Topoisomerase II minimizes DNA entanglements by proofreading DNA topology after DNA strand passage

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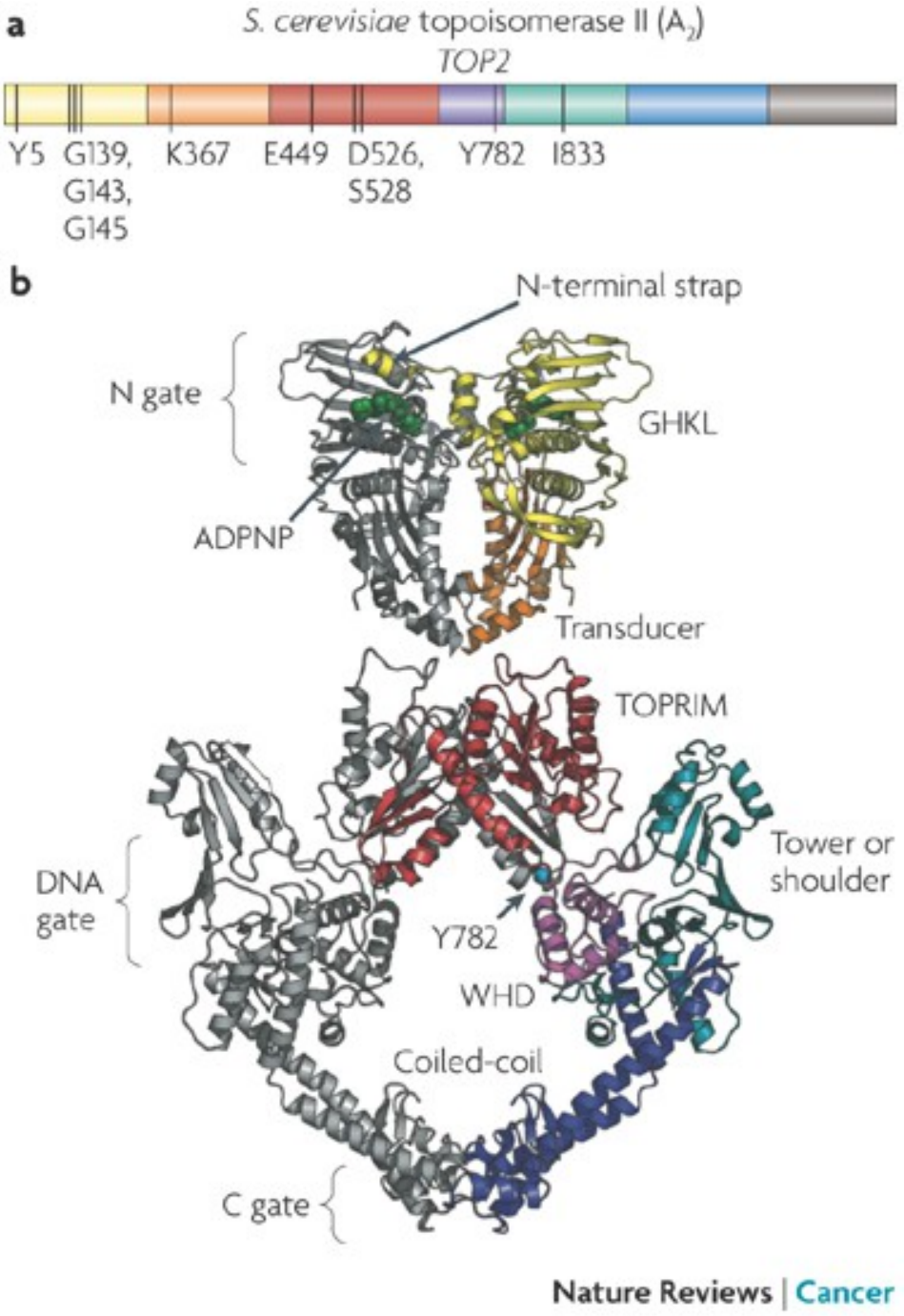
Topoisomerase II

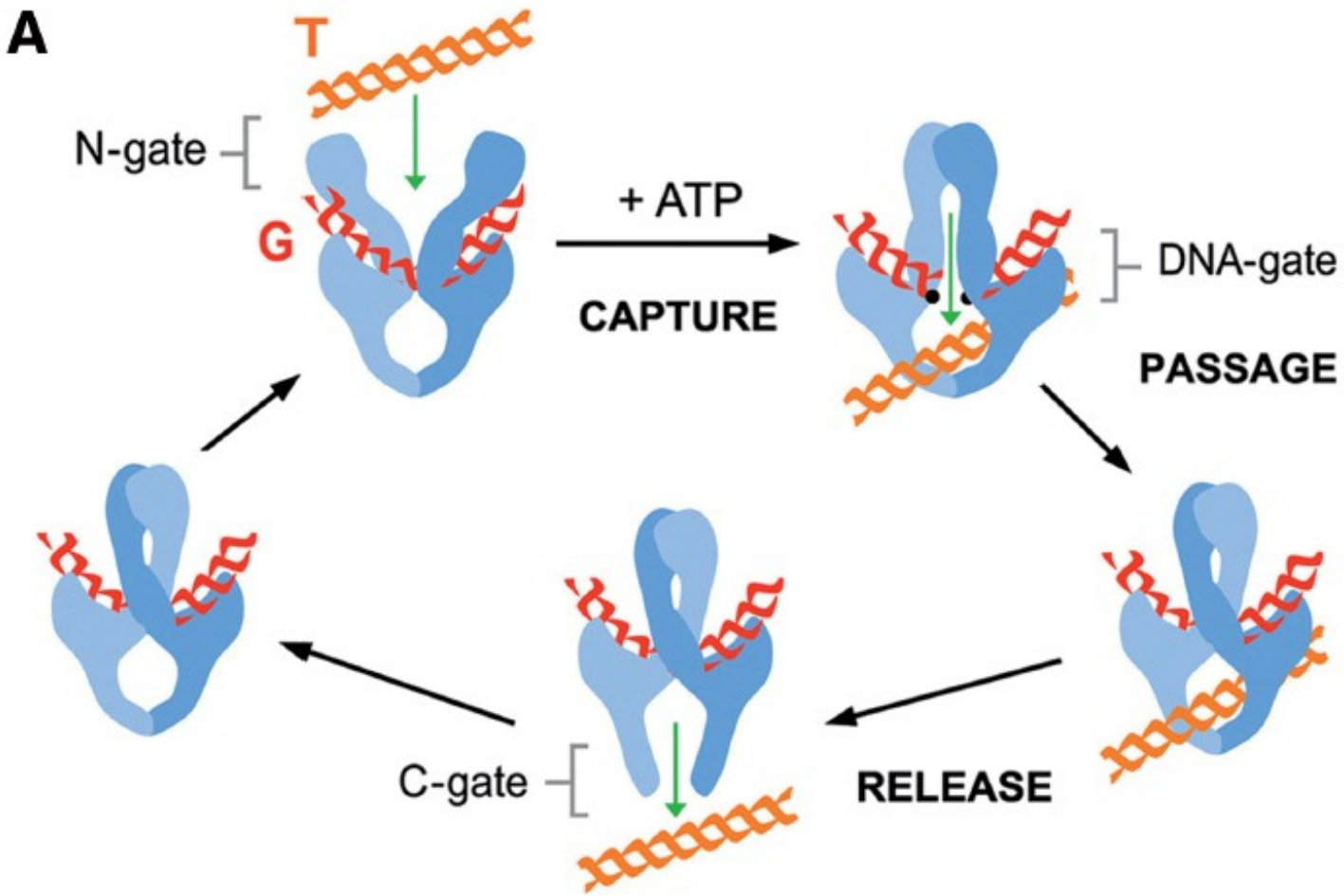
- Transport one DNA double-helix through another
- T – segment (intact)
- G – segment (temporarily broken)
- Reduces the concentration of catenates and supercoils below equilibrium values
- The selection of segments to process is enigmatic



Topoisomerase IIA

- Homodimer
- 4 functional domains
- N-gate – T-segment binding site
- DNA-gate
- C-gate – T-segment release site
- C-terminal domain

Topoisomerase II operation



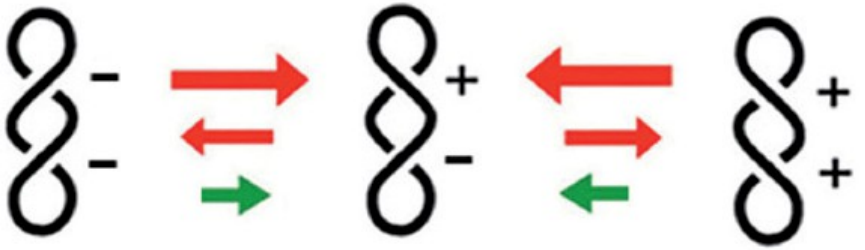
ATP hydrolysis completes with the opening of C-gate, T-segment dissociation and reopening of N-gate

TP2 operation

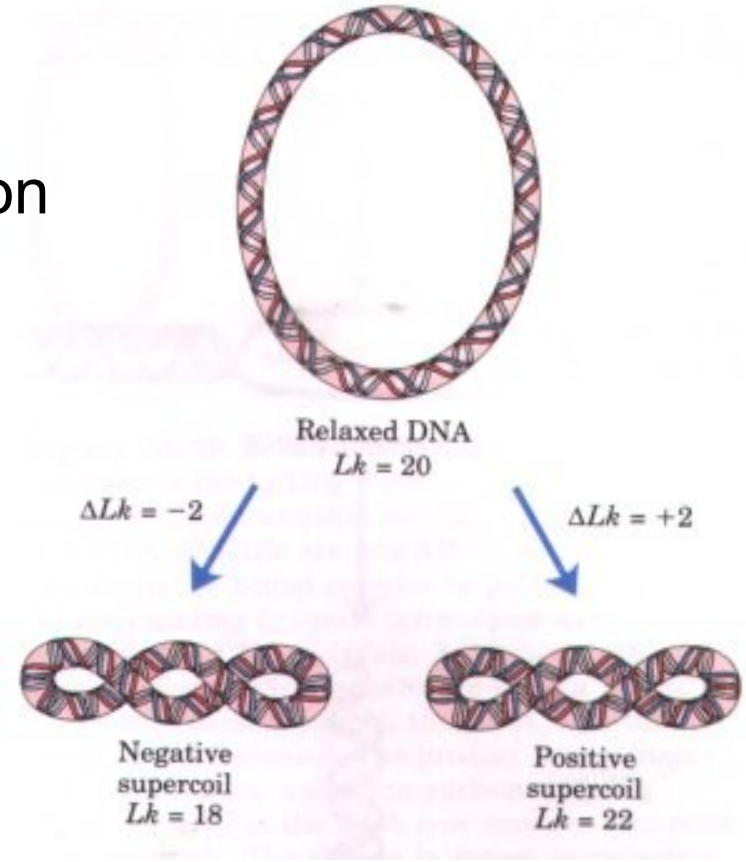
- Rybenkov et al 1997 – Topoisomerase II (T2) is able to produce steady-state fractions of catenane, knot and supercoil crossings that are many times lower than the corresponding equilibrium fractions
- I.e. T2 actively unties knots and avoids creating new ones
- Previously unknown how T-segments are selected
  - Active sliding (shotening of loop)
  - Kinetic proofreading (2 collisions)
  - G-segment hairpin (sharp turn in G-segment)
  - 3-segment interaction (chooses 1 of 2 T-segments)
  - Inter-hooked DNA
  - Proofreading (reversible transfer)

Methods

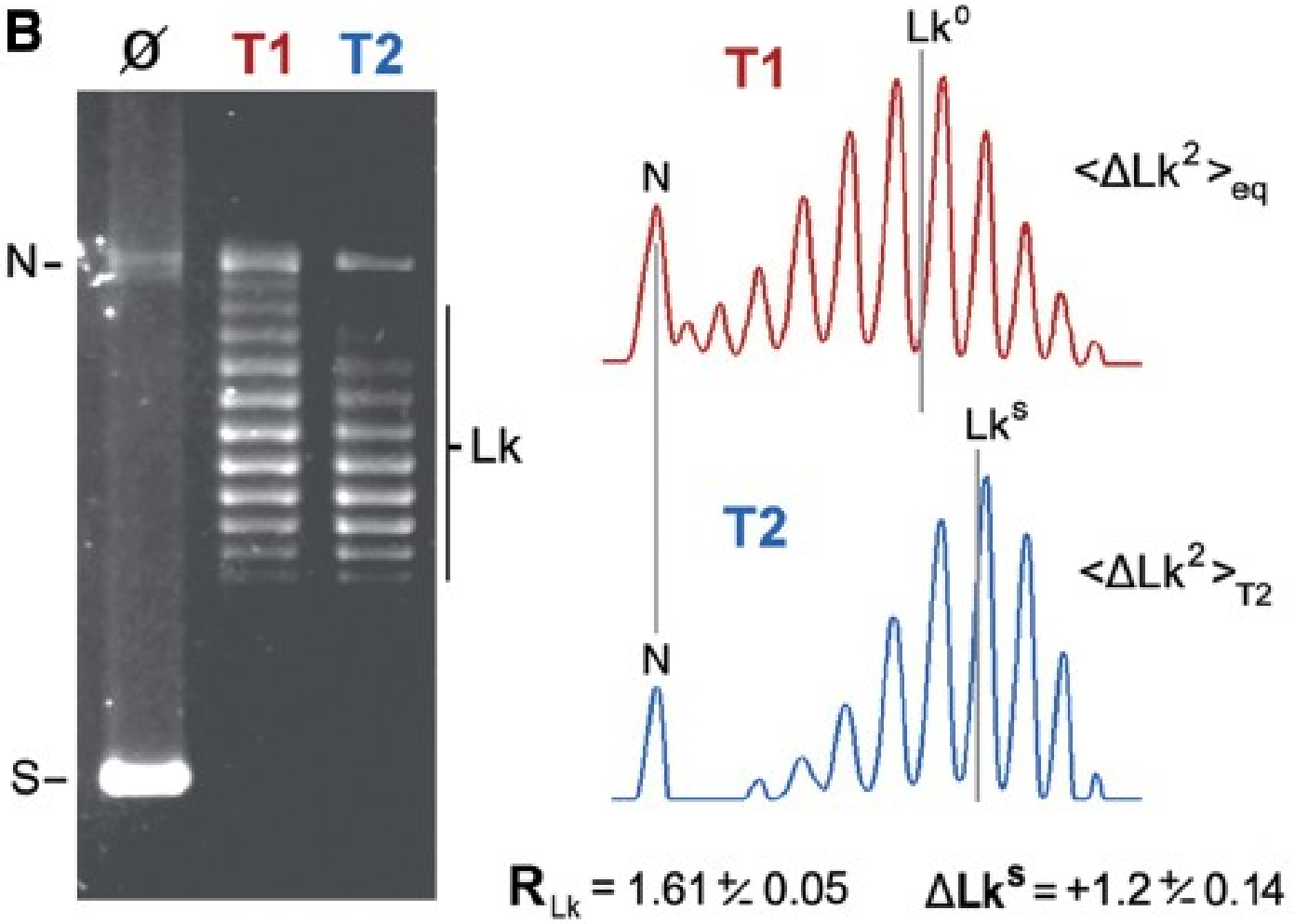
- Yeast T2 topoisomerase
- Supercoiled plasmid DNA
  - Lk topoisomers



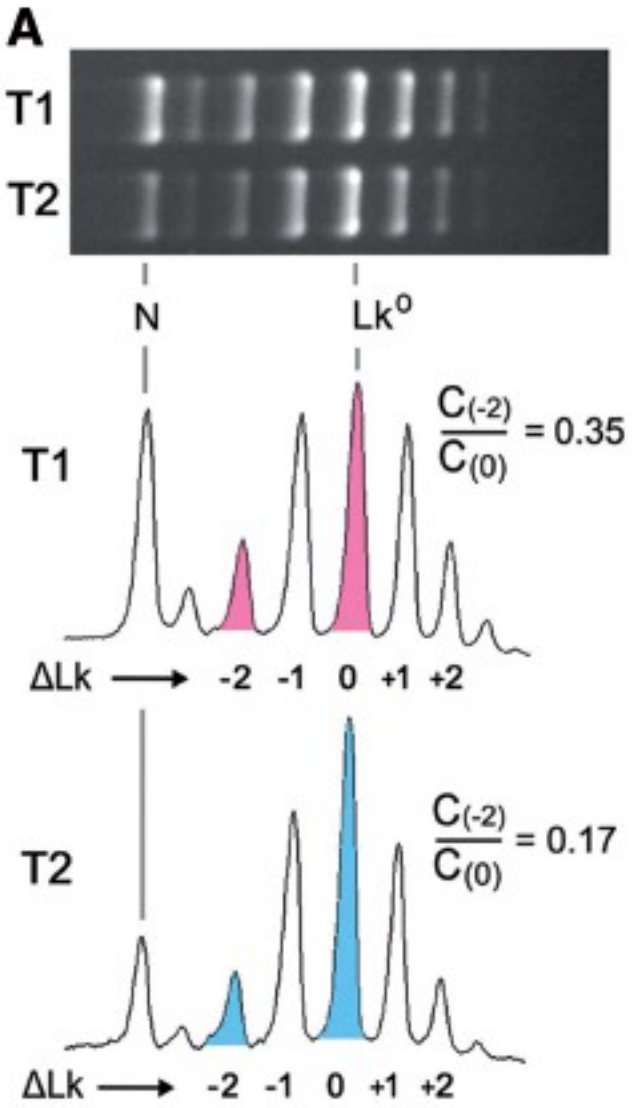
- T1 topoisomerase as control
- Lk^0 – equilibrium distribution center
- Lk^S – center of T2 generated distribution
- $\Delta Lk^S = Lk^S - Lk^0$
- $\langle \Delta Lk^2 \rangle$ variance of Lk distribution
- $R_{Lk} = \langle \Delta lk^2 \rangle_{eq} / \langle \Delta lk^2 \rangle_{T2} \approx 1.6$
- ΔLk^S depended on temperature (~0 at 25°C)

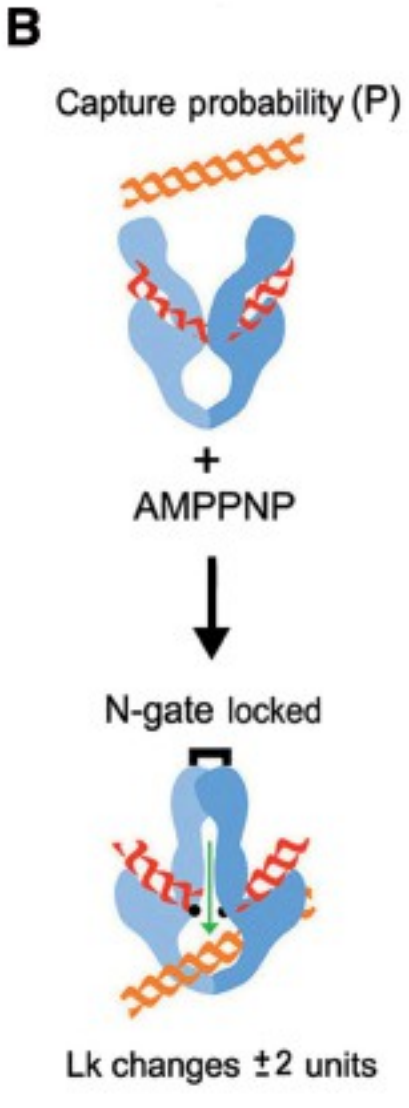


Distributions



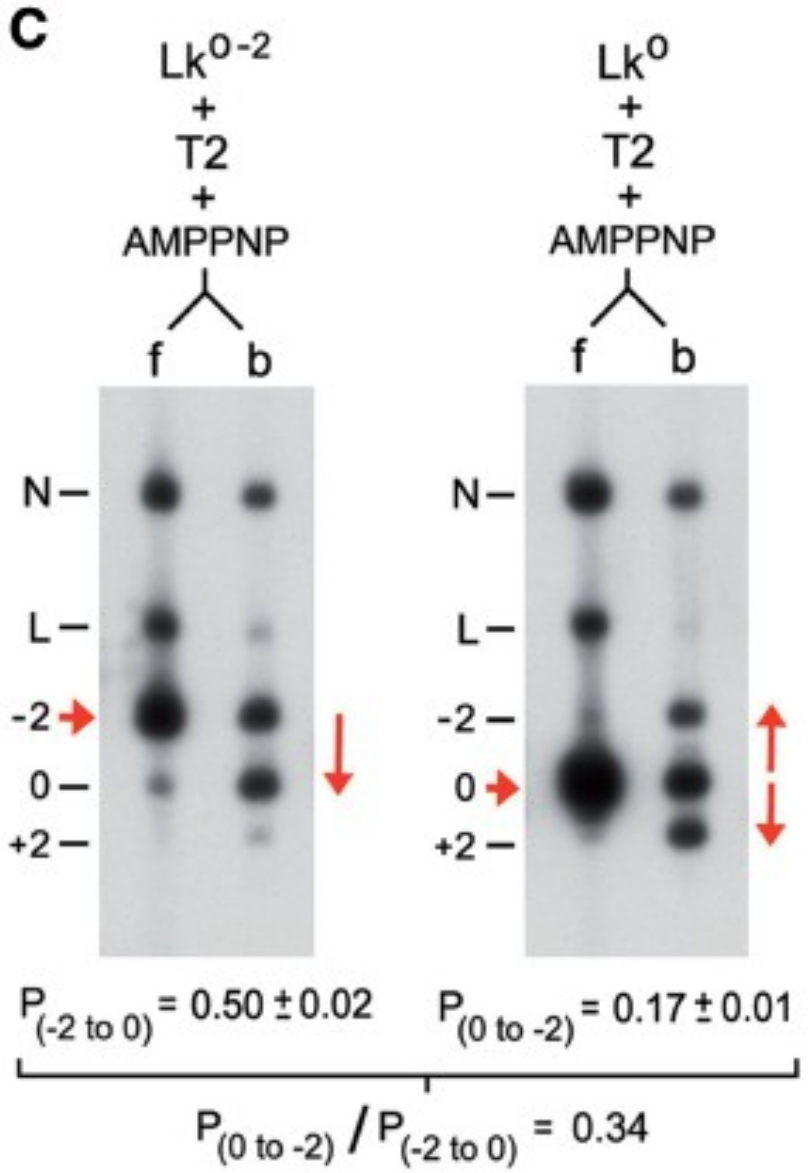
Capture probability model





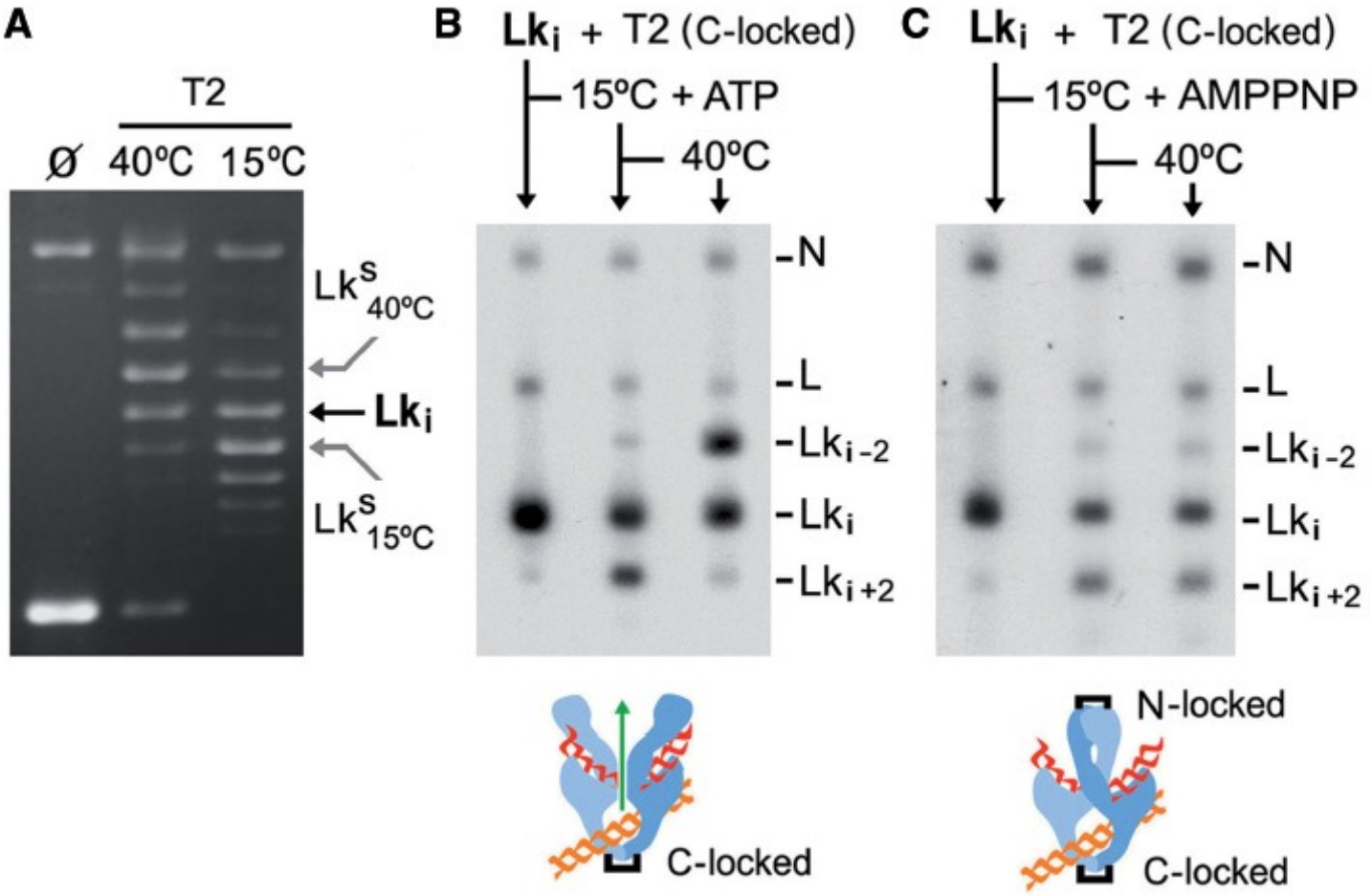
- C_i – the concentration of i-th topoisomerase
- $C_{-2}/C_0 = k_{(0,-2)} / k_{(-2,0)}$
- $k_{(i,j)}$ - rate constant of the conversion of Lk^i to Lk^j
- C_{-2}/C_0 of T2 ≈ 0.17
- C_{-2}/C_0 of T1 ≈ 0.35
- Each DNA transport event changes Lk
- $C_{-2}/C_0 = P_{(0,-2)} / P_{(-2,0)}$
- $P_{(i,j)}$ – probability of the capture of T-segment of respective topoisomere

Capture probability



- Added AMPPNP that allowed conversion to occur but kept C-gate closed
- DNA remained attached to T2
- The capture probabilities were similar to T1 (equilibrium model)
- $P_{(0,-2)} / P_{(-2,0)} \approx 0.34$
- Thus the mechanism that narrow equilibrium and relaxes DNA configuration does not depend on T-segment capture probability

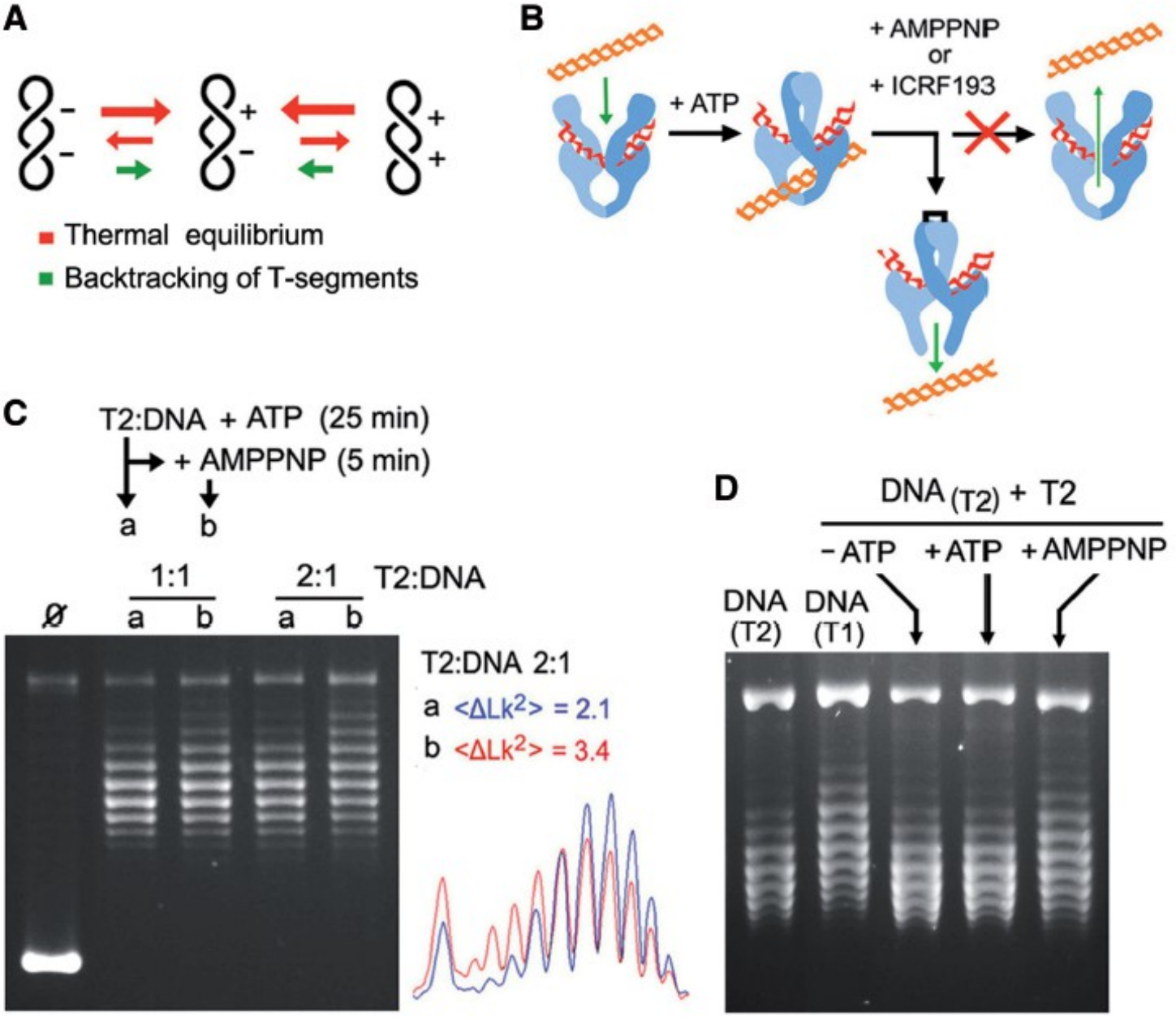
- If C-gate is blocked and N-gate opens, reaction may reverse
- Lk_i was chosen so that $Lk_i < Lk^S$ at 15°C and $Lk_i > Lk^S$ at 40°C
- Engineered T2 had with C-gate blocked
- ATP – allows N-gate reopening, AMPPNP keeps it closed



Blocking of N-Gate

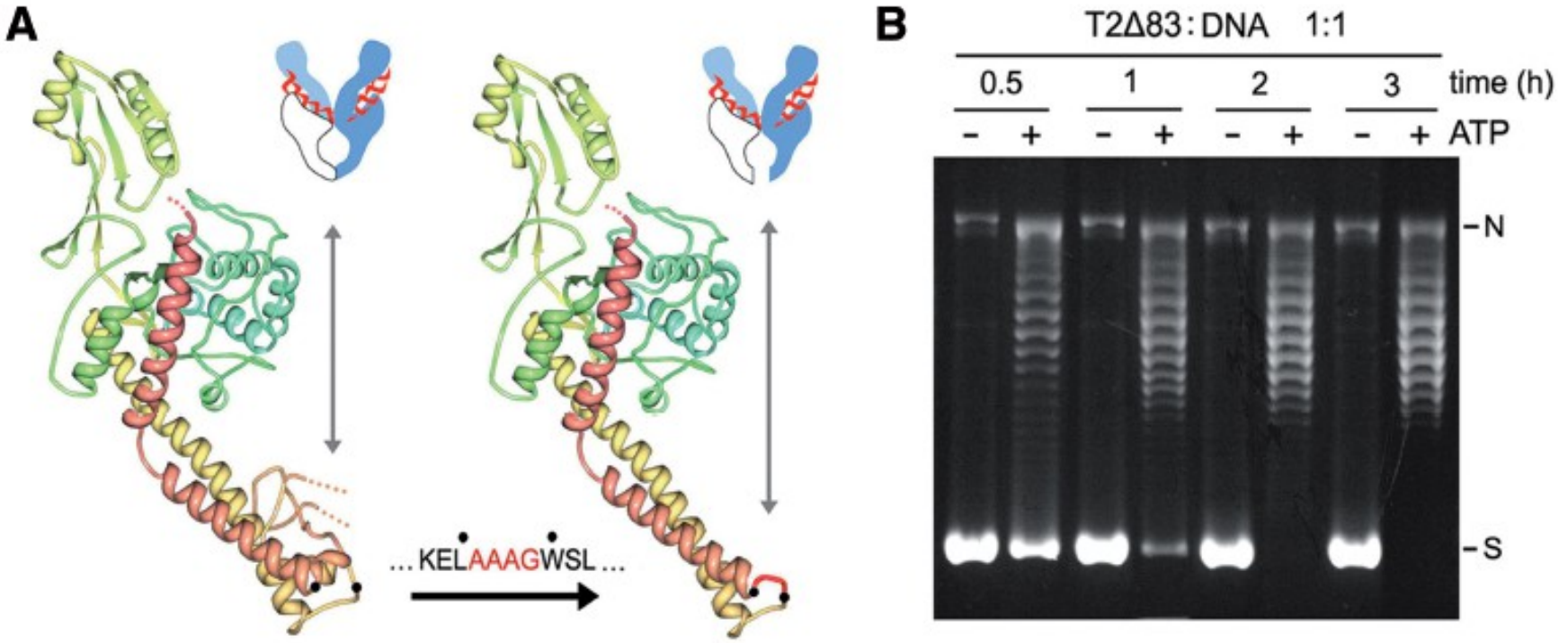
- If DNA topology is near equilibrium backtracking is possible
- The narrowing of distribution suggests, that topologies further away from equilibrium are more prone to backtrack
- Thus is N-does not reopen distribution should widen
- Supercoiled plasmid was relaxed with T2
- After adding AMPPNP the distribution widened (similar to equilibrium distribution)
- As each T2/AMPPNP complex performs only single event, the effect increases with the increase of T2/DNA ratio
- Different ways of blocking N-gate achieved the same result

Blocking of N-gate



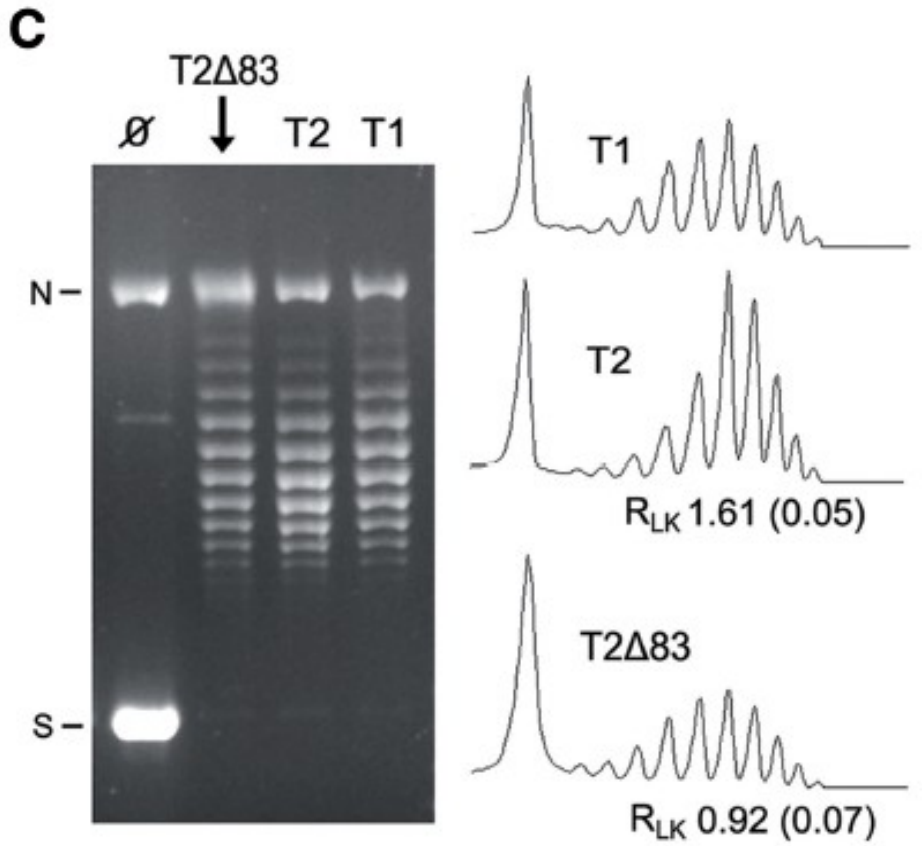
The role of C-gate

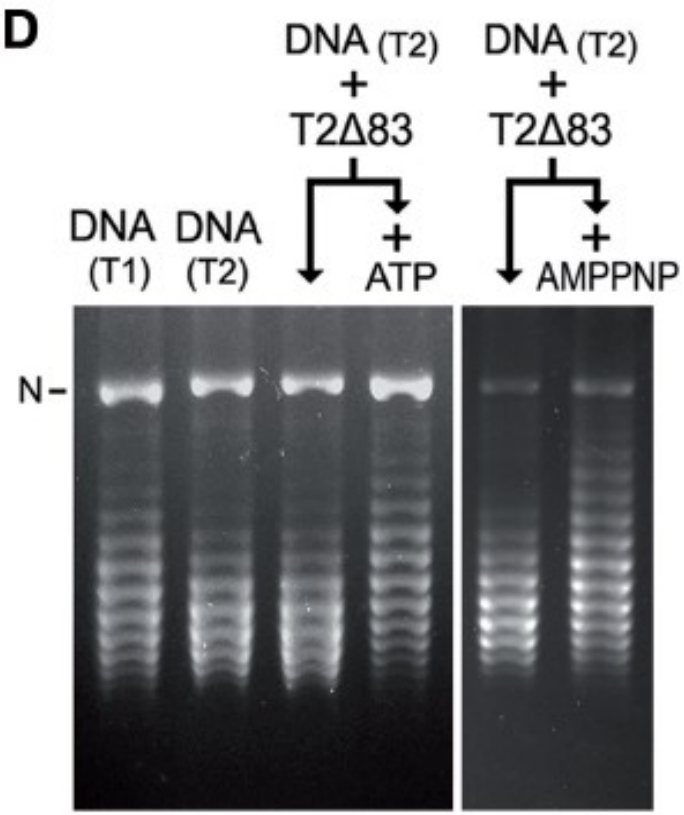
- If C-gate is deleted narrowing of distribution should not occur because DNA dissociates immediately after reaction
- T2Δ83 mutant
- Slow reaction speed



The role of C-gate

- The distribution of T2 relaxed plasmid widened





Discussion

- T-segment capture probability is determined mostly by DNA thermodynamics
- T segment can backtrack from DNA-gate and N-gate
- C-gate challenges the release of passed T-segments
- T-segment release is dissociation process whose rate is determined by molecular environment
  - Fast when DNA transport is energetically favourable
  - Slow if unfavourable
- In latter case, if N-gate opens before dissocation backtracking may occur
- T-segments that deviate topology from equilibrium are more likely to backtrack

Discussion

- The ancestral role of ATP hydrolysis is to coordinate gates to prevent DNA double-strand breaks
- PPR is implemented by different dissociation speeds
- Fast dissociation in case of topological stress
- Slow dissociation in case of molecular crowding
- T2 does not entangle chromosomes in crowded environment