twisting stress applies to both regions

plectoneme


catenane

Topoisomerases can change the DNA topology

**Bacteria**

- **TOPO Ia**: cuts a ssDNA for $\sigma < 0$
- **Gyrase**: unwinds DNA to maintain $\sigma < 0$
- **Topo IV**: relaxes $\sigma > 0$ and decatenates

**Eukaryot**

- **TOPO Ib**: cuts a ssDNA for $\sigma < 0$ and $\sigma > 0$
- **Topo II**: relaxes $\sigma > 0$ and $\sigma < 0$ and decatenates

The two substrats have different chirality

Topo II handles both but Topo IV strongly prefers (L) substrat

Paradox: Topo IV is known to decatenate!
Topo 1a

Prokaryote

- Agit sur une bulle de dénATuration
- Relaxe le sous-enroulement ($\sigma < 0$)

Topo 1b

Eukaryote

- Relaxe le sous-enroulement ($\sigma < 0$) et le sur-enroulement ($\sigma > 0$)
Topoisomérase Ib.

Friction and torque govern the relaxation of DNA supercoils by eukaryotic topoisomerase IB.
D.A. Koster, V. Croquette, C. Dekker, S. Shuman and N.H. Dekker
Nature (2005), in print...

Topoisomerase

Cleave

Religate

Measure $\Delta L_k$

Supercoil removal

Relaxation de la torsion de la molécule d'ADN.
Topoisomérase Ib.

Signal de Relaxation de la torsion de la molécule d'ADN.
Topoisomérase Ib.

Histogramme du nombre de tours enlevés par la topoisomérase Ib.
Topoisomérase Ib.

Modèle potentiel.
Action of topo II on a DNA molecule

ATP Concentration (20 μM).

Normal concentration of ATP

\[ \text{[Topo 2]} \text{ a few nM} \]
\[ \text{[ATP]} 300 \, \mu M \]

\[ T_{\text{pause}} \gg T_{\text{action}} \rightarrow \text{single molecule} \quad \delta h > 0.5 \, \mu m \rightarrow \text{Topo II is processive} \]
The enzyme velocity depends on [ATP] and F.

\[ V_{\text{max}} = \frac{V_{\text{sat}} [\text{ATP}]}{K_M + [\text{ATP}]} \]

\[ V_{\text{sat}} = 3.6 \pm 0.2 \text{ cycles/sec} \]
\[ K_M = 270 \pm 40 \mu\text{M} \]

\[ V_{\text{max}} = V_0 \exp\left( -\frac{F \Delta}{k_B T} \right) \]
\[ V_0 = 3.4 \text{ c/s} \]
\[ \Delta = 1 \text{ nm} \]

→ The enzymatic speed decreases exponentially with force → Arrhénius process.

→ Same reaction constant \( K_M \) than the one obtained in test tubes.

→ We measure the velocity of active enzymes.
Topo II without ATP

Question: what is the interaction mechanism of Topo II and DNA crossing?
Topo II binds to DNA crossing

The binding prevents the slidding of DNA strands.
Binding and unbinding of a Topo II

- Bille
- F = 1 pN
- ADN
- Topo II
- Surface

Graphs:
1. Extension de l'ADN vs. Temps (s)
2. Decroissance double-exponentielle
   - $P_1 = 0.7$, $\tau_1 = 20$ s
   - $P_2 = 0.3$, $\tau_2 = 260$ s