Chemie, struktura a interakce nukleových kyselin

2008 4.-5.EP/6-7.PŘEDNÁŠKA

Lokální struktury DNA a metody jejich analýzy

Critical Reviews in Biochemistry and Molecular Biology, 28(2):151–226 (1994)

Local Supercoil-Stabilized DNA Structures

Parametry různých typů ds DNA
Metody analýzy lokálních struktur DNA
Ohyby v DNA
Typy lokálních struktur stabilizovaných nadšroubovicovým vinutím
Strukturní rozhraní
Výskyt lokálních struktur DNA in vivo

E. Paleček

Max-Planck Institut für Biophysikalische Chemie, Göttingen, BRD and Institute of Biophysics, Czechoslovak Academy of Sciences, 61265 Brno, CSFR

Referee: James E. Dahlberg, Dept. of Physiological Chemistry, 587 Med. Sci. Bldg., University of Wisconsin, 1300 University Ave., Madison, WI 53706

ABSTRACT: The DNA double helix exhibits local sequence-dependent polymorphism at the level of the single base pair and dinucleotide step. Curvature of the DNA molecule occurs in DNA regions with a specific type of nucleotide sequence periodicities. Negative supercoiling induces *in vitro* local nucleotide sequence-dependent DNA structures such as cruciforms, left-handed DNA, multistranded structures, etc. Techniques based on chemical probes have been proposed that make it possible to study DNA local structures in cells. Recent results suggest that the local DNA structures observed *in vitro* exist in the cell, but their occurrence and structural details are dependent on the DNA superhelical density in the cell and can be related to some cellular processes.

KEY WORDS: supercoil-stabilized DNA structures, DNA double helix polymorphy, probing of DNA structure, DNA structure in cells.

I. INTRODUCTION

Until the end of the 1970s, it was generally accepted that the DNA double helix is very regular and independent of the nucleotide sequence.[1-3] This conclusion was based mainly on data obtained by means of the X-ray fiber diffraction technique that had been used to study DNA structure for more than 2 decades. During the 1960s and 1970s, evidence based chiefly on the results of empirical techniques gradually mounted,[4-10] e.g., suggesting that the structure of the DNA double helix is sequence dependent and influenced by environmental conditions.[10] In the early 1970s Bram[11,12] reached a similar conclusion based on his studies using X-ray fiber diffraction. Due to its limited resolution, this technique yields only an averaged DNA conformation; it cannot detect local variations in the double helix induced by the particular nucleotide sequence.[13] Using this technique and DNA samples with extremes of base composition, however, Bram[12] was able to predict an almost infinite polymorphy of DNA in the B state. At about the same time, Pohl and Jovin[14,15] obtained circular dichroism (CD) spectra of poly(dG-dC)·poly(dG-dC), which suggested that this polynucleotide at high salt concentrations assumes a structure differing from B-DNA and possibly left-handed.

The untenability of the single DNA structure conception became obvious in the mid-1970s. Based on results obtained with various techniques, it was suggested that the DNA double helix is polymorphic,[10,12] depending on the duplex nucleotide sequence and its anomalies as well as on environmental conditions.[10] This conclusion, however, received little attention at the time of its publication.

The situation changed dramatically by the end of the 1970s, when the first results from single-crystal X-ray analysis of short deoxyoli-

POLYMORFIE DVOJITÉ ŠROUBOVICE DNA

Až do konce 70. let bylo všeobecně předpokládáno, že DVOJITÁ ŠROUBOVICE DNA (DNA DOUBLE HELIX) je velmi pravidelná a nezávislá na sekvenci nukleotidů.

Tento názor byl založen především na výsledcích rtg.-strukturní analýzy VLÁKEN - metody, která byla používána po více jak 2 desetiletí k analýze struktury DNA.

V průběhu 60. a 70. let se však začaly hromadit výsledky empirických metod, nasvědčující tomu, že koncepce jedinečné (unique) struktury DNA je neudržitelná a že existuje vztah mezi sekvencí nukleotidů DNA a jejím prostorovým uspořádáním.

Začátkem 70. let **S. Bram** - rtg.-strukturní analýza VLÁKEN DNA s velmi rozdílným obsahem bazí

F. Pohl a T. Jovin - CD poly(dG)·(dC)

EP - elektrochemická analýza DNA

Koncem 70. let rtg.-strukturní analýza KRYSTALU

VISWAMITRA et al. d(pATAT)

A. Rich d(CGCGCG) d(CGCG) levotočivá Z-DNA

R. Dickerson d(CGCGAATTCGCG) pravotočivá B-DNA

Prokázána **závislost struktury na sekvenci nukleotidů,** která je velmi výrazná u B-DNA

Kromě sekvenční informace je možno uvažovat i informaci KONFORMAČNÍ

Polymorphy of the DNA double helix

B. sublilis and B. brevis DNAs have the same G+C content and different nucleotide sequence

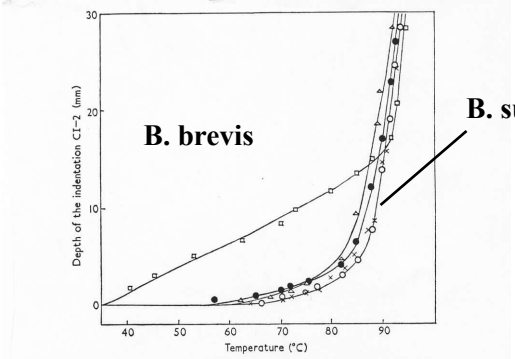


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●—●—, *B. subtilis* 168; —×—×—, *B. natto*; —○—○—, *B. subtilis* var. *niger*; —△—△—, *B. subtilis* var. *aterrimus*; —□—□—, *B. brevis* (ATCC 9999).
P 524 polaroscope, dropping mercury electron polarized with repeated cycles of A.C. The measurements were carried out in the laboratory of Prof. J. Marmur, Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.

Reprinted from:
RESS IN NUCLEIC ACID RESEARCH
MOLECULAR BIOLOGY, VOL. 18
© 1976
ACADEMIC PRESS, INC
York San Francisco London

Premelting Changes in DNA Conformation

E. PALEČEK

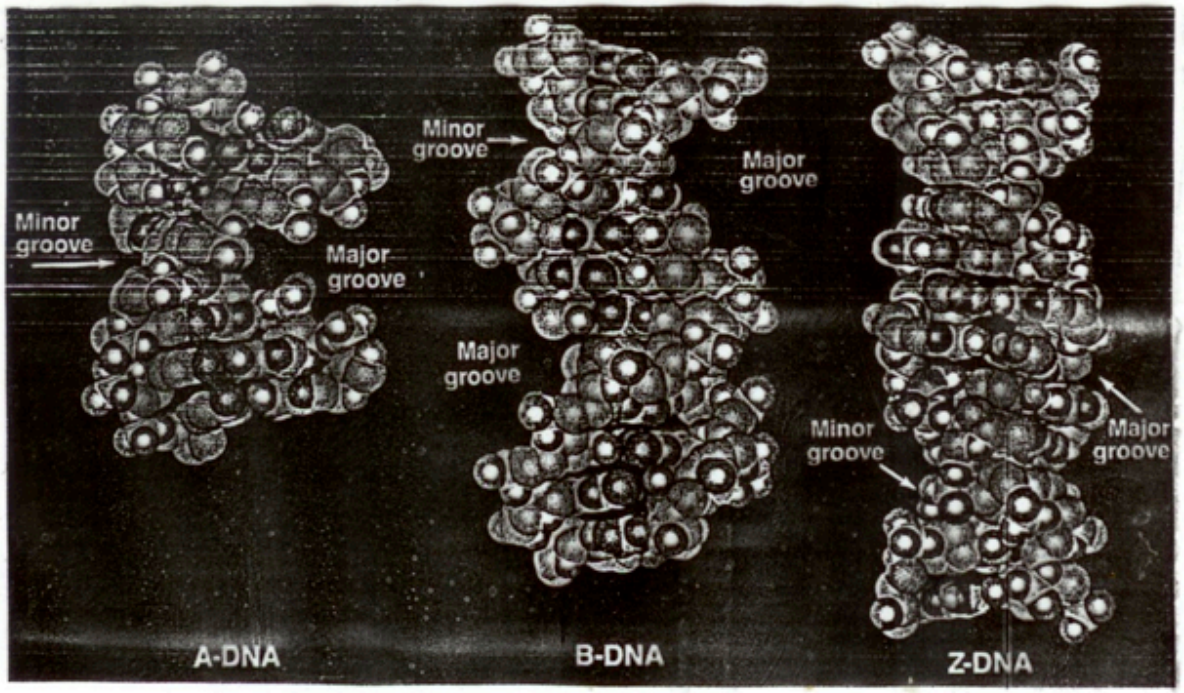
6. POLYMORPHY OF DNA SECONDARY STRUCTURE

On the basis of the preceding discussion, a schematic picture of the structure of natural linear DNA in solution under physiological conditions (e.g., at 36°C, moderate ionic strength, and pH 7) can be drawn. We can assume that the double-helical structure of the very long (A + T)-rich regions differs from the structure of the major part of the molecule and that some of the (A + T)-rich segments are open (Fig. 20). An open ds-structure can be assumed in the region of chain termini and/or in the vicinity of ss-breaks and other anomalies in the DNA primary structure. The exact changes in the open ds-regions will depend on the nucleotide sequence as well as on the chemical nature of the anomaly. Most of the molecule will exhibit an average Watson–Crick B-structure with local deviations given by the nucleotide sequence. Elevating the temperature in the premelting region (Fig. 20) is likely to lead to the opening of other regions and, eventually, to expansion of the existing distorted ds-regions and to further structural changes. Thus the course of the conformational changes as a function of temperature (premelting) will be determined by the distribution of the nucleotide sequences and anomalies in the primary structure, and may have an almost continuous character.

Consequently, even if we do not consider "breathing," not only the architecture of a DNA double-helical molecule, but also its mechanics or dynamics can be taken into account.

To determine whether, e.g., only the (A + T)-rich molecule ends will be open at a certain temperature or also long A + T regions in the center of the molecule, further experimental research with better-defined samples of viral and synthetic nucleic acids will be necessary. Further work will undoubtedly provide new information on the details of the local arrangement of nucleotide residues in the double helix, as well as on DNA conformational motility. Thus a more accurate picture of DNA structure will emerge, whose characteristic feature will be polymorphy of the double helix, in contrast to the classical, highly regular DNA structure models.

Dvojitá šroubovice DNA je POLYMORFNÍ

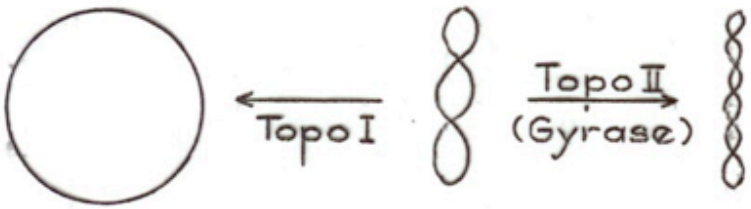


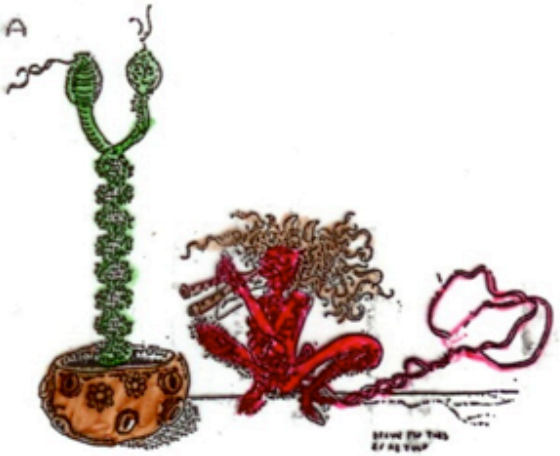
DNA structures from X-ray crystal analysis

DNA double helix is polymorphic depending on the nucleotide sequence

DNA se v BUŇKÁCH vyskytuje převážně v NEGATIVNĚ SUPERHELIKÁLNÍ (nadšroubovicové) formě

ÚROVEŇ SUPERHELICITY je homeostaticky KONTROLOVÁNA TOPOISOMERASAMI





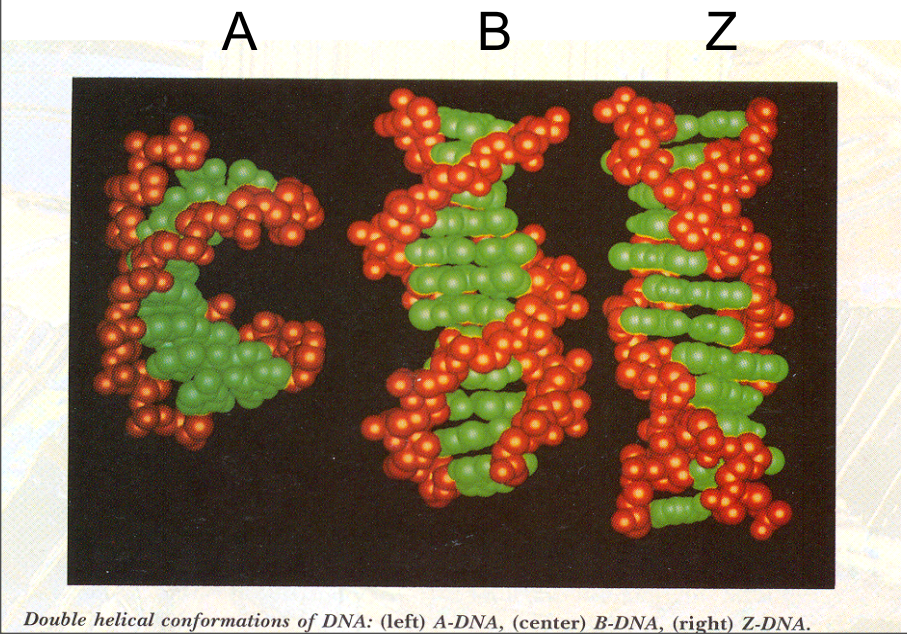
SUPERHELICITA DNA OVLIVŇUJE základní biochemické děje jako TRANSKRIPCI.

MICROHETEROGENEITY OF THE DNA DOUBLE HELIX FORMS

Studies of the detailed relationships between nucleotide sequence and DNA structure became feasible by the end of the 70s, when organic synthesis had been developed to the point where oligodeoxynucleotides (ODN) could be produced in the purity and quantity necessary for the preparation of single crystals for X-ray diffraction (and NMR) studies. Three main families of DNA forms were identified by crystallographic analysis of ODN: right-handed A and B-forms and the left-handed Z-form.

B-, A- and Z-helices

The A-, B- and Z-helices have distinctly different shapes which are due to the specific positioning and orientation of the bases with respect to the helix axis. In A-DNA, the base pairs are displaced from the helix axis, the major groove is very deep, and the minor groove is very shallow. In B-DNA the major and minor grooves are of similar depths and the helix axis is close to the base pair center. In Z-DNA the minor groove is deep and the major groove is convex. In A- and B-DNA a single nucleotide can be considered as the repeat unit, while in Z-DNA the repeat unit is a dinucleotide.



Double helical conformations of DNA: (left) *A-DNA,* (center) *B-DNA,* (right) *Z-DNA.*

In A-duplexes base pairs are heavily tilted in contrast to base pairs in B-duplexes which are almost perpendicular to the helical axis. (Table 1). Many of the structural differences between the helices arise from the puckering of the sugar ring; C3'-endo is typical for A-DNA, while in Z-DNA C3'-endo alternates with C2'-endo. In B-DNA sugar pucker tends to favor the C2'-endo or C1'-exo,but the distribution of conformations is much broader than in A- and Z-DNA.

The right-handed A- and B-forms have the anti glycosidic bond, whereas in the left-handed Z-helix the orientation alternates between syn (for purines) and anti (for pyrimidines). In the latter structure the orientation around the C4'-C5' bond with respect to the C3' atom alternates between gauche+ and trans conformations for cytidine and guanosine, respectively. The alternating features of Z-DNA result in the zig-zag shape of its sugar-phosphate backbone, from which the name was derived. The changes in the backbone and glycosidic-bond conformations are accompanied by substantial variations in the stacking interactions between successive base pairs in Z-DNA. Methylation or bromination of cytosines at position 5 (studied mainly in ODNs with alternating C-G sequence) stabilizes Z-DNA. Under certain conditions even non-alternating sequences of purines and pyrimidines can assume the conformation of Z-DNA with thymines in a syn orientation. The outer surface features of such a Z-helix are different at the non-alternating sites but the backbone is similar to that observed with alternating sequences.

TABLE 2
Average Helical Parameters for Selected Right-Handed Structures

	Helix twist (°)	Rise per base pair (Å)	Base pair tilt (°)	Propeller twist (°)	Groove width (Å)		Displacement Da (Å)
					Minor	Major	
A-form							
d(GGTATACC)	32	2.9	13	10	10.2	6.3	4.0
d(GGGCGCCC)	32	3.3	7	12	9.5	10.1	3.7
d(CTCTAGAG)	32	3.1	10	11	8.7	8.0	3.6
r(GCG)d(TATACGC)	33	2.5	19	12	10.2	3.2	4.5
r(UUAUAUAUAUAUAA)	33	2.8	17	19	10.2	3.7	3.6
Fiber A-DNA	33	2.6	22	6	11.0	2.4	4.4
B-form							
d(CGCGAATTCGCG)	36	3.3	2	13	5.3	11.7	−0.2
d(CGCGAATTBrCGCG)	36	3.4	−2	18	4.6	12.2	−0.2
Fiber B-DNA	36	3.4	2	13	6.0	11.4	−0.6

BrC = 5-bromcytosine

Adapted from Kennard, O. and Hunter, W. N., *Q. Rev. Biophys.*, 22, 327, 1989. With permission.

TABLE 1
Comparison of A-, B-, and Z-DNA

Helix sense	A-DNA[a] right-handed	B-DNA[a] right-handed	B′-DNA[b] right-handed	Z-DNA[c] left-handed
Base pairs per turn	11	10	10	12 (6 dimers)
Helix twist (°)	32.7	36.0	34.1, 36.8	−10, −50
Rise per base pair (Å)	2.9	3.4	3.5, 3.3	3.7
Helix pitch (Å)	32	34	34	45
Base pair tilt (°)	13	0	0	−7
P distance from helix axis (Å)	9.5	9.3	9.1	6.9, 8.0
Glycosidic orientation	*anti*	*anti*	*anti*	*anti*, *syn*
Sugar conformation	C3′-*endo*	Wide range	C2′-*endo*	C2′-*endo*, C3′-*endo*[d]

[a] Numerical values for each form were obtained by averaging the global parameters of the corresponding double-helix fragments.

[b] B′-DNA values are for a double helix backbone conformation alternating between conformational states I and II.

[c] The two values given correspond to CpG and GpC steps for the twist and P distance values, to cytosine and guanosine for the others.

[d] Two values correspond to the two conformational states. From Kennard, O. and Hunter, W. H., *Q. Rev. Biophys.*, 22, 3427, 1989. With permission.

Local DNA Structures and Nucleotide Sequence

The significant variations in some of the global parameters (Table 2), dependent on nucleotide sequence, result in local changes along the DNA double helix. Such relations have been analyzed in detail by several authors and reviewed by Shakked and Rabinovich. In A- and B-DNA these variations seem to be determined mainly by the specific interactions between the stacked base pairs and also to some extent by neighboring bases. In particular, homopolymer dinucleotide steps show a wide spectrum of stacking characteristics which are markedly neighbor dependent. On the other hand pyrimidine-purine steps in A-DNA (especially the C-G steps) often display a low twist and high slide that are only slightly dependent on neighboring steps. In Z-DNA the shape of the helix surface changes significantly due to deviations in the regular alternation of the purine-pyrimidine sequence while the sugar-phosphate backbone does not change. The effect of the nucleotide sequence on the fine geometrical features of each DNA form has been clearly demonstrated but not fully elucidated. The emerging rules should, however, be considered as tentative since they were based on a relatively small number of examples. The well-known "Calladine's rules" are now perceived to be incomplete and to neglect important factors other than the steric clash of purine rings.

DNA Hydration

Information about the organization of water molecules in DNA forms has been gained from X-ray diffraction analysis of crystals. Distinct hydration patterns were observed in the major and minor grooves and around the sugar-phosphate backbone. It was proposed that in DNA with a mixed nucleotide sequence hydration of the backbone is related to global conformation. In A- and Z-DNA a chain of water molecules can bridge the phosphate oxygens along the backbone. There are more water molecules around each phosphate group in the B-DNA but almost no water bridges between the phosphate oxygens, as the distances between phosphate oxygens in this DNA form are too great to be linked with a single water molecule. It appears that specific nucleotide sequences that create local changes in the DNA double helix may also affect the backbone hydration pattern. Even greater dependence of the hydration patterns on nucleotide sequence has been found in the DNA grooves. In A-DNA specific hydration patterns occur in the major grooves. A string of well ordered water molecules hydrogen bonded to oxygen and nitrogen atoms in the minor groove has been found in the central AATT sequence of the B-DNA dodecamer d(CGCGAATTCGCG). This specific hydration of B-DNA, called "spine of hydration", significantly contributes to DNA stability. Studies of further B-DNA helices revealed two ribbons of water molecules along the walls of wide regions of the minor groove while narrow regions of the minor groove contained an ordered zig-zag spine of hydration. It appears that the interdependence between nucleic acid structure and the solvent represents one of the bases for DNA double helix polymorphy.

METHODS OF ANALYSIS OF LOCAL DNA STRUCTURES

Metody analýzy lokálních stuktur DNA

1. ANALYSIS OF DNA ELECTROPHORETIC MOBILITY
two-dimensional gel electrophoresis

2. ANTIBODIES recognizing local DNA STRUCTURES
anti-Z-DNA, anti-cruciform, anti-triplex

3. ENZYMATIC PROBES
Nucleases and especially single-strand selective nucleases
nuclease S1, nuclease P1, mung bean nuclease, etc.

4. Ultraviolet radiation

5. CHEMICAL PROBES

a. Probes reacting with double-stranded DNA / FOOTPRINTING

i. Dimethyl sulfate (DMS) / G - N7, A - N3

ii. N-Ethyl-N-nitrosourea (ENU) / phosphates

iii. Probes with nuclease activities: 1,10-phenanthroline copper ion, methidiumpropyl-EDTA, Fe(II).EDTA - hydroxyl radical induced chain cleavage

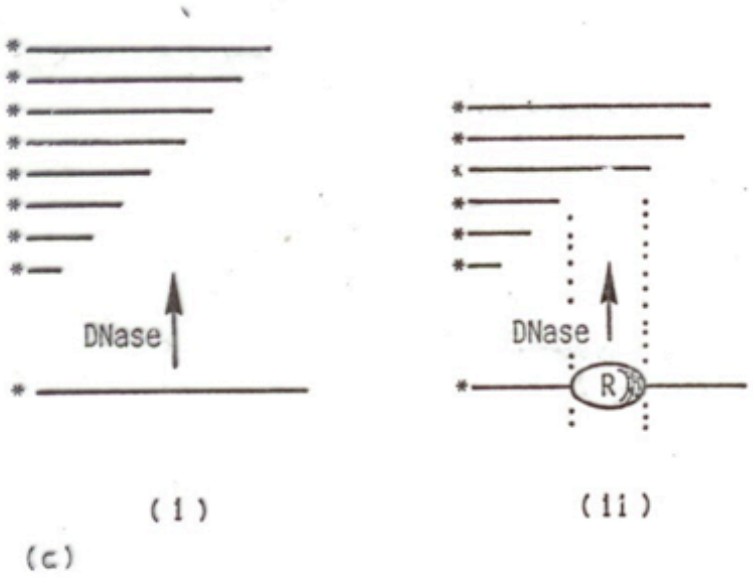
iv. Photochemical probes: Psoralens, acridines, EtdBr, uranyl salts, tris-phenanthroline transition metal complexes

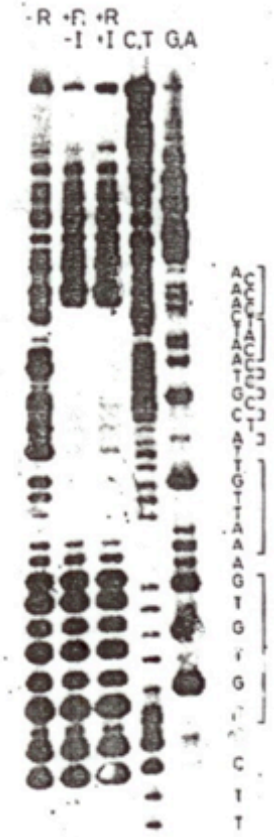
v. Complementary addressed modification and cleavage of DNA

b. Single-strand selective probes

DNA footprinting

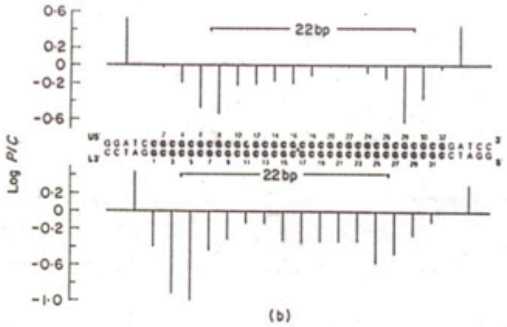
mapping of DNA interactions





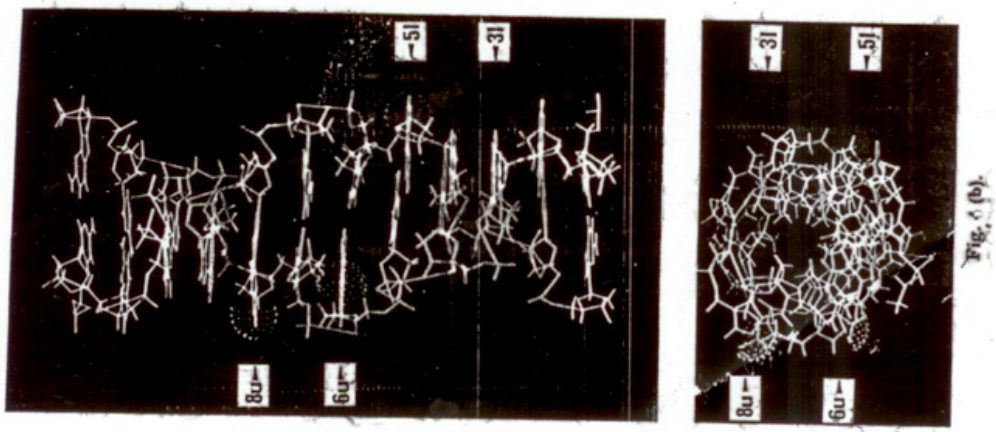
Enzymatic probe (DNAase I)

Fig. A.24 Example of DNase I footprinting. (a) Partial digestion of a piece of end-labelled DNA to which a protein (R) is bound (ii) results in the absence of the end-labelled fragments cleaved in this region but found in the digest (i) of the unprotected DNA; (b) the results of separation of the products of such an experiment on a polyacrylamide sequencing gel. The example is with *lac* operator DNA and the *lac* repressor (R). I is IPTG (0.3 M), which does not prevent the binding of a mutant repressor used in this study. C,T and G,A represent the results of Maxam-Gilbert C + T and A + G reactions on the undigested end-labelled fragment. Adapted from [105], with permission.



Chemical probe - DEPC

Figure 5. DEPC footprint of antibody Z22 over the extended $d(C\text{-}G)_{16}$ Z-DNA antigen. (a) Denaturing polyacrylamide gel analysis of DEPC footprinting patterns. Plasmid pLP32 was relaxed (lanes 1 and 2) or supercoiled to $-\sigma = 0{\cdot}060$ (lanes 3 and 4) and treated with DEPC in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of antibody Z22. The footprinting pattern on the upper strand (see(b)) is shown. The 3 sequencing lanes on the left are marked according to the Maxam & Gilbert (1980) sequencing reactions. Arrows as for Figs 1 and 2. (Note: the significance of the strong band at the bottom of lanes 2 to 4 is not understood at present.) (b) Graphical representation of antibody Z22 DEPC footprinting data along the $d(C\text{-}G)_{16}$ sequence. This figure is drawn in accordance with the explanations given for Fig. 3. Note that the spacing between the 2 regions of maximal protection measures 22 bp on the $d(C\text{-}G)_{16}$ Z-DNA antigen. Furthermore, the rotationally symmetric arrangement of the protection/induction pattern on the 2 strands should be noted.



Single-strand selective chemical probes of the DNA structure

CHEMICAL PROBES of the DNA STRUCTURE reacting preferentially with single-stranded and non-B DNA regions

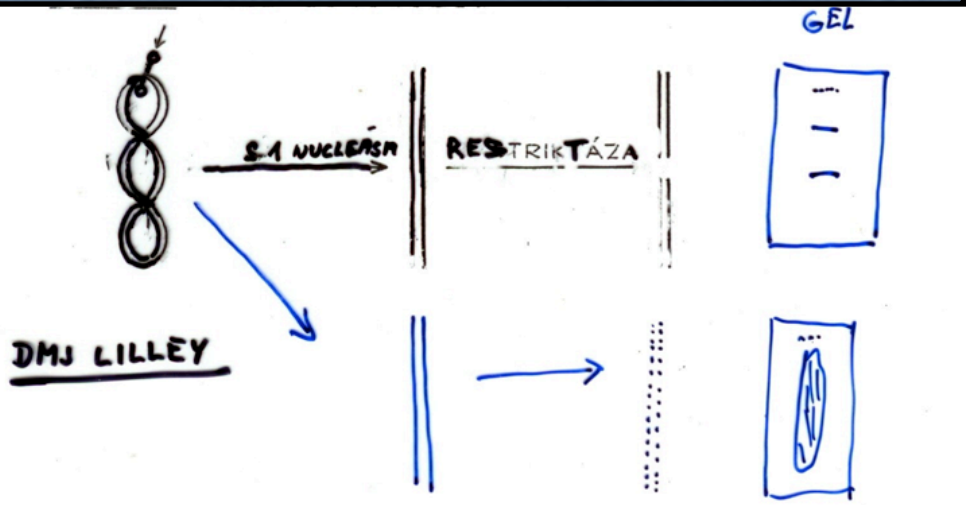
Base specificity	Probe
T>>C,G	OsO_4,py, OsO_4,bipy $KMnO_4$
T>>C	OsO_4 (alone)
A>G	DEPC*
A,C	BAA**, CAA**
G	glyoxal* N-hydroxylaminofluorene
C	$NaHSO_3$ hydroxylamine methoxylamine

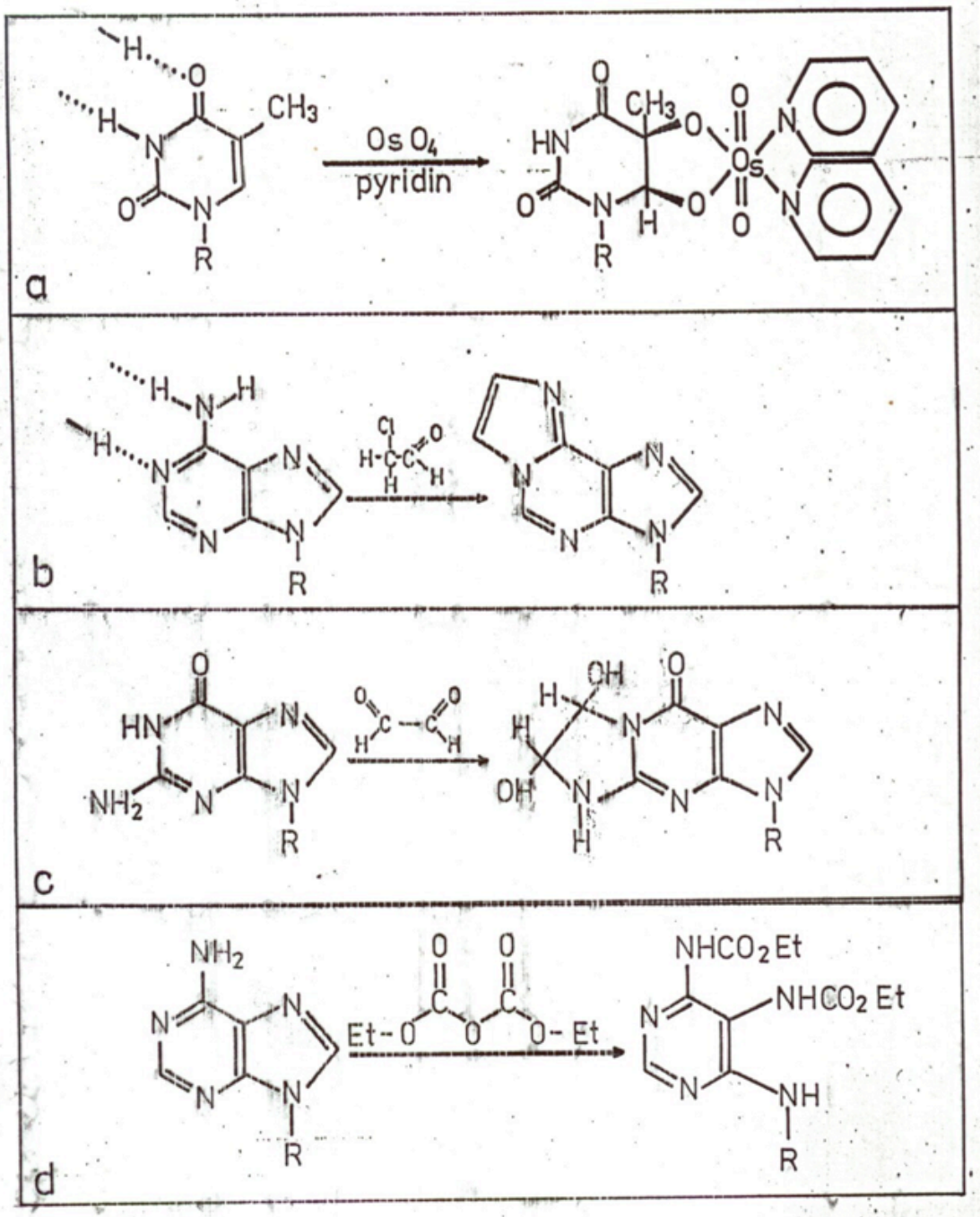
* reacts also with Z DNA (purines in syn conformation)

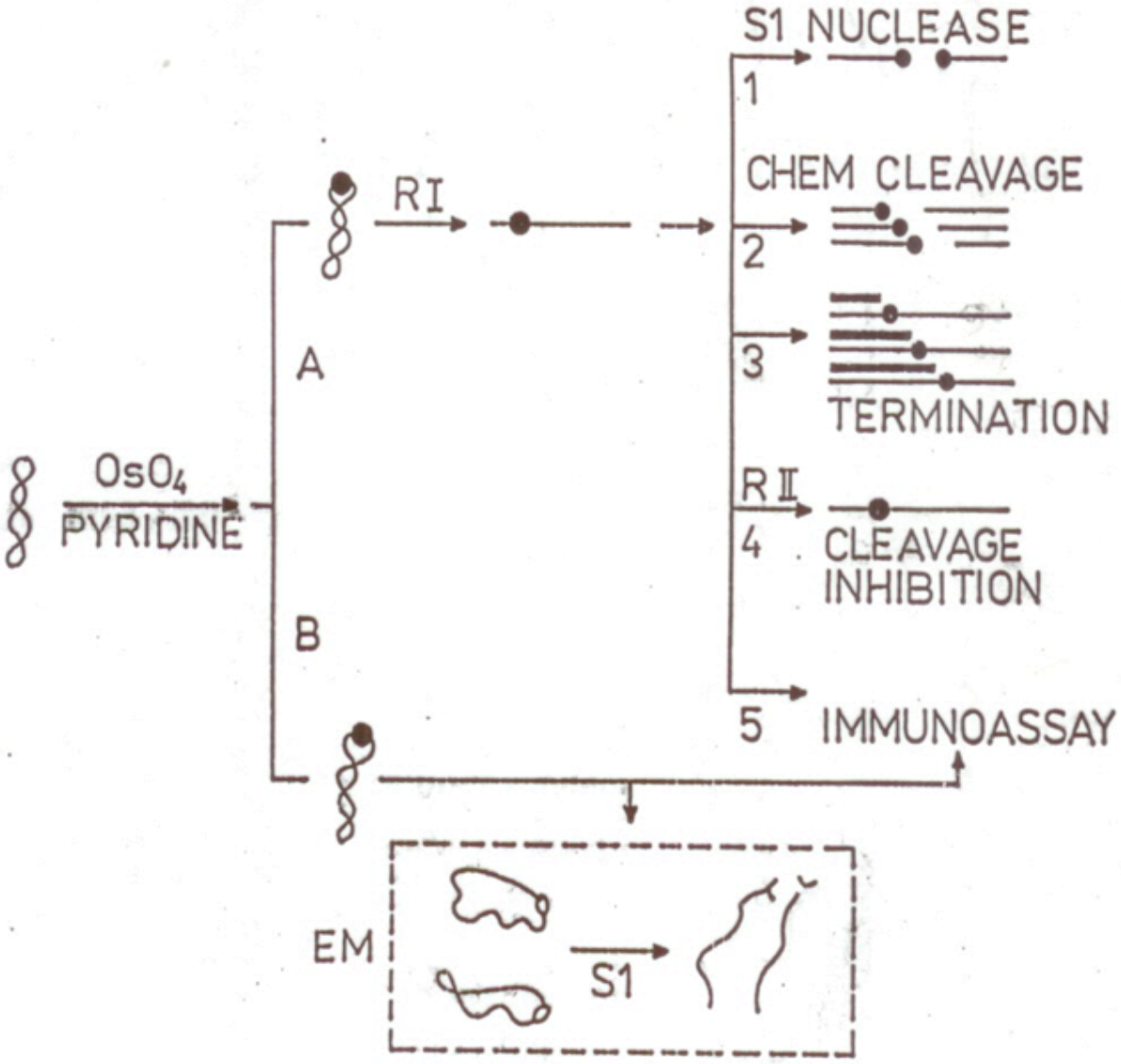
** disturbance of Watson-Crick pairing required

Discovery of the cruciform in sc DNA

D M J LILLEY, 1981

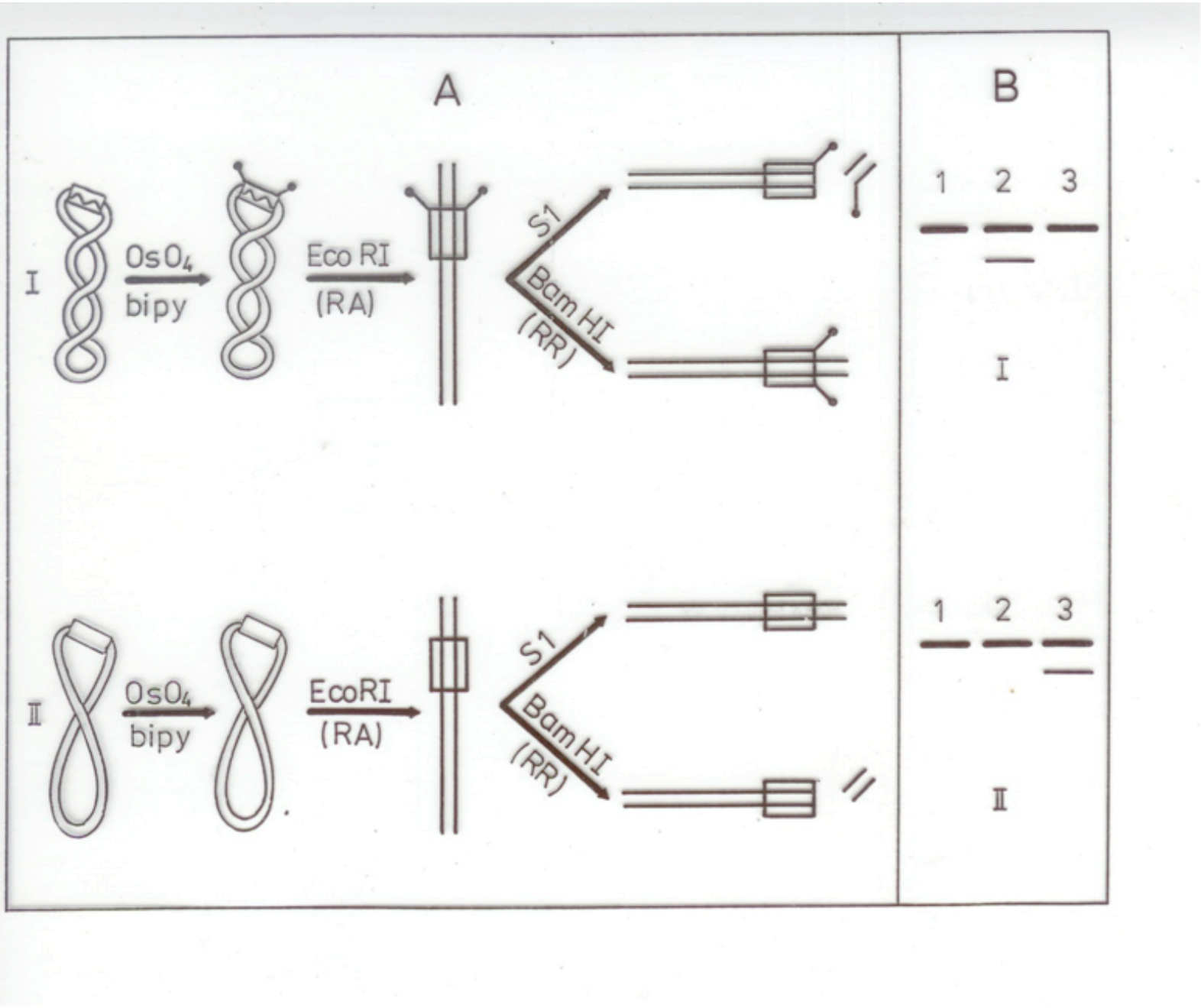


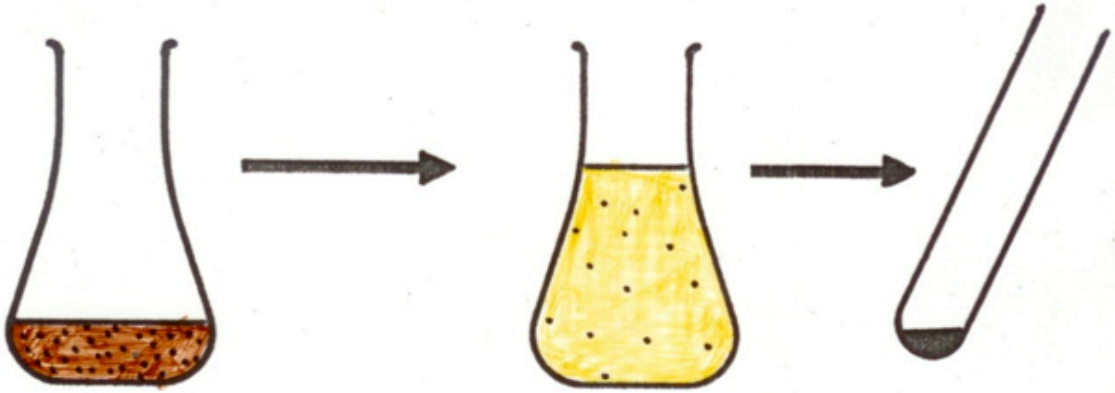

a
OsO_4
pyridin
b
c
d
$NHCO_2Et$
$NHCO_2Et$


S1 NUCLEASE
1
CHEM CLEAVAGE
RI
2
3
A
TERMINATION
OsO4
PYRIDINE
RII
4
CLEAVAGE
INHIBITION
B
5
IMMUNOASSAY
EM
S1

12.11. - 6. předn.

a. DR. L. HAVRAN: SYNTHESA OLIGONUKLEOTIDŮ (ca. 10 min)
b. Prof. M. VORLIČKOVÁ: CD DNA (a 40 min)


A
B
I
OsO4
bipy
Eco RI
(RA)
S1
Bam HI
(RR)
1
2
3
I
II
OsO4
bipy
EcoRI
(RA)
S1
Bam HI
(RR)
1
2
3
II

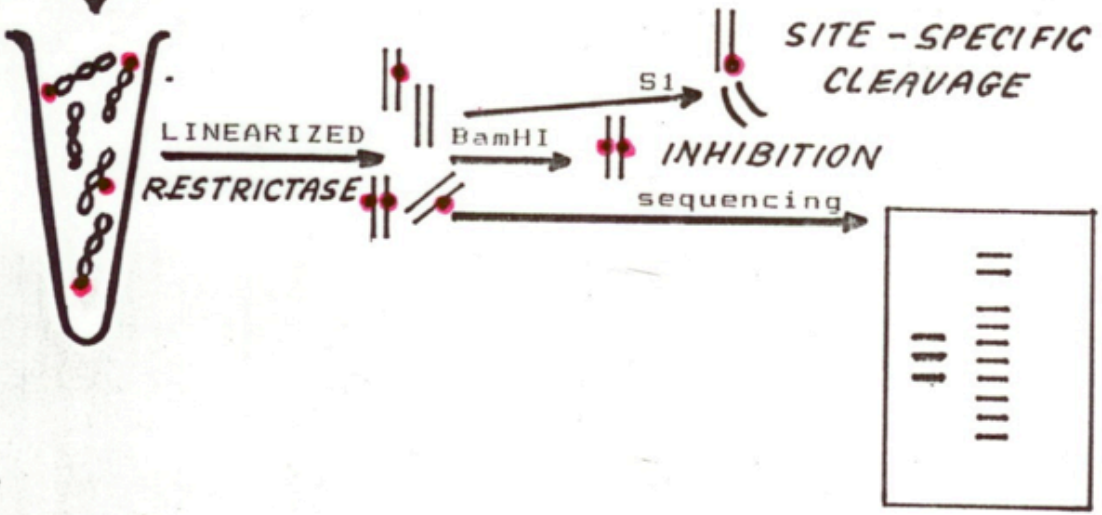


CELLS+Os,bipy
(1-2mM)
(e.g.in 0.5M
phosphate pH 7.4)
37° or 26 °C
10-60 min
2 mg cells/ml

10× DILUTION
0 °C

RINSED

DNA ISOLATION
(BOILING METHOD)



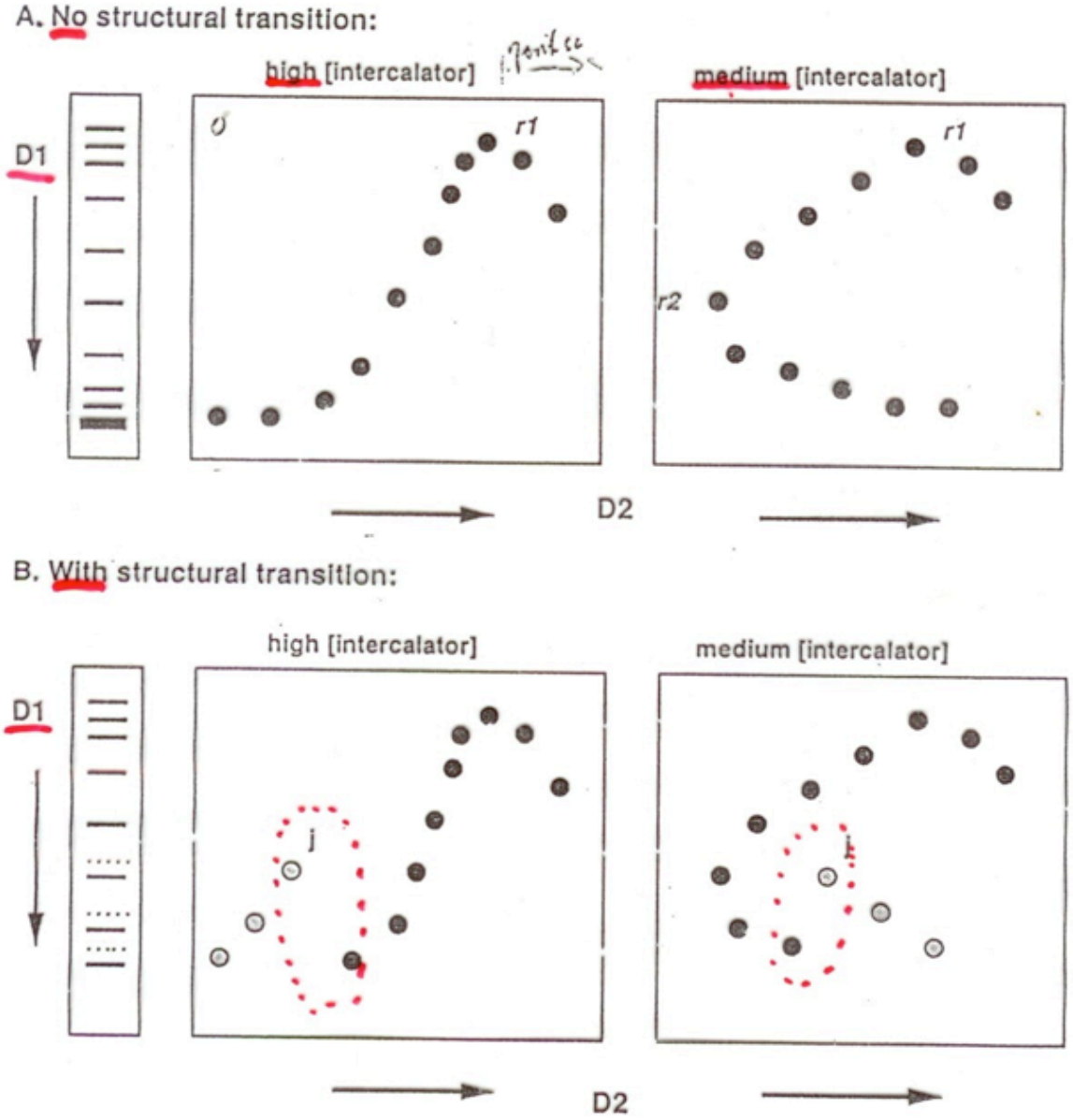


FIG. 2. Scheme to demonstrate the principle of two-dimensional gel electrophoresis. (A) Generation of two-dimensional gels from a circular DNA molecule that undergoes no structural transition. *Left*, One-dimensional gel electrophoresis (comparable to that in Fig. 1); *middle*, two-dimensional gel using a high chloroquine concentration in the second dimension; *right*, two-dimensional gel using a medium chloroquine concentration in the second dimension. (B) Equivalent to (A), except that we have now introduced a structural transition, leading to "jumps" of the topoisomer spots. See text for further details.

DNA CURVATURE AND BENDING
(OHYBY V DNA)

SEKVENCE — EXT. SÍLY

KORELAČNÍ ANALÝZA NUKLEOTIDOVÝCH SEKVENCÍ DNA CHROMATINU (TRIFONOV)

AA a TT se vyskytují v pravidelných intervalech korelujících se zdvihem šroubovice DNA

Snížená pohyblivost fragmentů DNA z kinotoplastů *Leishmania tarantolae* vysvětlena ohybem DNA

Osa helixu segmentu DNA může být jednosměrně ohnutá, jestliže se v něm vyskytují úseky adeninových zbytků v pravidelných intervalech, odpovídajích zdvihu šroubovice (10,5 bp). Max. efekt 4-6 A

Jiné sekvence

KINK

Wedge model (klín)

Junction model (rozhraní)

Sekvence tvořící ohyby DNA se vyskytují v různých organizmech ve funkčně důležitých oblastech

např. v blízkosti počátku replikace (origin of replication)

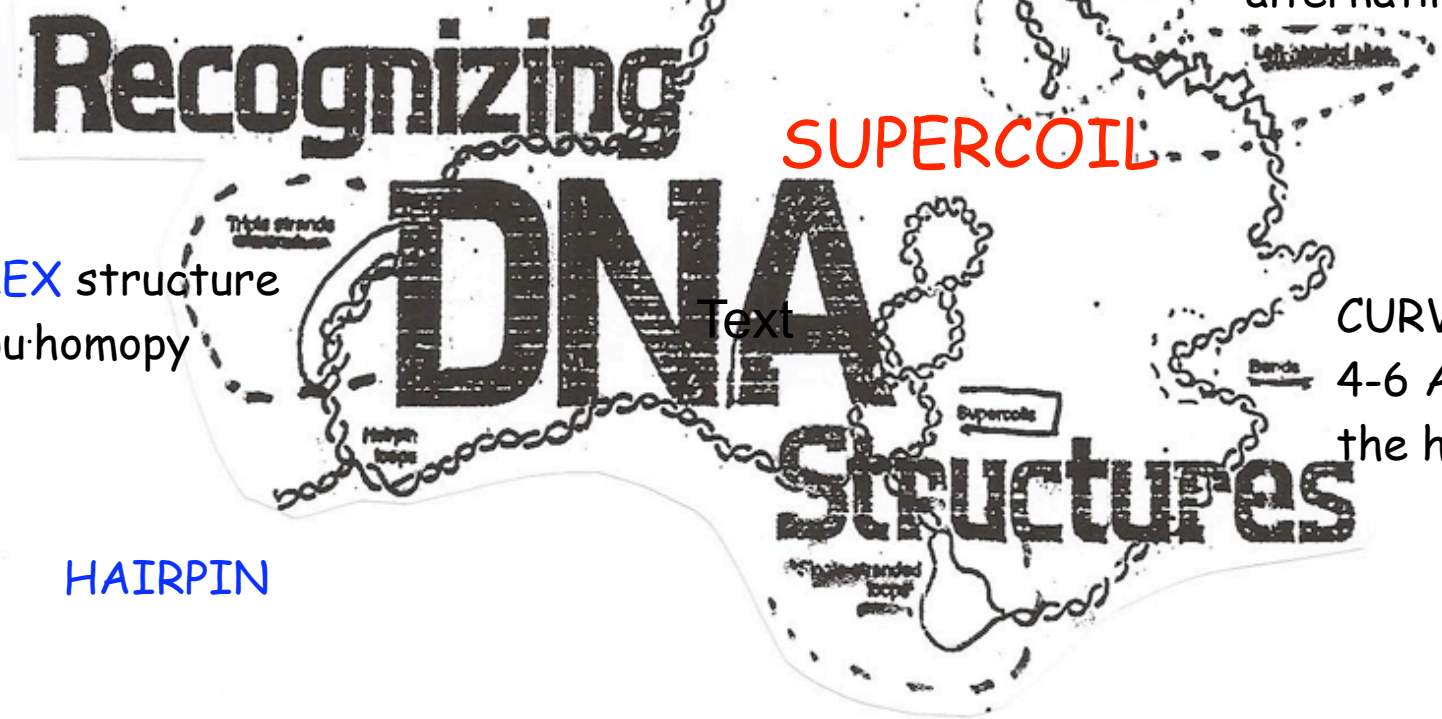
přítomnost **ohybu** v blízkosti **promotoru může ovlivnit transkripční aktivitu**

některé bílkoviny se vážou specificky na místa ohybu a příp. ohyb zvětšují, jiné svou vazbou ohyby vytvářejí

Negative SUPERCOILING stabilizes local DNA structures

CRUCIFORM
inverted repeat

LEFT-HANDED Z-DNA
alternating pu-py



SUPERCOIL

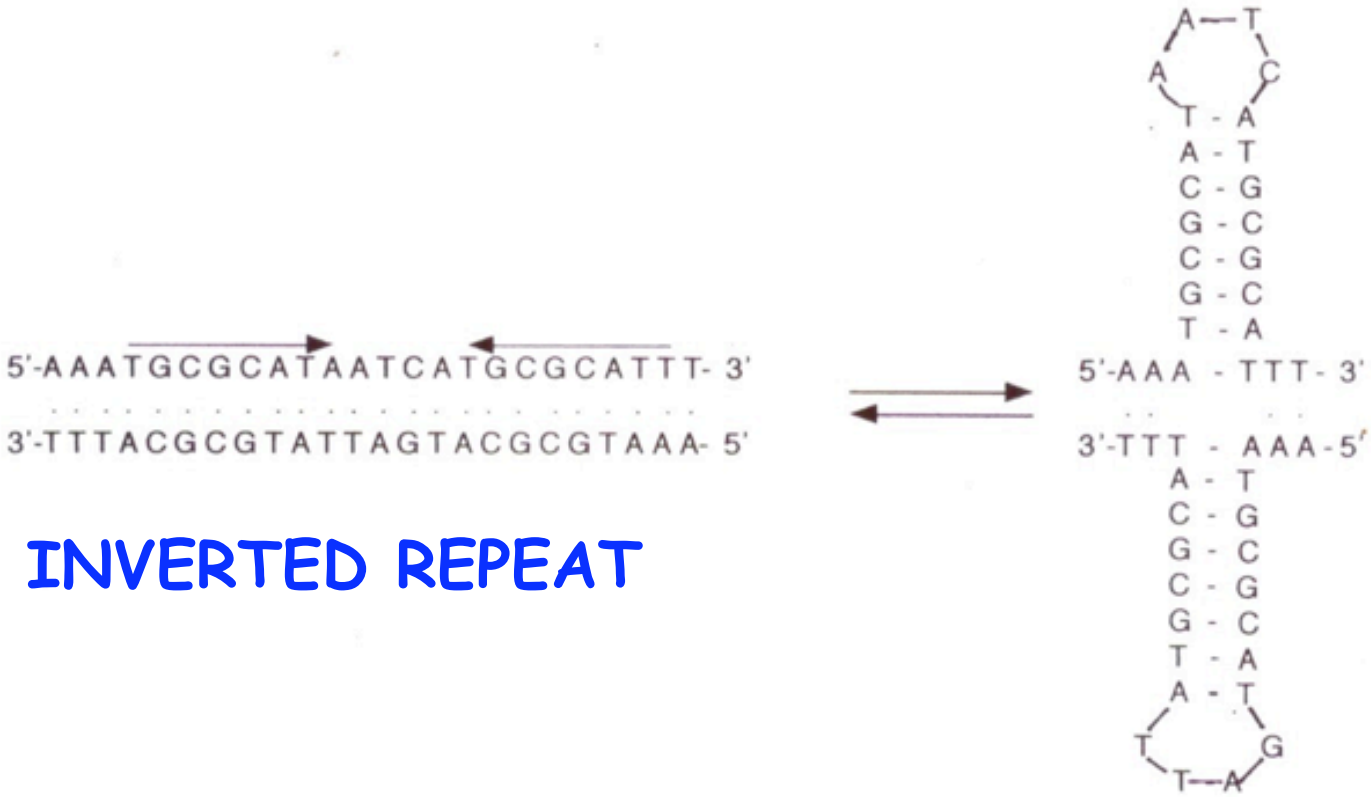
TRIPLEX structure
homopu·homopy

CURVATURE
4-6 A's in phase with the helix turns

HAIRPIN

SINGLE-STRANDED region
AT-rich

Physical methods such as NMR and X-ray analysis indispensable in the research of linear DNA structures are of limited use in studies of local structures stabilized by supercoiling



INVERTED REPEAT

Palindromes and cruciforms. The arrows indicate the palindromic sequence which can fold back on self to form the cruciform structure.

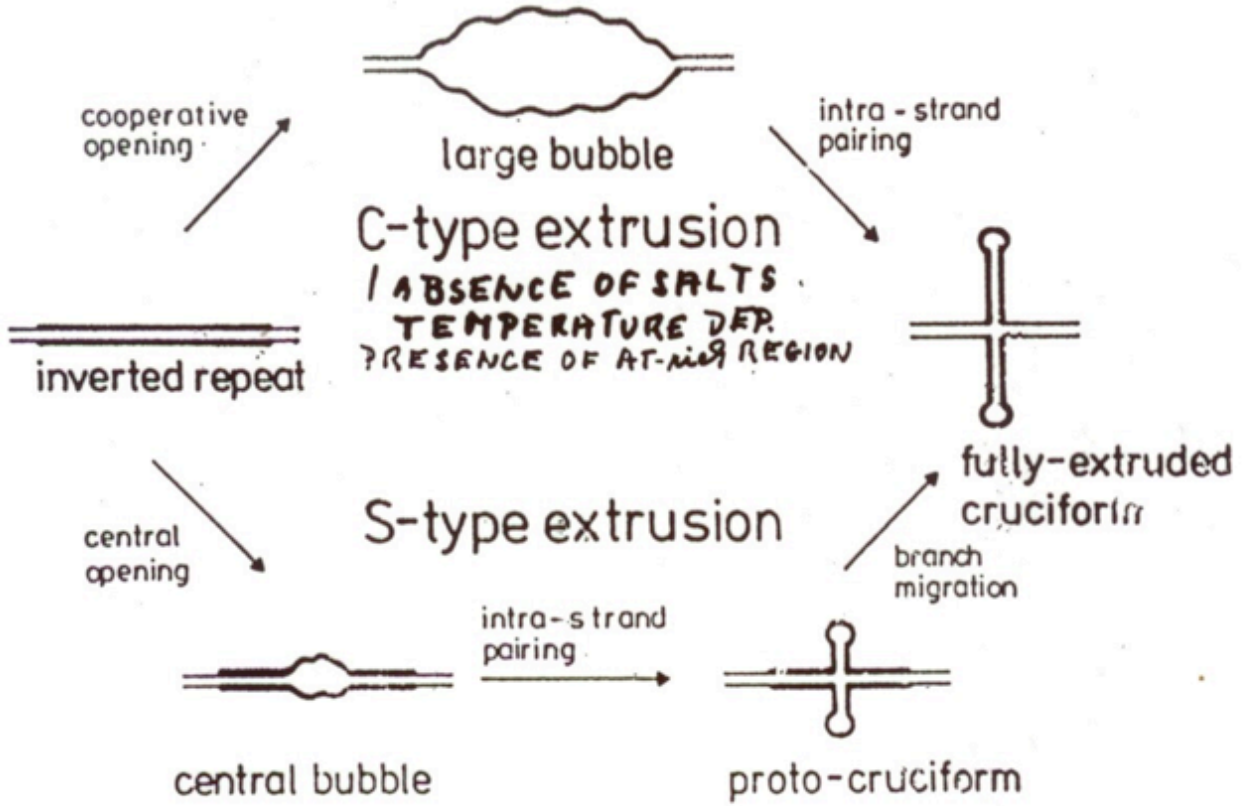


FIGURE 4. Mechanism of cruciform extrusion. The inverted repeat, represented by the thicker line, is shown in the unextruded form on the left. C-type cruciforms (top) initiate the extrusion process with a coordinate opening of many base pairs to form a large bubble. An intrastrand reassociation then forms the mature cruciform structure. The extrusion of S-type cruciforms (bottom), is initiated by a smaller opening event. Intrastrand pairing generates a smaller protocruciform, which may undergo branch migration. Base pairing is transferred from unextruded sequence to the growing cruciform stem in a multistep process to form the fully extruded structure. The principal differences between the two mechanisms lie in the initial opening and the degree of tertiary folding in the transition state. (From Lilley, D. M. J., *Chem. Soc. Rev.*, 18, 53, 1989. With permission.)

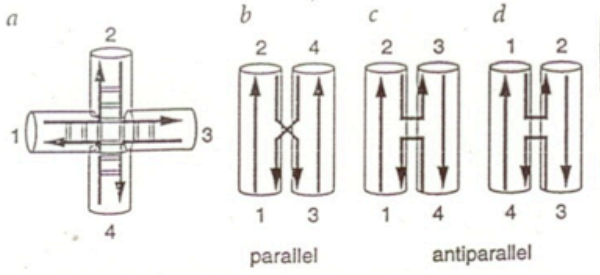


Fig. 1. Schematic of a four-way helical junction. *a*, The junction comprises four strands, indicated by different colors, forming four helical arms numbered 1–4. The polarity of the strand is indicated by the arrows drawn at the 3' termini. In this form the junction is unstacked with a square geometry. The junction can be folded by pairwise helical stacking of arms, as depicted in (*b*) through (*d*). *b*, A parallel-stranded form of the junction. In stacked forms there are two types of strand, continuous and exchanging, and in this structure the continuous strands are parallel to each other. *c*, An antiparallel structure, which can be obtained from (*b*) by a 180° rotation of the axis of the 3/4 helices relative to that of the 1/2 helices, thereby opening the center of the junction where the strands exchange between the helices. *d*, There are two conformers of these stacked forms, and the alternative form of the antiparallel structure is illustrated. In this form the stacking partners have exchanged, and thus helix 1 is now coaxially stacked on helix 4. Note that this changes the character of all the strands, so that the strands that were continuous are now exchanging, and *vice versa*.

nature structural biology • volume 6 number 10 • october 1999
897-900

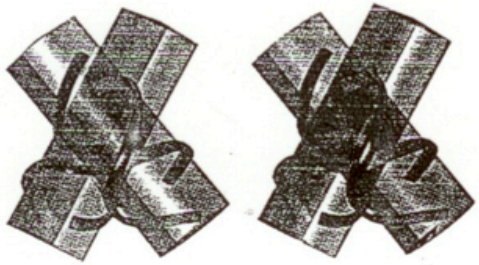


Fig. 3 The junction adopts a right-handed cross of helices, illustrated for the DNAzyme junction[3]. The view is that of the face of the junction, at 90° to that shown in Fig. 2b, for example. Cylinders have been fitted to the axes of the two pairs of stacked helices, and the path of the ribose-phosphate backbones shown by the ribbon as before. The angle between the axes is 55°, which compares well with previous biophysical estimates of ~60°. This angle should give optimal alignment between strands and grooves. However, the smaller angle found in the all-DNA junction[3] suggests that this angle may be easily altered, and perhaps crystal packing can distort this feature of the junction.

news and views

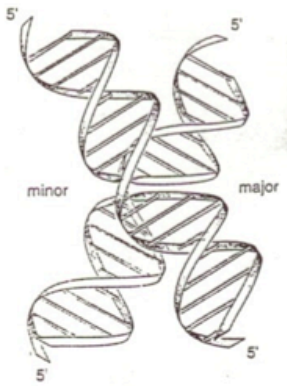


Fig. 4 The original proposal for the four-way junction structure was a stacked X-structure, as depicted here. The cartoon of the junction shows the face view, corresponding to that shown in Fig. 3. The view from the left would look into the minor groove, while that from the right looks into the major groove. The original cartoon was hand-drawn by H. Sebesse[3].

Levotočivá Z-DNA

Asi 8 let po uveřejnění CD spekter F. Pohla a T. Jovina (1971-72) byla vyřešena struktura levotočivé Z-DNA tvořené sekvencí (dC-dG)n pomocí rtg.-strukturní analýzy krystalu.

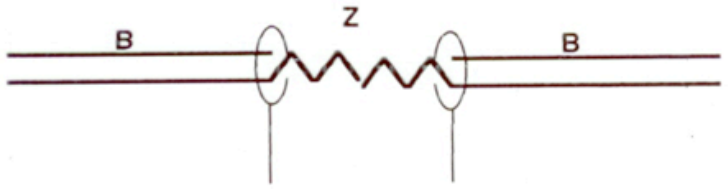
Z-DNA se stala středem zájmu vědců - v krátké době stovky publikací
Zprvu Z-DNA pozorována pouze za nefyziologických podmínek, brzy však zjištěno, v negativně superhelikální DNA se může Z-DNA vyskytovat za podmínek blízkým fyziologickým in vitro a také in vivo.

Přes toto velké úsilí její funkce není dosud zcela objasněna

Zde se dotkneme pouze několika problémů, studovaných v posledních 10-15 letech

1. Sekvence nukleotidů - levotočivá DNA v (C-G) x (A-T)
Max. 6 AT párů d(A-T)3 v Z-DNA tvořené (dC-dG)n
Specifický vliv iontů Ni^{2+}

2. Strukturní rozhraní mezi B a Z-DNA (B-Z junction)



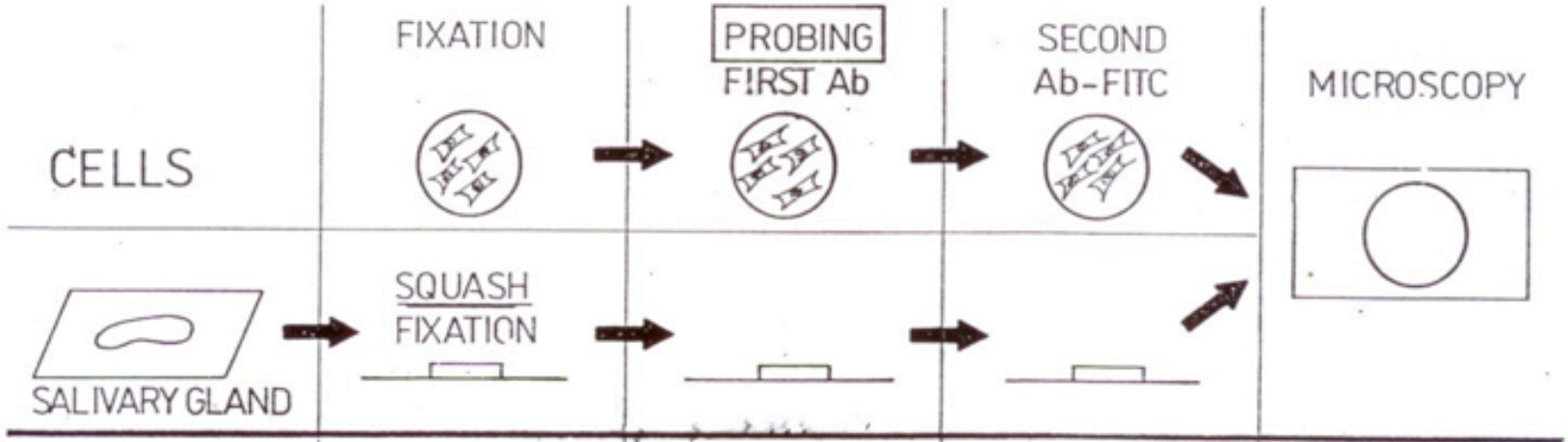
Strukturní rozhraní

(baze více dostupné pro interakce s okolím)

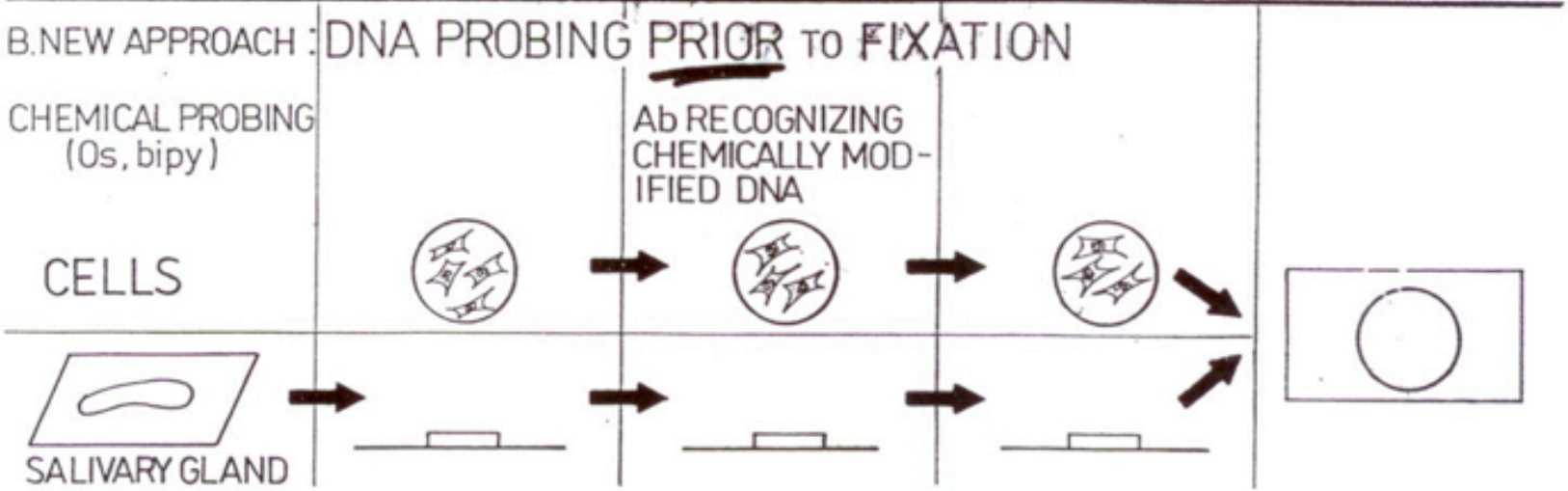
3. Existence Z-DNA in vivo

PROBING OF DNA STRUCTURE IN CELLS BY IMMUNOFLUORESCENCE

A. USUAL WAY: DNA PROBING AFTER FIXATION (M. ROBERT-NICOUD, T. JOVIN, GOTTINGEN)



B. NEW APPROACH: DNA PROBING PRIOR TO FIXATION



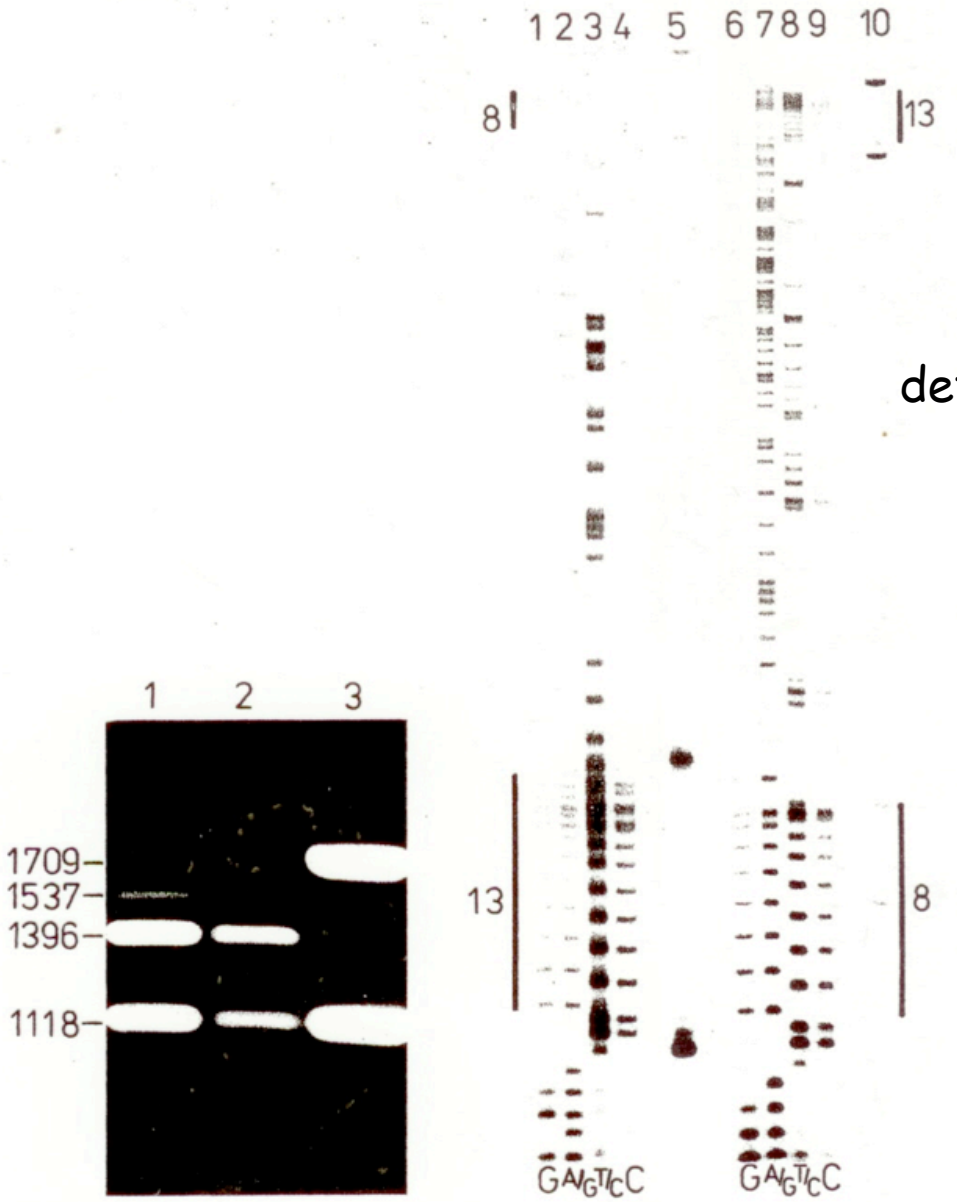


Figure 4: Sequence-mapping of osmium modified (15 min., 26°C) native pPK2 plasmid multiple cloning site (containing the insert, Figure 1). (A) DNA was digested with *Bgl*I and cleaved with either *Bam*HI (lane 1) or nuclease S1 (lane 3). Lane 2 contains the standard fragments derived by cleavage of unmodified pPK2 DNA with *Bgl*I plus *Bam*HI. (B) DNA was cleaved by *Hind*III and *Eco*RI restrictases, the *Eco*RI-*Hind*III fragment was handled according to Materials and Methods. The left part of the autoradiograph (lanes 1-5) contains the sequencing of the bottom strand (OsO_4 modified sample in lane 5), the right half (lanes 6-10) deals with the upper strand (OsO_4 modified sample in lane 10). The vertical lines span the $(dC\text{-}dG)_n$ segments, the adjoining numbers stand for *n* values in respective segments.

DNA B-Z junction

Strukturní rozhraní mezi B- a Z-DNA
detekce pomocí chemické strukturni sondy

konec/12.11.08

17.18. J. Šponer - mat. modelování NA (ca 45 min)

INTRAmolecular

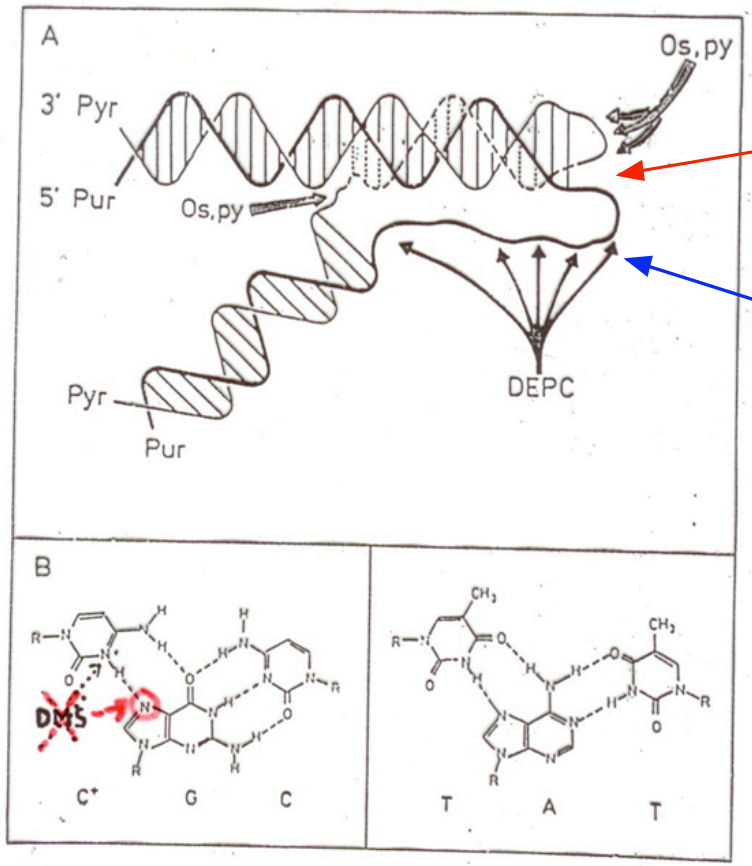


FIGURE 5. Structure of H-DNA. (A) Schematic representation of H-DNA in $(TC{\cdot}AG)_{10}$, with 3′-half of the pyrimidine (dT-dC) repeat donated to the triplex, forming the H-y3 conformer. The 5′-half of this repeat, plus the complementary 3′-half of the $(dA\text{-}dG)_n$ polyurine repeat, act as the acceptor helix in this conformation. The two halves of the polypyrimidine strand in the triplex (——— and – – – –) are antiparallel. Watson-Crick base pairs are shown as lines, Hoogsteen base pairs as dots.

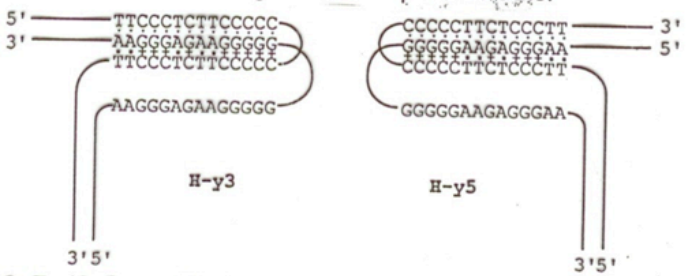


Figure 5 Two isoforms of H-DNA (91). Watson-Crick hydrogen bonds are labeled by points, nonprotonated Hoogsteen hydrogen bonds are shown by squares, and protonated Hoogsteen hydrogen bonds are shown by plus symbols.

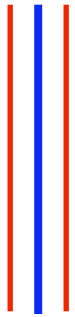
py

DNA triplexes

their identification by chemical probes

pu

INTERmolecular



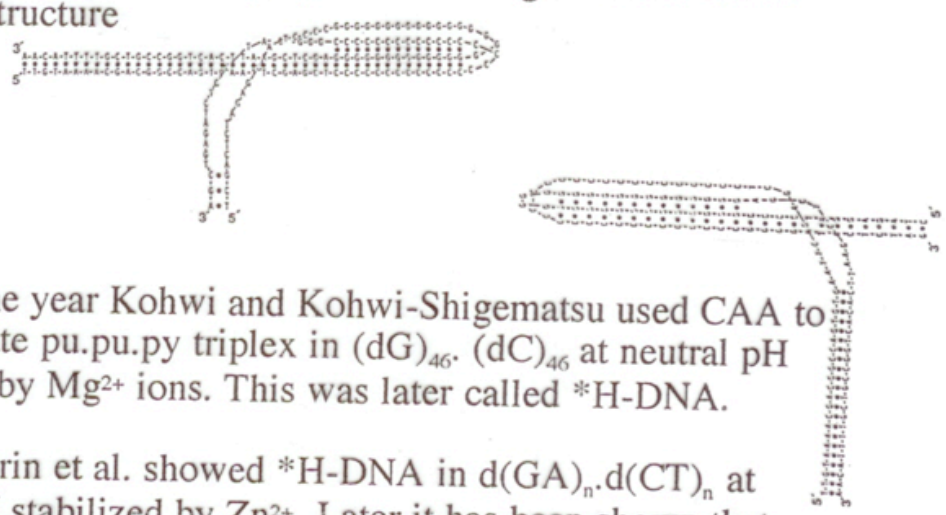
THE TRIPLEX STORY

Since 1982 (Larsen & Weintraub; Hentschel) hypersensitivity to nuclease S1(associated with homopurine homopyrimidine sequences) has been reported in active chromatin (mainly within the regulatory regions) and in supercoiled plasmids. Various structures were proposed to explain the S1 sensitivity

1986: M. Frank-Kamenetskii et al. showed by 2-D gel electrophoresis of pEJ4 plasmid containing $d(GA)_{16}.d(CT)_{16}$ sequence that the structure is stabilized by hydrogen ions and proposed a model of H-DNA triplex

1987: Vojtíšková and Paleček applied for the first time single-strand selective chemical probes to study at low resolution the H-DNA in pEJ4 and confirmed the Frank-Kamentskii model

1988: Several laboratories (Dahlberg, Frank-Kamenetskii, Johnston, Paleček, Wells) applied this approach at single-nucleotide resolution bringing a convincing evidence of the H-DNA structure



In the same year Kohwi and Kohwi-Shigematsu used CAA to demonstrate pu.pu.py triplex in $(dG)_{46}.(dC)_{46}$ at neutral pH stabilized by Mg^{2+} ions. This was later called *H-DNA.

1989: Azorin et al. showed *H-DNA in $d(GA)_n.d(CT)_n$ at neutral pH stabilized by Zn^{2+}. Later it has been shown that *H-DNA can be formed in various sequences and can be stabilized by various bivalent ions

1990 existence of triplex H-DNA in cells was demostrated in Paleček's laboratory

Homopurine.homopyrimidine mirror repeat sequence
(H palindrome) is necessary for the formation of H-DNA.

Mirror repeat (H palindrom)

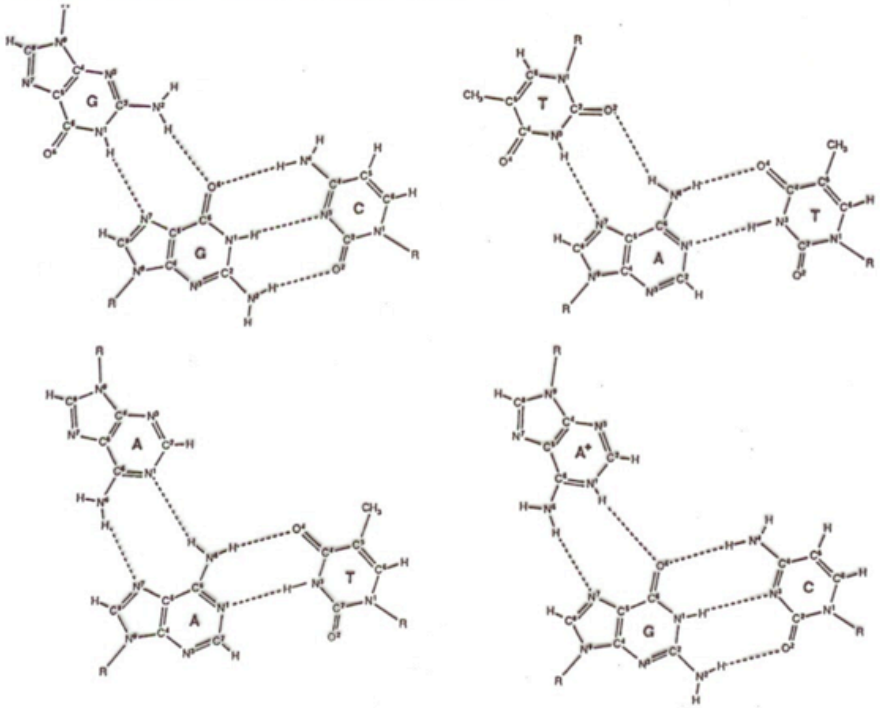
AAGGGAGAAGGGGG TATA GGGGG AAGAGGGAA
TTCCC TCTTCCCCC ATAT CCCCCTTCT CCCTT
** * * **

H-DNA: CG*C$^+$ and TA*T

---

*H-DNA is more versatile as compared to H-DNA with respect sequence requirements.

*H-DNA: CG*G and TA*A but also TA*T, CGA$^+$ and less stable TA*C and GC*T



therefore *H-DNA can be formed by sequences that are neither homopurine.homopyrimidine nor mirror repeat

Homopy.homopy sekvence se zrcadlovou symetrii je potřebná pro vznik triplexu H-DNA

*H-DNA může vzniknout i v jiných sekvencích

E. Trifonov, Weizmann Inst, Rehovot
M. Frank-Kamenetskii, Boston Univ
S. Mirkin, Univ. Illinois, Chicago
D. Lyamichev .....

všichni původně v Moskvě

G-quartet

G-quartet

This structure (**Figure 11a**) can be formed by direct repeats containing tandemly arranged runs of guanines. The building elements are stacked G4 runs that are stabilized by certain monovalent cations (**Figure 11b**). This structure is definitely formed by single-stranded G-rich direct tandem repeats (such as telomeric repeats in eukaryotes) and is extensively characterized at atomic resolution. However, there are only fragmentary indications that it exists in superhelical DNA.

DOPLNĚK k předn. prof. M.Vorlíčkové

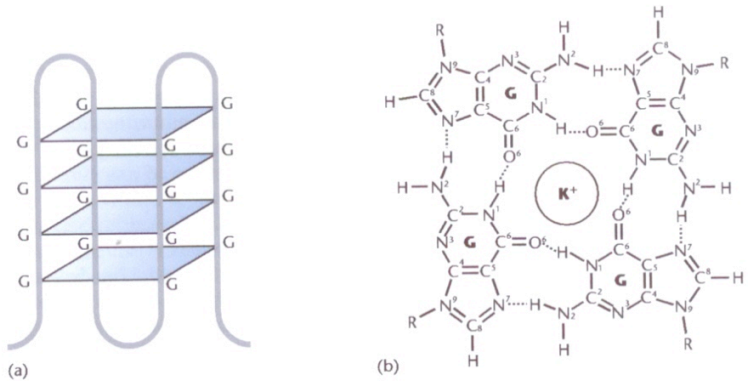


Figure 11 G-quartet. (a) General overview. The black line is the DNA strand and the purple rectangles are the stacked G-quartets. (b) Structure of a G-quartet.

$(C_4A_2)_n \cdot (T_2G_4)_n$ TETRAHYMENA $n = 50$ and > 50
$(C_4A_4)_n \cdot (T_4G_4)_m$ OXYTRICHA E. BLACKBURN

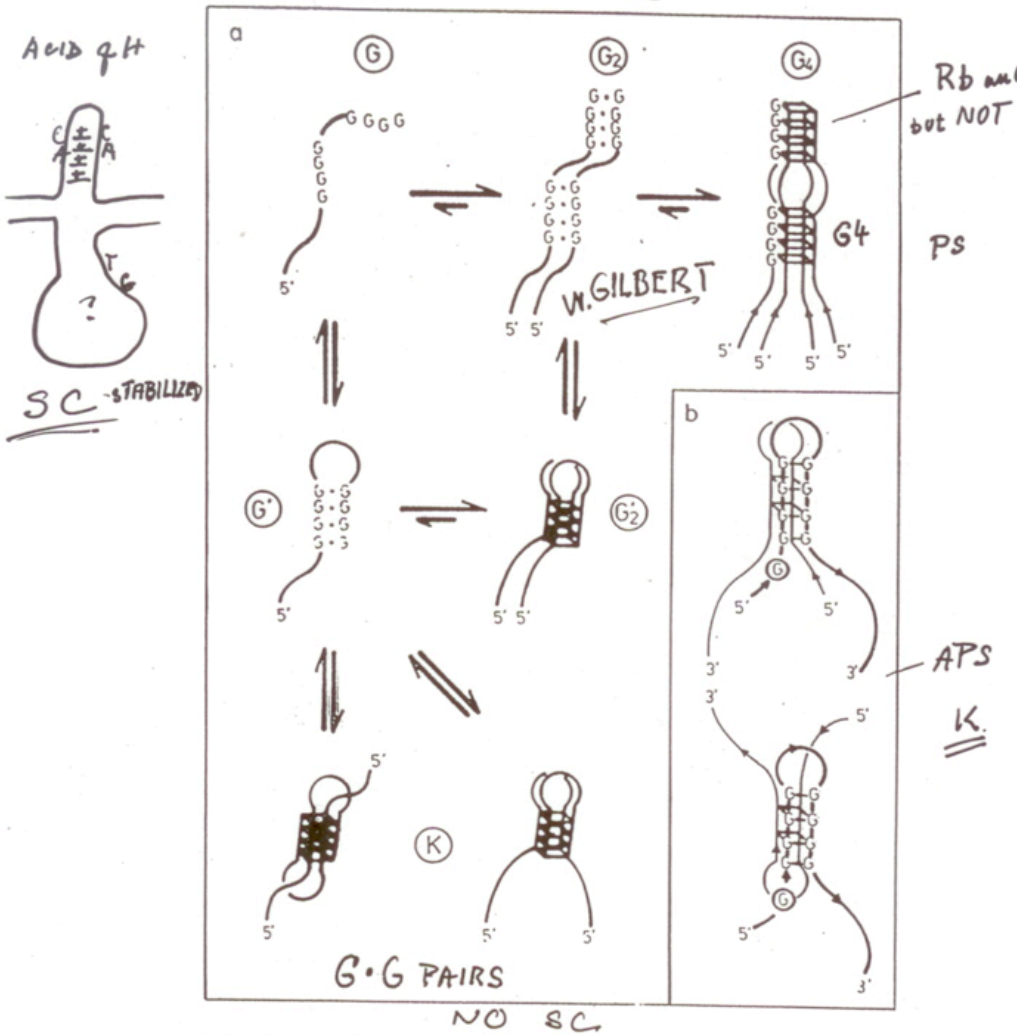


FIGURE 6. (a) Scheme for the formation of G_4-DNA. The formation of the dimer structure G_2 must be rate-limiting, and it must be rapidly converted into G_4. Only three possible structures (including K and G_2') of fold-back intermediates are shown, but other structures may also be formed. (b) Structures of product K most compatible with its methylation-protection pattern. The methylation-enhanced guanine is circled. The arrows indicate the 5′-3′ direction of the sugar-phosphate backbone. (Reprinted by permission from Sen, D. and Gilbert, W., *Nature*, 244, 410, 1990.)

TELOMERS - REPLICATION AND MAINTENANCE OF CHROMOSOMAL ENDS

Oblasti s nespárovanými bazemi
[Base unpaired (unwound) regions]

v sekvencích bohatých páry AT, stabilizovány superhelicitou (supercoil-stabilized)

Paralelní řetězce (parallel-stranded, ps) DNA
připraveny synteticky jako např. vlásenkové struktury (hairpin) v nichž smyčky (loops) smyčky s fosfodiesterickými vazbami 5'-5' nebo 3'-3' stabilizovali paralelní orientaci řetězců

triplexy, kvadruplexy

Unusual structures - jsou opravdu neobvyklé?
Společným rysem lokální struktur stabilizovaných superhelicitou je jejich spojení s otevřenými oblastmi - baze dostupné pro interakci s okolím "open DNA structures".

Výskyt lokálních struktur in vivo

Některé sekvence potřebné pro vznik těchto struktur jsou abundantní

Metodické prostředky pro analýzu struktur DNA in vivo jsou omezené (ve srovnání s analýzou in vitro)

Prokázána existence křížových forem, Z-DNA i triplexů in vivo.

ZÁVĚRY

PROSTOROVÉ USPOŘÁDÁNÍ DNA JE ZÁVISLÉ NA SEKVENCI NUKLEOTIDŮ, PODMÍNKÁCH PROSTŘEDÍ A HUSTOTĚ NADŠROUBOVICOVÉHO VINUTÍ

CHEMICKÉ SONDY JSOU V POSLEDNÍCH LETECH STÁLE VÍCE POUŽÍVÁNY PŘI STUDIU LOKÁLNÍCH STRUKTUR DNA STABILIZOVANÝCH NADŠROUBOVICOVÝM VINUTÍM (na př. KŘÍŽOVÉ FORMY, TRIPLEXY A SEGMENTY LEVOTOČIVÉ DNA)

KOMPLEXY OXIDU OSMIČELÉHO PATŘÍ K NEJČASTĚJI POUŽÍVANÝM CHEMICKÝM SONDÁM STRUKTURY DNA

K JEJICH VÝHODÁM PATŘÍ:

1. SNADNÁ DETEKCE MÍSTA VAZBY SONDY V POLYNUKLEOTIDOVÉM ŘETĚZCI NA ÚROVNI ROZLIŠENÍ JEDNOTLIVÝCH NUKLEOTIDŮ

2. JEDNODUCHÁ PŘÍPRAVA SOND S RŮZNÝMI VLASTNOSTMI, ZÁMĚNOU DUSIKATÝCH LIGANDŮ

3. DOSTUPNOST POLYKLONÁLNÍCH A MONOKLONÁLNÍCH PROTILÁTEK S VYSOKOU SPECIFICITOU VŮČI ADUKTŮM SOND S DNA

4. POUŽITELNOST KE STUDIU STRUKTURY DNA *in vivo* (PŘÍMO V PROKARYOTNÍCH A EUKARYOTNÍCH BUŇKÁCH)

VÝZKUM STRUKTURY DNA PŘÍMO V BUŇKÁCH (POMOCÍ CHEMICKÝCH SOND) OTEVÍRÁ NOVÉ MOŽNOSTI STUDIU VZTAHŮ MEZI SEKUNDÁRNI A TERCIÁRNÍ STRUKTUROU DNA na straně jedné a JEJÍ FUNKCÍ na straně druhé

Výskyt lokálních struktur DNA v prokaryotních a eukaryotních buňkách

Superhelical torsion in cellular DNA responds directly to environmental and genetic factors

(DNA supercoiling/cellular DNA topology/cruciform/topoisomerase/osmium tetroxide)

JAMES A. MCCLELLAN*, PAVLA BOUBLÍKOVÁ†, EMIL PALEČEK†, AND DAVID M. J. LILLEY*‡

*Department of Biochemistry, The University, Dundee DD1 4HN, United Kingdom; and †Institute of Biophysics, Czechoslovak Academy of Sciences, Kralovopolska 135, 612 65 Brno, Czechoslovakia

Communicated by I. Tinoco, Jr., July 13, 1990

ABSTRACT Superhelical tension of DNA in living bacteria is believed to be partially constrained by interaction with proteins. Yet DNA topology is a significant factor in a number of genetic functions and is apparently affected by both genetic and environmental influences. We have employed a technique that allows us to estimate the level of unconstrained superhelical tension inside the cell. We study the formation of cruciform structures by alternating adenine-thymine sequences in plasmid DNA by *in situ* chemical probing. This structural transition is driven by superhelical torsion in the DNA and thus reports directly on the level of such tension in the cellular DNA. We observe that the effect of osmotic shock is an elevation of superhelical tension; quantitative comparison with changes in plasmid linking number indicates that the alteration in DNA topology is all unconstrained. We also show that the synthesis of defective topoisomerase leads to increased superhelical tension in plasmid DNA. These experiments demonstrate that the effect of environmental and genetic influences is felt directly at the level of torsional stress in the cellular DNA.

Proc. Natl. Acad. Sci. USA
87 (1990) 8373-8377

Chemická modifikace DNA v buňkách pomocí komplexu OsO_4 (Os,bipy) a její využití pro testování -σ DNA

Strukturní přechod DNA duplex – křížová forma v buňce může informovat o superhelikální hustotě DNA a o jejich změnách působených změnami prostředí nebo genetickými faktory

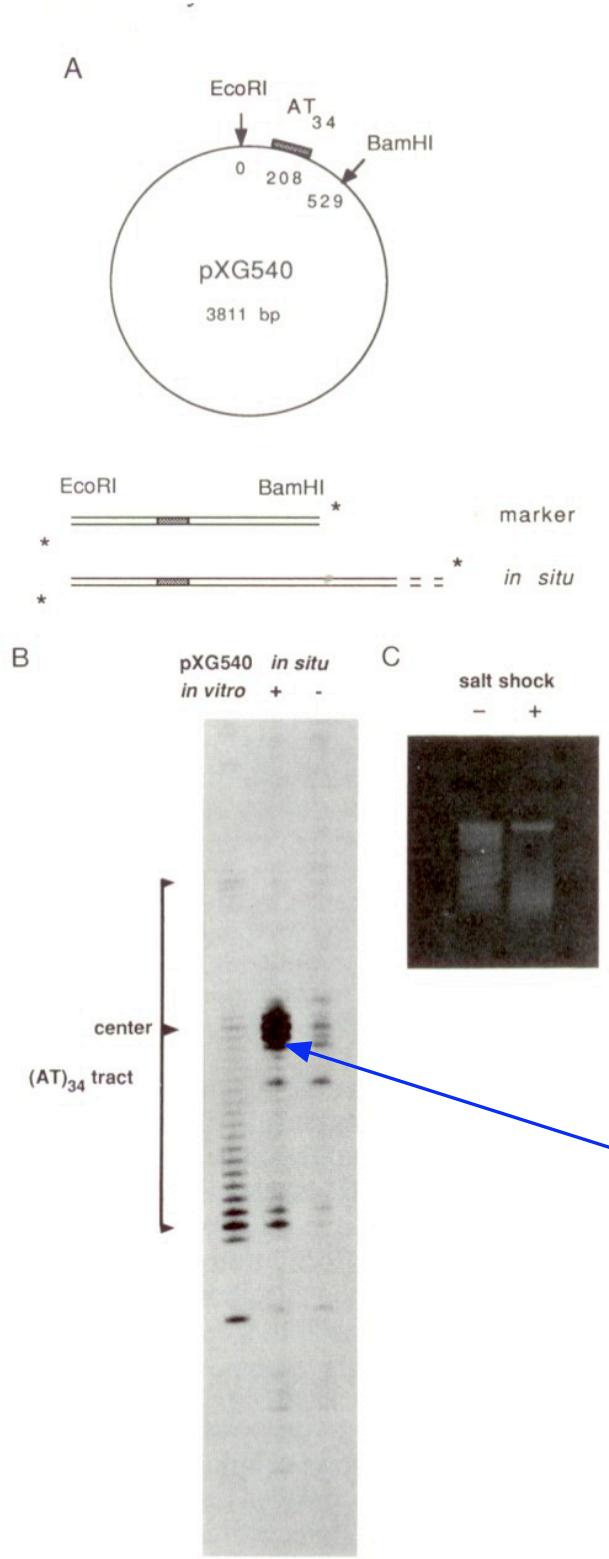


FIG. 1. Chemical modification of a cruciform structure adopted by $(A\text{-}T)_{34}$ sequence present in *E. coli*. (*A*) Map of pXG540 showing the $(A\text{-}T)_{34}$ sequence (stippled). In the *in situ* modification experiments, the plasmid was cleaved with *Eco*RI and radioactively labeled at the resulting 3′ termini. For *in vitro* modification of pXG540 as a marker, the DNA was cleaved with *Eco*RI and *Bam*HI and labeled at the resulting 3′ termini. (*B*) *In situ* modification of pXG540 with and without salt shock. The DNA in the left-most lane was obtained by *in vitro* modification of pXG540 with osmium tetroxide under conditions where there is uniform reactivity of all thymine bases in the $(A\text{-}T)_{34}$ tract (43) and can be used to identify the extent of the alternating sequence, indicated by the arrowheads on the left side. Note the strong modification at the center of the $(A\text{-}T)_{34}$ tract by osmium tetroxide, provided that the cells were subjected to the salt-shock procedure. The middle and right lanes show *in situ* modification with (+) and without (−) salt shock. (*C*) Effect of salt shock on the linking number of plasmid extracted from *E. coli*. Cells were grown as above, with and without salt shock, and pXG540 DNA was prepared. This was electrophoresed in a 1% agarose gel in 90 mM Tris·borate (pH 8.3), 10 mM EDTA, and chloroquine (4 μg/ml). Note that the salt-shocked DNA is more supercoiled than that of the untreated cells, by approximately six turns.

We conclude that these differences reflect different extents

Salt shock

Cruciform structures in E. coli cells

TRIPLEX DNA V BUŇKÁCH PROKÁZANÝ POMOCÍ Os,bipy

P. Karlovský, P. Pecinka, M. Vojtísková, E. Makaturová, E. Palecek,
FEBS Letters 274 (1990)39-42

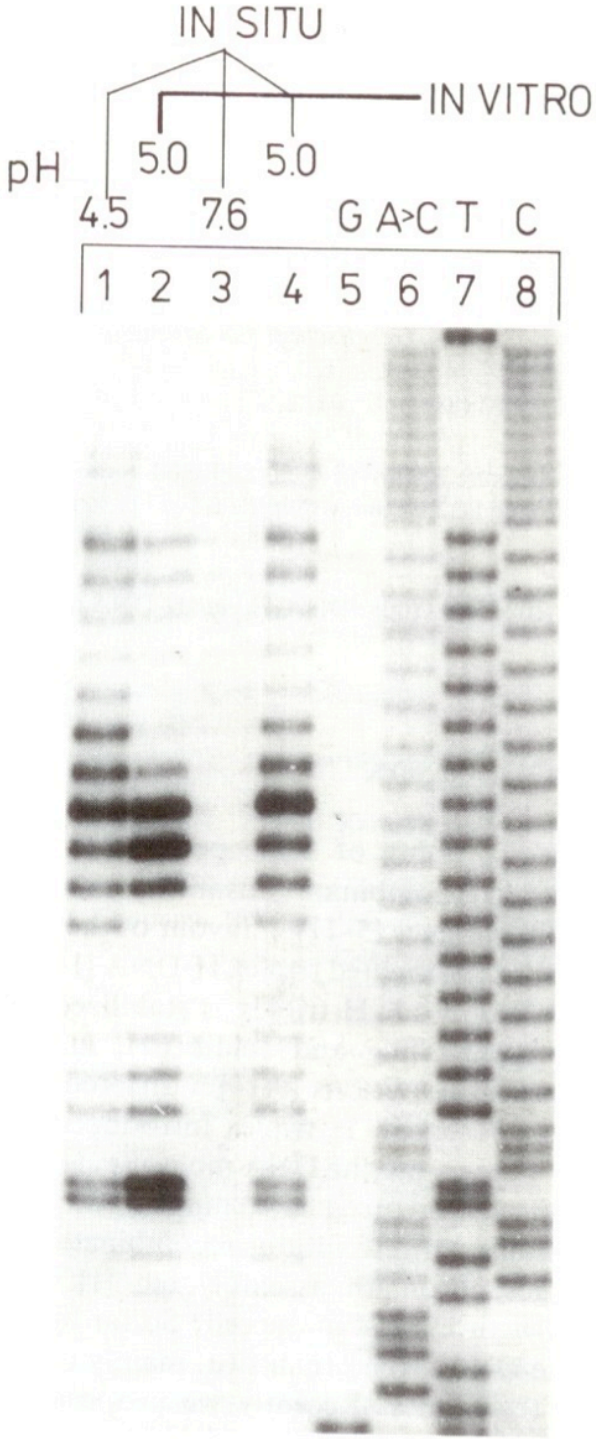


Fig. 2. Osmium binding sites in the homopurine–homopyrimidine region of pL153. *E. coli* JM109 (pL153) treated with Os,bipy at 4.5 (lane 1), pH 5.0 (lane 4) and pH 7.6 (lane 3). pL153 DNA modified in vitro at pH 5.0 (lane 2).

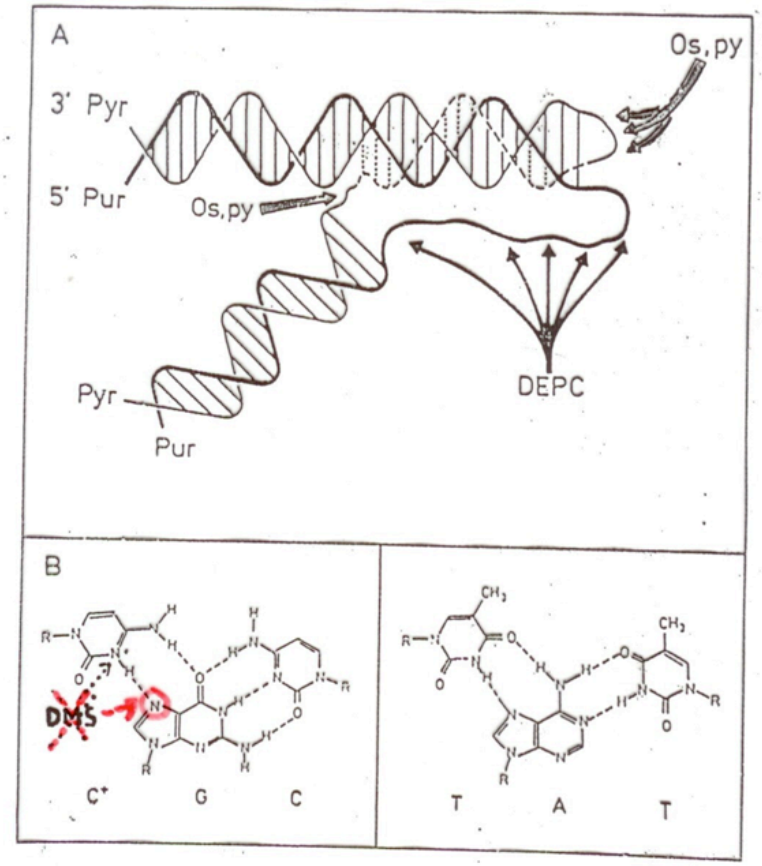


FIGURE 5. Structure of H-DNA. (A) Schematic representation of H-DNA in $(TC{\cdot}AG)_{10}$, with 3′-half of the pyrimidine (dT-dC) repeat donated to the triplex, forming the H-y3 conformer. The 5′-half of this repeat, plus the complementary 3′-half of the $(dA\text{-}dG)_n$, polyurine repeat, act as the acceptor helix in this conformation. The two halves of the polypyrimidine strand in the triplex (——— and – – – –) are antiparallel. Watson-Crick base pairs

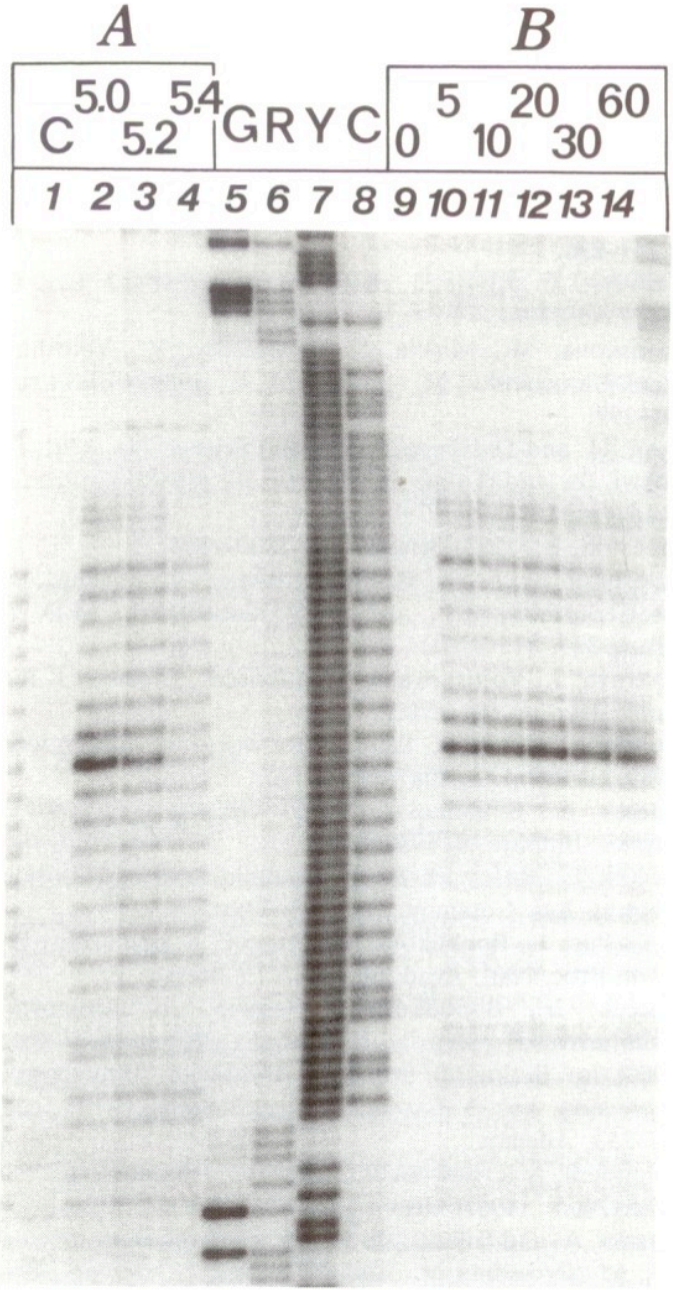


Fig. 3. Osmium binding sites in the homopurine–homopyrimidine sequence of pL153 present in *E. coli* cells. (A) pH dependence: cells were treated with Os,bipy at pH 5.0 (lane 2), pH 5.2 (lane 3) and pH 5.4 (lane 4). Lane 1, unmodified control. (B) Time dependence: cells were treated with Os,bipy at pH 5.0 for 0 (lane 9), 5 (lane 10), 10 (lane 11), 20 (lane 12), 30 (lane 13) and 60 min (lane 14). R, purine; Y, pyrimidine.

Konformer H-y5
pravděpodobně
v buňkách převažuje

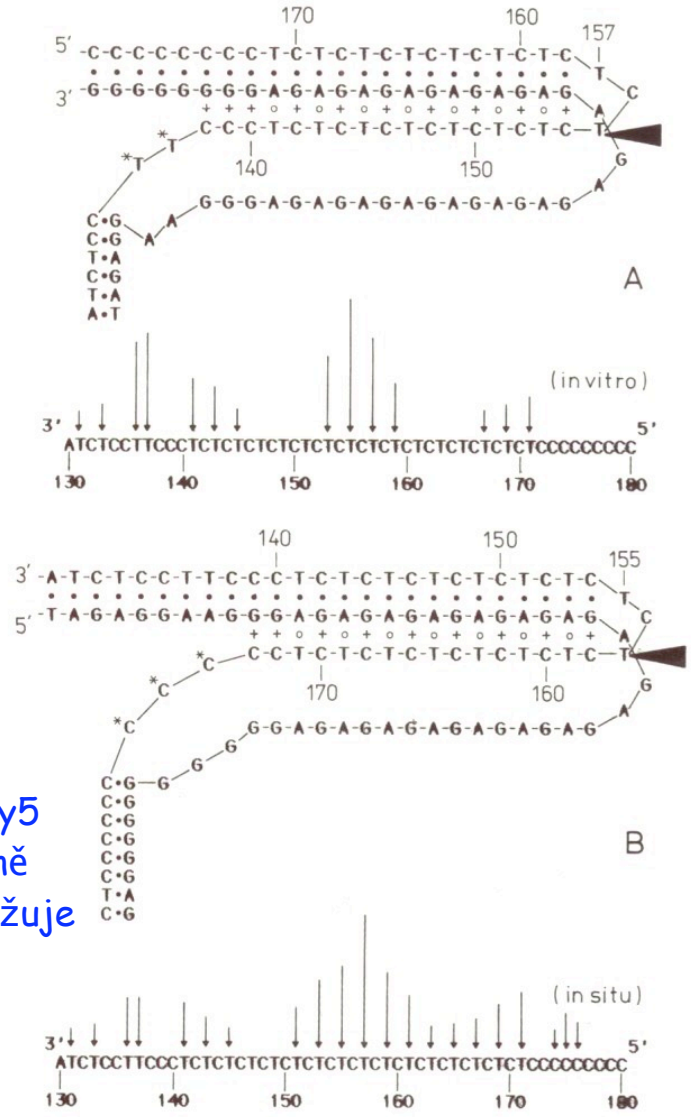


Fig. 4. (A) H-y3 and (B) H-y5 conformers [23] of the H-DNA triplex [4] and the Os,bipy modification of bases in the polymyrimidine tract of the pL153 insert (A), in vitro and (B), in situ. The lengths of the vertical arrows in the nucleotide sequence represent the relative intensities of the bands on the sequencing gel (Fig. 2) obtained by densitometric tracing, (A) after Os,bipy treatment of the supercoiled plasmid pL153 DNA in vitro (Fig. 2, lane 2) and (B) after treatment of cells with Os,bipy at external pH 5.0 (Fig. 2, lane 4). The main element of both H conformers is a triple helix [4] which includes the Watson-Crick duplex (•) associated with the homopyrimidine strand by Hoogsteen base pairing (o, +) where cytosines are protonated. The triangle shows the strongest modified base in the triplex and the asterisks denote the modification at the B–H junctions.

Unconstrained supercoiling in eukaryotic cells

In difference to the prokaryotic genome the **eukaryotic genome was for years believed not to be under the superhelical stress due to the accommodation of the DNA writhing around histone octamers in nucleosomes** [Pearson, 1996 #96; van Holde, 1994 #72] . The **actively transcribing portion of the eukaryotic genome was, however, shown to contain unconstrained supercoiling, part of which can be attributed to the process of transcription *per se*** [van Holde, 1994 #72; Chen, 1994 #101; Cook, 1994 #100; Pearson, 1996 #96].

Using prokaryotic cells it has been recently shown [Krasilnikov, 1999 #93] that the effects of transcriptionally driven supercoiling are remarkably large scale in vivo (in a kbp range). Similarly to the **transcription effects, in DNA replication intermediates supercoils are formed both behind and in front of the replication fork and superhelical stress is distributed throughout the entire partially replicated DNA molecule** ([Peter, 1998 #87] and references therein) . **A number of additional processes may operate creating transient and localized superhelical stresses in eukaryotic DNA** [van Holde, 1994 #72] . These may include **binding of various proteins such as transcription factors and helix-tracking proteins, looping of DNA by protein binding at two locations, nucleosomal displacement, histone acetylation and gyrase activity of topoisomerases** .

Unconstrained **negative supercoiling stabilizes local DNA structures such as cruciforms, Z-DNA segments and intramolecular triplexes. Mounting evidence of the existence of these structures in vivo both in prokaryotic and eukaryotic cells** has been reviewed [Pearson, 1996 #96; van Holde, 1994 #72; Palecek, 1991 #80; Herbert, 1996 #68]. **It appears that alternative DNA structures are located in extranucleosomal regions such as linkers and DNase-hypersensitive sites** [van Holde, 1994 #72] **but probably not within the DNA wrapped around DNA octamer** .

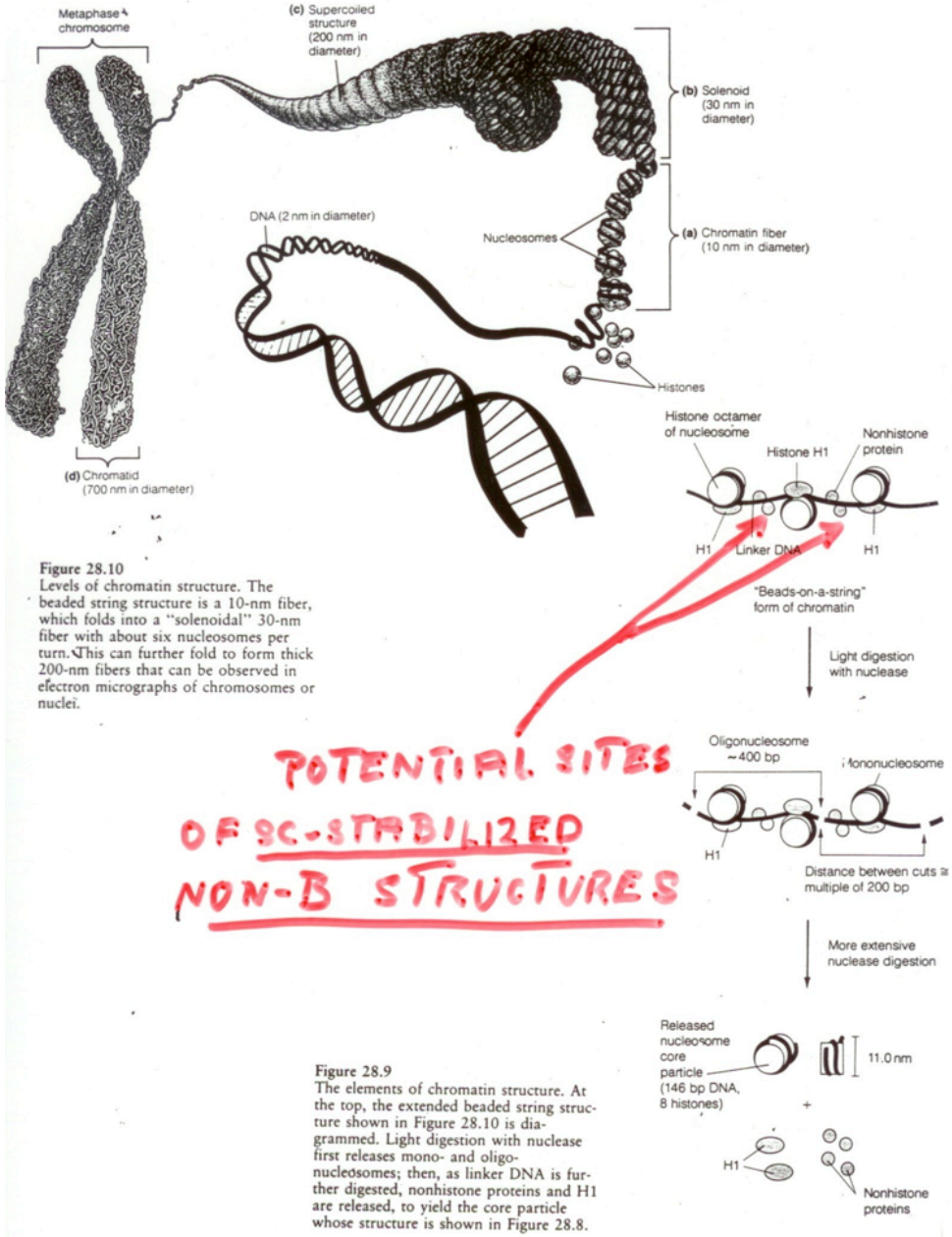


Figure 28.10
Levels of chromatin structure. The beaded string structure is a 10-nm fiber, which folds into a "solenoidal" 30-nm fiber with about six nucleosomes per turn. This can further fold to form thick 200-nm fibers that can be observed in electron micrographs of chromosomes or nuclei.

POTENTIAL SITES OF SC-STABILIZED NON-B STRUCTURES

Figure 28.9
The elements of chromatin structure. At the top, the extended beaded string structure shown in Figure 28.10 is diagrammed. Light digestion with nuclease first releases mono- and oligonucleosomes; then, as linker DNA is further digested, nonhistone proteins and H1 are released, to yield the core particle whose structure is shown in Figure 28.8.

Recently **mechanisms by which the effect of supercoil-stabilized non-B DNA structures on transcription can be exerted in eukaryotic cells have been proposed** [van Holde, 1994 #72] .

If the promoter region of the gene is blocked by a nucleosome and sites for transcription factors are inaccessible, nucleosome dissociation can result in transcriptional activation provided transcription factors are available; such dissociation is accompanied by formation of negative supercoils capable to induce alternative structure in a linker. Similar transcriptional activation may be produced by binding of a specific protein, shifting the equilibrium in favor of an alternative DNA structure, followed by formation of positive supercoils and loss of a nucleosome. For this mechanism, denominated as *conformational compensation* , precise location or orientation c the potential non-B sequence is not critical.

Another mechanism based on binding of proteins to potential non-B sequences has been proposed by Hatfield at al. [Sheridan, 1999 #70; Sheridan, 1998 Parekh, 1996 #71].

Supercoiling can locally destabilize B-DNA structure and drive transitions to other structures at susceptible sequences. In principle the supercoil driven local structural transitions can be either inhibited or facilitated by proteins that bind at or near potential transition sites [Sheridan, 1999 #70; Sheridan, 1998 #69; Parekh, 1996 #71]. **If a DNA segment, susceptible to forming a supercoil-induced alternative structure, is stabilized in the B-form by a DNA-binding protein, the propensity of this segment for structural transition will be transferred to another site within the same DNA domain. Positioning of this site in the promoter region may facilitate open complex formation and activation of gene expression.**

Transkripce může být aktivována vazbou specif. proteinu (např. Z-binding) posunující rovnováhu ve prospěch lokální struktury, následované vznikem pozitivní nadšroubovice a ztrátou nukleosomu

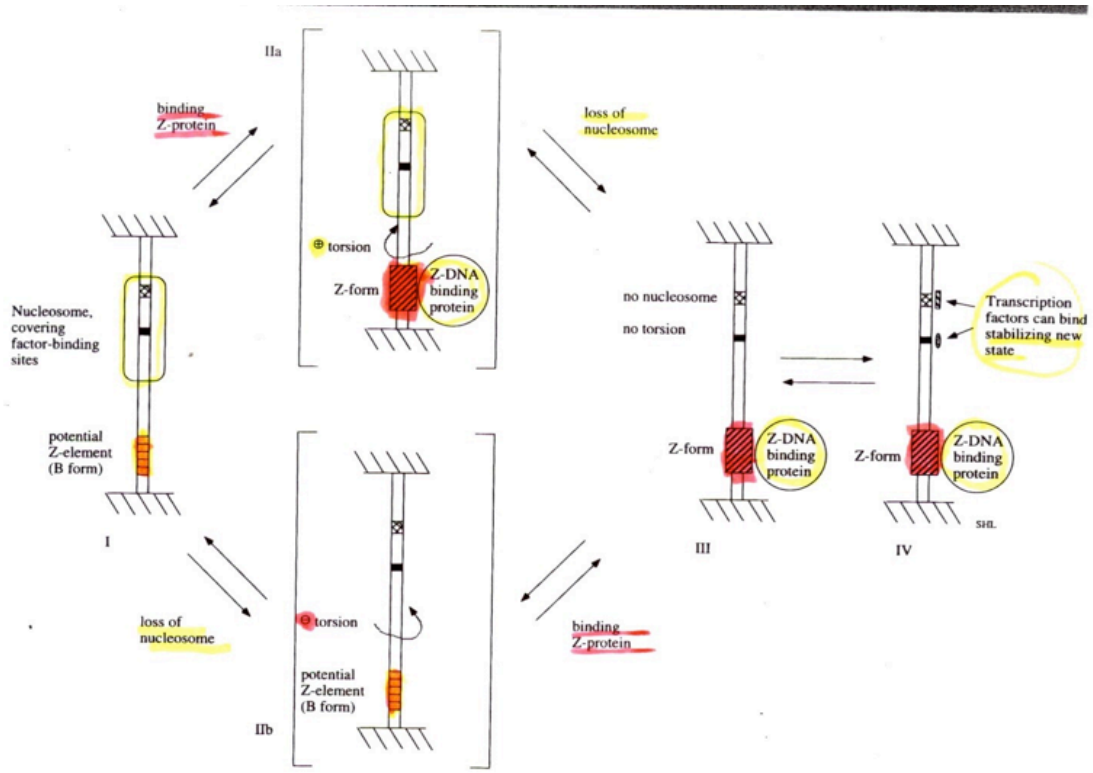


Fig. 2. Conformational compensation as a potential model for gene activation. A region of chromatin is constrained by surrounding structures in the nucleus. Here for specificity we imagine a segment containing one nucleosome in the promoter regions of a gene with a potential Z-element in an adjacent linker (state I). Either binding of a Z-protein or transient dissociation of the nucleosome lead to energetically unfavorable transition states (IIa or IIb) with DNA under torsion. This can be relaxed as shown in III, a state which could then be stabilized by the binding of transcription factors to uncovered sites (state IV).

Disociace nukleosomu vede k aktivaci transkripce a tvorbě negativní nadšroubovice (supercoil) schopné indukovat lokální struktury.

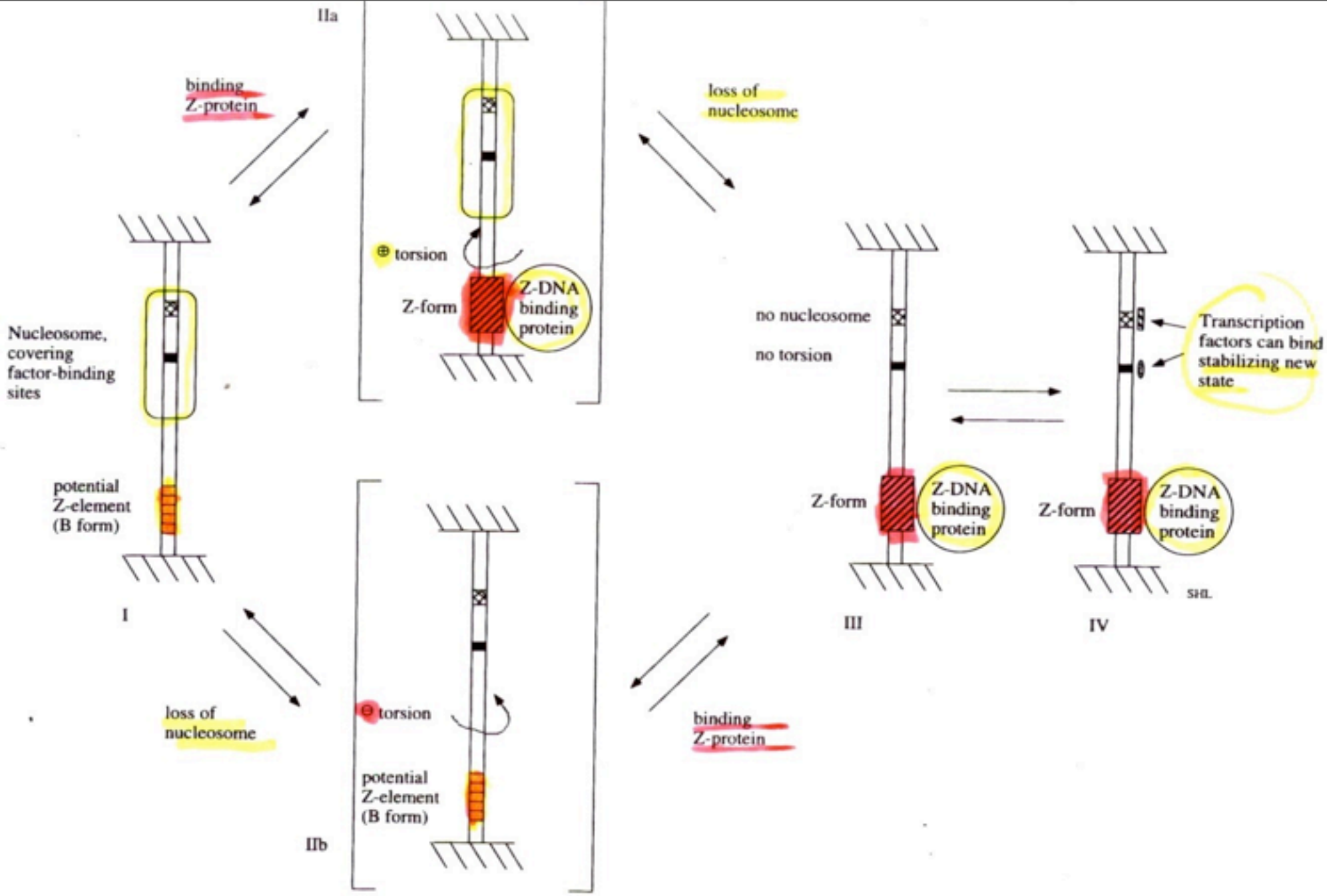


Fig. 2. Conformational compensation as a potential model for gene activation. A region of chromatin is constrained by surrounding structures in the nucleus. Here for specificity we imagine a segment containing one nucleosome in the promoter regions of a gene with a potential Z-element in an adjacent linker (state I). Either binding of a Z-protein or transient dissociation of the nucleosome lead to energetically unfavorable transition states (IIa or IIb) with DNA under torsion. This can be relaxed as shown in III, a state which could then be stabilized by the binding of transcription factors to uncovered sites (state IV).

Merry Christmas and
a Happy New Year
2009!