DNA Bending Associated With Protein Binding
-DNA bending proteins either recognize, exacerbate, or create bends in DNA
-often influence transcription
-effects may be due solely to induced DNA bending, or may also require protein:protein interactions that are facilitated by DNA bending
-eg. CRP can substitute for IHF in a recombination reaction, but IHF cannot substitute for CRP in transcription activation, which involves bending the DNA AND interacting with the α subunit of RNAP
-DNA bending proteins vary in DNA binding motif, sequence specificity

HU
-small, dimeric DNA-binding protein, highly conserved
-works like eukaryotic histones, wraps DNA
-no nucleotide specificity; binds preferentially to kinked DNA
-facilitates binding of transcription factors that require DNA looping or bending (eg. Lac repressor, CAP) can act like a chaperone (is lost after other proteins bind DNA)

IHF
-small, dimeric protein related to HU
-specific binding sites
-binding induces bend of 140°
-architectural factor that facilitates construction of DNA-protein structures (eg. brings together activators bound at a distance and RNAP)
FIS
-small, dimeric protein
-weak consensus sites
-induces bends of up to 90°

H-NS
-small, abundant protein
-avidly binds bent DNA
-generally functions as a repressor by inhibiting RNAP binding or strengthening repressor:DNA complexes

Effects of DNA Curvature & Bending on Transcription
-transcription initiation affected in several ways by DNA bending

**strengthening RNAP:DNA contacts**
-DNA thought to wrap around RNAP
-bent DNA could facilitate this
-eg. UASs confer increased RNAP footprints

**docking sites for proteins that stimulate or repress RNAP-promoter interactions**

**increase isomerization from closed ➔ open complex**
-torsional stress resulting from conformational changes in DNA:protein complexes facilitates DNA melting

**bringing together distantly bound transcription factors**
-LOOPING
-eg. lac operon
-3 operators; O₁ overlaps promoter, O₂ 401 bp downstream in lacZ, O₃ 92 bp upstream
-pairs of operators give greater repression than single operators
-looping can be visualized by EM
-functions to block promoter in case of lac, also cases where looping brings together activator and RNAP to facilitate transcription
-some loops require DNA bending proteins

**DNA SUPERCOILING**

-results from overwinding (POSITIVE SUPERCOILING) or underwinding (NEGATIVE SUPERCOILING) of DNA molecules whose ends are constrained (by protein or in a circular molecule)
-important for DNA bending and processes in which the two strands of DNA must be melted (replication, recombination, transcription)
-in B-form DNA, strands wrapped around each other once every 10.5 base pairs
-POSITIVE SUPERCOILING = more than once every 10.5 bp
-NEGATIVE SUPERCOILING = less than once every 10.5 bp
-supercoiling is modulated in the cell by TOPOISOMERASES
-Most topoisomerases relax supercoiling
-some only relax negative supercoils, some relax both positive and negative supercoils

-TYPE I: cut one strand of DNA, pass other strand through break and reseal; major bacterial type I topoisomerase removes negative supercoils (i.e. winds up DNA)
-TYPE II: cut both strands of DNA-topoisomerase I and gyrase dynamically control a balanced level of supercoiling in cell
-DNA gyrase from E. coli only topoisomerase known that uses ATP to induce negative supercoiling

Towards DNA supercoiling

The Degree of Supercoiling can be Altered

i) drugs – novobiocin and coumermycin inhibit gyrase and cause relaxation of DNA
ii) mutations – in genes for topoisomerase I (\textit{topA}) or gyrase (\textit{gyrA} and \textit{gyrB})

iii) cellular ATP:ADP ratio – gyrase requires ATP, therefore a decrease in this ratio will lead to a decrease in negative supercoiling

iv) environment – elevated temperature leads to a relaxation in DNA supercoiling; some ions alter supercoiling eg. supercoil-sensitive \textit{proU} promoter, which directs synthesis of transport system for osmoprotective substances, is activated by high osmolarity that alters supercoiling

\textbf{TRANSCRIPTION INITIATION}

\textbf{TRANSCRIPTION}

-synthesis of an RNA chain representing coding strand (identical to) of DNA
-begins when RNAP binds to promoter region at start of gene
-promoter proximal to STARTPOINT of transcription, where transcription begins (NOT TO BE CONFUSED WITH TRANSLATION START POINT!)
-RNAP moves along template, synthesizing RNA until it reaches a terminator sequence
Supercoiling Affects Transcription

-transcription of many genes affected by changes in supercoiling in different ways
-three mechanisms proposed:

i) negatively supercoiled DNA is underwound, and this facilitates promoter melting

ii) binding of regulatory proteins can be affected by changes in supercoiling

iii) changes in helix twist about promoter regions alter the orientation of the –10 and –35 to either activate or repress transcription

-angular orientation of –35 and –10 region will vary depending on length of spacer region
changes in twist conferred by local alterations in supercoiling will either optimize or worsen this orientation for RNAP binding
-eg. optimal spacing = 17 bp; 16 bp spacer underwound, 18 bp overwound
-eg. binding of mercury to MerR transcription activator causes a conformational change that alters the twist at promoter of mer operon encoding mercury resistance proteins by underwinding it

**Transcription Affects DNA Supercoiling**

-translocating RNAP causes overwinding of DNA in front of transcription complex (POSITIVE SUPERCOILING) and underwinding behind it (NEGATIVE SUPERCOILING)
-topoisomerase I and gyrase are required to relieve the torsional strain caused
-termed the TWIN-SUPERCOILED DOMAIN MODEL
-can explain how nearby promoters (divergently or convergently) can affect transcription from one another
-eg. mutant leu500 promoter (A → G transition in –10)
-inhibited in isomerization reaction
-inhibition relieved in topA mutant, but ONLY at native location on chromosome → ???
-initiation dependent on negative supercoils generated by transcription from divergent ilvH promoter 1.9 kb away