Closing the Loop on Protein-DNA Interactions

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Acknowledgments

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DNA Biophysics in the 21st Century

- nanoscale
- mesoscale
- picoscale
Outline

• Role of DNA looping in biology
• General principles of DNA looping deduced from statistical mechanics
• Applications to loop-mediated gene repression: the *lac* operon
• Looping in site-specific recombination and implications for DNA topology
DNA Looping in Biology

• Gene regulation
  - Repression: *ara, deo, gal, lac* operons
  - Activation: *glnALG* operon
  - Many eukaryotic examples (e.g., β-globin locus)

• Intramolecular site-specific recombination (Int, Cre, Flp, Gin/Hin, etc.)

• Transposition (Mu)

• Type-II restriction enzymes (*SfiI, NgoMIV*)

• DNA mismatch repair (*MutHSL*)

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Courtesy of Mike White, UCSD
Relationship to DNA Cyclization

- $J$ drops precipitously with DNA size for small DNAs
- Near 200 bp, $J$ varies by ~100-fold over a full helical turn
- $J$ values are extremely sensitive to the presence of intrinsic bends

Solving the DNA-looping Problem

Hamiltonian: \[ \beta H = \sum_{i=1}^{N-1} \sum_{j=1}^{3} \frac{(x_{ij} - \bar{x}_{ij})^2}{\sigma_{ij}^2} \]

- \( x_{ij} \) = instantaneous rotation angle (tilt, roll, or twist) of the \( i \)-th rigid body relative to \( (i-1) \)-st
- \( \sigma_{ij}^2 \) = variance of rotation angle
- \( \bar{x}_{ij} \) = corresponding mechanical-equilibrium angle

Non-linear constraints: \[ f^{(k)}\left(\{x_{ij} : i = 1,\ldots,N-1; j = 1,\ldots,3\}\right) = 0; \quad k = 1,\ldots,6 \]

Harmonic-approximation solution for the J factor: \[ J = \frac{8\pi^2 e^{-E_s}}{\sqrt{\pi^6 \det(A) \det(F)}} = \exp\left[-\frac{\Delta G_{\text{loop}}}{k_B T}\right] \]

- The J factor is:
  - Proportional to \( K_{eq} \) for the formation of a closed loop from an open chain
  - Effective concentration of one loop end in the vicinity of another
  - Ratio of statistical-mechanical partition functions for closed and open loops
Parameterization of DNA Conformations

- Tilt: $\theta$
- Roll: $\phi$
- Twist: $\tau$
$\phi_{DP}$, $\tau_{PP} \approx 0$

$\phi_{PD}$

$\tau_{PP} = -60^\circ$

Coupling of Twist and Writhe in DNA Looping

Two distinct looped conformations contribute to the J factor.
Dramatically reduced phase dependence – cannot rule out looping.

Summary – Part I

• Numerical approach to computing looping free energies based on harmonic approximation is $10^4$-fold more efficient than Monte Carlo simulation

• DNA looping is distinguished from cyclization by strong coupling of twist and writhe

• Relationship between $Tw$ and $Wr$ in small loops can generate phase shifts such that the most energetically favorable loops involve non-integral numbers of helical turns

• Negligible helical-phase dependencies do not necessarily imply absence of DNA looping
Architectural DNA-bending Proteins in Genome Organization and Regulation

E. coli genome
4.6 \cdot 10^6 \text{ bp}
L = 1.6 \text{ mm}
V ≈ 4 < S^2 >^{3/2}/3
= 6 \cdot 10^{-11} \text{ cm}^3

Intact E. coli cell
V ≈ 1 \text{ fL} = 1 \cdot 10^{-12} \text{ cm}^3

E. coli architectural DNA-bending proteins:
HU ≈ Fis > IHF > H-NS > StpA > Dps

HU-DNA cocrystal structure
DNA Looping and Regulation of the *lac* Operon

- Looping between the primary operator, O₁, and auxiliary operators, O₂ and O₃, enhances repression by increasing the effective concentration of LacR at the promoter.

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Structure of the LacR Tetramer

“V-shaped” tetramer

“Extended” tetramer

137-bp DNA loop mediated by extended tetramer
The “V-shaped” Repressor Forms
Multiple Loop Geometries

"WT" 179 bp

\[ J = 0.42 \text{ nM} \]
\[ \Delta G_{\text{loop}} = 53.5 \text{ kJ mol}^{-1} \]

\[ \text{WT} \]

179 bp

\( \Delta G_{\text{loop}} = 53.5 \text{ kJ mol}^{-1} \)

\( \Delta G_{\text{loop}} = 62.8 \text{ kJ mol}^{-1} \)

\[ \text{(+)}, \ J = 0.01 \text{ nM} \]
\[ \Delta G_{\text{loop}} = 62.8 \text{ kJ mol}^{-1} \]

\[ \text{(+)}, \ J = 0.78 \text{ nM} \]
\[ \Delta G_{\text{loop}} = 52.0 \text{ kJ mol}^{-1} \]

\( \text{WA} \)

179 bp

\[ \text{WA} \]

163 bp

\( \text{LB} \)

163 bp

\[ (-), \ J = 15 \text{ nM} \]
\[ \Delta G_{\text{loop}} = 44.6 \text{ kJ mol}^{-1} \]

\( (-), \ J = 0 \text{ nM} \)
\[ \Delta G_{\text{loop}} \approx 100 \text{ kJ mol}^{-1} \]

\[ (-), \ J = 0.75 \text{ nM} \]
\[ \Delta G_{\text{loop}} = 52.0 \text{ kJ mol}^{-1} \]

The Extended LacR Tetramer is the Dominant Form in Small Loops

Thermodynamic Model for LacR Repression

\[
d_c = \frac{\lambda J P_t}{K_1 K_2 + (K_1 + K_2 + \lambda J + P_t) P_t}
\]

\[
E_{\text{loop}} = \frac{K_1 (K_2 + P_t)}{K_1 K_2 + (K_1 + K_2 + \lambda J + P_t) P_t}
\]

\[
E_{\text{noloop}} = \frac{K_1}{K_1 + P_t}
\]

\[
R = \frac{E_{\text{noloop}}}{E_{\text{loop}}} = 1 + \frac{\lambda J P_t}{(K_1 + P_t)(K_2 + P_t)} \equiv 1 + \Gamma J
\]

\[
\Gamma = \frac{\lambda P_t}{(K_1 + P_t)(K_2 + P_t)} \text{ (known)}
\]

\[
J = J(h_0, \text{DNA flex.}, \text{protein flex.}) \text{ (4 params)}
\]

Analyzing Experimental Data

Data of Müller et al.

Data of Becker et al.

### Analyzing Experimental Data (cont’d)

<table>
<thead>
<tr>
<th>Data Set</th>
<th>No. of data points, $N_d$</th>
<th>$\Gamma \times 10^2$</th>
<th>Fitting error</th>
<th>Persistence length, bp</th>
<th>Torsional rigidity, $10^{-19}$ erg cm</th>
<th>Helical repeat, bp turn$^{-1}$</th>
<th>Protein flexibility, deg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Müller et al. [5]</td>
<td>51</td>
<td>1.17b</td>
<td>1.0</td>
<td>95 (± 1)</td>
<td>1.1 (± 0.1)</td>
<td>11.60 (± 0.01)</td>
<td>20.7 (± 0.5)</td>
</tr>
<tr>
<td>Becker et al. [18] WT</td>
<td>26</td>
<td>4.66bc</td>
<td>1.1</td>
<td>95 (± 3)</td>
<td>0.7 (± 0.1)</td>
<td>11.08 (± 0.04)</td>
<td>19 (± 1)</td>
</tr>
<tr>
<td>Becker et al. [18] ΔHU</td>
<td>25</td>
<td>4.66bc</td>
<td>1.0</td>
<td>128 (± 2)</td>
<td>0.8 (± 0.1)</td>
<td>10.95 (± 0.03)</td>
<td>16 (± 1)</td>
</tr>
</tbody>
</table>

Looping, LacR flexibility, and HU-dependent Bending Dramatically Increase Repression Efficiency

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• Small loops (≤ 200 bp) between lac operators are predominantly mediated by the extended LacR tetramer conformation

• LacR-dependent regulation *in vivo* is facilitated by enhanced DNA flexibility in the presence of HU protein

• HU binding and protein flexibility are both important factors that promote DNA looping over short distances
Mechanism of Tyrosine Site-specific Recombinases

- Cleavage, strand exchange
- Isomerization (?), HJ resolution
- Holliday-junction intermediate
DNA Knotting via Site-specific Recombination

Electron micrograph of a (+3) DNA knot

Electron micrograph of a (+3) DNA knot

Tsen & Levene, unpublished

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Topology of Cre Recombination is Inconsistent with a Planar Intermediate

Cre-HJ cocrystal structure
Atomic-force Microscopy of Cre Synaptic Complexes

Conclusions

• Development of comprehensive theory for DNA looping that accounts for DNA and protein conformation, protein flexibility, thermal fluctuations, and helical phasing

• Small regulatory loops (≤ 200 bp) in the lac operon are mediated by the extended LacR tetramer conformation and regulation *in vivo* is facilitated by enhanced DNA flexibility in the presence of HU

• Understanding DNA looping is vital for rigorously interpreting results of topological experiments

• Loop-closure kinetics is an emerging tool for analyzing the structure of complex nucleoprotein assemblies
Nick Cozzarelli
1938 - 2006