GENOME ORGANIZATION IN PROKARYOTES

Prokaryotes and Eukaryotes genome

Prokaryotes	**Eukaryotes**
Single cell	Single or multi cell
No nucleus	Nucleus
One piece of circular DNA	Chromosomes
No mRNA post transcriptional modification	Exons/Introns splicing

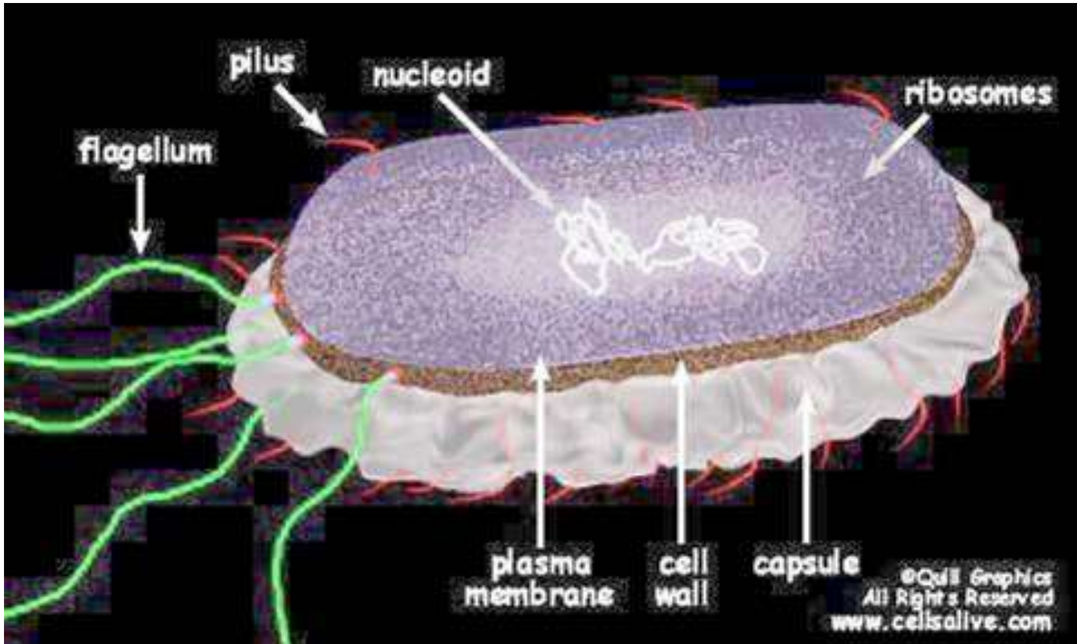
Prokaryotes

- The genome of *E. coli* contains amount of $4X10^6$ base pairs

- Lacks a membrane-bound nucleus.
  - Circular DNA and supercoiled domain, **nucleoid**.

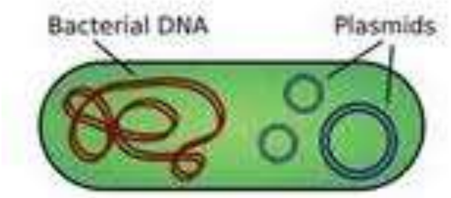
- > 90% of DNA encode protein

- Histones not present



- Prokaryotic genomes generally contain one large circular piece of DNA referred to as a “Chromosome" (not a true chromosome in the eukaryotic sense).

- Some bacteria have linear "chromosomes".



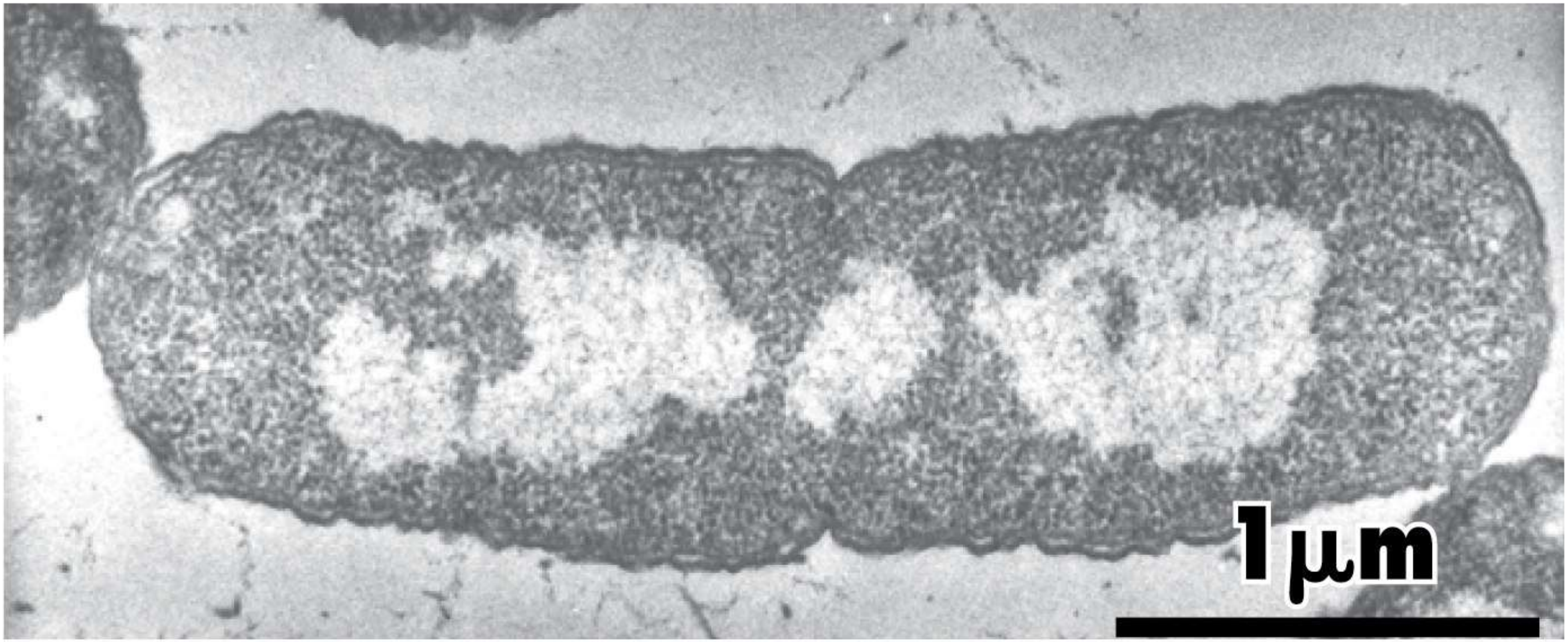
- Many bacteria have small circular DNA structures called plasmids which can be swapped between neighbors and across bacterial species.

- Some types of plasmid are able to integrate into the main genome, but others are thought to be permanently independent.

- Carry genes - usually not present in the main chromosome – mostly non-essential.

Type of plasmid	Gene functions	Examples
Resistance	Antibiotic resistance	Rbk of *Escherichia coli* and other bacteria
Fertility	Conjugation and DNA transfer between bacteria	F of *E. coli*
Killer	Synthesis of toxins that kill other bacteria	Col of *E. coli*, for colicin production
Degradative	Enzymes for metabolism of unusual molecules	TOL of *Pseudomonas putida*, for toluene metabolism
Virulence	Pathogenicity	Ti of *Agrobacterium tumefaciens*, conferring the ability to cause crown gall disease on dicotyledonous plants

The *Escherichia coli* nucleoid:
Transmission electron micrograph of dividing cell

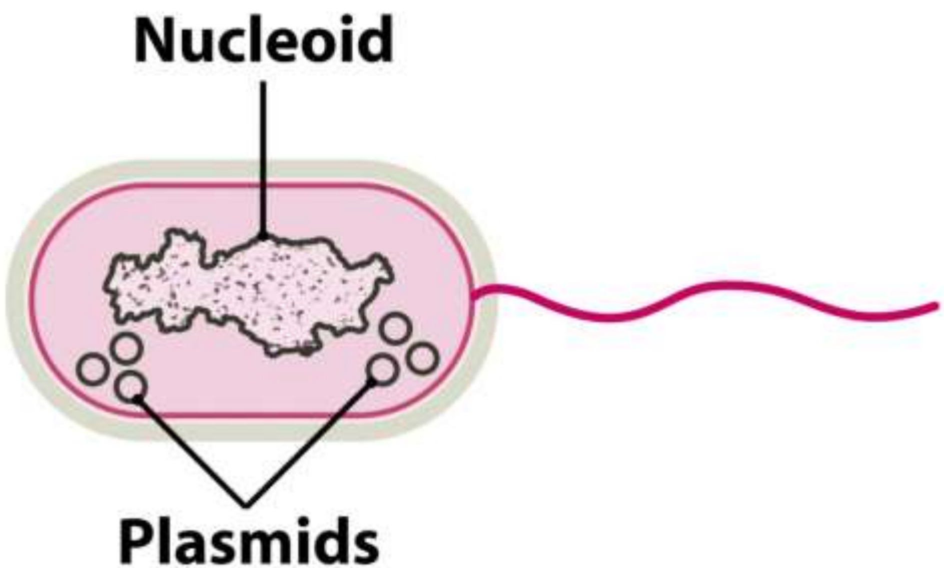


Plasmid

- The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952.

- A **plasmid** is separate from, and can replicate independently of, the chromosomal DNA.

- Plasmid size varies from 1 to over 1,000 (kbp).



Species	Genome organization DNA molecules	Size (Mb)	Number of genes
Escherichia coli K12	One circular molecule	4.639	4405
Vibrio cholerae El Tor N16961	Two circular molecules		
	Main chromosome	2.961	2770
	Megaplasmid	1.073	1115
Deinococcus radiodurans R1	Four circular molecules		
	Chromosome 1	2.649	2633
	Chromosome 2	0.412	369
	Megaplasmid	0.177	145
	Plasmid	0.046	40
Borrelia burgdorferi B31	Seven or eight circular molecules, eleven linear molecules		
	Linear chromosome	0.911	853
	Circular plasmid cp9	0.009	12
	Circular plasmid cp26	0.026	29
	Circular plasmid cp32*	0.032	Not known
	Linear plasmid lp17	0.017	25
	Linear plasmid lp25	0.024	32
	Linear plasmid lp28-1	0.027	32
	Linear plasmid lp28-2	0.030	34
	Linear plasmid lp28-3	0.029	41
	Linear plasmid lp28-4	0.027	43
	Linear plasmid lp36	0.037	54
	Linear plasmid lp38	0.039	52
	Linear plasmid lp54	0.054	76
	Linear plasmid lp56	0.056	Not known

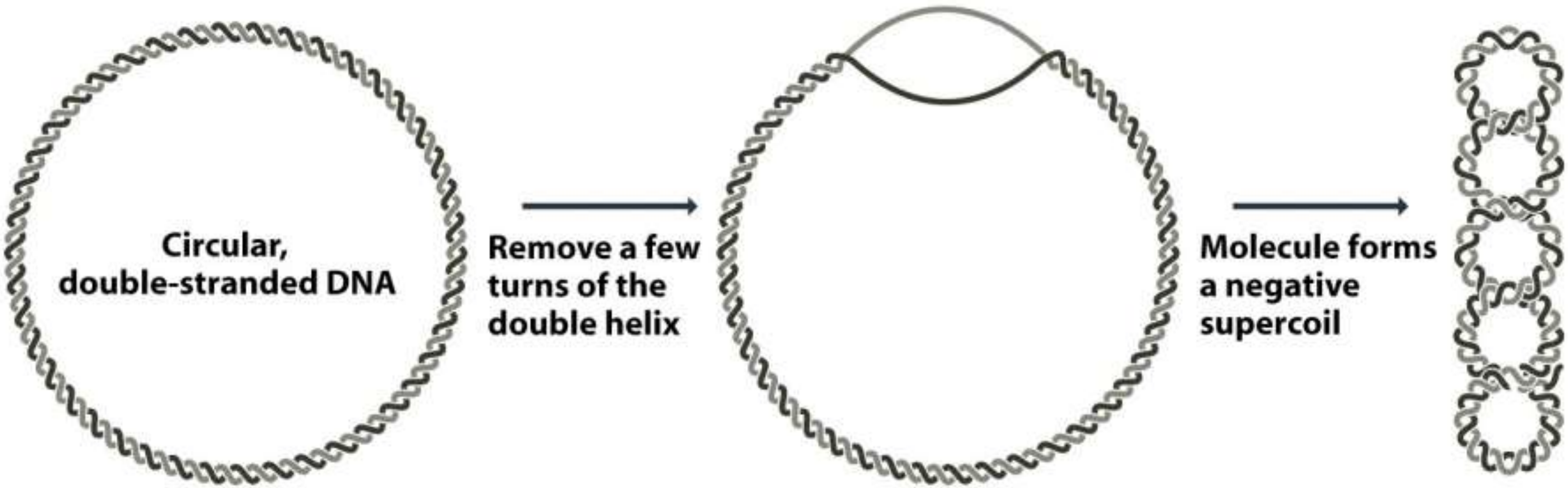
E. coli chromosome

- Circular & supercoiled
- Circumference of 1.6 mm
- *E. coli* cell is just 1.0 - 2.0 µm

Supercoiling:

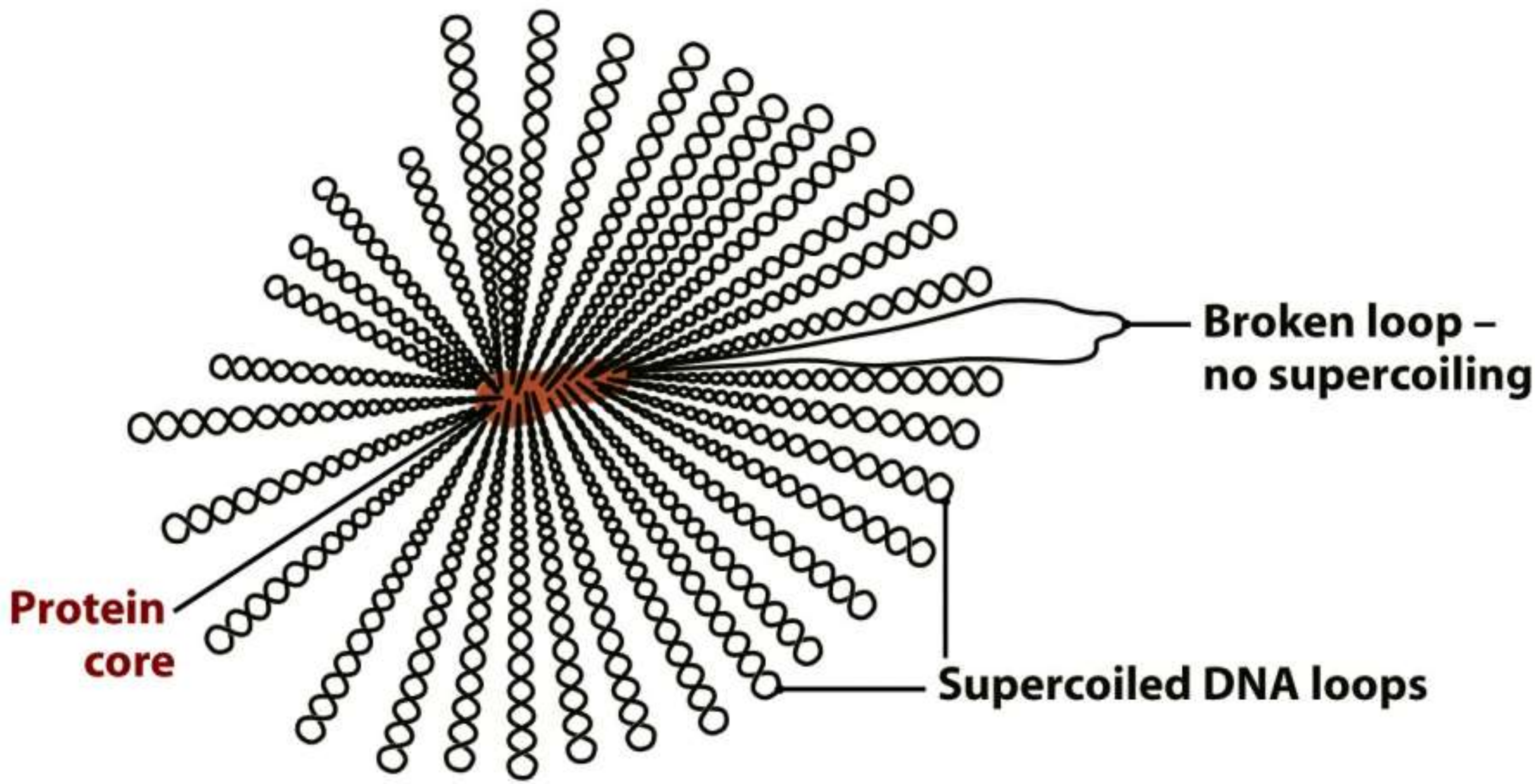
- Additional turns are introduced into the DNA double helix (**positive supercoiling**) or
- If turns are removed (**negative supercoiling**)
- Ideal way to package a circular molecule into a small space.
- Generated and controlled by DNA gyrase and DNA topoisomerase I.

Supercoiling:
Underwinding a circular, doublestranded DNA molecule results in negative supercoiling.



E. coli chromosome

- Studies of isolated nucleoids - *E. coli* DNA molecule does not have unlimited freedom to rotate once a break is introduced.
- Bacterial DNA is attached to proteins that restrict its ability to relax.
- Break a single site results in loss of supercoiling from only a small segment of the molecule
- Proved by Experiments - trimethylpsoralen to distinguish between supercoiled and relaxed DNA.

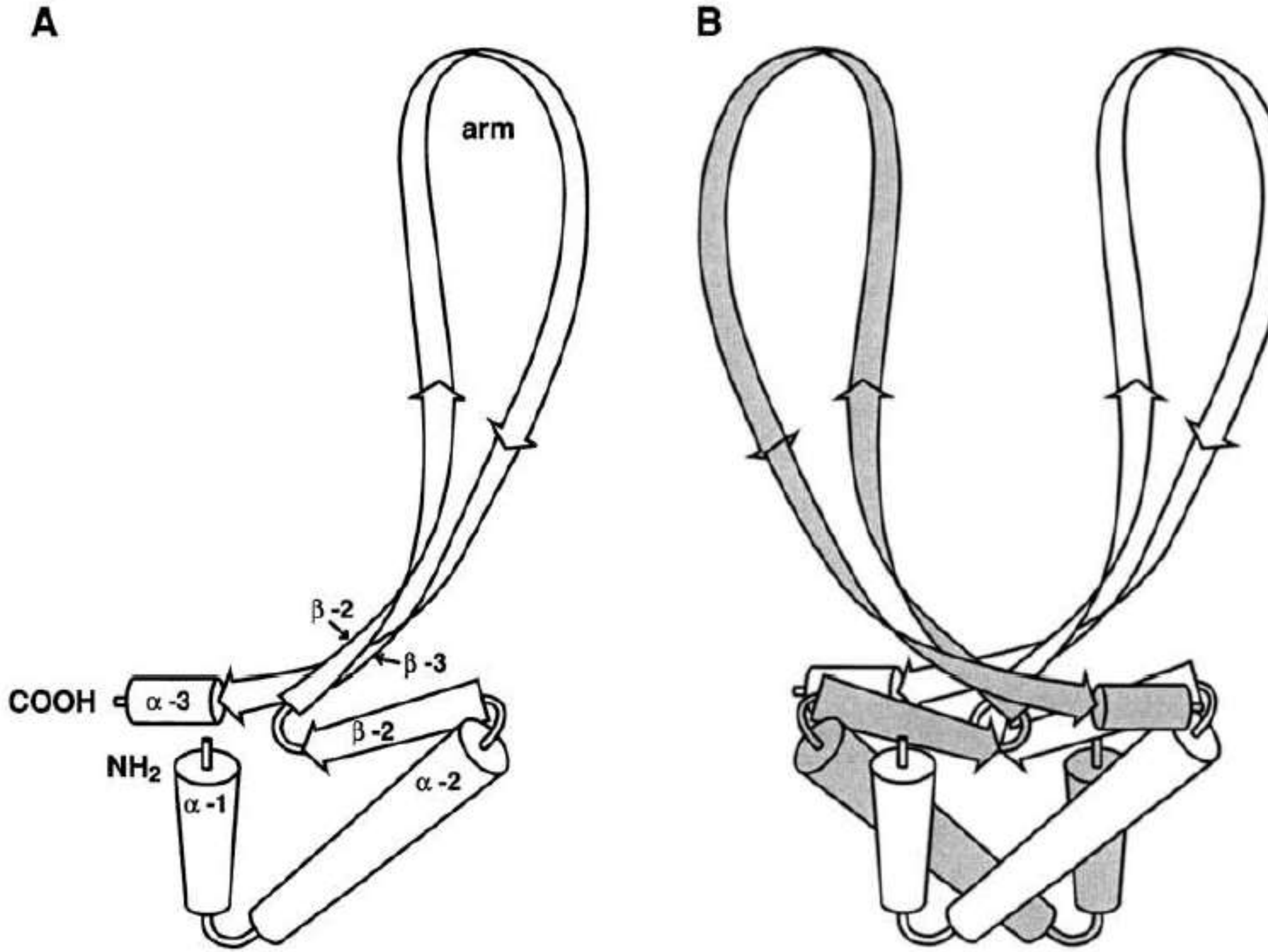

Broken loop –
no supercoiling
Protein
core
Supercoiled DNA loops

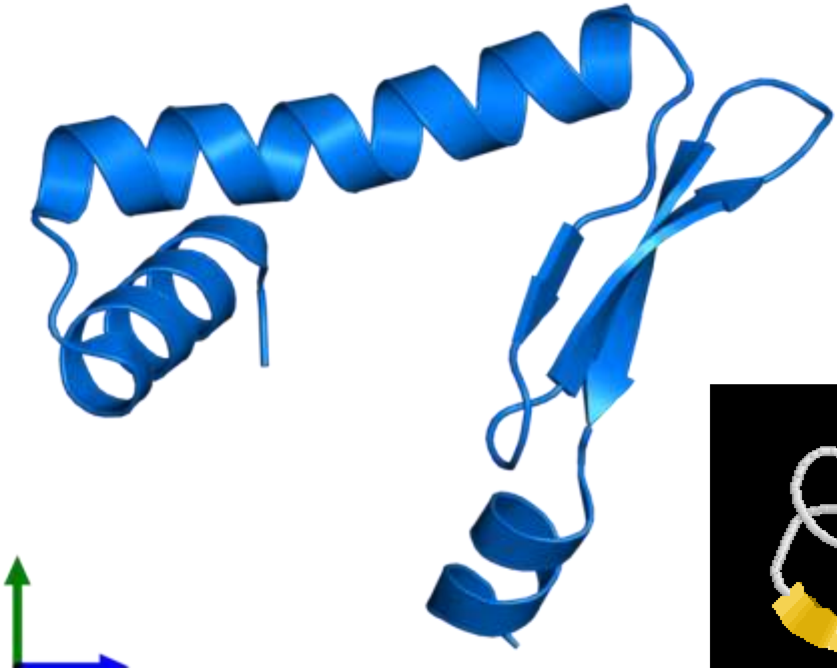
E. coli chromosome

- *E. coli* DNA attached to a protein core from which 40–50 supercoiled loops (~100kbp each) radiate out into the cell.
- Protein component of the nucleoid includes DNA gyrase and DNA topoisomerase I.
- Most abundant packaging proteins is HU – di/tetramer – 60bp DNA
- 60000 HU in single cell of EC.
- Other H-NS - may be involved in chromosome organization - act alone / interact with the HU protein to organize the chromosome *in vivo*.

HU PROTEIN

- Small basic protein of 18,000 daltons.
- Exists as a heterodimer of two nearly identical subunits (HU-1 and HU-2).
- Utilize coiling as a mechanism of DNA compaction.
- Wrapping DNA around HU proteins in prokaryotes or around histones in eukaryotes influences the level of DNA supercoiling and the amount of free energy potentially available for biological reactions.
- HU binds weakly to DNA - rapidly dissociable binding – advantageous - most of the genome must be accessible.
- HU Bends DNA – difficult to bent DNA fragments in absence of HU.


A
B
arm
β -2
β -3
COOH
α -3
β -2
NH2
α -1
α -2



- In Archaea – packaging proteins are much more similar to histones – tetramer - ~80bp DNA.

- Linear DNA
  - *Borrelia burgdorferi*
  - *Streptomyces coelicolor*
  - *Agrobacterium tumefaciens*
  - These chromosomes require terminal structures equivalent to the telomeres.

- *Vibrio cholerae* - two circular DNA molecules - one of 2.96 Mb and the other of 1.07 Mb - most of the genes for the central cellular activities are located on larger molecule

- **Integron**—a set of genes and other DNA sequences that enable plasmids to capture genes from bacteriophages and other plasmids