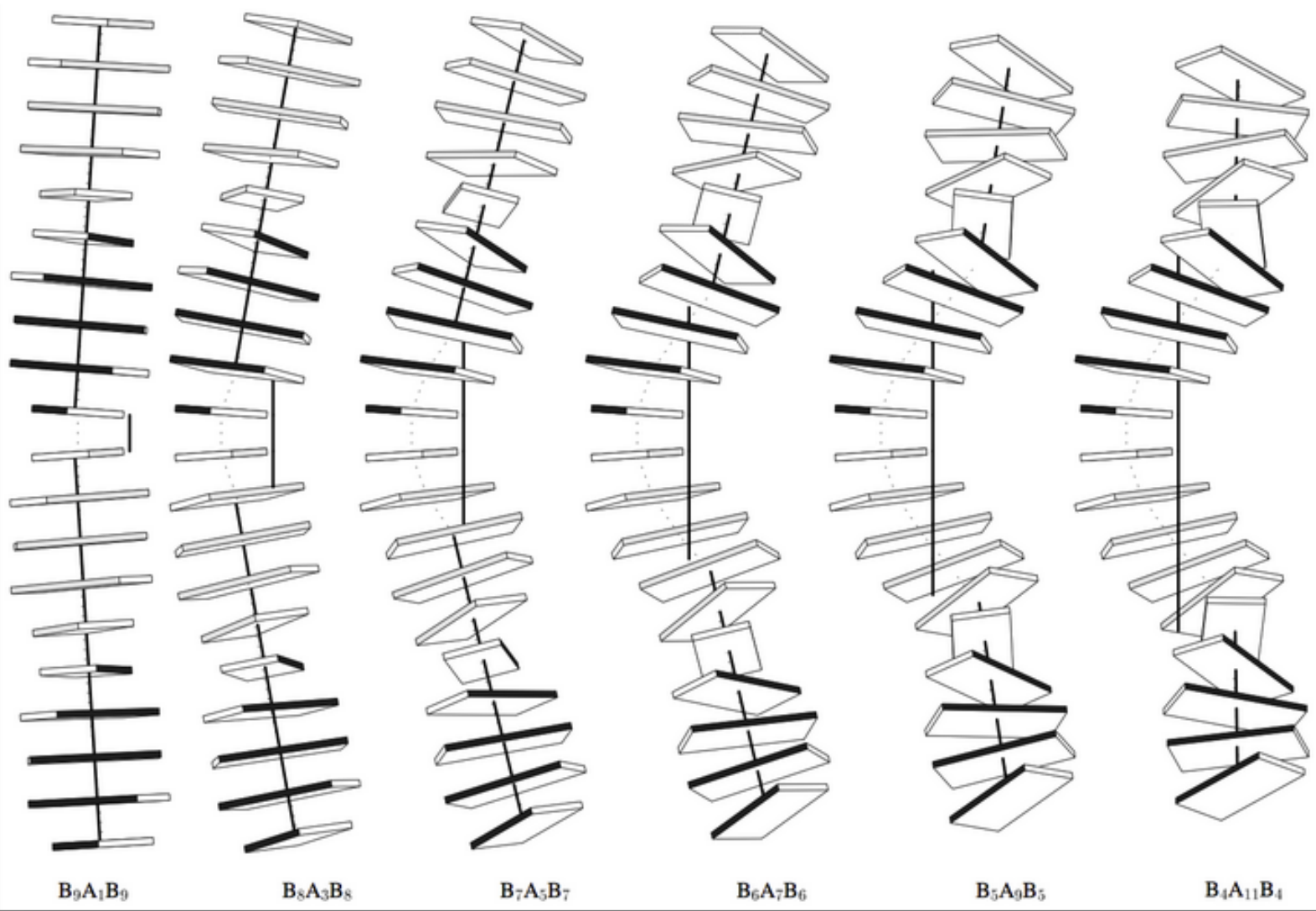


La curvatura mediata da IHF può facilitare il legame della Proteina Int con siti di DNA distanti tra loro.



# Intrinsically curved DNA





# FIS Factor for Inversion Stimulation

## Caratteristiche

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

W TA  
R GA  
K GT  
Y CT

Struttura

pM

geni

OMODIMERO

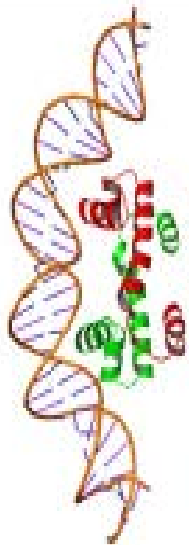
FIS

2x 11.5 kDa

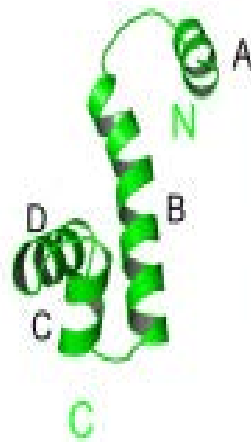
fis (27.4 min)

# FIS

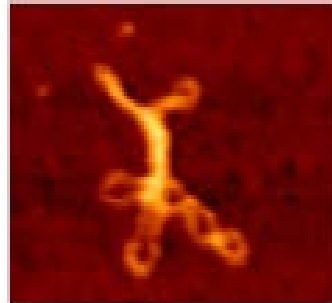
H



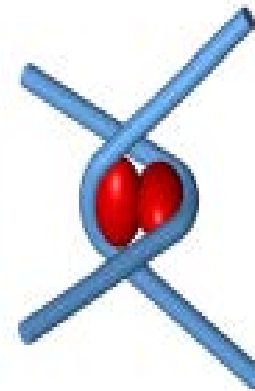
I



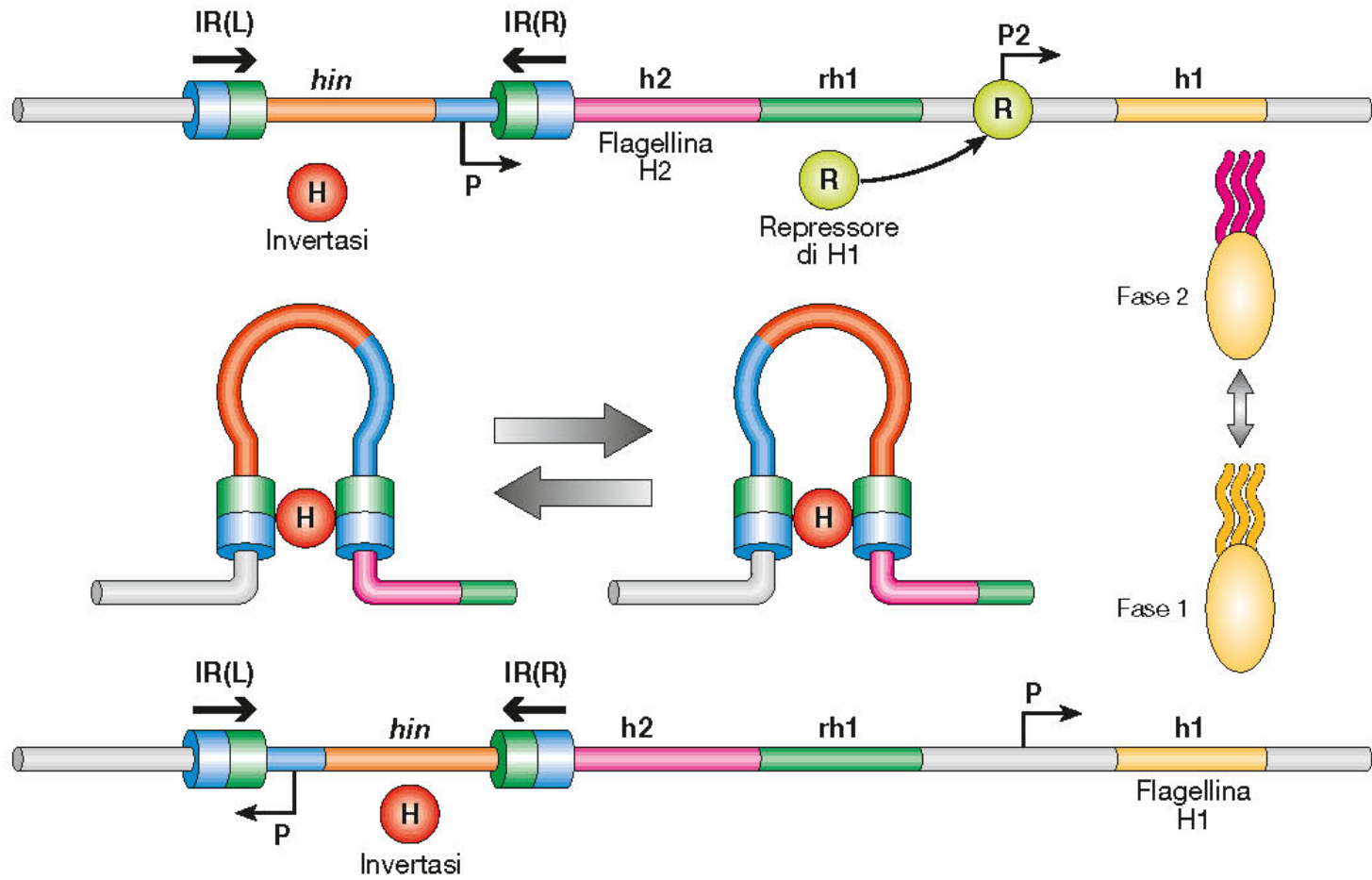
J

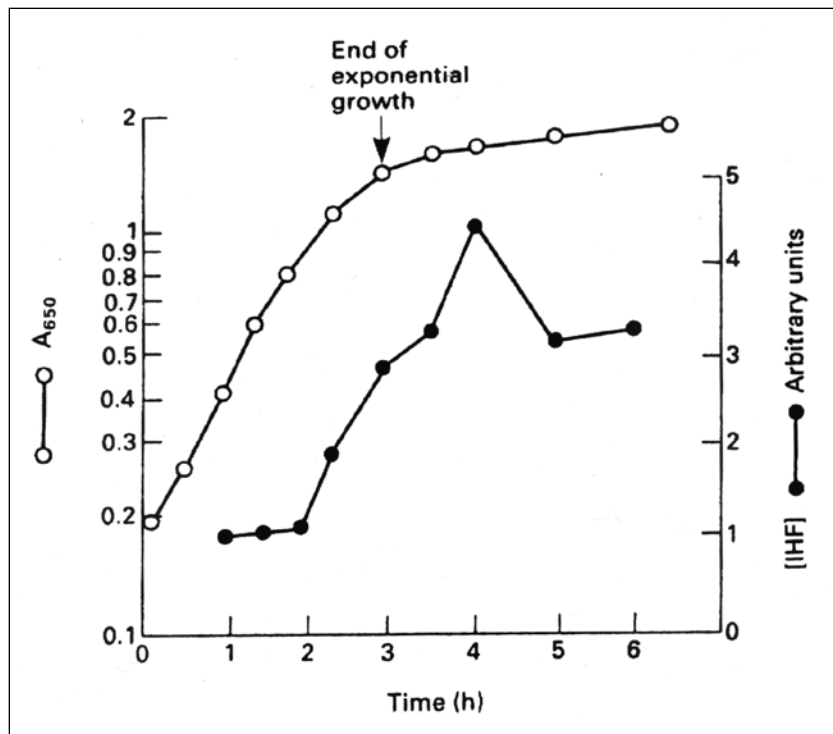


K



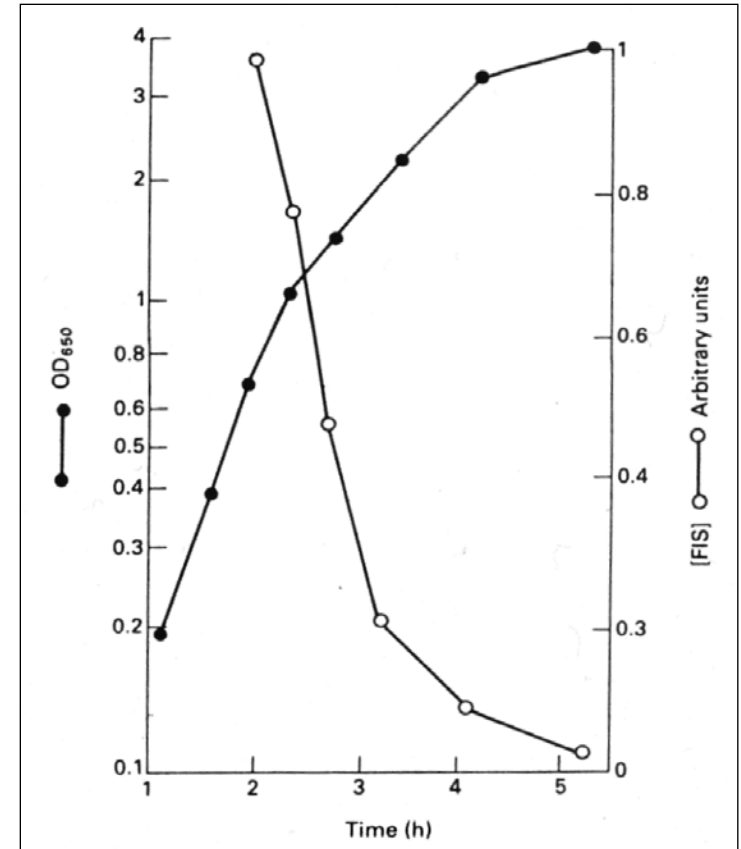






La proteina FIS è invece molto abbondante all'inizio della fase esponenziale

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

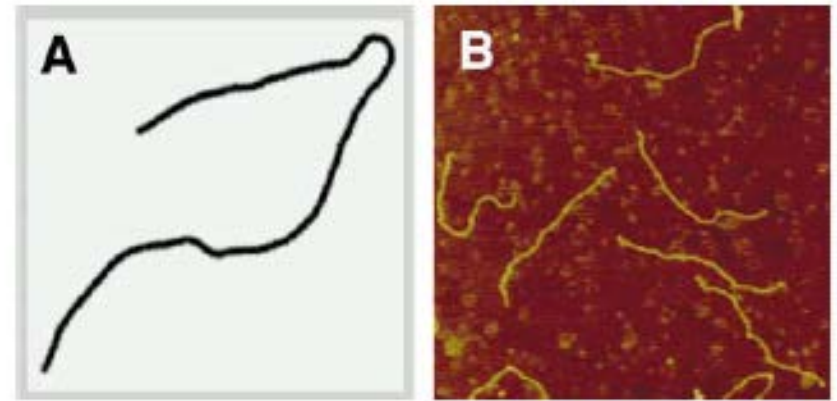


# H-NS

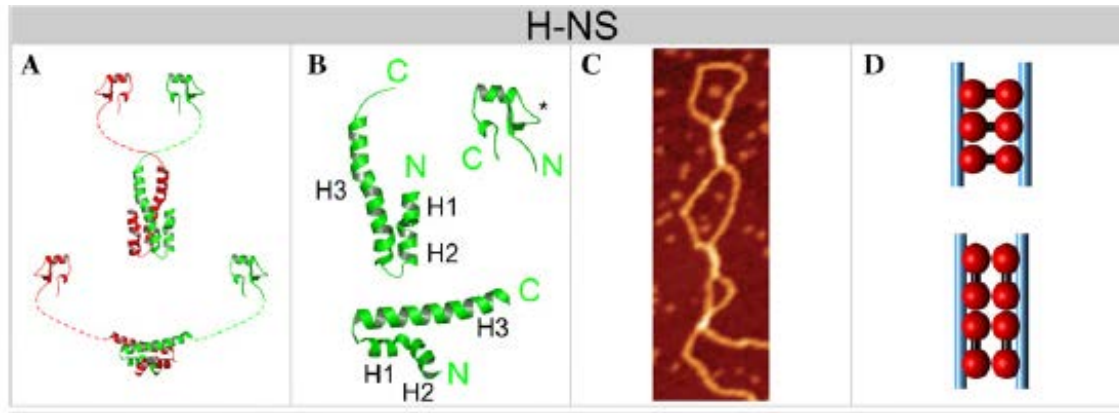
(Histone-like Nucleoid Structuring protein)

- 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



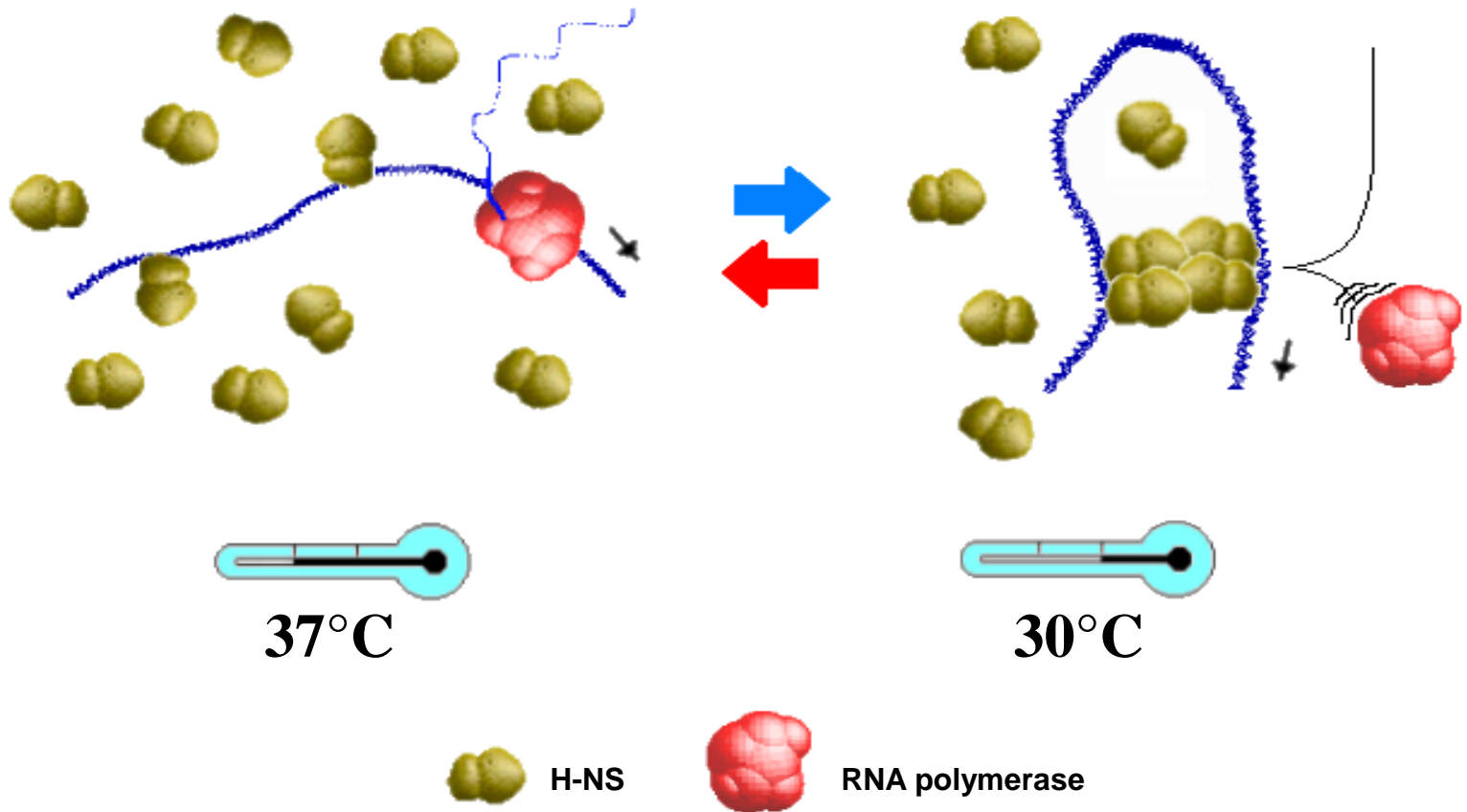
**H-NS viene considerata una proteina in grado di formare ponti**

- 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

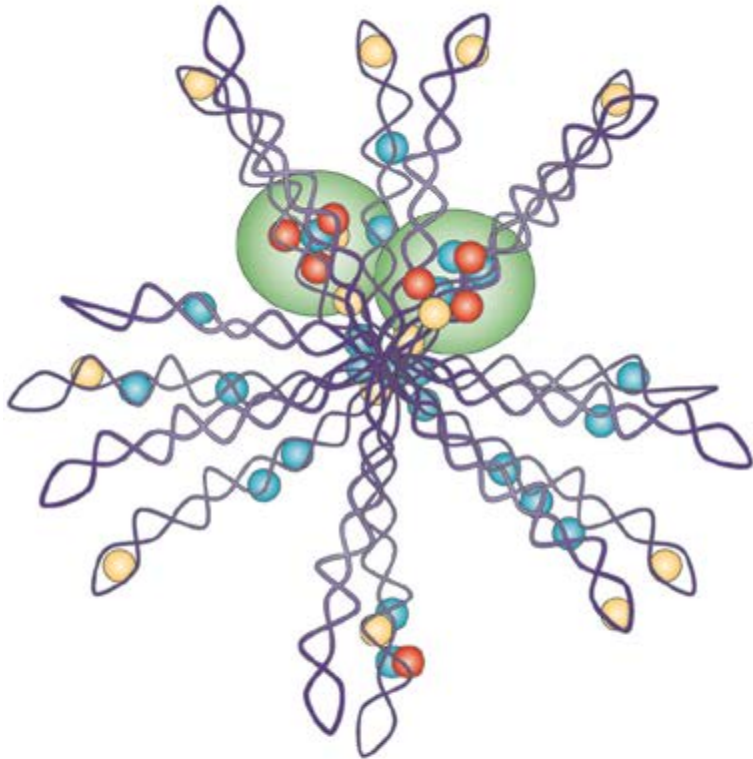
Altre proteine NAP che formano ponti su LRP e MuKB

# Temperature-dependent *virF* expression

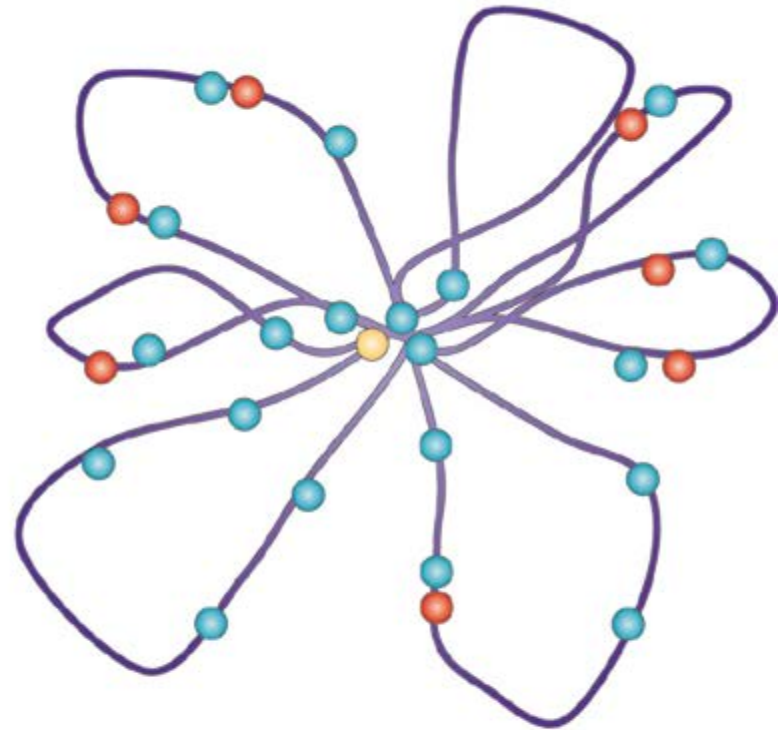
Working model

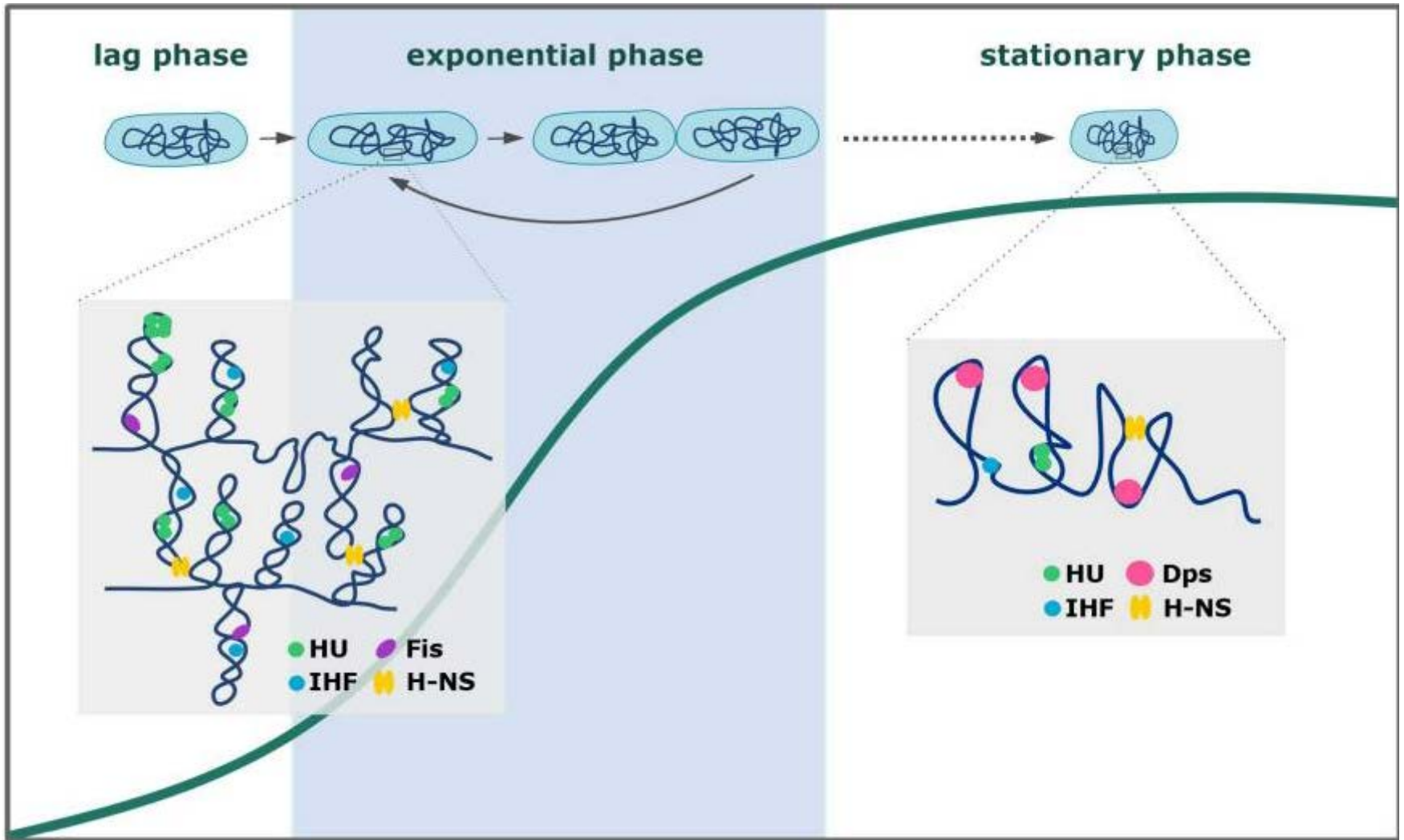


**a Exponential phase of growth**



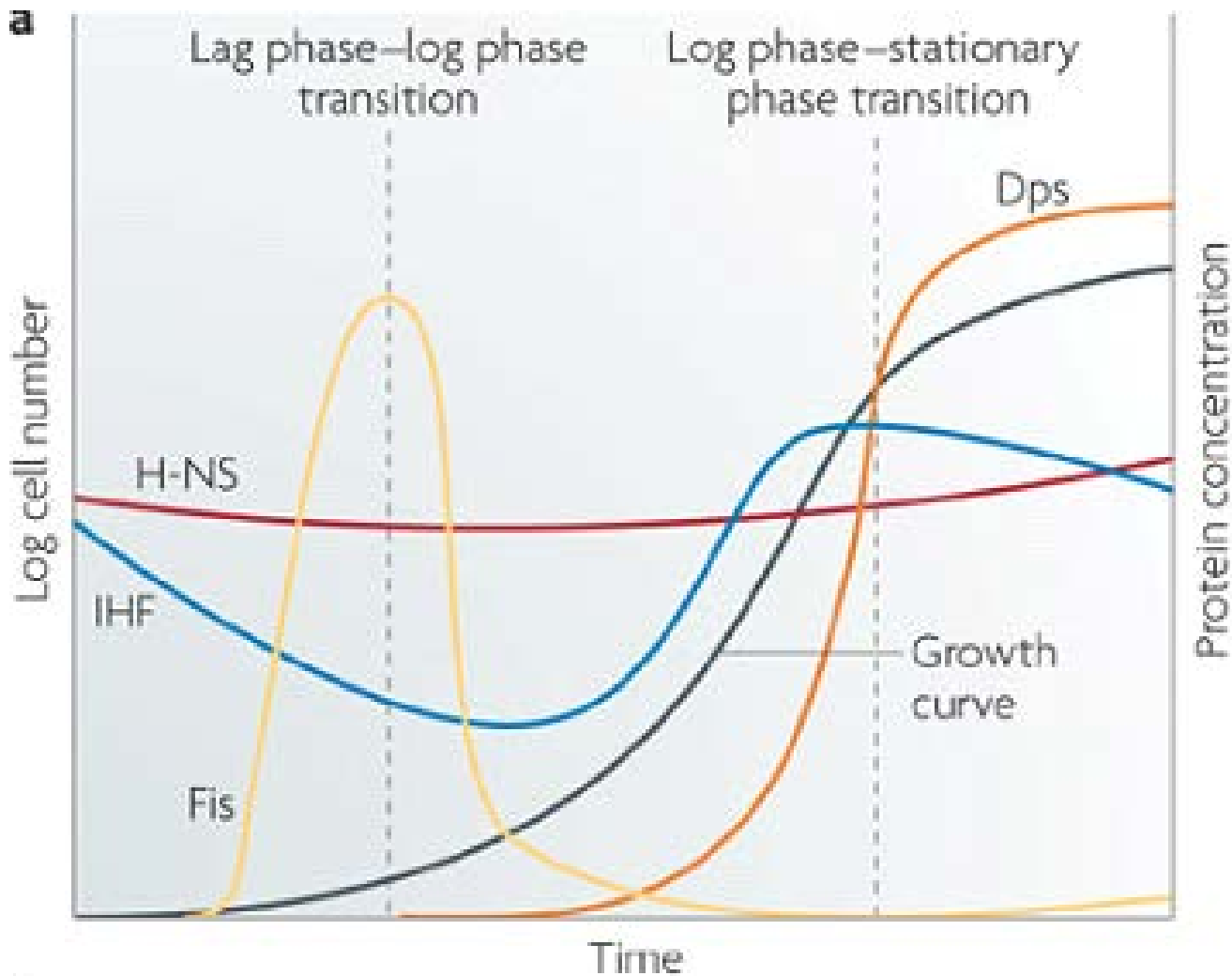
**b Stationary phase of growth**





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

## La proteina Dps ( Dna Binding protein from starved cells)

forma un complesso costituito da 12 monomeri di 19 KDa

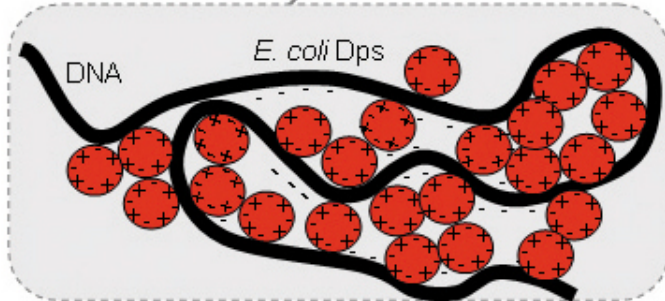
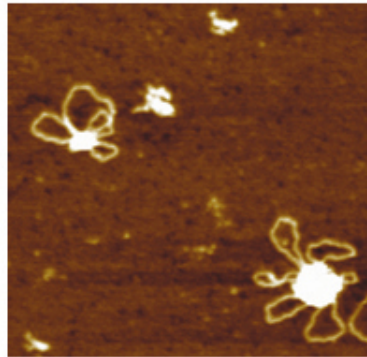
Il complesso Dps contiene uno ione Fe e rende il DNA resistente allo stress ossidativo

E' presente in alto numero di copie circa 20.000

Si lega al DNA a livello di sequenze non specifiche



DNA looping and condensation



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

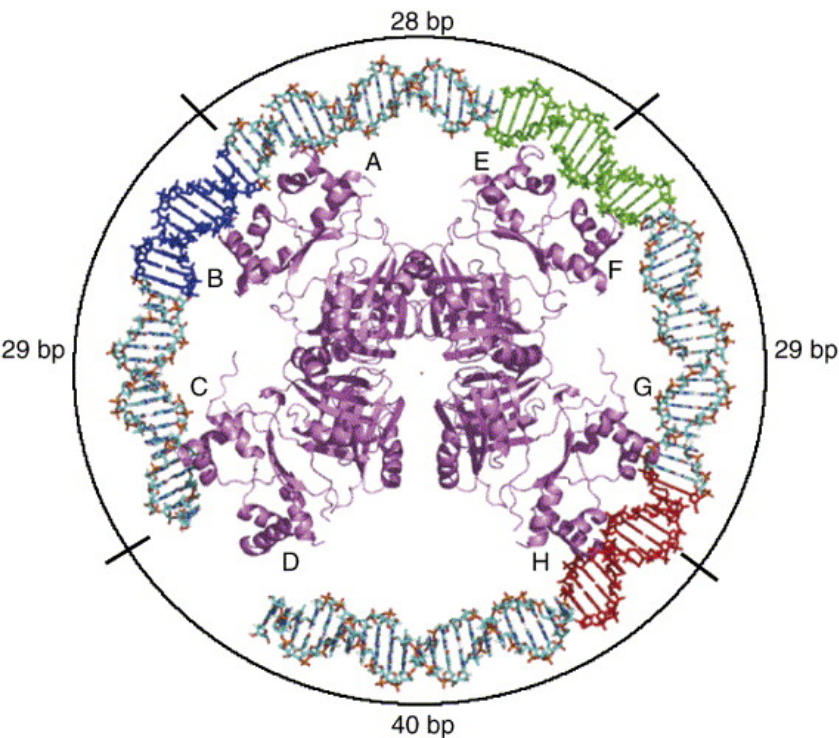
## Lrp Leucine responsive regulatory protein

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.

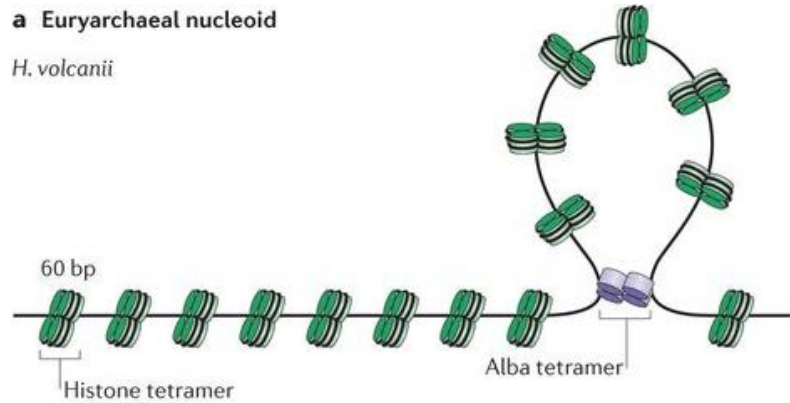
Esiste in diversi stati oligomeric, dimero, ottamero o esadecamero.



Ottamero di LRP

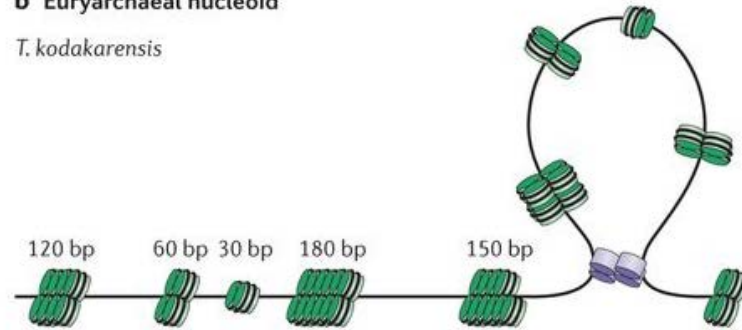
### a Euryarchaeal nucleoid

*H. volcanii*



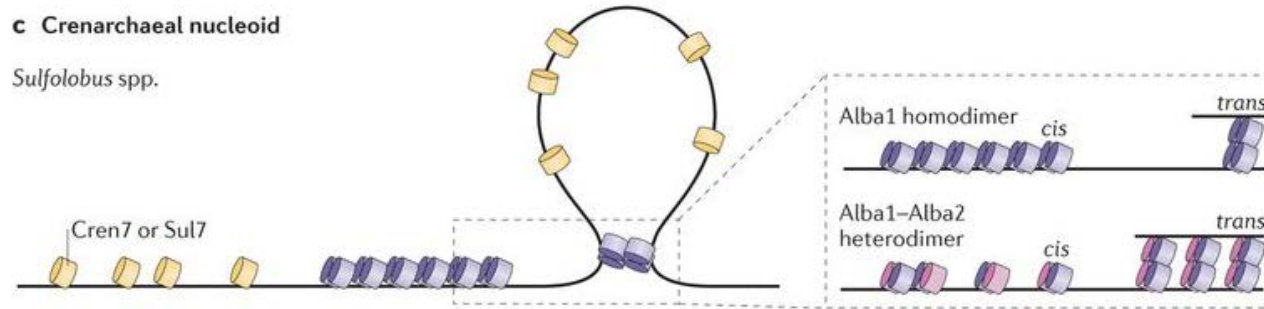
### b Euryarchaeal nucleoid

*T. kodakarensis*



### c Crenarchaeal nucleoid

*Sulfolobus* spp.



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<sup>15</sup>. Alba seems to have an ancient evolutionary history and considerable functional plasticity<sup>16</sup>. Most Alba proteins interact with RNA in addition to binding to double-stranded DNA (dsDNA) and have been suggested to function in RNA metabolism. In euryarchaeal methanogenic archaea, Alba proteins are low-abundance, sequence-specific dsDNA-binding proteins<sup>19</sup>, whereas in crenarchaeal organisms, it was shown that Alba is a highly abundant cellular protein that binds to dsDNA without apparent sequence specificity<sup>15,20</sup>. Alba assembles into dimers, which are homodimeric or heterodimeric depending on whether paralogues are encoded and on their relative amounts.

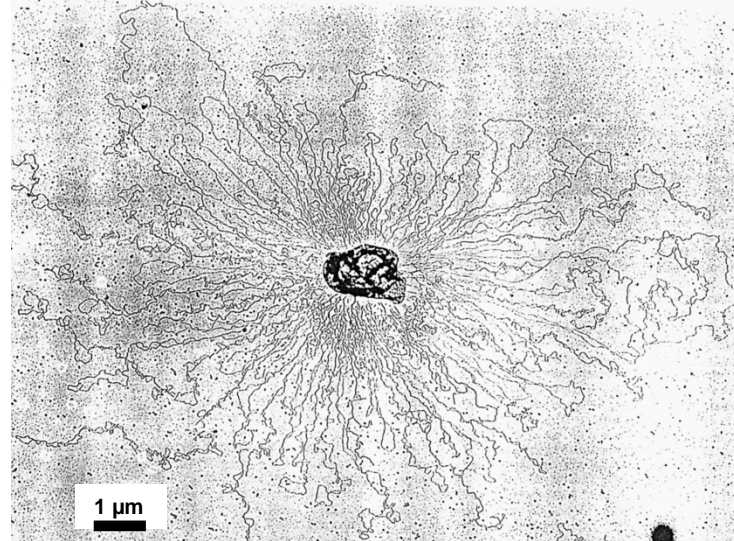
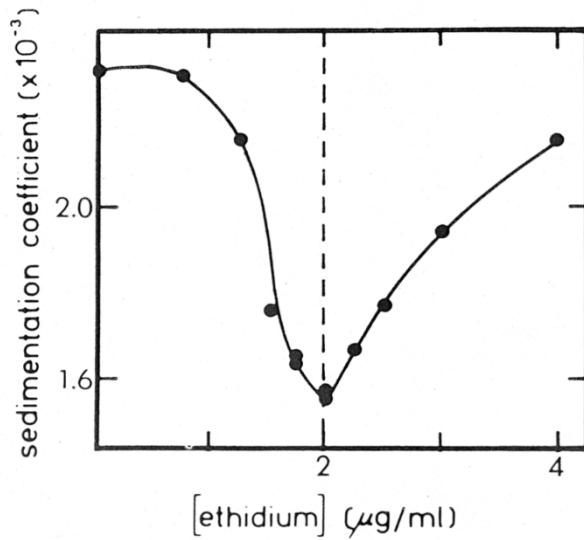
Fig. 1. Architectural properties of nucleoid-associated proteins: DNA bridging proteins. (A–D) H-NS. (A) High resolution models of dimeric H-NS based on the structure of the *Escherichia coli* H-NS C-terminal DNA binding domain (PDB: 1HNH—residues 80–136) (Shindo et al., 1995) and two different structures of the N-terminal dimerization domain (PDB: 1N18—residues 1–46 (*Escherichia coli*) (Bloch et al., 2003) and 1LR1—residues 1–57 (*Salmonella typhimurium*) (Esposito et al., 2002)). (B) Close-up of the monomeric constituents of the H-NS dimer. The positively charged surface (amino acids A80–K96 and T110–T117) (Shindo et al., 1999) of the C-terminal domain is indicated by an asterisk. (C) SFM image of a DNA loop formed as a consequence of DNA duplex bridging. Image size: 110 × 340 nm. (D) Low resolution models for DNA duplex bridging mediated by H-NS. (E–H) SMC/MukB. (E) High resolution model of closed dimeric SMC based on the structure of the *Pyrococcus furiosus* SMC interacting isolated head domains (PDB: 1XEX) (Lammens et al., 2004). (F) Close-up of the monomeric SMC head domain. (G) SFM image of *Bacillus subtilis* SMC that forms a rosette-like structure presumably by joining the head domains (courtesy of Peter Graumann) (Mascarenhas et al., 2005). Image size: 170 × 150 nm. (H) Low resolution models for DNA duplex linking due to gathering or trapping by SMC. (I–L) Lrp. (I) Structure of octameric Lrp from *Pyrococcus furiosus* (PDB: 1H1G) (Leonard et al., 2001). (J) Close-up of a monomeric Lrp subunit (within an Lrp octameric context). (K) EM image of the *Bacillus subtilis* LrpC–DNA complex (Beloin et al., 2003) (courtesy of Eric Le Cam). Image size: 160 × 120 nm. (L) Low resolution model for DNA duplex bridging and wrapping mediated by Lrp.

Fig. 2. Architectural properties of nucleoid-associated proteins: DNA bending proteins. (A–G) IHF/HU. (A) Structure of an IHF–DNA complex (PDB: 1IHF) (Rice et al., 1996). (B) Close-up of a monomeric IHF subunit. (C) SFM image of an IHF–DNA complex (Dame et al., 2005b). (D) Structure of an HU–DNA complex (PDB: 1P51) (Swinger et al., 2003). It should be noted that the DNA substrate used for the HU–DNA co-crystals was pre-distorted and that the observed bend does not necessarily reflect the extent of bending induced in native DNA. (E) Close-up of a monomeric HU subunit. (F) SFM images of HU–DNA complexes. HU bends (top) DNA at low concentrations and induces the formation of rigid filaments (bottom) at high HU concentrations (van Noort et al., 2004). Image size: 200 × 200 nm (top)/70 × 70 nm (bottom). (G) Low resolution model for DNA compaction by the binding of multiple HU/IHF molecules and for the formation of HU–DNA filaments. A filamentous structure of this kind has also been proposed to occur at high IHF/DNA ratio (Dame, 2005). (H–K) Fis. (H) High resolution model of a Fis–DNA complex (Pan et al., 1996) (courtesy of Reid Johnson). (I) Close-up of a monomeric Fis subunit. (J) SFM image of Fis–DNA complexes with Fis bound at the nodes of supercoiled pUC18 plasmids (courtesy of Georgi Muskhelishvili) (Schneider et al., 2001). Image size: 700 × 500 nm. (K) Low resolution model for node formation due to Fis–Fis interactions.

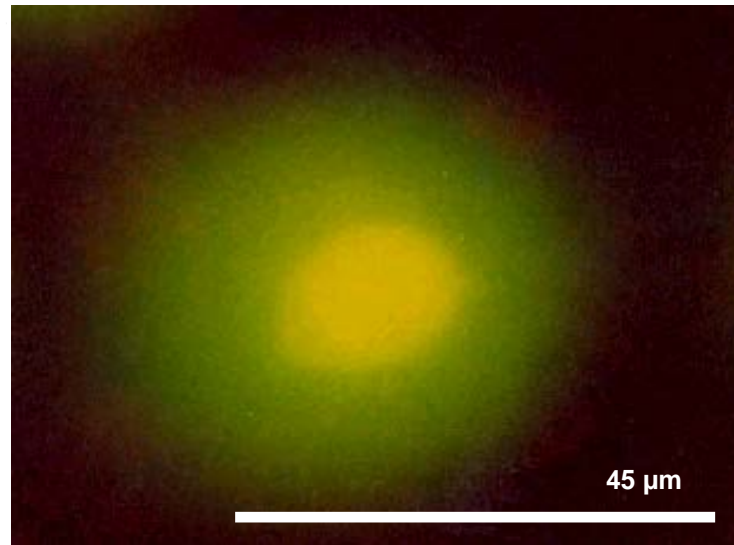
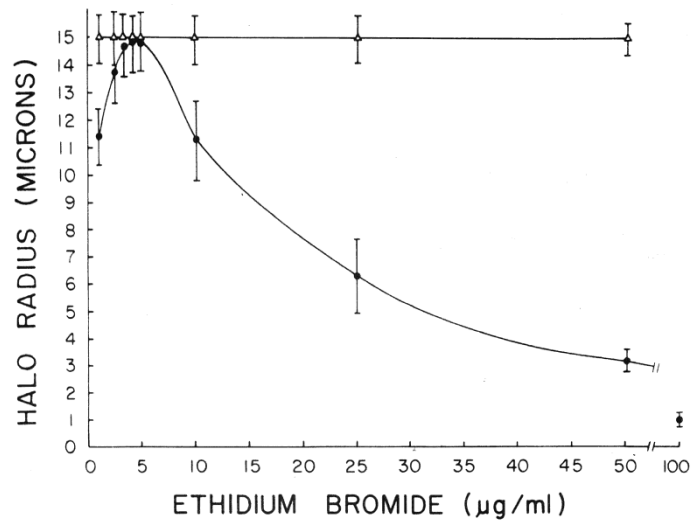


# Confronto tra l'organizzazione ad anse ...

... nel nucleo batterico ...



... e nel nucleo eucariote





# Proteine del nucleotide come regolatori globali

