DNA unlinking in bacteria

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DNA in chromosomes is organized into several levels of compaction

- Total length of human DNA – 1 meter
- Diameter of a human cell nucleus – 10 μm

(Random coil ~ 150 μm)

How does the cell package DNA into its tiny confines?
Chromatin is organized into several levels of compaction

1. Wrapping around nucleosomes
   packing ratio ~7: ~160bp/6nm
2. Nucleosome compaction into 30 nm structure
   packing ratio ~40: ~1.2kb/11nm
3. Radial loops ~100 kb
4. Association of anchoring elements (or chromosome scaffold) into chromosome axis

In bacteria: nucleosomes – No; supercoiling & looping - Yes
Chromatin structure is highly dynamic

Chromosome condensation during cell division ensures faithful segregation of genetic material
Histone depleted metaphase chromosomes retain their shape.
Bacterial chromosome is organized into ~100 supercoiled loops.
**Lk** – Linking Number - cannot be changed unless DNA is broken

\[ \Delta Lk = Lk = Lk_0 \] – Linking Number Deficit – a measure of supercoiling

\[ \sigma = \Delta Lk / Lk_0 \] – Degree of Supercoiling

Inside the cell, \( \sigma = -0.04 \) to \(-0.08 \)
Gel mobility of supercoiled DNA
Topological challenges to DNA replication

Topological links must be removed:

- **Fast**
  - to support replication
  - to maintain supercoiling elsewhere

- **Completely**
  - Catenation between daughter chromosomes results in ds breaks
Topoisomerases untangle DNA

Chris Ullsperger
Measure of DNA compactness: number of links between sister chromosomes
Computer modeling can be used to estimate length dependence of catenation and catenation between two supercoiled DNAs

\[ P_{\text{cat}} = B \cdot c \]

DNA length dependence:
\[ B \sim L^{1.7} \]

*Alex Vologodskii, unpublished*
Topoisomerases simplify topological equilibrium in DNA
Topo IV is an efficient decatenase
Topo-2s simplify all aspects of topological equilibrium.
Correlation between preferential decatenation, unknotting and relaxation
Maxwell’s Demon model for topology recognition

Typical (frequent) DNA conformations

Atypical (rare) DNA conformations

Work
Topo-2s hydrolyze two ATPs per strand transport

First ATP is hydrolyzed fast; the second ATP hydrolysis is slow.

Non-hydrolyzable ATP analogs promote equilibrium catenation

Does Topo IV hydrolyze only 2 ATPs per strand transport?
Single turnover kinetics: vanadate, phosphate analog, traps Topo IV in ADP-bound form

Burst phase kinetics: first enzyme turnover is faster than the rest

Open clamp (active)  Closed clamp (inactive)

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\[
\begin{align*}
E & \rightarrow E \cdot ATP \\
E \cdot ATP & \rightarrow E^* \cdot ADP \cdot P_i \\
E \cdot ADP & \rightarrow E \cdot ADP \cdot V_i
\end{align*}
\]
ATP, but not AMP-PNP supports selective transport

Two roles of ATP hydrolysis:
- topology recognition (before strand transport)
- reactivation enzyme (after strand transport)
Length dependence of Topo IV efficiency supports “local” models of topology recognition.
Expected number of links between sister *E. coli* chromosomes

<table>
<thead>
<tr>
<th>Relaxed random coil</th>
<th>Relaxed loops</th>
<th>Supercoiled; equilibrium</th>
<th>Supercoiled; steady state</th>
<th>Chromosome missegregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^6</td>
<td>10^4</td>
<td>10^1</td>
<td>10^0</td>
<td>10^{-5}</td>
</tr>
</tbody>
</table>

Diagram showing the process involving gyrase, topo IV, and condensins.
SMC (Structural Maintenance of Chromosome) proteins:

- Are found in all kingdoms of life
- Have distinctive structure
- Are required for diverse global chromatin functions
- Act in complex with non-SMC subunits
- Alter DNA shape in vitro and in situ
- Require ATP for function and activity

Kimura et al, 1997
Several kinds of SMC complexes

- Chromosome condensation (Condensins): intramolecular DNA condensation
- Chromosome cohesion (cohesins): intermolecular DNA condensation
- Recombinational repair (e.g. Rad50, SbcC): DNA end binding
- Dosage compensation (compensins): intramolecular DNA condensation?

How do SMCs recognize DNA substrates? What determines specificity of reaction?
DNA reshaping by bacterial condensin MukBEF

Inactivation of MukB, MukE or MukF produces the same phenotype: chromosome decondensation and cutting, anucleate cells.
MukB recognizes global DNA shape

Predominant formation of 3- and 5-noded knots suggests solenoidal DNA supercoiling.

Intramolecular condensation + Muk + Topo II + ATP

Condensins + Cohesins

Predominant formation of 3- and 5-noded knots suggests solenoidal DNA supercoiling.
Table of knots with less than 9 minimal crossings

Knots can be classified according to the minimal number of crossings in a projection.

Note *twist* *(circled; step of 1)* and *torus* *(boxed; step of 2)* families of knots
Chiral trefoil knots suggest solenoidal, looped DNA

Topology of more complex knots is also consistent with looped DNA
DNA reshaping by MukB

- MukB stabilizes right handed coils in DNA
- DNA coils are arranged in space - and thereby limit excessive knotting – *protein-DNA filament*?
DNA with modified extremities can be attached to a bead and a surface.

XYZ position of the bead can be followed ±10 nm.
DNA condensation by MukB in real time

Crystallographic DNA length

Buffer

MukB

$F = 0.5 \text{ pN}$
DNA stretching confirms multiprotein MukB-DNA complex
Summary of DNA reshaping by MukB (E.coli) vs. 13S condensin (frogs)

<table>
<thead>
<tr>
<th></th>
<th>13S condensin</th>
<th>MukBEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA knotting</td>
<td>(+)trefoils</td>
<td>(+)trefoils</td>
</tr>
<tr>
<td>Right handed DNA looping</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Superciling</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>ATP dependence of DNA reshaping</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Non-SMC subunits</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Why do we need MukEF?
MukEF inhibits DNA binding by MukB
Summary of DNA reshaping by MukBEF

- MukBEF reshapes DNA in vitro by introducing right-handed loops
- MukB is the DNA reshaping end of MukBEF
- MukEF inhibits MukB

In vitro artifact?
Biochemistry in vivo:
Overproduction of either MukBEF or MukB condenses nucleoids of live cells

Blue – SyproOrange (cells)  Red – DAPI (DNA)

Wang et al., J. Bacteriol. 2006
Overproduction of either B or BEF results in ~3 fold reduction of nucleoid area
MukBEF has additional attachment sites within the cell

MukBEF copurifies with chromatin scaffold

- Only a subset of proteins co-purifies with the Scaffold fraction of nucleoids.
- These proteins are believed to be a part of chromatin scaffold.
- Most of endogenous but not of overproduced MukB is in Scaffold.
- Excess (or absence) of MukEF disrupts association of MukB with scaffold.
Our current view of condensins

- MukBEF is a condensin: in vitro and in vivo
- Right-handed DNA looping is highly conserved between species
- MukB is the DNA reshaping end
- MukEF is needed for chromosome organization and association with chromatin scaffold.
Summary

Chromatin organization in Eukaryota:
- Chromatin scaffold
- Loops
- Nucleosome packing (30-nm fiber)
- Wrapping around nucleosomes

Chromatin organization in Bacteria:
- Chromatin scaffold
- Loops
- DNA supercoiling
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