Edward N. Trifonov

GENETIC CODES

„Вот послушай. Я уж знаю: скучно не будет.
А заскучаешь, значит, полный ты м.....
и ни ... не петришь в биологии молекулярно

(Юз Алешковский,
„Николай Николаевич")

"Listen. I know it's not going to be boring.
And if you'll get bored, then you are
f....ng fool with no idea what molecular
biology is about"

(Y. Aleshkovsky,
„Nikolai Nikolaevich")

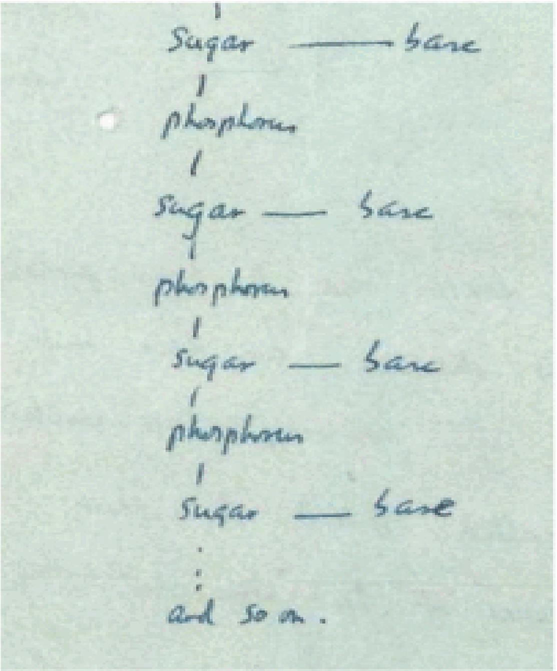
19 Portugal Place
Cambridge
19 March '53

My Dear Michael,

Jim Watson and I have probably made a most important discovery. We have built a model for the structure of des-oxy-ribose-nucleic-acid (read it carefully) called D.N.A. for short. You may remember that the genes of the chromosomes -- which carry the hereditary factors -- are made up of protein and D.N.A.

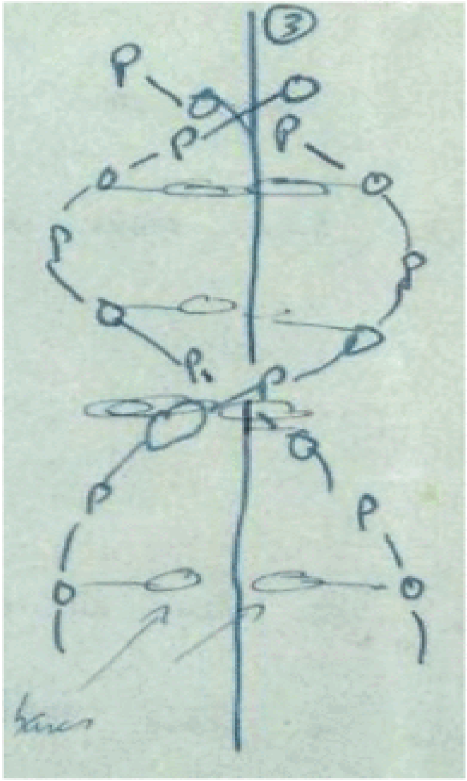
Our structure is very beautiful. D.N.A. can be thought of roughly as a very long chain with flat bits sticking out. The flat bits are called the "bases". The formula is rather like this.

```
[diagram]
       :
       I
sugar -- base
       I
phosphorus
       I
sugar -- base
       I
phosphorus
       I
sugar -- base
       I
phosphorus
       I
sugar -- base
       .
       :
and so on.
```



```
 |
Sugar ——— base
 |
phosphorus
 |
Sugar —— base
 |
phosphorus
 |
Sugar —— base
 |
phosphorus
 |
Sugar —— base
 .
 :
and so on.
```

Now we have two of these chains winding round each other -- each one is a helix -- and the chain, made up of sugar and phosphorus, is on the outside, and the bases are all on the inside. I can't draw it very well, but it looks like this



[drawing of double helix showing base pairings on inside]

The model looks much nicer than this.

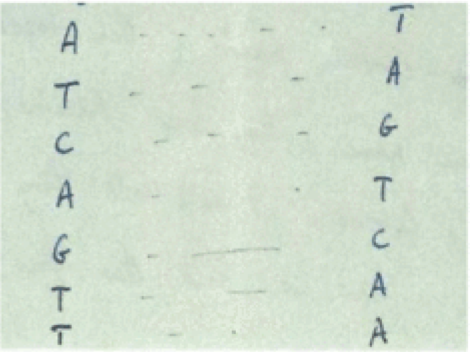
Now the exciting thing is that while these are 4 different bases, we find we can only put certain pairs of them together. Thee bases have names. They are Adenine, Guanine, Thymine & Cytosine. I will call them A, G, T and C. Now we find that the pairs we can make -- which have one base from one chain joined to one base from another -- are
only A with T

and G with C.

Now on one chain, as far as we can see, one can have the bases in any order, but if their order is fixed, then the order on the other chain is also fixed. For example, suppose the first chain goes

> then the second must go

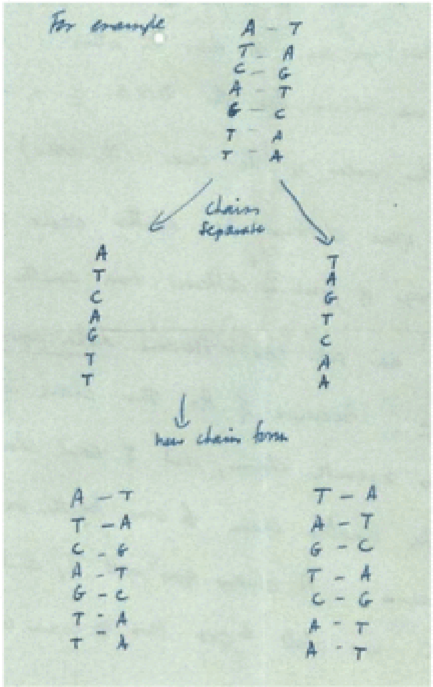
A - - - - - - - - - - - - - - - - - T
T - - - - - - - - - - - - - - - - - A
C - - - - - - - - - - - - - - - - G
A - - - - - - - - - - - - - - - -- - T
G - - - - - - - - - - - - - - - - - C
T - - - - - - - - - - - - - - - - - A
T - - - - - - - - - - - - - - - - - A


A - - - T
T - - - A
C - - - G
A - - - T
G - - - C
T - - - A
T - - - A

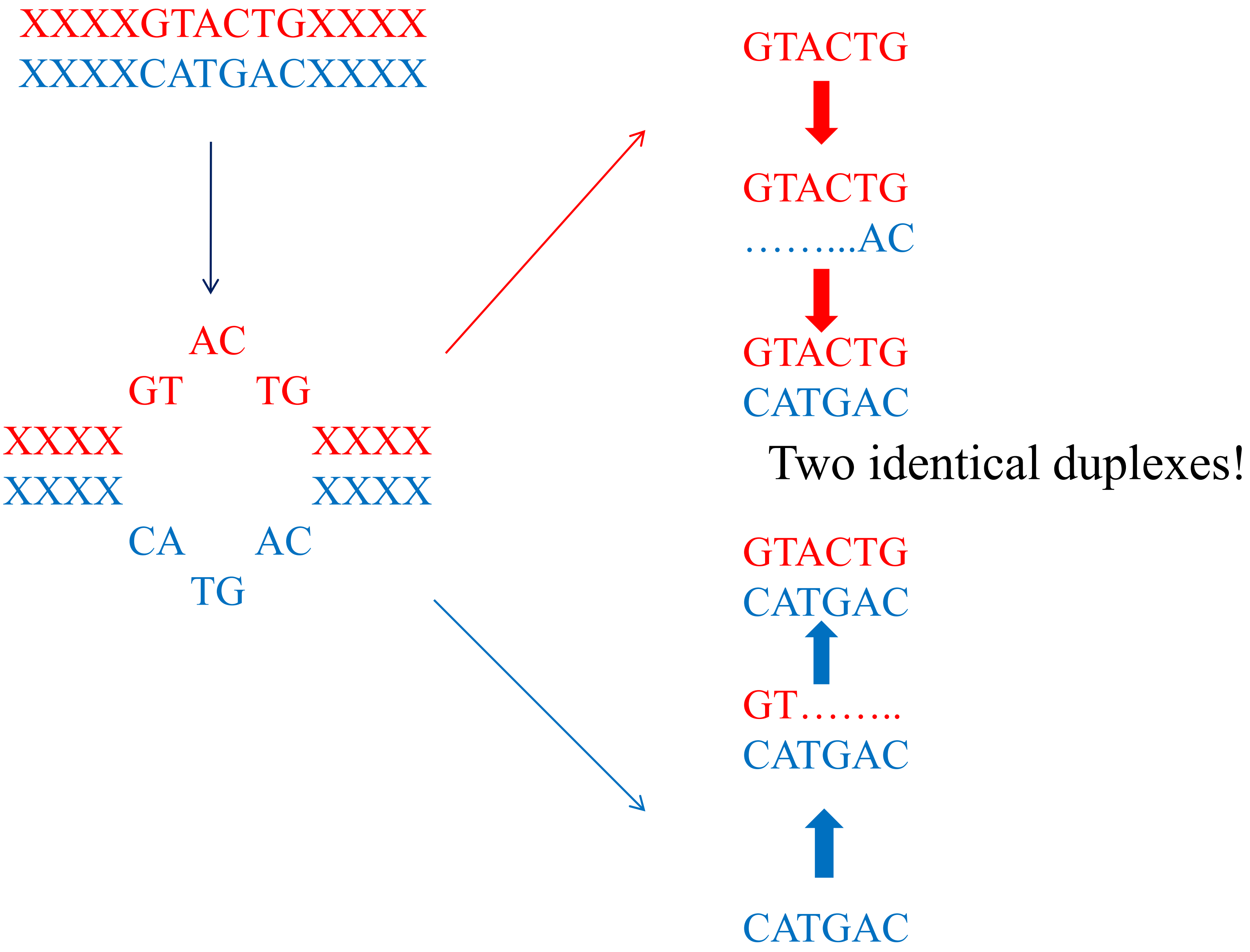
It is like a code. If you are given one set of letters you can write down the others.

Now we believe that the D.N.A. is a code. That is, the order of the bases (the letters) makes one gene different from another gene (just as one page of print is different from another). You can now see how Nature makes copies of the genes. Because if the two chains unwind into two separate chains, and if each chain then makes another chain come together on it, then because A always goes with T, and G with C, we shall get two copies where we had one before.

For example



[diagram showing chains separate into two newly formed chains]



XXXXGTACTGXXXX
XXXXCATGACXXXX

AC
GT TG
XXXX XXXX
XXXX XXXX
CA AC
TG

GTACTG

GTACTG
.........AC

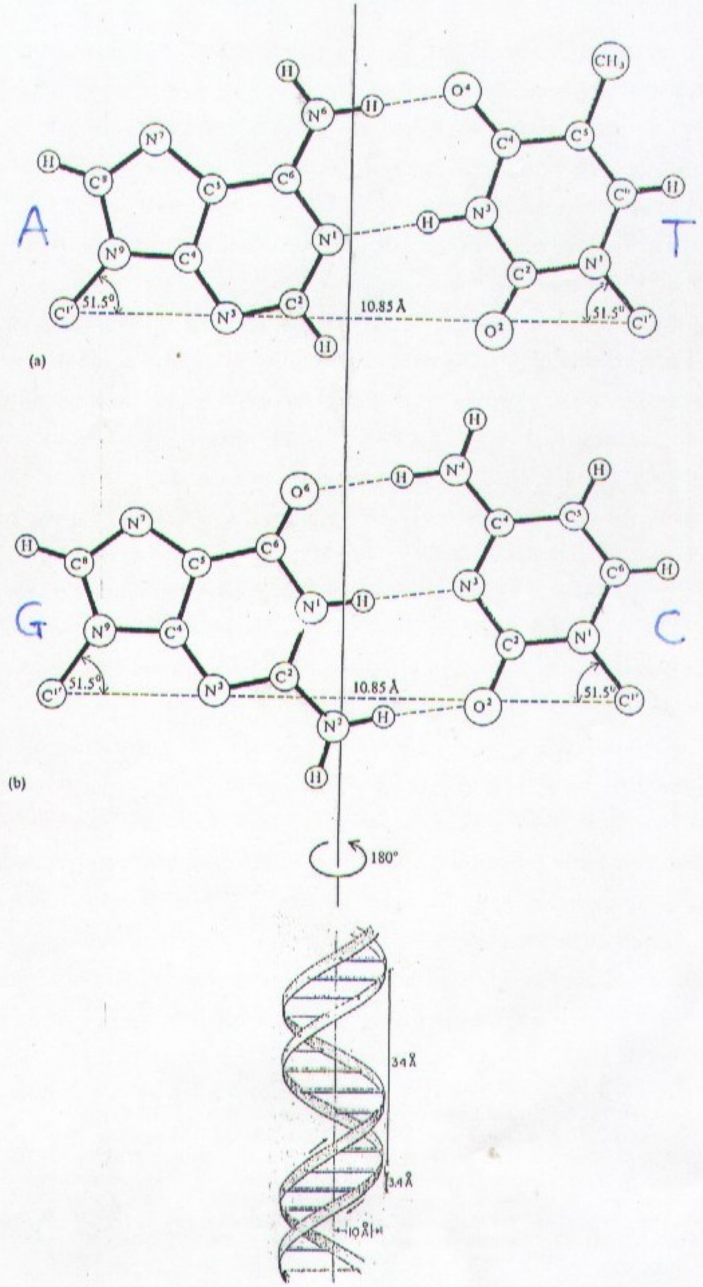
GTACTG
CATGAC

Two identical duplexes!

GTACTG
CATGAC

GT........
CATGAC

CATGAC



A
T
G
C
(a)
(b)
10.85 Å
51.5°
180°
3.4 Å
3.4 Å
10 Å

The paper of
Rosalind Franklin and Wilkins
with x-ray diffraction of A-DNA

appeared in the same issue of Nature
as the paper by Watson and Crick.

Watson, Crick and Wilkins received
Nobel Prize several years
after Franklin died from cancer

Prehistory of the discovery

Friedrich Miescher looked for hereditary material in sperm

and discovered DNA (1869).

He thought (1882) that the genetic information
may exist in the form of a molecular text,
a linear sequence of chemical symbols,

"just as the words and concepts of all languages
can find expression in twenty-four to thirty
letters of the alphabet"

For a long time (1906-1948) DNA was viewed as monotonous repetition of

identical tetranucleotide units

(Steudel, 1906; Levene and Simms, 1925)

Astbury and Bell (1938)
discovered
3.3 Å periodicity in the fiber
x-ray diffraction of DNA –

-stacking of flat DNA bases

They also hypothesized that the
bases
"form the long scroll on which
is written the pattern of life".

The idea on

molecular complementarity
in macromolecular interactions

was outlined by
Linus Pauling and Max Delbruck
in 1940

Nature 371, 285, 1994

Transforming activity of DNA

was first demonstrated by
O. Avery, S. MacLeod and M. McCarty
in 1944

Erwin Chargaff established the “Chargaff’s rule” in 1952:

$$A = T, \quad \text{and} \quad G = C$$

He was at the very doors of the discovery of DNA duplex structure.
Ruining the tetranucleotide theory, he was cautious with the obvious speculation, fearing to get in the shoes of Steudel and Levene,

…and missed the great discovery.

To the end of his days he was openly very bitter about that.

Many scientists have become "zombies":
they do not need to think
about important biological problems anymore,
instead, they simply go to the laboratory
and use the technical facilities available
to collect large quantities of data.

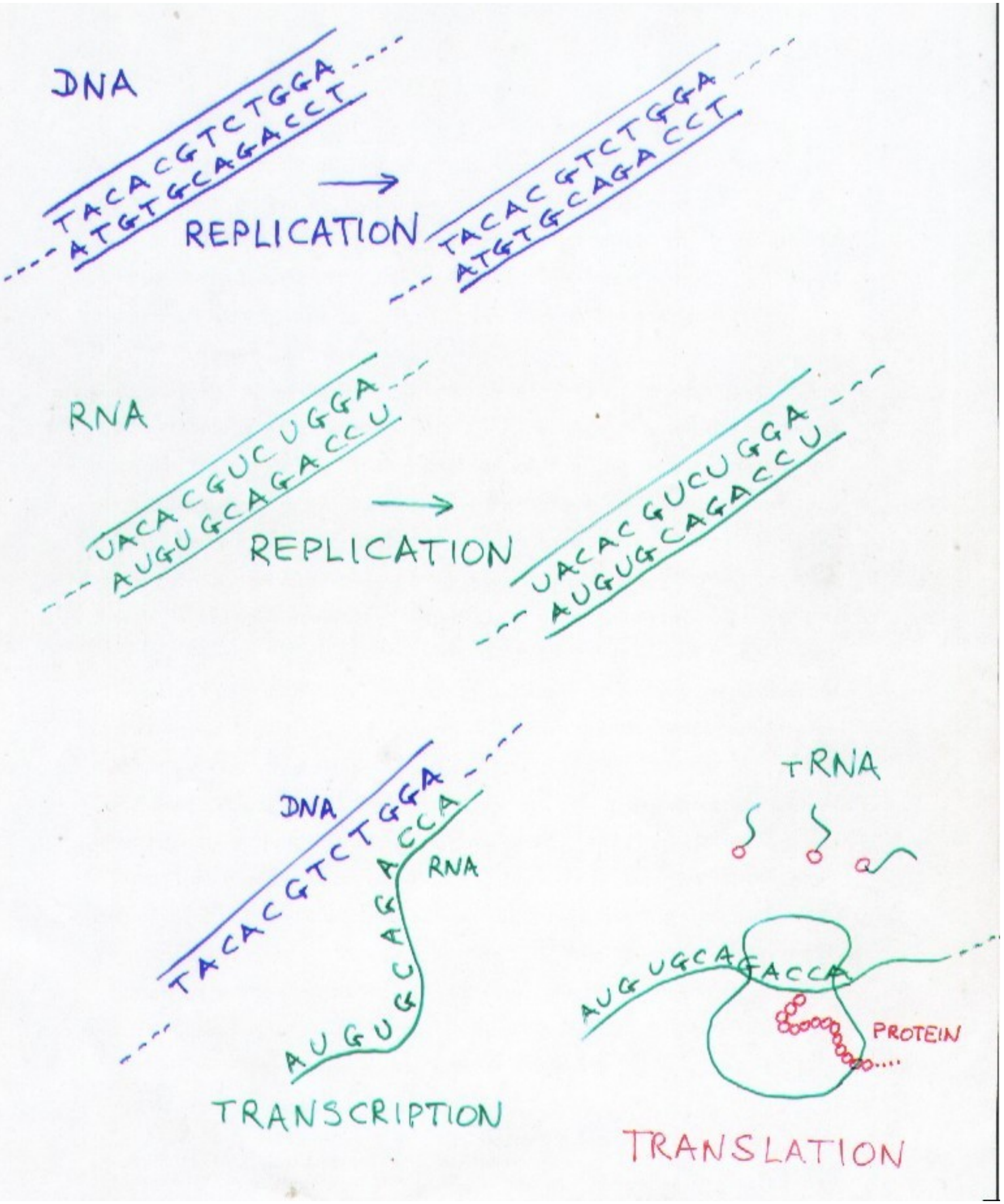
(Sidney Brenner)

“Now we believe that the D.N.A. is a code.”

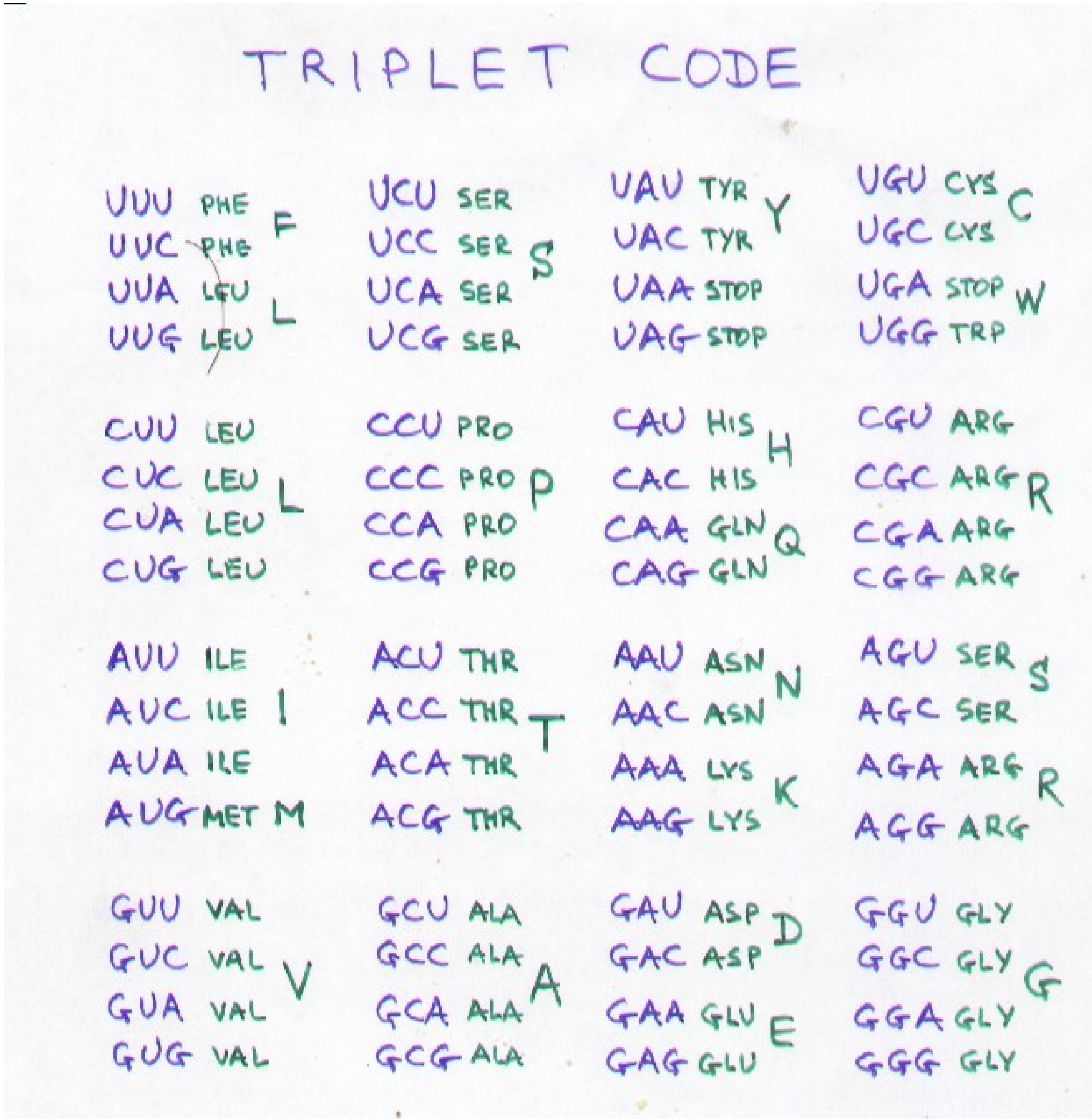
Historically, thus,
the Watson-Crick DNA complementarity code, or

DNA replication code is
the first DNA code deciphered.

Although traditionally, the triplet code
is considered as the first genetic code



DNA
TACACGTCTGGA
ATGTGCAGACCT
REPLICATION
TACACGTCTGGA
ATGTGCAGACCT
RNA
UACACGUCUGGA
AUGUGCAGACCU
REPLICATION
UACACGUCUGGA
AUGUGCAGACCU
DNA
TACACGTCTGGA
AUGUGCAGACCA
RNA
TRANSCRIPTION
tRNA
AUGUGCAGACCA
PROTEIN
TRANSLATION



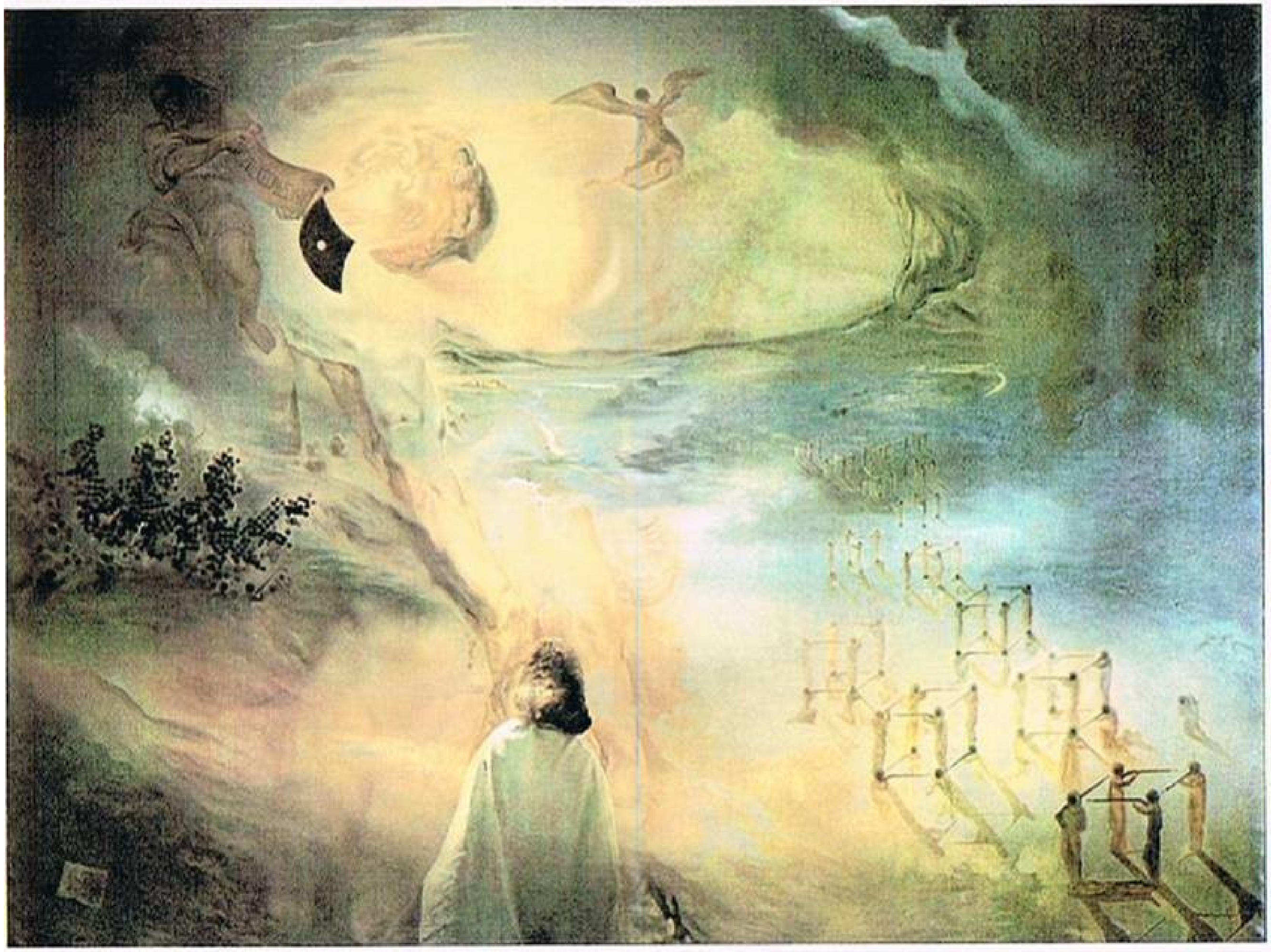
TRIPLET CODE

UUU	PHE	F	UCU	SER	S	UAU	TYR	Y	UGU	CYS	C
UUC	PHE		UCC	SER		UAC	TYR		UGC	CYS	
UUA	LEU	L	UCA	SER		UAA	STOP		UGA	STOP	W
UUG	LEU		UCG	SER		UAG	STOP		UGG	TRP	
CUU	LEU	L	CCU	PRO	P	CAU	HIS	H	CGU	ARG	R
CUC	LEU		CCC	PRO		CAC	HIS		CGC	ARG	
CUA	LEU		CCA	PRO		CAA	GLN	Q	CGA	ARG	
CUG	LEU		CCG	PRO		CAG	GLN		CGG	ARG	
AUU	ILE	I	ACU	THR	T	AAU	ASN	N	AGU	SER	S
AUC	ILE		ACC	THR		AAC	ASN		AGC	SER	
AUA	ILE		ACA	THR		AAA	LYS	K	AGA	ARG	R
AUG	MET	M	ACG	THR		AAG	LYS		AGG	ARG	
GUU	VAL	V	GCU	ALA	A	GAU	ASP	D	GGU	GLY	G
GUC	VAL		GCC	ALA		GAC	ASP		GGC	GLY	
GUA	VAL		GCA	ALA		GAA	GLU	E	GGA	GLY	
GUG	VAL		GCG	ALA		GAG	GLU		GGG	GLY	

Artist`s impression

“And now the announcement of Watson and Crick about DNA. This is for me the real proof of the existence of God”

Salvador Dali



GALACIDALACIDESOXIRIBUNUCLEICACID
(HOMAGE TO CRICK AND WATSON)
Oil on Canvas 120"x 161½" 1962-63

Sequences (introductory)

```
tgccattgcg ctccaaaaaa aaaaaaaaaa aagacattaa cataaattta aatattttat      2580
aatgacaatc cacattaact acttaaagca taagctattt tccaggagag gcagcaagtg      2640
cattctactc ccatgcccaa gaagaaagga gcgtgacttt ggtgggagta ctaggagttt      2700
ctactggagc acttgcccgc agagtgagaa acgttcctag agaggaagtt atacctgctg      2760
tggaatttaa gagaatcttg tcatattttg acaagttttt tgagatggaa gtctcactct      2820
gtcgcccagg ctggagtgca gtggcgcaat ctcagctcac tgcagcctgc acctcctcgg      2880
ctccagctat tctcttgtct cagcctcctg agtaactggg attacaggcg cccgccacta      2940
cgcctggcta atttttgtat ttttagtaga aatggggttt taccatgttg gccagactgg      3000
tctcaaactc ccgacctcag gtgatctgcc tgcctcagcc tcccaaagtg ctggaattac      3060
aggcgtgtgc cactgcgcct ggctaatttt tttttttttt tttttttagt agagacggtg      3120
gtttcaccat gtcatccagg ctggtctcaa actcctgacc tcaggtgatc cacccacctt      3180
ggtctaccaa agtgctcgga ttacaggcat gagccaccag gcccagtcaa cgtgatgtgt      3240
tttggaaccc tgaattcctt ggcttgcccg gagggttttc tttttgttaa tatctttgct      3300
tgctttctag tatttaaaaa attgtgtttt gctctaacta tgcaatggct ttaagtctta      3360
```

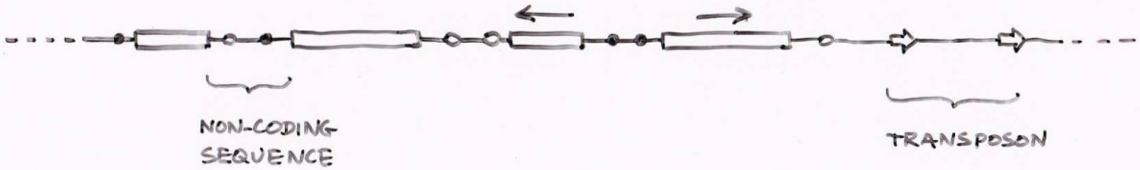
Sequence fragment from rDNA spacer of *Arabidopsis thaliana*

```
MSVNYMRLLCLMACCFSVCLAYRPSGNSYRSGGYGEYIKPVETAEAQAAALTNAAGAAASS
AKLDGADWYALNRYGWEQGKPLLVKPYGPLDNLYAAALPPRAFVAEIDPVFKRNSYGGAYG
ERTVTLNTGSKLAVSAAIGREAIVGAGLQGPFGGPWPYDALSPFDMPYGPALPAMSCGAGS
FGPSSGFAPAAAYGGGLAVTSSSPISPTGLSVTSENTIEGVVAVTGQLPFLGAVVTDGIFP
TVGAGDVWYGCGDGAVGIVAETPFASTSVNPAMSKSGVPRLLTASERERLEPIDQIHYSPR
ADDEYEYRHMLPKAMLKAIPTDYFNPETGTLRILQEEEWRGLGITQSGWEMYEVHVPEPHI
LLFKREKDYQMKFSQQRGGMLLNRTSFVTLFAAGMLVSALAQAHPKLVSSTPAEGSEGAAP
AKIELHFSENLVTQFSGAKLVMTAMPGMEHSPMAVKAAVSGGGDPKTMVITPASPLTAGTY
KVDWRAVSSDTHPITGSVTFKVKMSSQQQKQPCTLPPQLQQHQVKQPCQPPPQEPCVPKTK
EPCQPKVPEPCQPKVPEPCQPKVPEPCQPKVPQPCQPKVPEPCQPKVPEPCQPKVPEPCQP
KVPEPCQSKVPQPCQPKVPEPCQTKQKMADNLSQSFDKSAMTEEERRHIKKEIRKQIVAFA
LMIFLTLMSFMAVATDVIPRSFAIPFIFILAVIQFALQLFFFMHMKDKDHGWANAFMISGI
FITVPIAALMLLLGVNKISKIVKFLKELATPSHSMEFFHKPASNSLLASELNFVRRNIKRE
DFGHEVLTGAFGTLKSPVIVSIFHSRIVACEGGDGEEHDILFHTVAEKKPTICLDGQVFKL
KHISSEGEVMYYMFRQCAKRYASSLPPNALKPAFGPPDKVAAQKFKESLMATEKHAKDTSN
MWVKISVWVALPAIALTAVNTYFVEKEHAEHREHLKHVPDSEWPRDYEFMNIRSKPFFWGD
GDKTLFWNPVVNRHIEHDDQSTVHIVGDNTGWSVPSSPNFYSQWAAGKTFRVGDSLQFNFP
ANAHNVHEMETKQSFDACNFVNSDNDVERTSPVIERLDELGMHYFVCTVGTHCSNGQKLSI
NVVAANATVSMPPPSSSPPSSVMPPPVMPPPSPS
```

PROKARYOTIC GENOME

1–2 CIRCULAR CHROMOSOMES 400 kbp – 4000 kbp

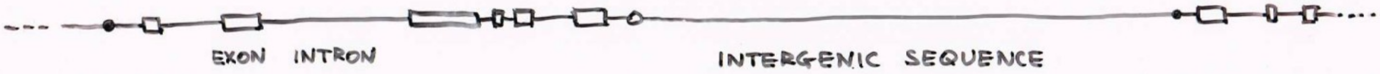
PLASMIDS, 1–50 COPIES/CELL 1 kbp – 100 kbp



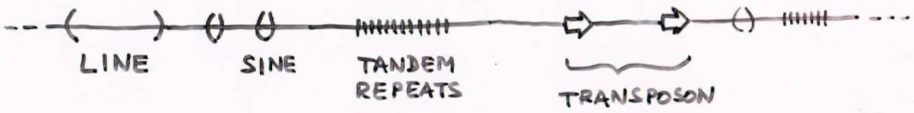
PROTEIN-CODING SEQUENCES : ~ 80%

EUKARYOTIC GENOME

4-200 CHROMOSOMES	500 000 kbp – 5 000 000 kbp
MITOCHONDRIA, CHLOROPLASTS	10 kbp – 200 kbp
EXTRACHROMOSOMAL CIRCULAR DNA	1 kbp – 20 kbp



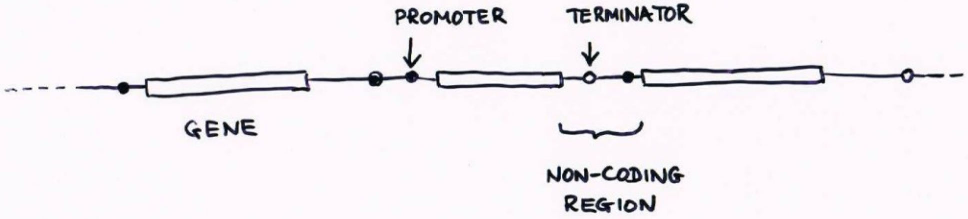
INTRONS & INTERGENIC SEQ-S:



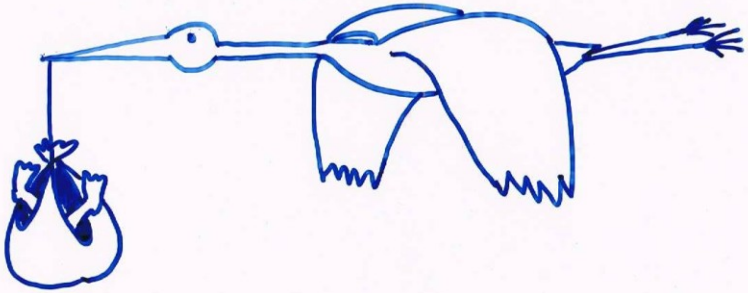
EXONS, rRNA GENES ; iRNA	1-10%
TRANSPOSONS & REPEATS :	20-40%
INTRONS & UNASSIGNED SEQ-S :	50-70%

VIRAL GENOME

1 ÷ 20 DNA or RNA SEGMENTS („CHROMOSOMES") 0.2 — 200 kbp



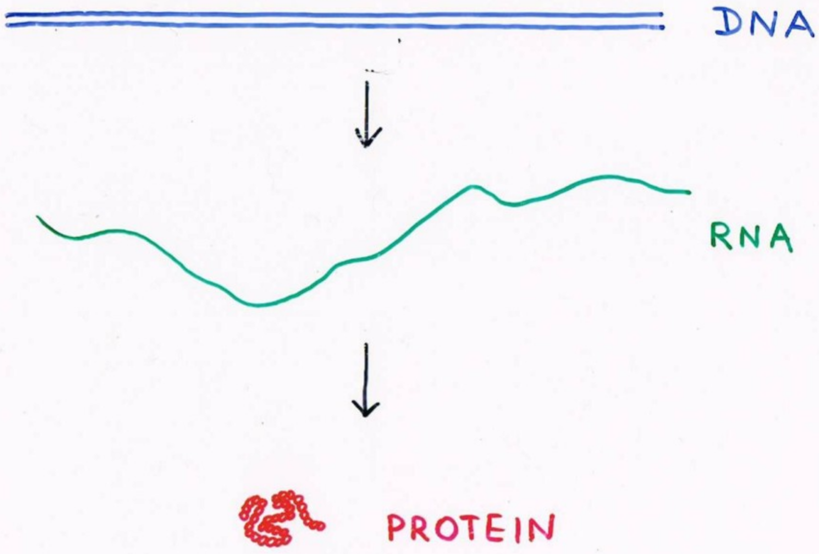
CODING REGIONS : ~ 80%

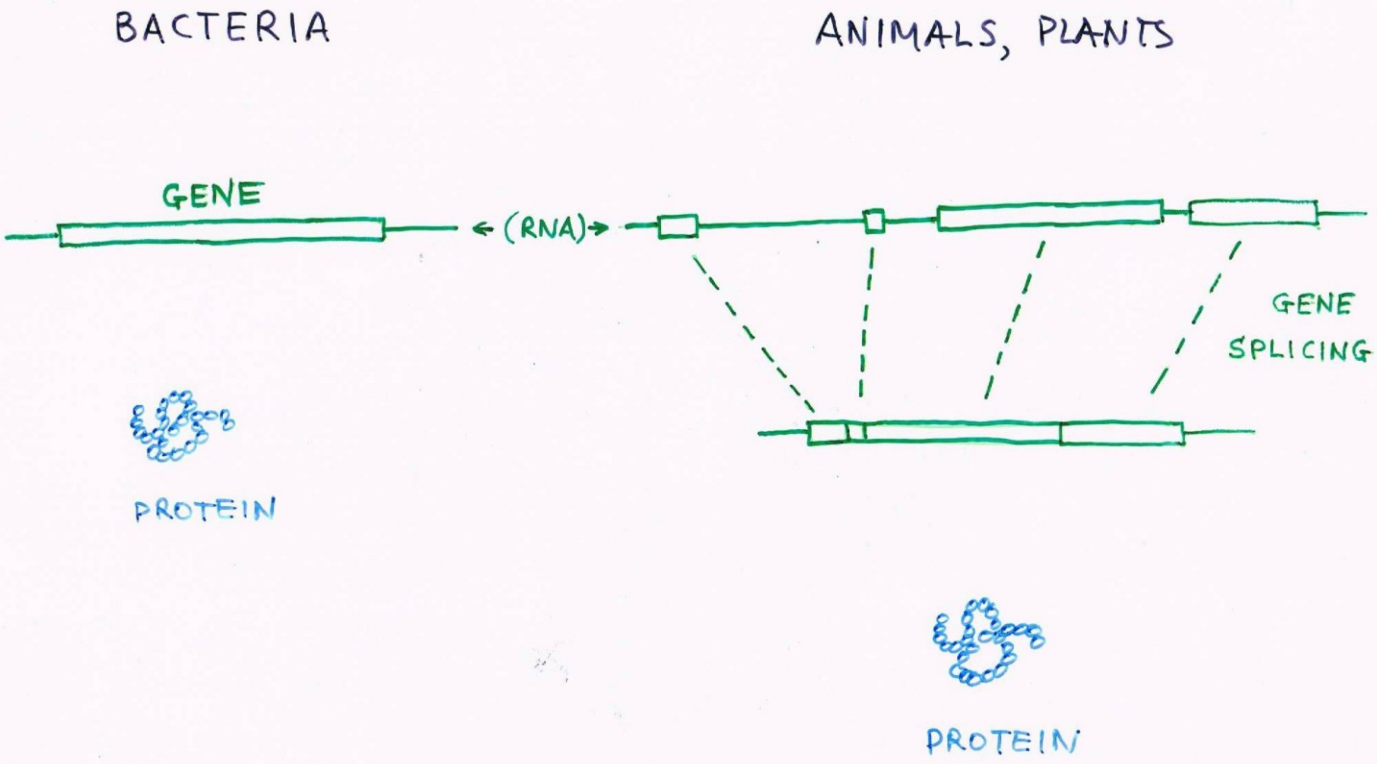


“What is true for E. coli is also true for the elephant”

(Jacque Monod)

Jacque Monod died in 1976
Gene splicing was discovered in 1977


DNA
RNA
PROTEIN


BACTERIA
ANIMALS, PLANTS
GENE
←(RNA)→
GENE SPLICING
PROTEIN
PROTEIN

The sequences carry endless surprizes (and new codes to be discovered)

Definition of the sequence code:

Any sequence pattern or bias responsible for specific biological or biomolecular function

(ENT, 1989)

There are, thus, many codes

Definition of language code:

A rule that transforms one sequence of symbols (letters, often meaningless) into another one, with a meaning

Any bilingual dictionary serves as a code, to translate
a text written in one language to text in different language

The spy code (secret dictionary) is another example

From Mexican military code “Temascaltepec”, 1907

49	A	351	Administrator
73	B	355	Capitan
49	ab	379	Secretario
50	ac	381	Soldado
		383	Suprema Corte de Justicia
100	cra	390	Visitador
101	cre	410	Mexico
102	cri	436	Municipalidad de
257	po		
258	pu		
259	pa		

The course GENETIC CODES has been given by ENT in 15 Universities of 8 countries, since 1981

1981-2000 The Weizmann Institute of Science, Israel
1987 University of North Carolina, Chapel Hill, USA
1988 University of Wuerzburg, Germany
1989 Research Computer Center, Pushchino, Russia
1990 Yale University, New Haven, USA
1990 Pauling Inst. of Science and Medicine, Palo Alto
1992, 95, 97 Bar-Ilan University (Tel-Aviv, Israel).
1993, 95 University of San Francisco, USA
1999 Lomonosov Moscow State University, Russia
2000 University Paris Sud, Orsay, France
2000 Murdoch University, Australia
2002-2012 University of Haifa
2005, 2009 University of Rome "Sapienza“, Italy
2007-2014 Masaryk University, Brno, Czech Republic

and yet, the community of molecular biologists
still lives with concept of single genetic code,
repeatedly bumping into yet another “second genetic code”

Trifonov, E. N.,
Structure of DNA in chromatin.
In: "International Cell Biology 1980-1981" (Ed. H. Schweiger),
Springer-Verlag, Berlin, **1981**, pp. 128-138.

- Second code of chromatin DNA

Trifonov, E. N.,
The multiple codes of nucleotide sequences.
Bull. Math. Biol. 51, 417-432 (**1989**)

Trifonov, E. N.,
Sequence codes.
In: "Encyclopedia of Molecular Biology",
T. E. Creighton, Ed., John Wiley & Sons, Inc., New York, **1999**, p. 2324-2326

Linguistics of genetic sequences (introductory)

One finds in human texts
A variety of hidden meanings (codes) –
rythms,
rhymes,
acrostichs,
repeats,
palindromes,
symmetries,
etc.

Aus der Harzreise, 1824,
Heinrich Heine.

Auf die Berge
Will ich steigen,

Wo die dunkeln
Tannen ragen,

Bäche rauschen,
Vögel singen,

Und die stolzen
Wolken jagen.

Acrostic of Guido d’Arezzo (1025)

(on the hymn to St. John the Baptist)

Do (**Ut** in France)	***Ut*** *queant laxis*
Re	***Re***_sonare fibris_ (vocal chords)
Mi	***Mi***_ra gestorum_
Fa	***Fa***_muli tuorum_
Sol	***So***_lve polluti_
La	***La***_bii reatum_ (tight lips)

Russian physicist Yakov Zeldovich,
being in quarrel with Arkady Migdal,
published the following achrostic:
(Uspekhi Fizicheskikh Nauk, 1976)

Могучий	МИГДАЛ ТЫ ИОПА	Almighty
И	(Migdal you asshole)	And
Громадный		Huge,
Далёк		Remote is
Астральный		Celestial
Лад.		Tune.
ТЫ		YOU
Ищешь		Look for
Объясненья –		Explanation -
Познай		Cognize the
Атомосклад		Star depot

NOW NO SWIMS ON MON

NOW NO SWIMS ON MON

- sign of dyad symmetry

G G A T C C

C C T A G G

Bam H1 restriction site

When placed in one sequence

….GGATCCxxxxxxxxxxGGATTC….

the Bam H1 sites will make a hairpin with xxxxxxxxxx in a loop

The best for a loop is mirror-symmetrical sequence, e.g.

G G A T C C   C C T A G G

It can not possibly make a hairpin

Such mirror-symmertrical sequences (texts, words) are called palindromes, e.g.

AMORE ROMA

НАЖАЛ КАБАН НА БАКЛАЖАН

GOD DAMN I AM A MAIN MAD DOG (V. Ivanov)

S	A	T	O	R	Founder
A	R	E	P	O	Crawl
T	E	N	E	T	Hold
O	P	E	R	A	Effort
R	O	T	A	S	Wheel

Two-dimensional palindrome
discovered under ashes in Pompei

ABRACADABRA
ABRACADABR
ABRACADAB
ABRACADA
ABRACAD
ABRACA
ABRAC
ABRA
ABR
AB
A

Amulet against malaria

The same string may carry another message, read in different way:

DORMITORY	DIRTY ROOM
MOTHER IN LAW	WOMAN HITLER
TWELVE + ONE	ELEVEN + TWO

http://i.imgur.com/BVvCZG8.png

Various sequence types may be characterized

by so-called contrast words –

the words that expand uniquely

from inside of the word,

but continue randomly outside

RAT

OPERATOR
OPERA TALENTS
CAR AT THE GATES

SEIZURE

Multiple overlapping codes

in the biological sequences

```
MnnnnnMnnnMMnnnnMnnMMMnnnMMnnnnnMnnMnnnnn  No.1
|     |   ||    |  |||   ||     |  |
MnnnMnMnnnMMnMnnMnnMMMnMnMMnnnMnMnMMnnMnn  No.1 and No.2
    | |      |  |   || |  |   |   |   |      superimposed
nnnnMnMnnnnnnMnnMnnnMMnMnnMnnnMnnnMnnnMnn  No.2
```

The sequences between genes (intergenic sequences),
and those between exons (intervening sequences)
are called "non-coding sequences" ,
that is non-coding for proteins.

They, actually, carry an unknown number
of other (mostly unknown) codes,
not related to proteins

Those people who don`t like anything unknown
call the sequences various names
with different degrees of disdain:

Garbage,
Junk (S. Ohno),
Selfish DNA (F. Crick),
Polite DNA (E. Zuckerkandl)

One should not consider a book garbage
only because one does not know the language

Sidney Brenner:

The non-coding sequences
could not have been called "garbage“
instead of "junk", since
the garbage is to throw away
while the junk is to carry with.

GG× CU× AC× GU× AGY GC× ...
GLY LEU THR VAL SER ALA

TRIPLET CODE

G×× G×× G×× G×× G×× G×× ...

FRAMING CODE

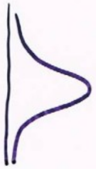
AG×××××××× AG×××××××× AG×× ...
AAA××××××× AAA××××××× AAA× ...
GC×××AG×CG××CT××××TT×××× ...

DNA
SHAPE
CODE

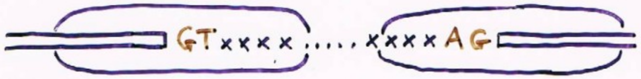
RR×××YY×××RR×××YY×××RR×× ...

CHROMATIN CODE

TGTG×××××××××××× ...
TGTGTG×××××××××× ...
TGTGTGTG×××××××× ...
TGTGTGTGTG×××××× ...
TGTGTGTGTGTG×××× ...

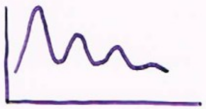


MODULATION CODE



GENE
SPLICING
CODE

MET××××××...××MET××××××...××MET××...



GENOME
SEGMENTATION
CODE

Trifonov, E. N.,
Structure of DNA in chromatin.
In: "International Cell Biology 1980-1981" (Ed. H. Schweiger),
Springer-Verlag, Berlin, **1981**, pp. 128-138.

Second code of chromatin DNA

1981

[second!] **Second Genetic Code Deciphered**

The New York Times **May 13, 1988**

reported in today's issue of nature,
by Ya-Ming Hou and Paul Schimmel
(aa tRNA synthase/tRNA recognition)

1988

DNA methylation, DNA's *[third !]*Second Code,

It is often featured as such in literature since 2001.
It was used first under this name by Orion Genomics Company in 2001,
after publication: Martindale, Diane; "Genes Are Not Enough,"
Scientific American, 285:22, October 2001; and is broadly accepted since then.

See, e. g.:

Crack the **Second Code**: Methylated DNA Sequencing for Epigenetic Analysis
ETON Bioscience Inc 2003;

Imprinted Genes Offer Key to Some Diseases and to Possible Cures. By Sharon Begley,
Wall Street Journal. 24 June 2005.

2nd genetic code could provide clues to schizophrenia, bipolar disorder
March 12, 2008, **CBCNews**

2001

Packaging proteins may be
[fourth!] **second genetic code**

NewScientist 09 August 2001 by Emma Young

(T. Jenuwein & C. D. Allis, histone modifications,
Science (vol 293, from p 1068)

2001

I′ m done with seconds, can I have a third?

As an aside, the authors of the editorial summary coined the work as the second genetic code. I find this amusing, because this would be the third second genetic code. The aminoacyl tRNA code was also coined the second genetic code, but people must have forgotten that, because another second genetic code was proposed in 2001. This genetic code describes how methylated DNA sequences regulate chromatin structure and gene regulation.

(*Todd Smith* , FINCHTALK Journal Club, May 11, 2010)

Cracking the ***[fifth !]*** **Second Genetic Code**: Sequence Patterns in Noncoding DNA

Jeff Elhai

(intragenomic recombination sites in *Nostoc*)

Virginia Commonwealth University BBSI
Symposium 1, 2003

2003

Genome`s ***[sixth!]*** **second code**

Allende ML et al., Methods 39, 212, 2006

(highly conserved enhancers across species)

2006

A genomic code for nucleosome positioning

Eran Segal, Yvonne Fondufe-Mittendorf, Lingyi Chen, AnnChristine Thastrom, Yair Field, Irene K. Moore, Ji-Ping Z. Wang & Jonathan Widom

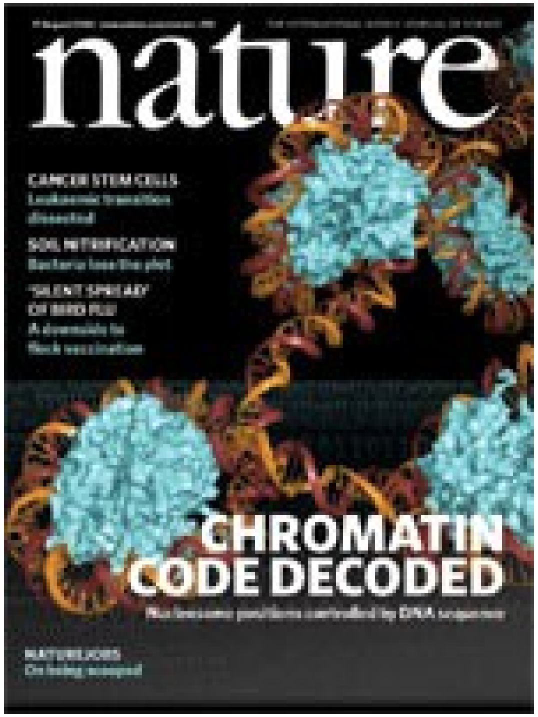
nature 442, 772-778, 2006

“a *[seventh !]***second code** in DNA

in addition to the genetic code”

The New York Times July 25, 2006

2006


nature
CANCER STEM CELLS
Leukaemic transition dissected
SOIL NITRIFICATION
Bacteria lose the plot
'SILENT SPREAD' OF BIRD FLU
A downside to flock vaccination
CHROMATIN CODE DECODED
Nucleosome positions controlled by DNA sequence
NATUREJOBS
On being scooped

2006

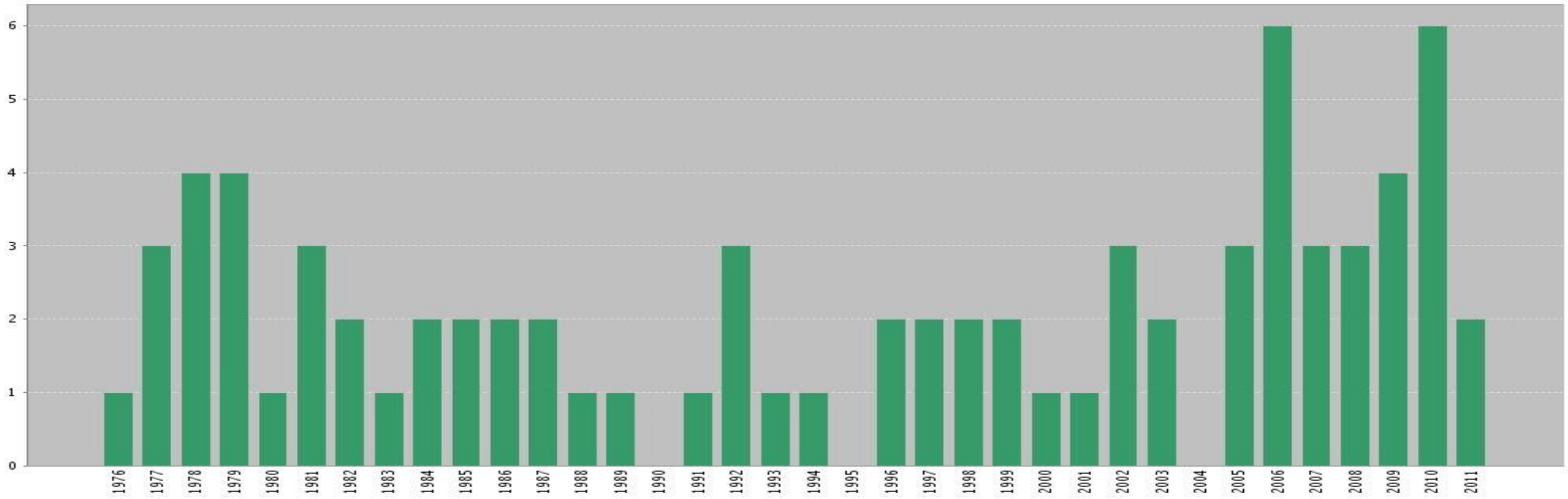
The tendency of the dinucleotides to fit to … 10.5 or so base frame … can be considered as another message… two codes …

Trifonov, Nucl. Acids Res. 1980

"Second code of chromatin DNA" –

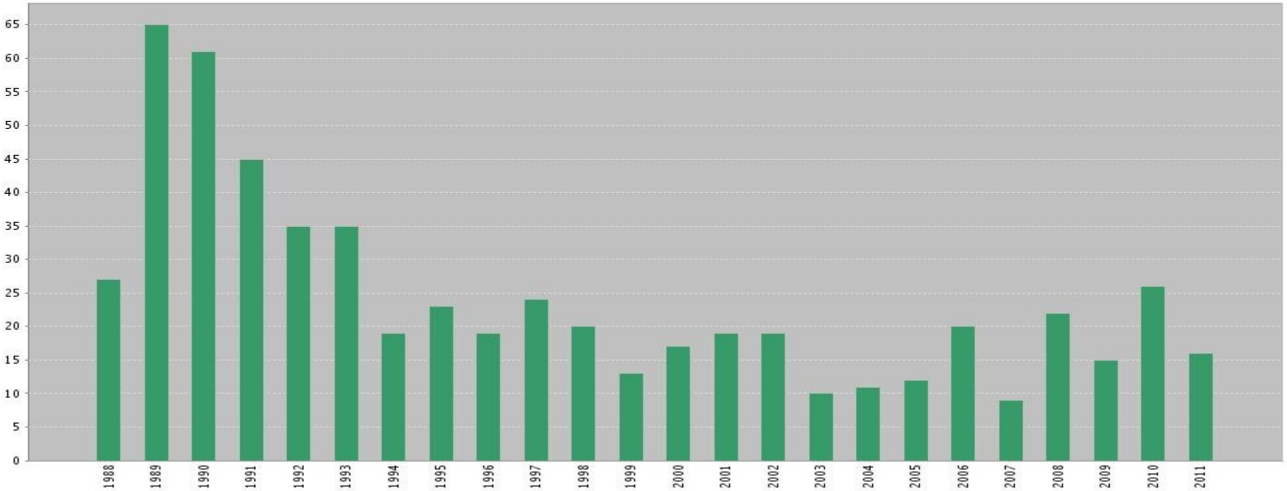
chapter by Trifonov in
"International Cell Biology 1980-1981"

Zuckerkandl, J Mol Evol 1977

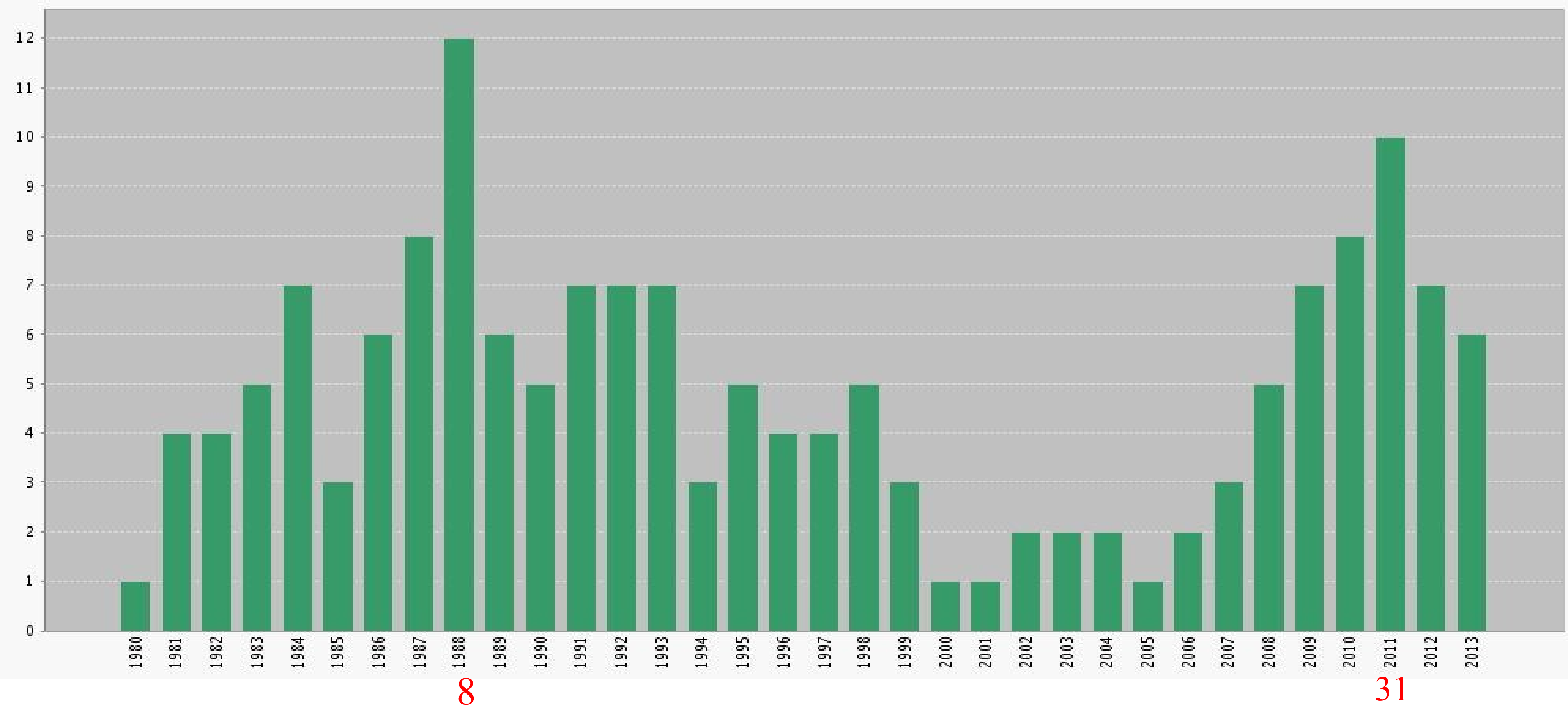


34

Holliday R, Science 1987

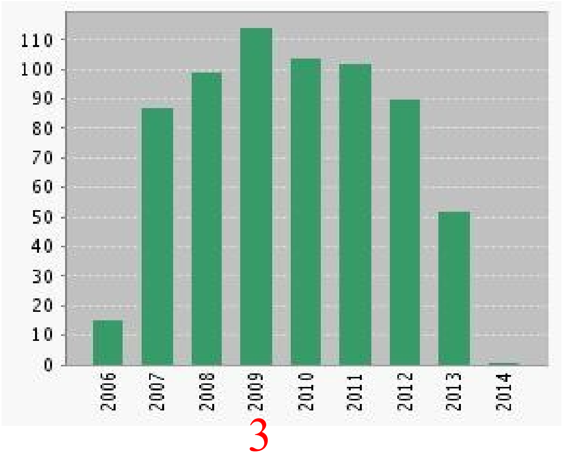


21



E. N. Trifonov,
Nucl Acids Res, 1980
"second genetic code"-
Chromatin code

E. Segal et al,
Nature, 2006
(Sixth) "second genetic code"-
Chromatin code



If I am able to generate just one good idea – let it be stolen

Fritz Pohl, codiscoverer of left-handed DNA,
(from personal conversation)

“Cracking the *[eighth !]* Second Genetic Code”

T.R. Hughes et al., 21st Intl Mammalian Genome Conference, 2007, abstract:

“relationship between transcription factors and cis-regulatory elements has been termed the second genetic code”,

also
Tim Hughes, *The FASEB Journal*. 2008;22:262.2

2007

“protein structure prediction” is a long-last difficult problem called “cracking the ***[ninth !]*** **second genetic code**”

In:

Quantum bio-informatics: from quantum information to bio-informatics
Eds: L. Accardi,W. Freudenberg,Masanori Ohya, World Scientific, 2008 (p. 441)

2008

Two previously declared second genetic codes – DNA methylation (2001) and histone modification (2001) are combined now in one:

Epigenetics:

The ***[tenth !]*** **Second Genetic Code**

(N. M. Springer and S. M. Kaeppler. Advances in Agronomy 100, 59-80, 2008)

2008

Deciphering the splicing code

Yoseph Barash, John A. Calarco, Weijun Gao, Qun Pan, Xinchen Wang, Ofer Shai, Benjamin J. Blencowe & Brendan J. Frey

Breaking the *[eleventh !]* second genetic code

J. Ramón Tejedor and Juan Valcárcel

nature, May 6, 2010

2010

Duons: Researchers Find *[twelfth !]* Second Code Hiding within DNA

Dec 13, 2013 by Sci-News.com, about paper in Science
(2013: Vol. 342 no. 6164 pp. 1367-1372,
by A.B. Stergachis, …, J.A. Stamatoyannopoulos),
on overlapping of factor binding sites with protein-coding sequences

2013

twelve SECOND CODES:

three in nature,
two in Science,
one in Scientific American,
one in The FASEB Journal
five in other sources

Chronology of 12 Second Genetic Codes

1981 •

1988 •

2001 • •

2003 •

2006 • •

2007 •

2008 • •

2010 •

2013 •

The truth is that there are MANY codes in the sequences:

		discovered	cracked
1.	DNA replication code (Watson/Crick)	(1953)	(1953)
2.	RNA-protein translation (triplet) code	(1961)	(1961)
3.	Genomic code (isochores)	(1973)	(1973-1990)
4.	Chromatin (nucleosome positioning) code	(1980,1981)	(1980-2009)
5.	DNA shape code (curved DNA)	(1980,1981)	(1980-1996)
6.	Gene splicing code (Chambon rules)	(1981)	not yet
7.	N-end rule (protein lifetime)	(1986)	(1986-1996)
8.	Translation framing code	(1987)	(1987)
9.	Fast adaptation (modulation) code	(1989)	(1989)
10.	Genome segmentation code	(1994)	not yet
11.	Codes of small RNAs	(1998)	(1998)
12.	Translation pausing code	(2002)	(2002)
13.	Proteomic code (proteins)	(2003)	(2003-2008)
14.	Genome inflation code	(2010)	(2010)

....

Several more sequence patterns are known, that qualify as general codes:

Transcription initiation code (promoters)
Transcription termination code (terminators)
Poly-adenylation code

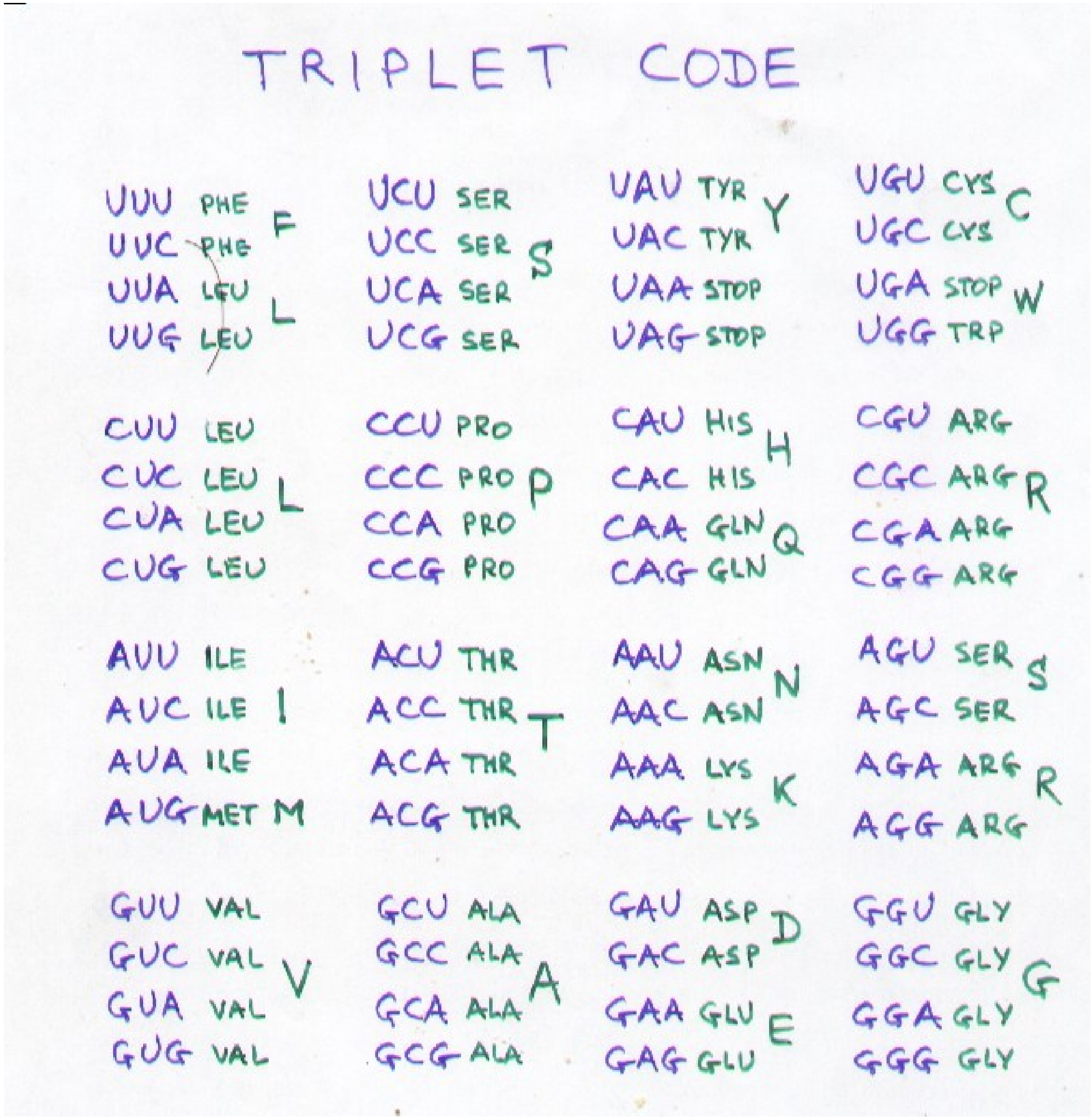
And this is common knowledge, essentially, since 1989:

Trifonov, E. N., Bull. Math. Biol. 51, 417-432 (1989)
Trifonov, E. N., Sequence codes. In: "Encyclopedia of Molecular Biology", 1999

Those many codes do not have to be called all as “Second genetic codes”.

Also, there is no need to number them

Triplet code
(RNA-protein translation code)



TRIPLET CODE

UUU	PHE	F	UCU	SER	S	UAU	TYR	Y	UGU	CYS	C
UUC	PHE		UCC	SER		UAC	TYR		UGC	CYS	
UUA	LEU	L	UCA	SER		UAA	STOP		UGA	STOP	
UUG	LEU		UCG	SER		UAG	STOP		UGG	TRP	W
CUU	LEU	L	CCU	PRO	P	CAU	HIS	H	CGU	ARG	R
CUC	LEU		CCC	PRO		CAC	HIS		CGC	ARG	
CUA	LEU		CCA	PRO		CAA	GLN	Q	CGA	ARG	
CUG	LEU		CCG	PRO		CAG	GLN		CGG	ARG	
AUU	ILE	I	ACU	THR	T	AAU	ASN	N	AGU	SER	S
AUC	ILE		ACC	THR		AAC	ASN		AGC	SER	
AUA	ILE		ACA	THR		AAA	LYS	K	AGA	ARG	R
AUG	MET	M	ACG	THR		AAG	LYS		AGG	ARG	
GUU	VAL	V	GCU	ALA	A	GAU	ASP	D	GGU	GLY	G
GUC	VAL		GCC	ALA		GAC	ASP		GGC	GLY	
GUA	VAL		GCA	ALA		GAA	GLU	E	GGA	GLY	
GUG	VAL		GCG	ALA		GAG	GLU		GGG	GLY	

Experiment of Nirenberg and Matthaei (1961):

```
UUU UUU UUU UUU UUU UUU UUU UUU UUU UUU
 F   F   F   F   F   F   F   F   F   F
```

After random "mutations", incorporation of C instead of U, expected NEW triplets: CUU, UCU, UUC.
Three or less NEW aminoacids expected in the product

Only two new aminoacids detected:
serine (S) and leucine (L)

```
UUU UCU UUU CUU UUU UUU UCU UUU UUC UUU
 F   F   F   F   F   F   F   F   F   F
    or      or          or      or
     S       S           S       S
    or      or          or      or
     L       L           L       L
    or      or          or      or
   none    none        none    none
Final answer:  CUU L
               UCU S
               UUC F
```

Note to degeneracy of triplet code

```
Original sequence:   TACTCGCTAACCGTAGGGGCCCGG
       Sequence I:   T  T  C  A  G  G  G  C
      Sequence II:    A  C  T  C  T  G  C  G
     Sequence III:     C  G  A  C  A  G  C  G
```

It turned out that
the third position sequence
is the most deviant from random)

(Sasha Rapoport, 2008)

OUT-OF-CONTEXT SEQUENCES I, II and III

```
original seq.  ACC GCU AUA CAG AUG UGU CAU ACC GCC CAU GAC GGC ACU UGC AAU GCA CGU UUA
    I          A   G   A   C   A   U   C   A   G   C   G   G   A   U   A   G   C   U
    II          C   C   U   A   U   G   A   C   C   A   A   G   C   G   A   C   G   U
    III          C   U   A   G   G   U   U   C   C   U   C   C   U   C   U   A   U   A
```

original seq.   ACCGCUAUACAGAUGUGUCAUACCG**CCC**AUGACGGCA**CUU**GCAAUGCACG**UUU**A

I   AGACAUCAGCGGAUAGCU

II   **CCU**AUGACCAAGCGACGU

III   CUAGG**UUCCUCCUCU**AUA

A. Rapoport, 2008

```
(a)
. . . G A G T C G T G G C A A G A A T A C C A A G A G T T C C T C G G T T T G C C A G T T . . .

      G A   T C   T G   C A   G A   T A   C A   G A   T T   C T   G G   T T   C C   G T    1) Gene TRP1
      glu   ser   trp   gln   glu   tyr   gln   glu   phe   leu   gly   leu   pro   val

      G                       G                 G                 G                 G    2) framing of TRP1

      G A G               A A G A       C C   A G A G     C C T C             C C        3) nucleosome

(b)
. . . A C A G T T G T G A C G C T G A T T G G T G T C G T T A C A A T C T A A C G C . . .

      A C   G T   G T   A C   C T   A T   G G   G T   G T   A C   A T   T A A      1) end of frdD gene
      thr   val   val   thr   leu   ile   gly   val   val   thr   ile   term

            G     G                       G     G     G                            2) framing of frdD

         T T G   C A                                   T A   A A T                 3) promoter P1
                                                                                      of ampC gene

(c)
. . . T C G A A G T G G A C T G C T G G T G G A A A A T C A G C A A A T T C A A . . .

      T C   A A   T G   A C   G C   G G   G G   A A   T G A                       1) Gene A,A*
      ser   lys   trp   thr   ala   gly   gly   lys   term

                              G     G     G                                         2) framing of A,A*

        C G   A G   G G   C T   C T   G T   G A   A A   G A   G A   A T   C A      3) Gene K
        arg   ser   gly   leu   leu   val   glu   asn   glu   glu   ile   gln

                    G                 G     G           G     G                     4) framing of K

                                                   A T G A G   A A   T T   A A      5) Gene C
                                                   fmet  arg   lys   phe   asn
```

Translation framing code

....GCCAGCAGCCTAGCAGCCAGTCAGCTTGCC GCCGGC GGCCAA GCA GCC AACC ATGCTCAACTTC

GGTGCCTCTCTCCAGCAGACTGCG .....TCGAAGTGGACTGCTGGTGGAAAA TGA GGAAATTCAA.....

Atkins JF, Elseviers D, Gorini L,

Low activity of beta-galactosidase in frameshift mutants of Escherichia coli.

PNAS 69, 1192-1195, 1972

Despite various measures to exclude contamination
by wild type strain the effect persisted.

All arguments discussed in the paper seem to "invalidate
any hypothesis attempting to explain frameshift leakiness
by postulation of a ribosomal slippage along the message"

But, as it turned out, the leakiness was caused, indeed, by the ribosomal slippage

Distribution of bases in three codon positions

	I	II	III	I	II	III	I	II	III
A	2442	2756	1290	1212	1243	766	557	488	481
C	2005	1900	2999	859	1032	1316	194	486	475
G	2723	1618	2688	1257	780	1036	561	344	180
U	1612	2508	1805	772	1045	982	395	389	571
		Human			Mouse			Ciliates	
A	538	495	478	1496	1573	1044	660	830	606
C	263	470	317	561	1271	1229	503	517	666
G	575	290	98	1690	652	848	798	373	490
U	383	504	866	1063	1314	1689	396	637	595
		Dictyostelium			Yeast			Plants	
A	4933	6064	3608	662	824	603	463	569	323
C	4723	4479	5586	401	535	450	480	479	600
G	7314	3497	5311	773	359	550	729	340	595
U	2767	5697	5232	449	567	682	312	596	466
		E. coli			Bacilli			*S. typhimurium*	
A	387	455	242	4701	3025	6212	1273	1355	1555
C	382	385	575	3121	3620	3917	985	1339	951
G	599	312	459	3173	1808	749	1990	1100	681
U	241	457	333	3597	6139	3714	1290	1744	2351
		Rhizobiaceae			Mitochondria			Chloroplasts	
A	551	596	495	682	705	556	861	916	793
C	292	380	238	657	738	721	410	462	546
G	547	316	353	912	569	849	641	311	390
U	354	452	658	474	713	599	391	614	574
		SV40			RSV			CMV	
A	1048	1119	958	945	1162	653	641	688	499
C	490	712	419	662	691	924	557	586	625
G	1107	547	380	1164	594	828	880	494	736
U	620	887	1508	554	878	920	461	771	679
		T4			T7			Transposons	
A	883	948	906	660	685	571	25595	26496	22639
C	209	418	157	551	617	674	18305	21117	23385
G	684	348	185	841	459	584	28958	15111	17990
U	614	676	1142	464	755	687	17209	27343	26053
		Plasmid K1			Plasmid Ti			Total	

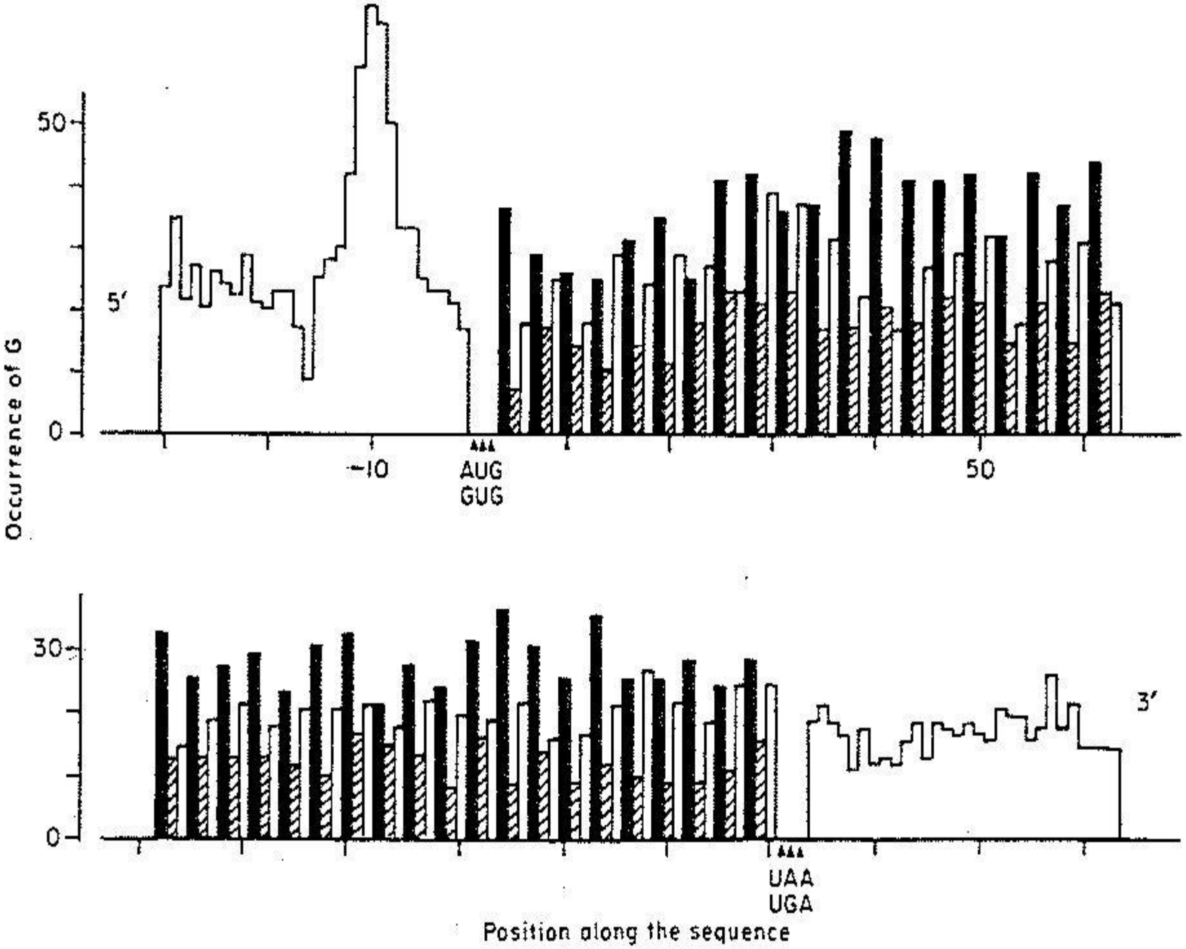


Figure 1. Distribution of guanines along *E. coli* mRNA. Filled bars, first positions of the codons; hatched bars, second positions. Only the first and last 60 bases of the coding regions are presented.

The three-base periodicity suggests that the ribosome may recognize correct reading frame far away from initiation triplet AUG.

Why that would be needed?

Does ribosome always move by exactly three steps?

It does not!

Occasionally, ribosome makes mistakenly two base steps instead, or 4 base steps.

That is, the ribosome may spoil the reading frame,
and synthesize protein with wrong sequence,
starting from the site of the mistake.

Frameshift mutation,
and translational frameshifting
are **different phenomena.**

First is a mishap caused by insertion/deletion
(gene sequence changed)

Second is a mishap (or happy accident)
caused by failure of the ribosome
to correctly count triplets
(no change in the gene sequence)

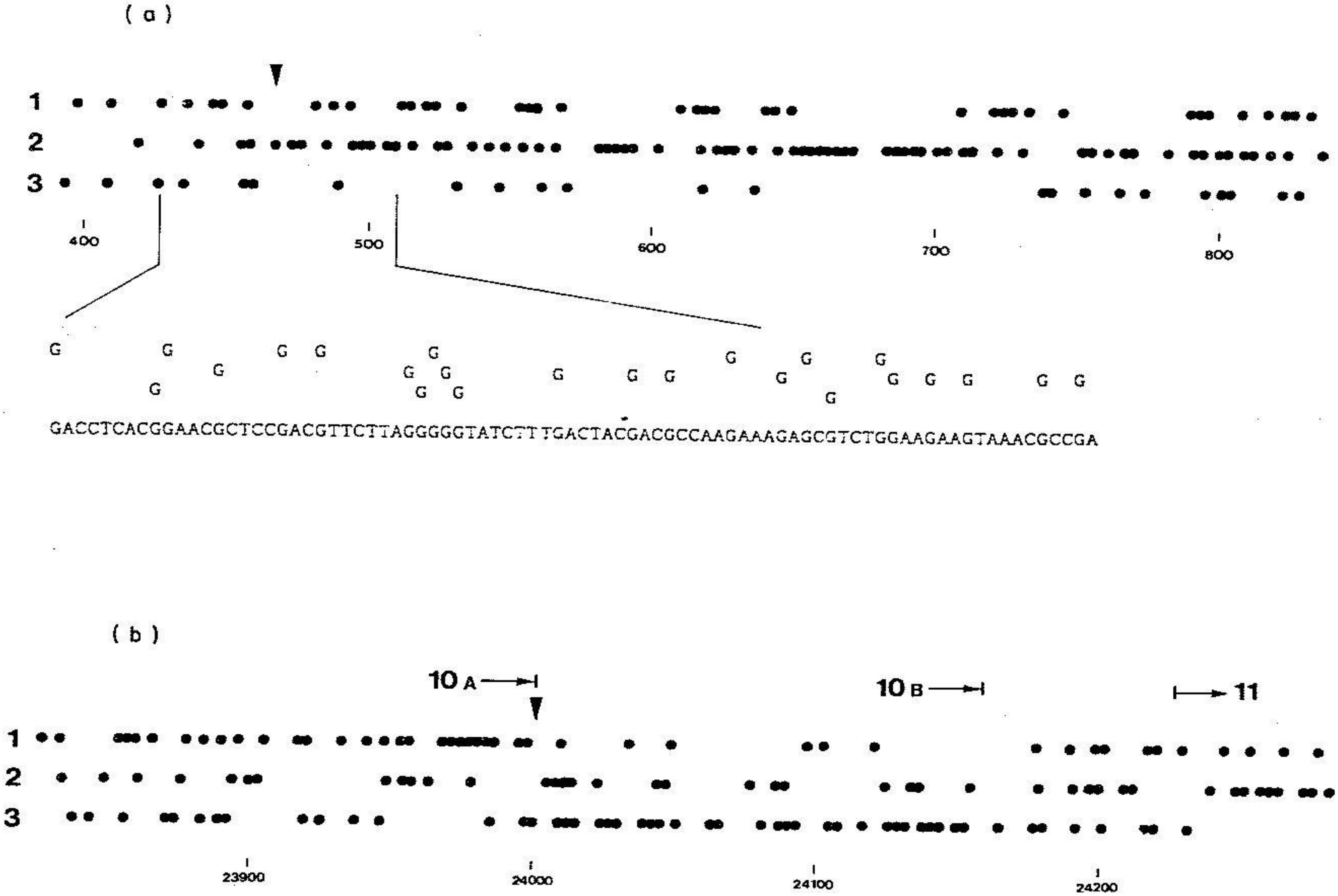
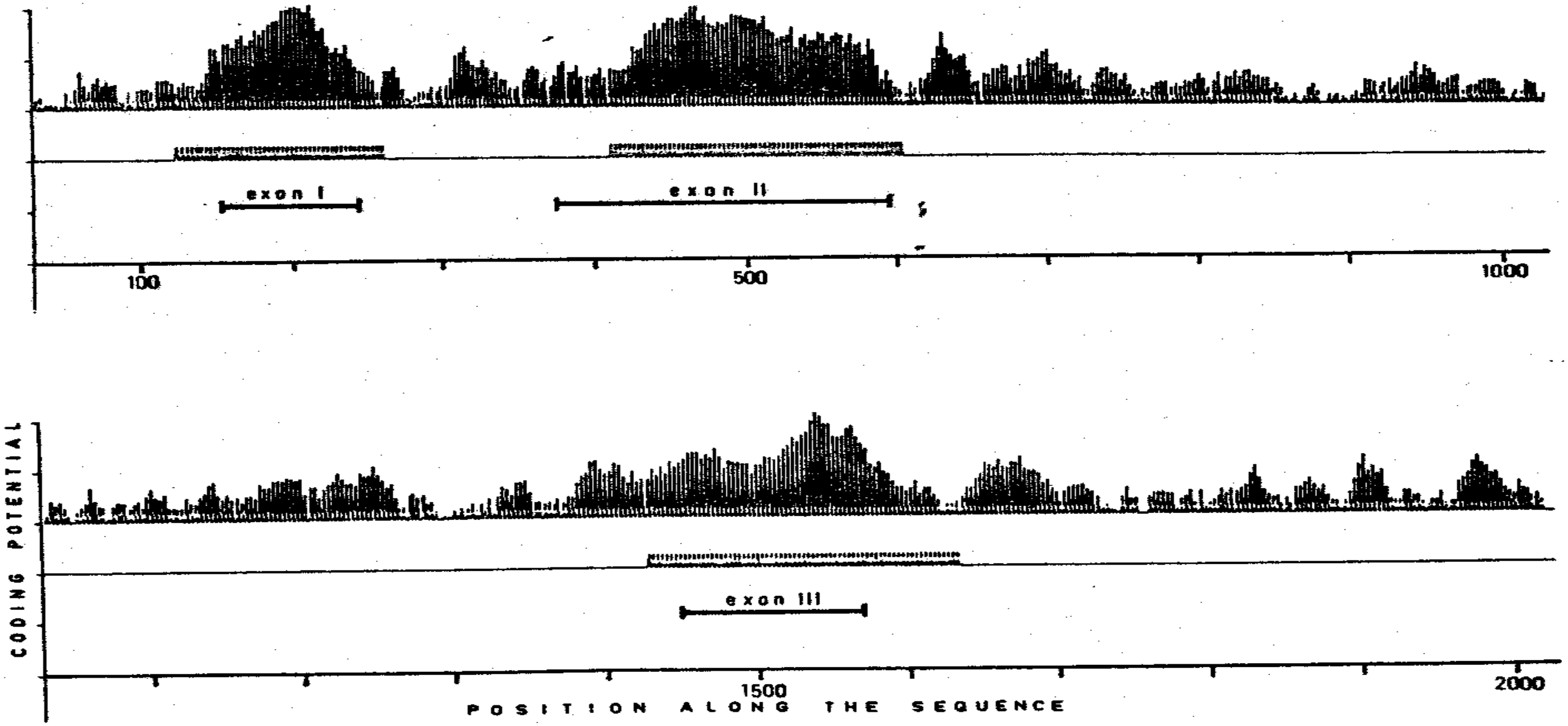


Figure 3. Actual distribution of guanines in 3 frames of the *RF-2* gene of *E. coli* (a) and the *10A,B* gene of bacteriophage T7 (b). The sequence around the ribosome slippage site is also shown (a). Every occurrence of G is indicated by a dot. Arrowheads indicate positions of ribosome frameshifting. Sequence co-ordinates correspond to those in original papers (Craigen *et al.*, 1985; Dunn & Studier, 1983).


exon I
exon II
100
500
1000
CODING POTENTIAL
exon III
1500
2000
POSITION ALONG THE SEQUENCE

Potential mRNA binding sites in 16 S rRNA

$(NNC)_n$ sites	Stickiness to *E. coli* $(GNN)_n$ mRNA	Exposed loops
(1395)caCacCucC	1·19	+
(517)gcCagCagCcgC	1·17	+
(629)aaCugCauC	1·15	
(499)agCacCggC	1·13	
(1061)guCguCagC	1·13	
(803)guCcaCgcC	1·11	
(306)acCagCcaC	1·11	
(1312)guCugCaaC	1·10	
(874)guCgaCcgC	0·97	
(1531)auCacCucC	0·96	+
(891)uaCggCcgC	0·92	
(993)gaCauCcaC	0·89	
(1095)ucCcgCaaC	0·88	
(1257)agCgaCcuC	0·80	
(730)ggCggCccC	0·73	
(1320)cuCgaCucC	0·52	
(337)gaCucCuaC	0·44	

mRNA binding sites in 16 S rRNA

(517)G C $\overset{\bullet}{\text{C}}$ A G $\overset{\bullet}{\text{C}}$ A G $\overset{\bullet}{\text{C}}$ C G $\overset{\bullet}{\text{C}}$ G G U A A U(534)

(1392)G U A C A $\overset{\bullet}{\text{C}}$ A C $\overset{\bullet}{\text{C}}$ G C $\overset{\bullet}{\text{C}}$ C G U C A(1408)

(1530)G A U $\overset{\bullet}{\text{C}}$ A C $\overset{\bullet}{\text{C}}$ U C $\overset{\bullet}{\text{C}}$ U U A(1542)

mRNA consensus (J. Lagunez-Otero, 1992)

$(GHN)_n$ - obvious pattern (1987)

$(GHU)_n$ - normalized base distributions

$(GCU)_n$ - dinucleotide preferences

$(GCU)_n$ - avoidance of bad mismatches

$(GCU)_n$

```
5'-U GCU GCU GCU GCU G   mRNA consensus
   • ••• ••• ••• ••• •
3'-A UGG CGC CGA CGA C   525 site of 16S rRNA
                          (proof-reading site)
```

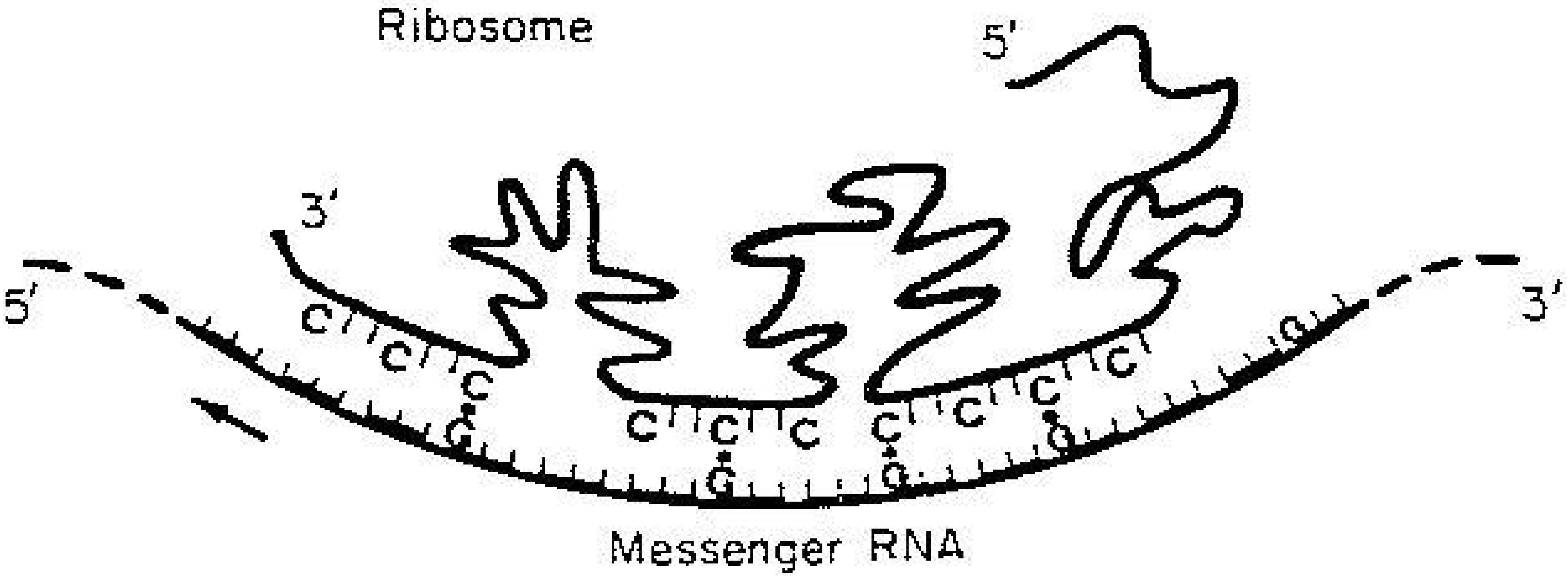
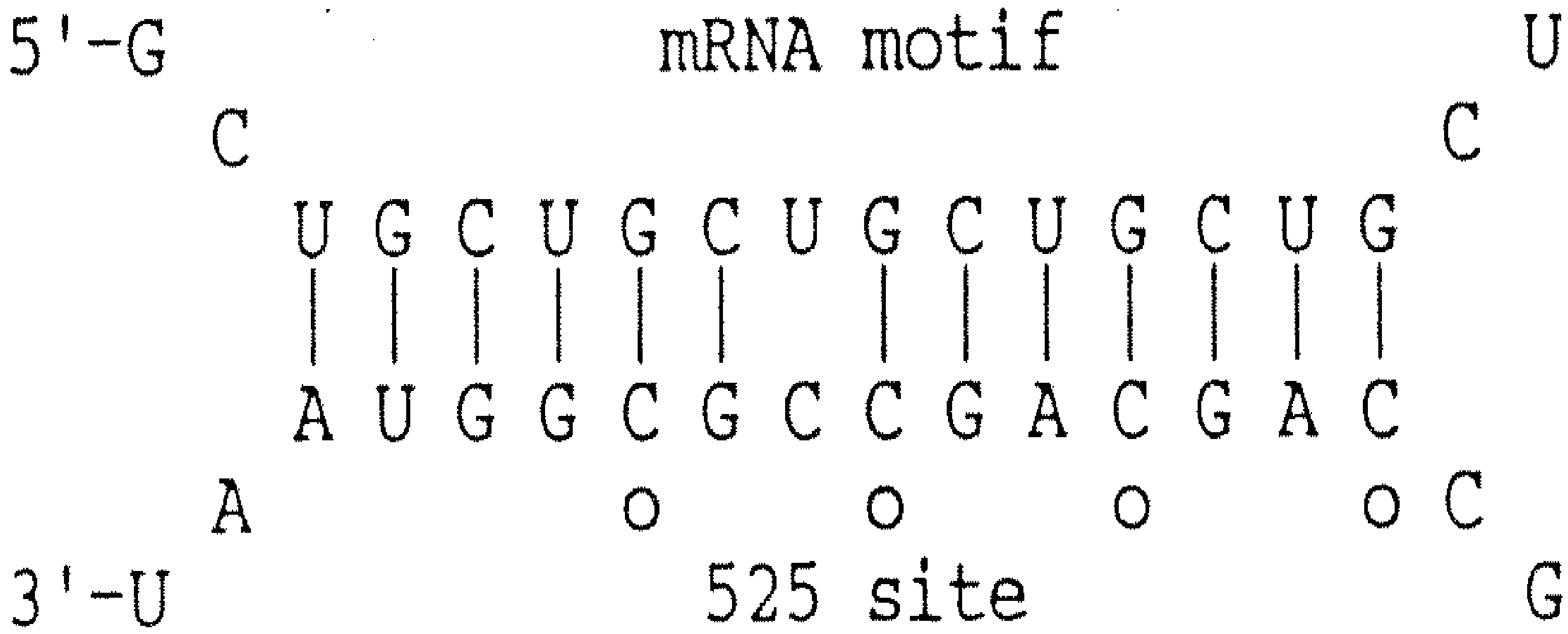


Figure 4. Scheme of the translation frame-monitoring mechanism.

ENT, 1987

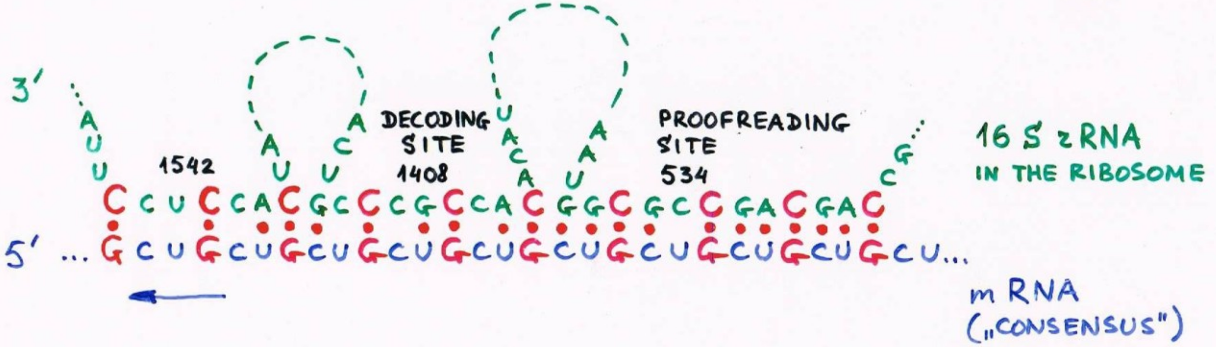


```
5'-G              mRNA motif               U
    C                                    C
      U G C U G C U G C U G C U G
      | | | | | |   | | | | | | |
      A U G G C G C C G A C G A C
    A         o     o     o     o C
3'-U              525 site                 G
```

Which one is more ancient?

TRANSLATION FRAMING CODE

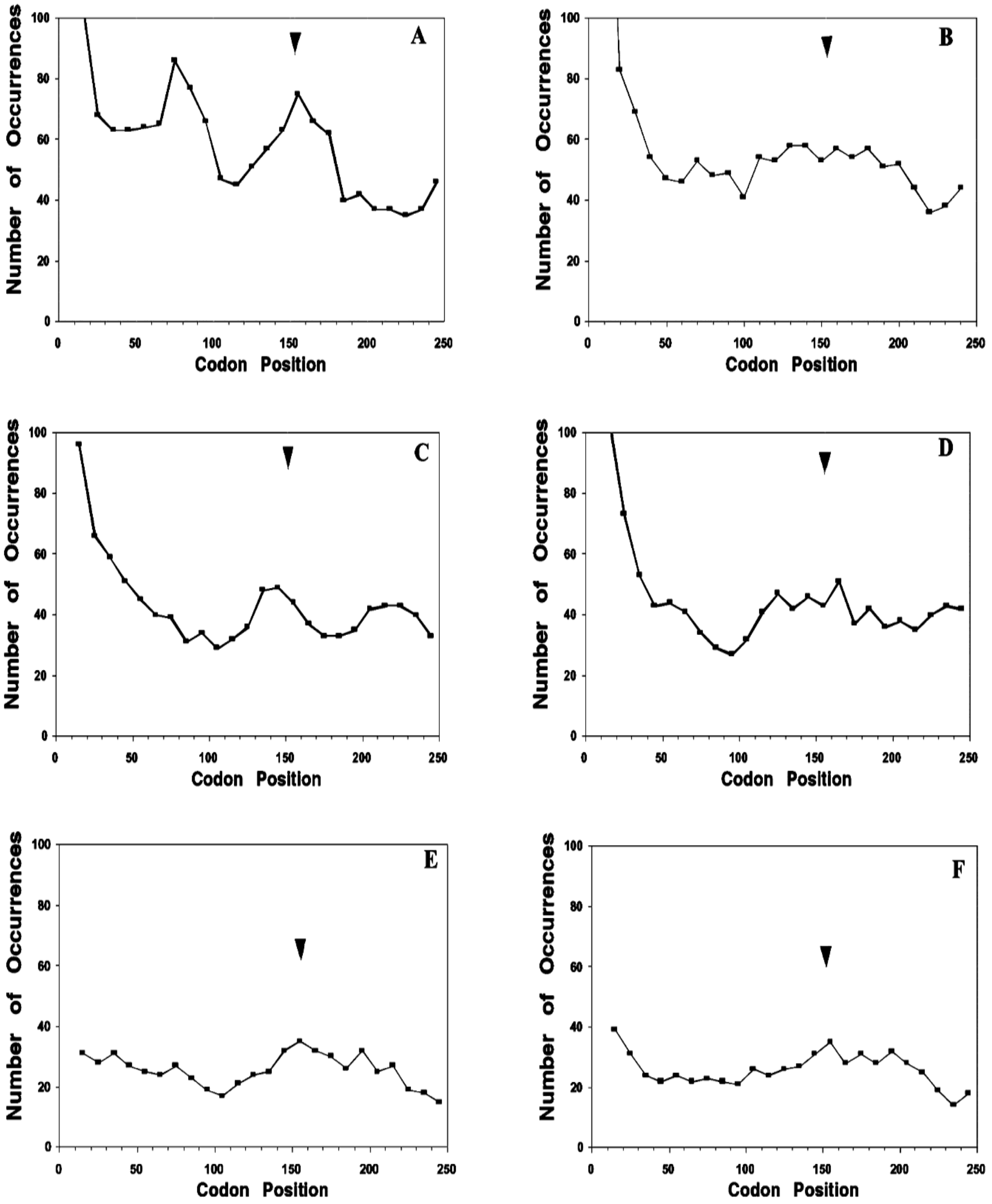
$(GCU)_n$ - mRNA „CONSENSUS"
(J. Lagunez-Otero,
E. Trifonov)
1992

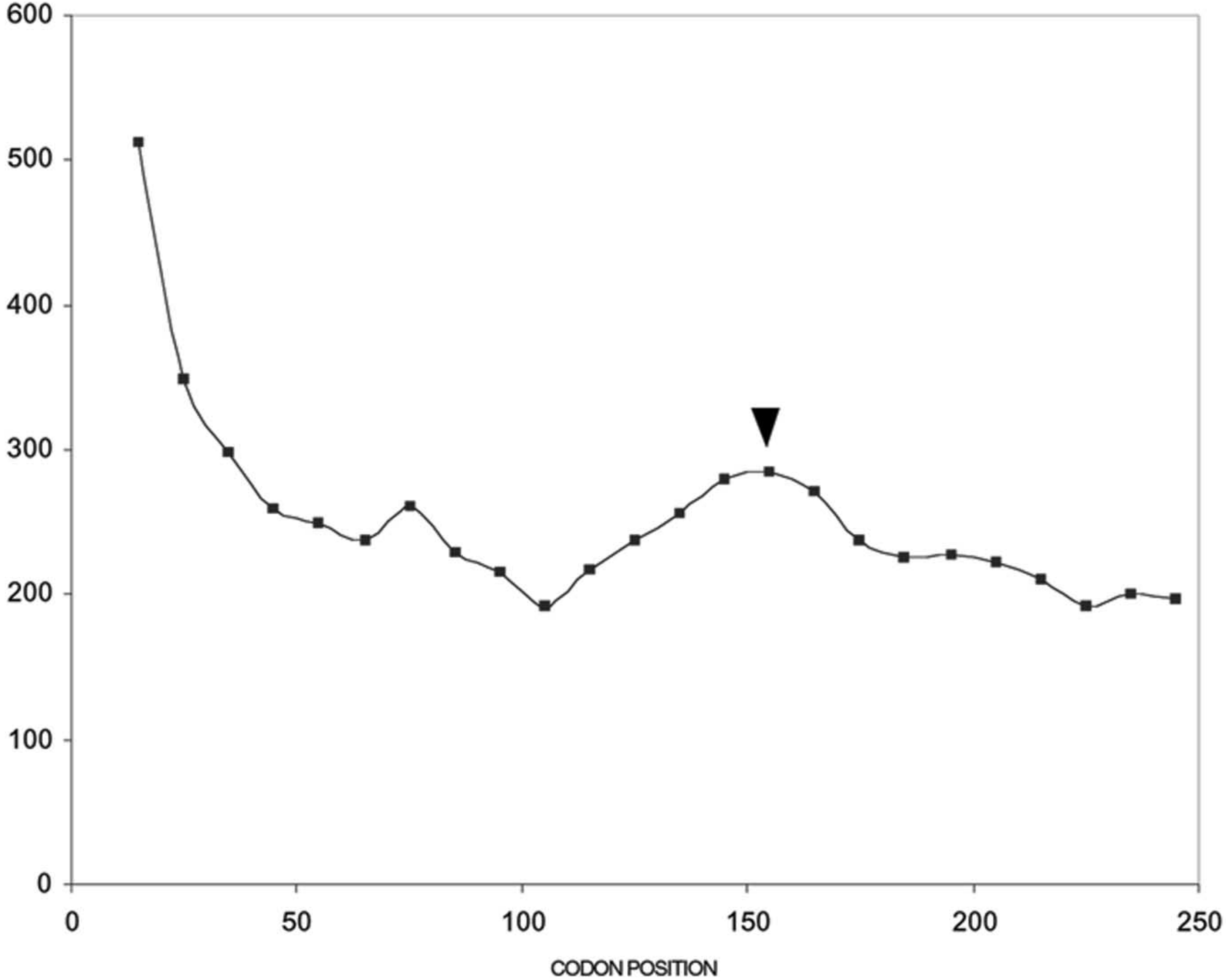


THE IN-FRAME COMPLEMENTARITY
PREVENTS RIBOSOME SHIFTING TO WRONG FRAME

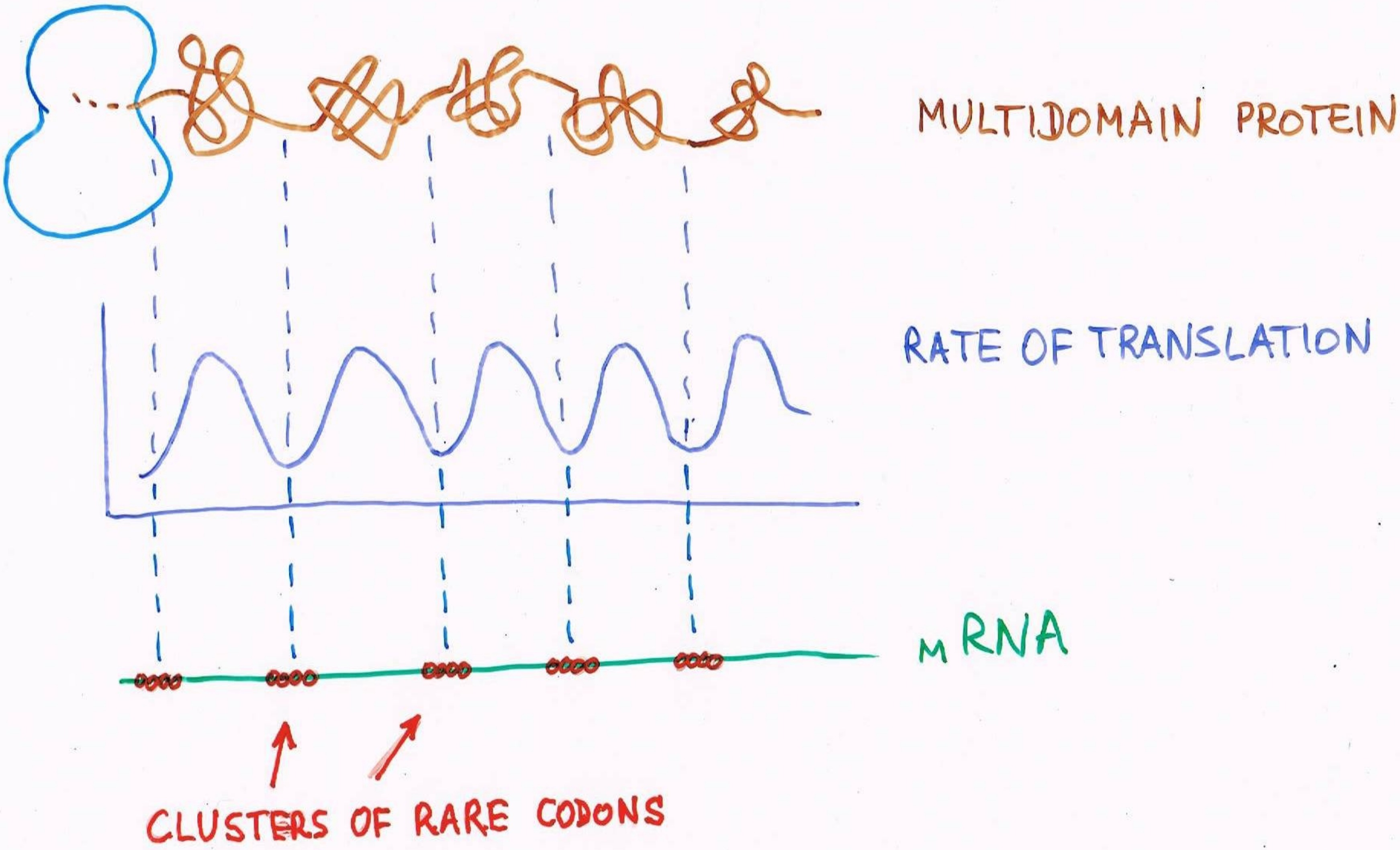
THIS IS IMPORTANT FOR LARGE PROTEINS

Translation pausing code

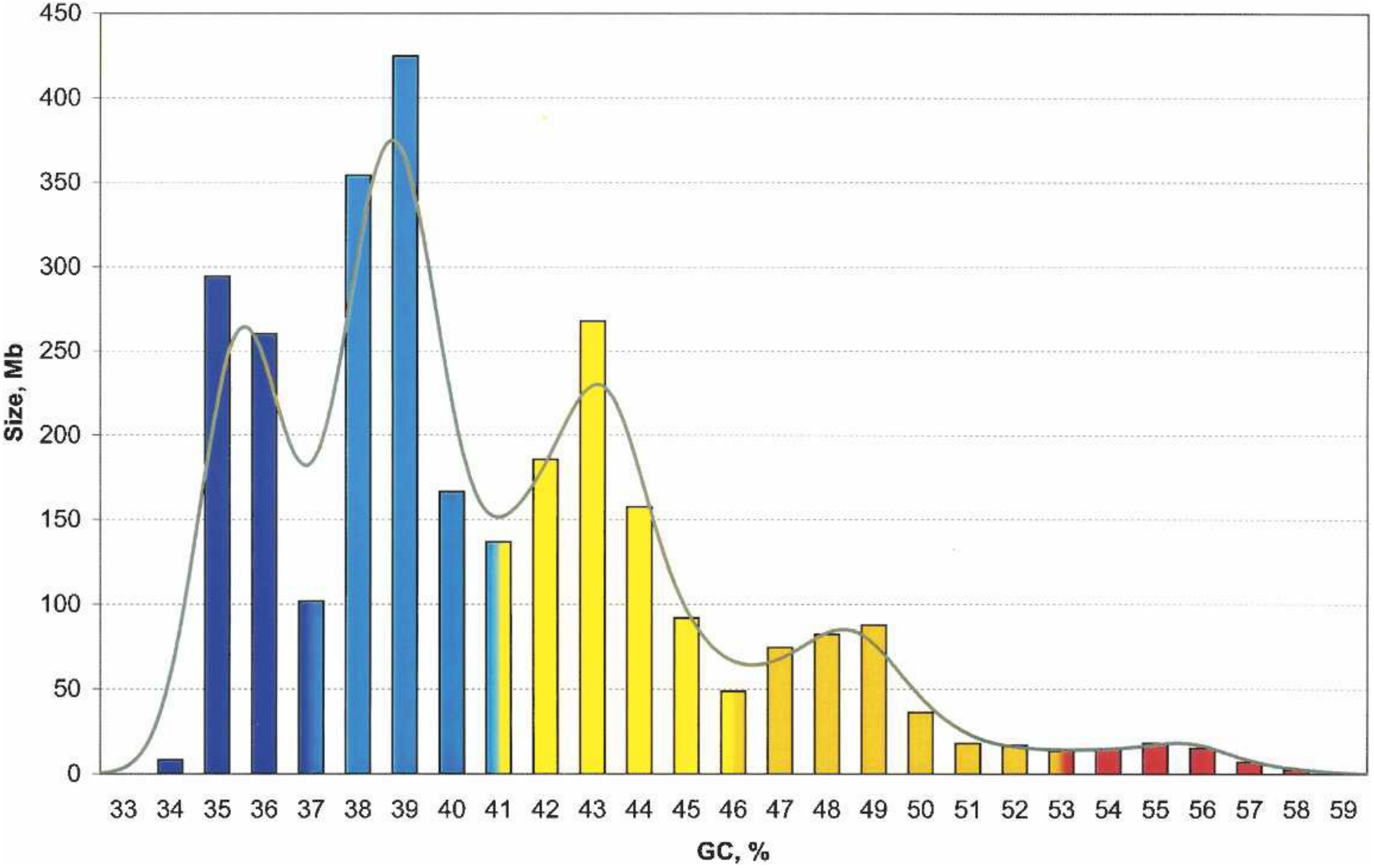

A
B
C
D
E
F
Number of Occurrences
Codon Position
0
20
40
60
80
100
0
50
100
150
200
250


600
500
400
300
200
100
0
0
50
100
150
200
250
CODON POSITION

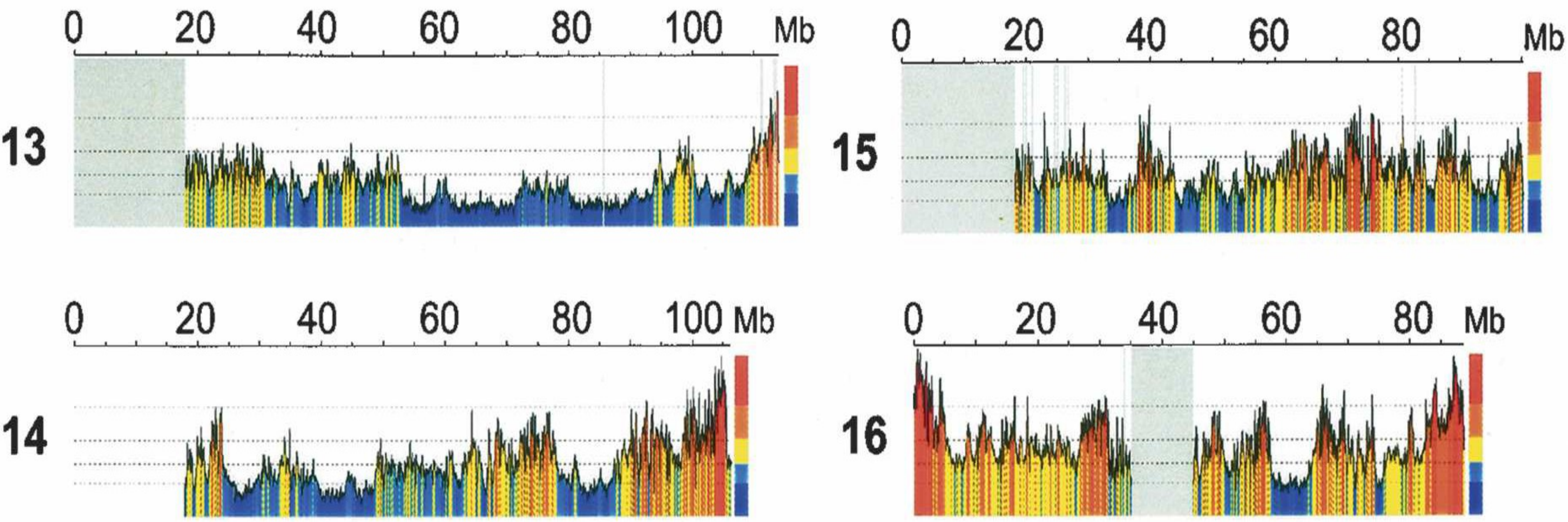
TRANSLATION PAUSING CODE



Genomic code (isochores)


450
400
350
300
250
200
150
100
50
0
Size, Mb
33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59
GC, %

H3	🟥	>53
H2	🟧	46-53
H1	🟨	41-46
L2	🟦	37-41
L1	🟦	<37



Isochores

Lab of G. Bernardi, 2006

Transcription factor binding sites
in G+C rich isochores are G+C rich as well

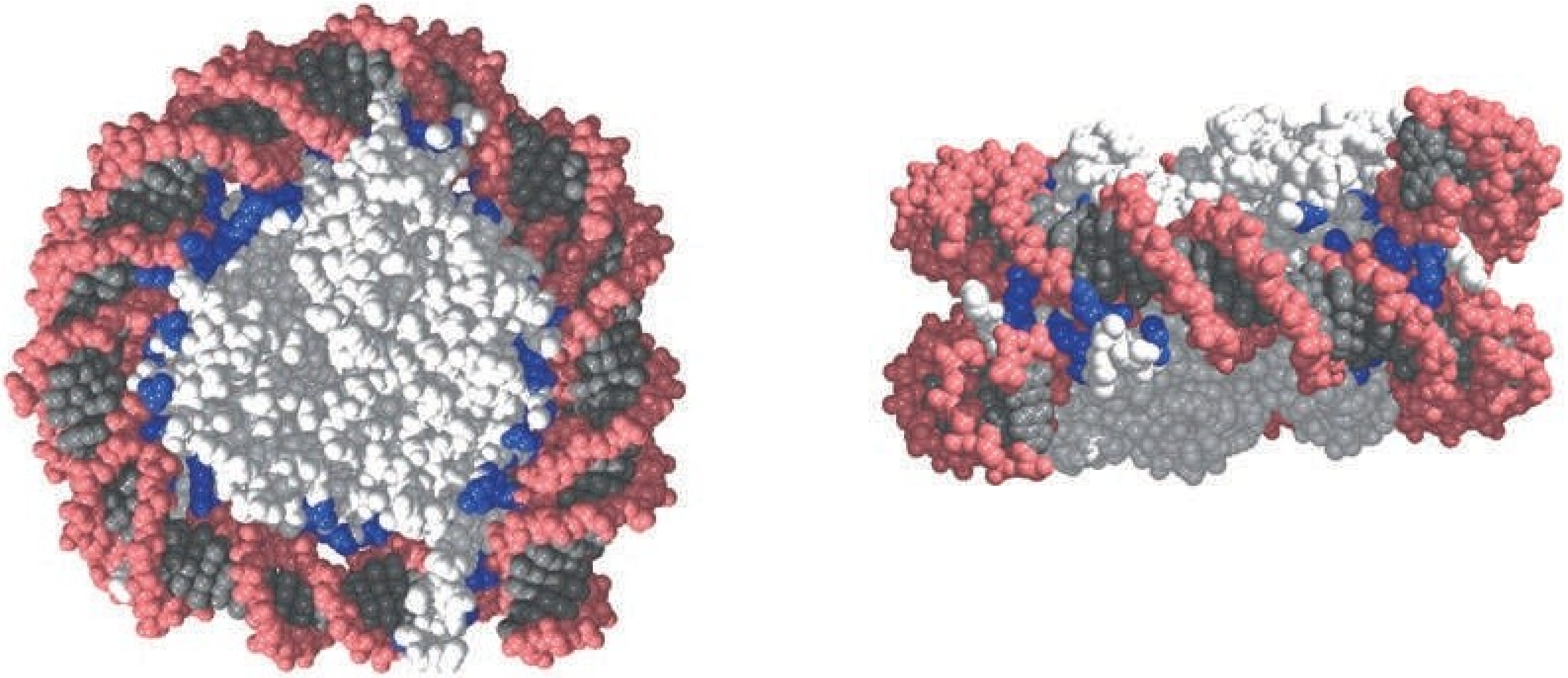
This results in different usage of transcription factors
in different isochores

In other words, each isochore type in the genome
is under isochore-specific separate regulatory system

In that sense isochores appear as individual mini-genomes
within the genomes

Apparently, modern eukaryotic genomes are mosaics of
many fused small ancestral genomes

DNA SHAPE CODE
(CURVED DNA)



S. Tan, Pennsylvania State University, USA.

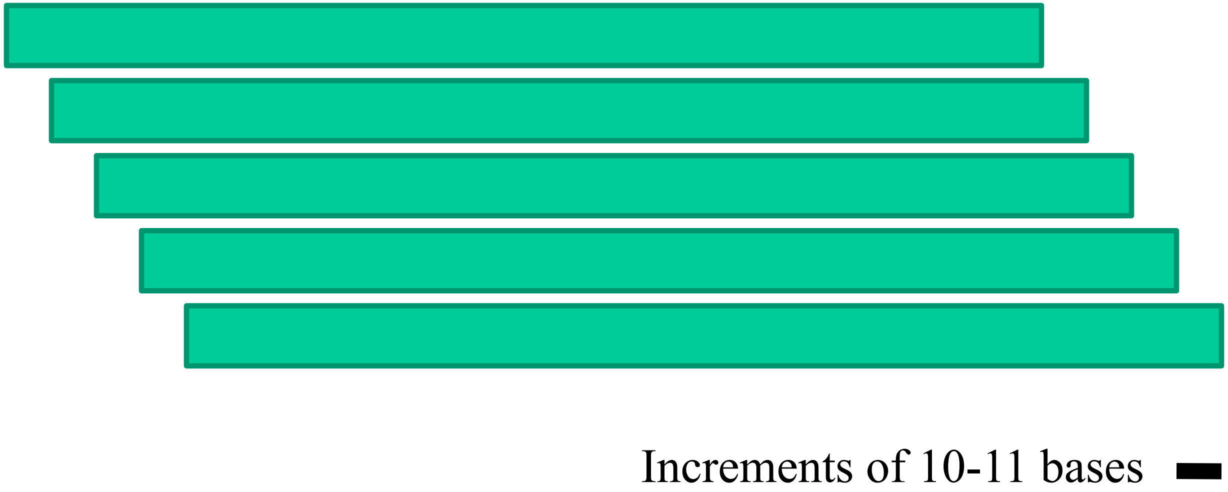
Since 1974 the experimental evidence started to accumulate suggesting that

1. Nucleosomes prefer some specific sequences

2. Comparisons of the sequences do not show anything in common

3. Often there are several alternative nucleosome positions on the same sequence

4. The alternative positions are separated by 10-11 bases



Separation of the nucleosome positions by 10-11 bases
(one structural period of DNA helix)
means that

The DNA molecule binds to histone octamers by one side

Physically, there are two ways to make DNA sided:

1. DNA may have the curvilinear shape, with arc-like axis – **Curved DNA**

2. DNA (straight DNA) could be easier bent in certain direction – **Bent DNA**

One is arc-like because it has that shape (like banana) – no force applied (curved DNA)

Another one is arc-like because the bending force is applied to it (bent DNA)

There is a wide-spread confusion on the name
of the DNA that has curvilinear shape

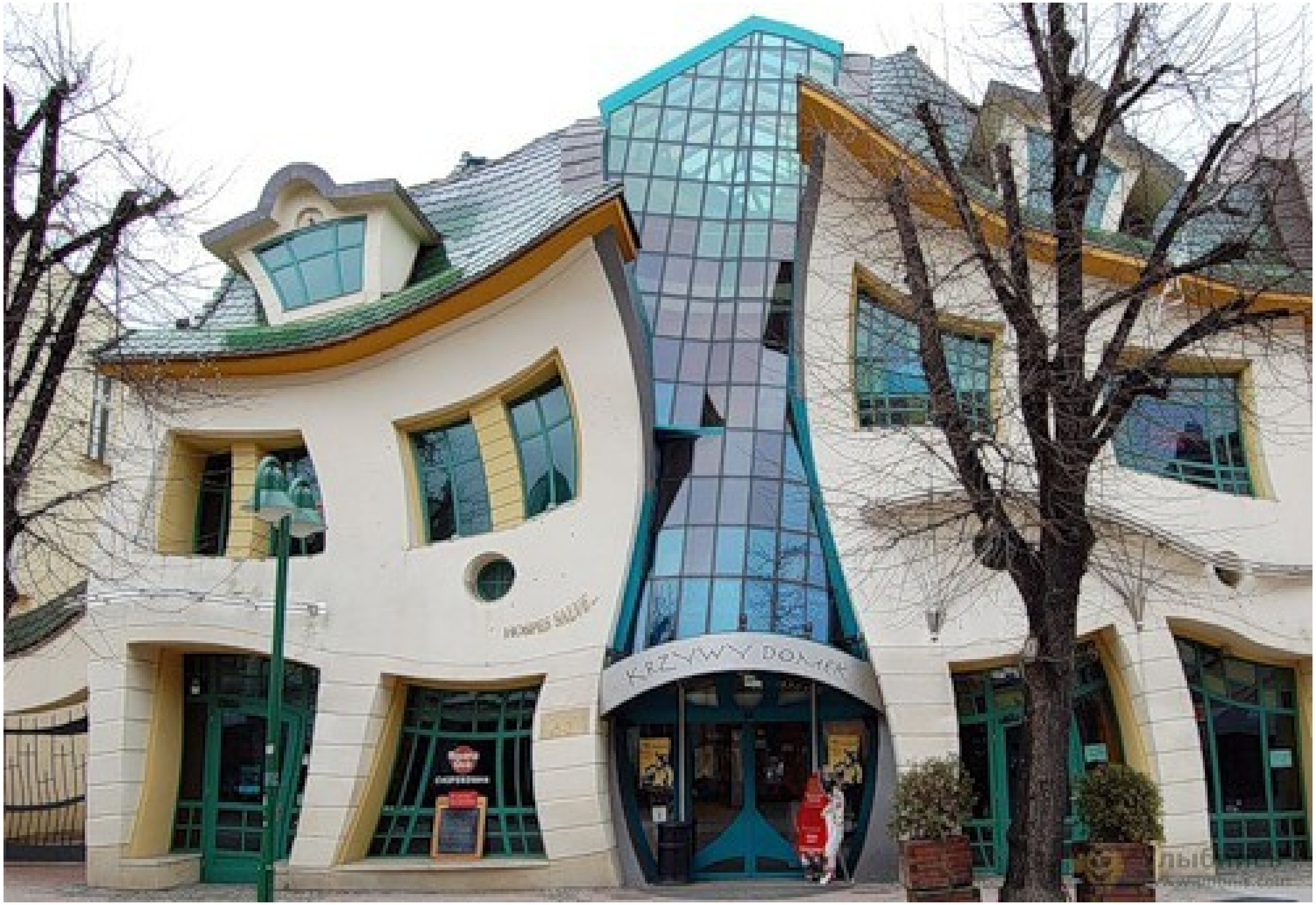
Original name (Trifonov, 1980) was
CURVED DNA.

But soon instead another name was introduced
by Crothers (1982): BENT DNA

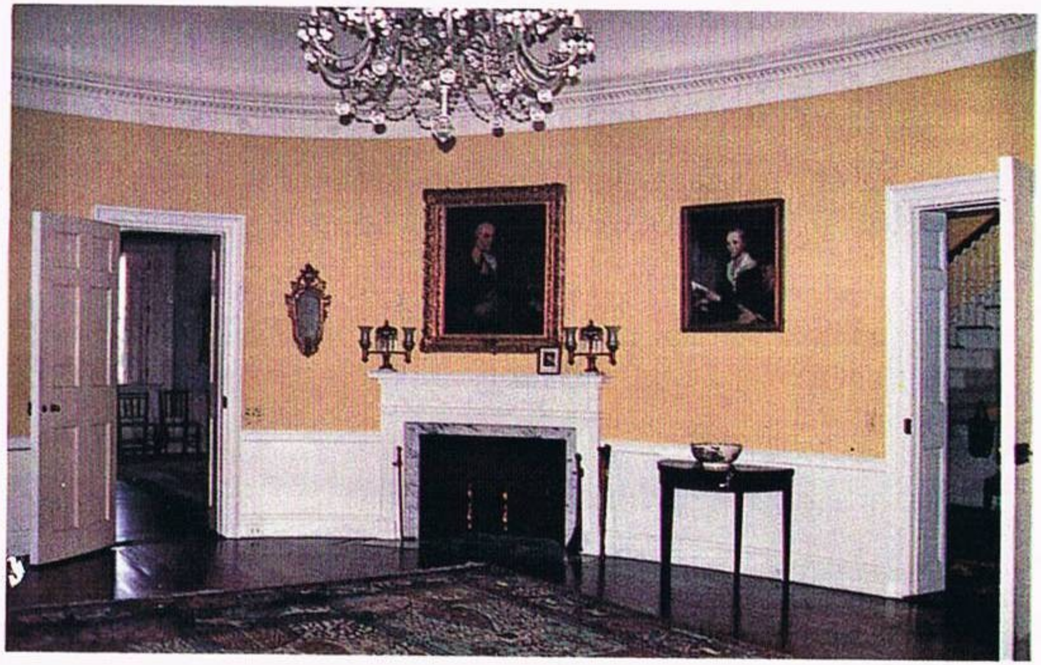
It was accepted by English speaking community
since both “curved” and “bent” are passive terms in English,
contrary to other languages, and “bent” is more frequently used

Object of arc-like shape is called

	≠		(Hebrew)
Кривой	≠	Согнутый	(Russian)
Křivý	≠	Ohnutý	(Cžech)
Krzywy		?	(Polish)
Krumm		?	(German)
Curved	≈	Bent,	(English)
↑		↑	
no force applied		**actively deformed**	



Krzywy domek (Curved house), Sopot, Poland



From Google :

“Curved DNA” is used ~ 40%
“Bent DNA” is used ~ 60%

As Mendel said once:

“My time will yet come”
(“Nash chas eshche pride” in Czech)

One innocent way to “hijack” somebody`s idea
is to describe the same idea by using different terms.

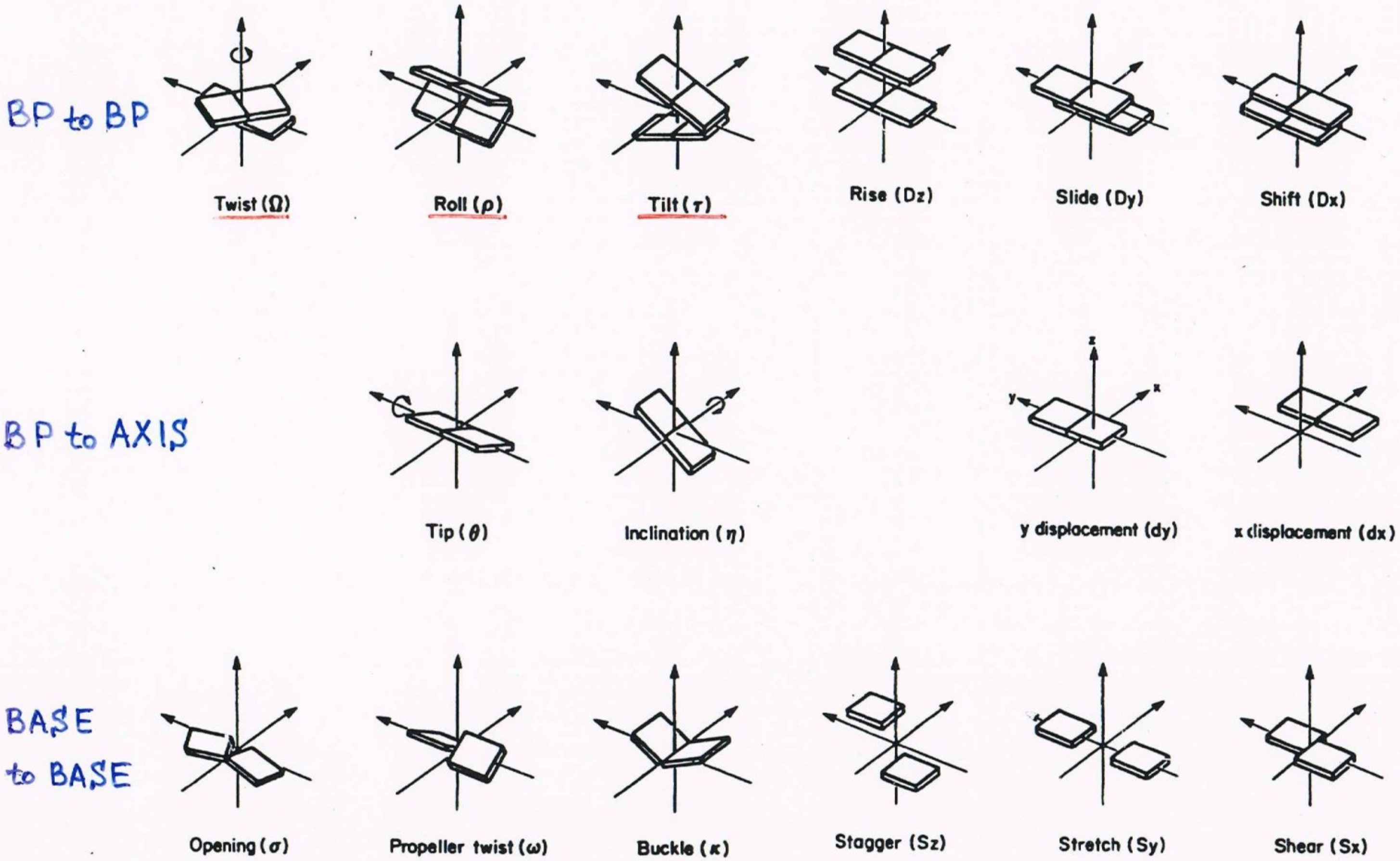
Before historians of science will establish true priority,
the hijacker will enjoy credit for “his” idea.

And he is not to blame. After all, he just suggested
to call the thing differently.

CURVATURE and	BENDABILITY	
Curved DNA	Bent DNA	} DIFFERENT THINGS
(with no strain)	(force applied)	

Strongest nucleosome motif: GAAAATTTTC

Strongest curvature motifs: AAAAATGACT
and AAAAACGCGA


BP to BP
Twist (Ω)
Roll (ρ)
Tilt (τ)
Rise (Dz)
Slide (Dy)
Shift (Dx)
BP to AXIS
Tip (θ)
Inclination (η)
z
y
x
y displacement (dy)
x displacement (dx)
BASE to BASE
Opening (σ)
Propeller twist (ω)
Buckle (κ)
Stagger (Sz)
Stretch (Sy)
Shear (Sx)

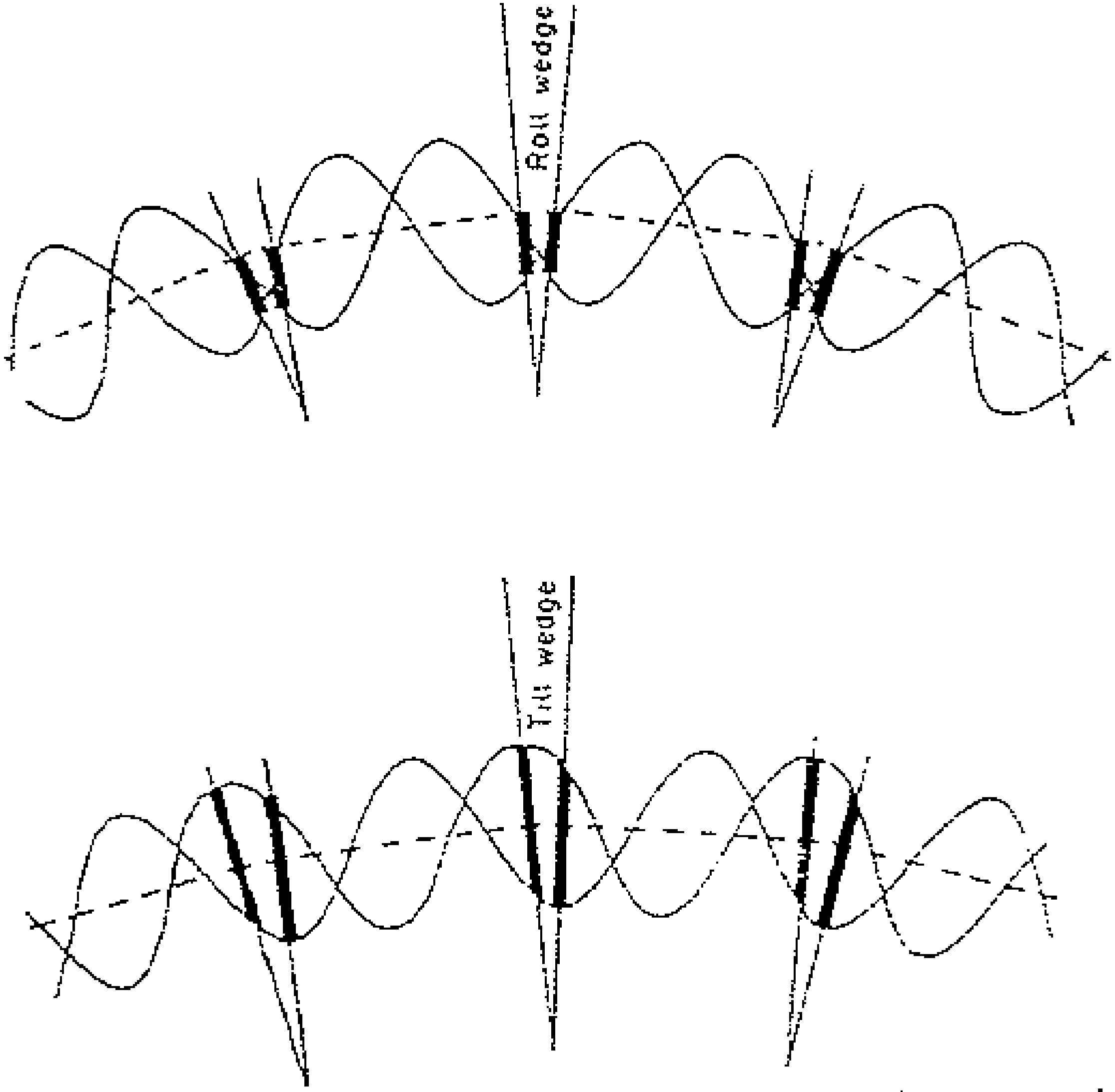
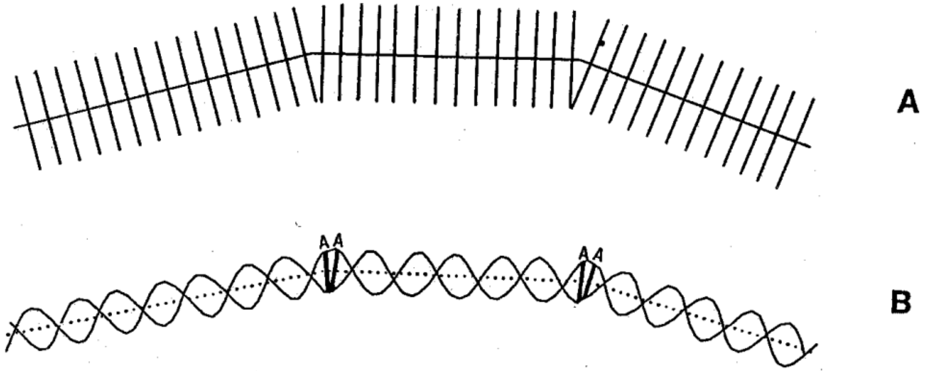


Figure 2. Wedge components of curved DNA (scheme). two interwound strands of double helical DNA molecule are presented by their sinusoidal projections. Only those base-pairs are shown which are non-parallel making the coresponding angles in their in-plane projections (From Ulanovsky and Trifonov, 1987, with permission).


A
AA
AA
B

Prediction:

If the static DNA curvature is good for the nucleosomes,
some sequence elements (dinucleotides)
would have tendency to be at one or more period distances
from one another

Checking the prediction:

List all distances between the same type dinucleotides
and see whether they like to be at ~ 10, 20, 30,… bases
one from another.

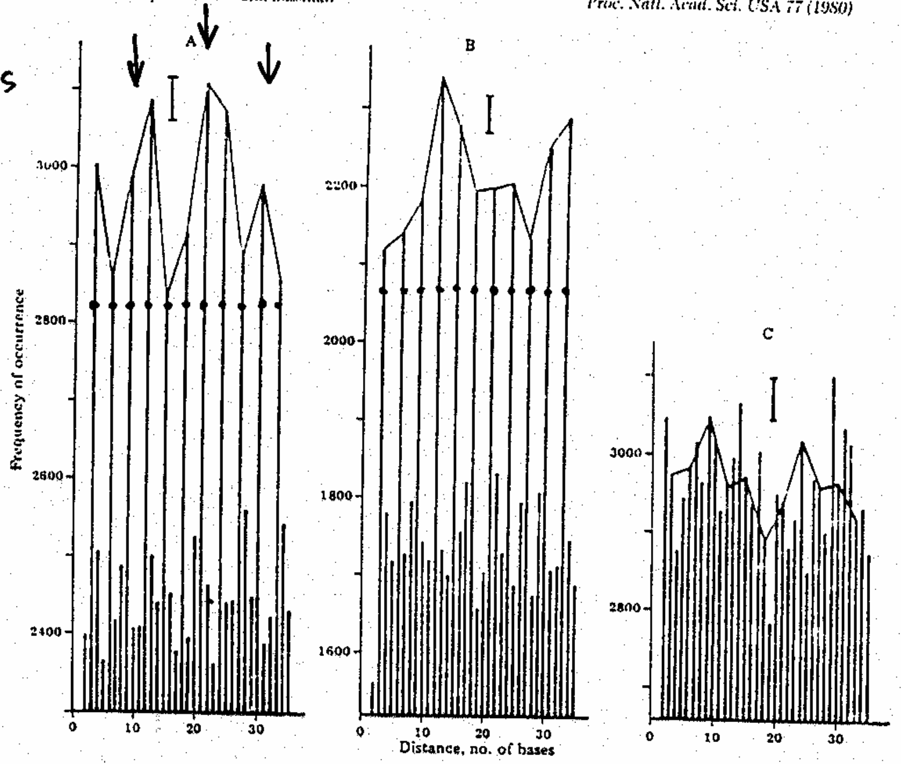
This is called distance analysis, or
positional autocorrelation analysis

```
aacaagctaagtaccgtactgaagcgcattttaattacgataaggcttatcttaatttcgccgatggcaatgaatgacgtaagcttac
.  .    .            .          .        .           .              .   .       .
0  3    8            21         32       41          53             68  72      80
   0    5            18         29       38          50             65  69      77
        0            13         24       33          45             60  64      72
                      0         11       20          32             47  51      59
                                 0        9          21             36  40      48


                      *          *
    * *  ** * *    * **  *    *  **  * * **   * ** ** *
.........................................................
0         10        20        30        40        50


aacgaacgatccgcaattaagtcgcgtctggtgcaagggtacttaacagattggaagtaaccgtaactgtcaggaacgtaaggtccat
.   .         .   .               .         .         .   .     .         .    .
0    4        14  18              34        44        54  58    64        74   79
     0        10  14              30        40        50  54    60        70   75
               0   4              20        30        40  44    50        60   65
                   0              16        26        36  40    46        56   61
                                   0        10        20  24    30        40   45

                                        *
                              *         *
    *     *   *     *         *         *   *     *     *
    *     *   * * * *   * *   *   * *   *   ***   *     *
................................................... ......
0         10        20        30        40        50
```

TRIFONOV, SUSSMAN, 1980

3818 Biochemistry: Trifonov and Sussman

Proc. Natl. Acad. Sci. USA 77 (1980)



~ 10.5 BASES

3 BASES

EUKARYOTES

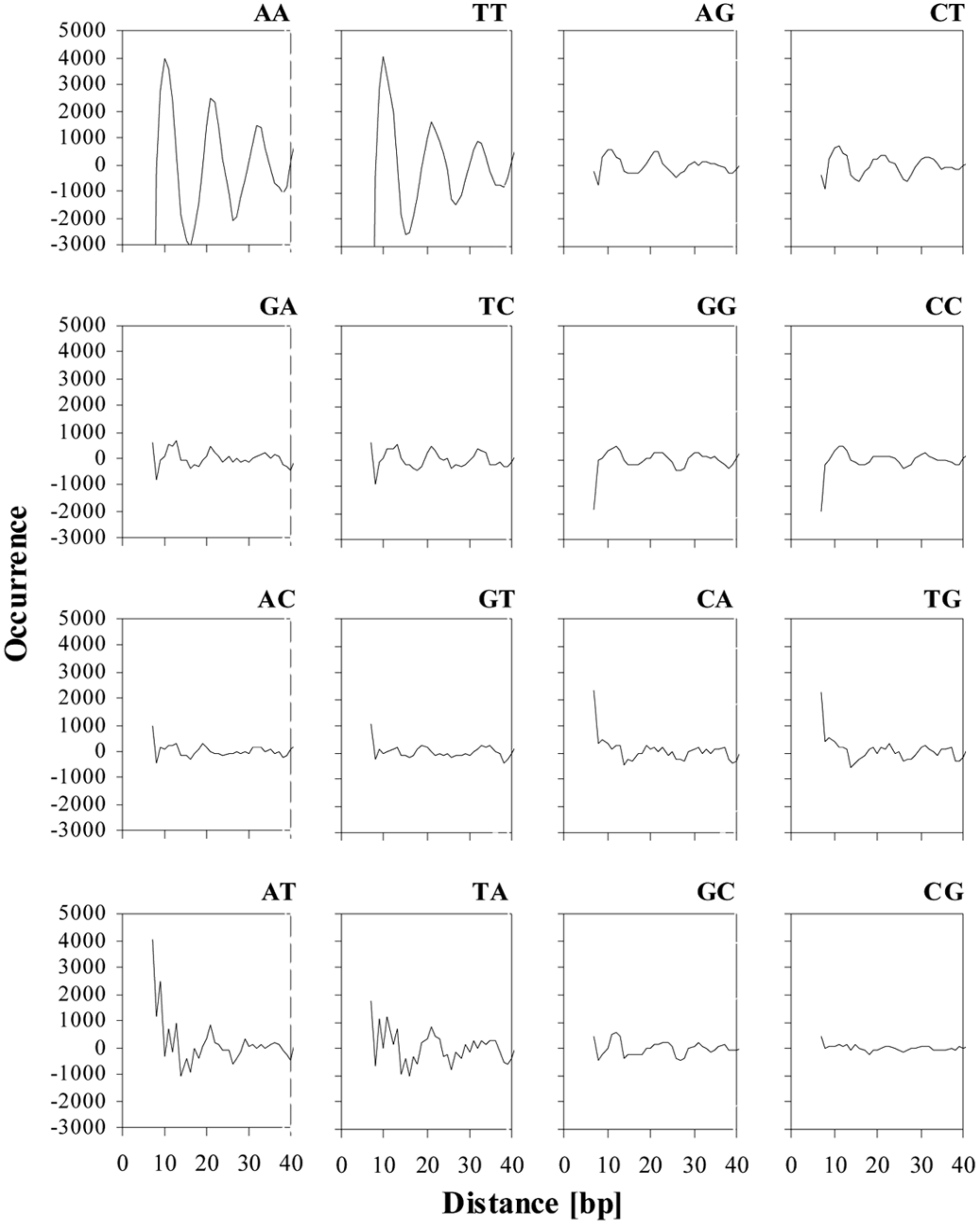
PROKARYOTES

RANDOM

~30 000 BASES

The signal thus detected was so small (~3.5 STD),
that many questioned this result,

until much stronger oscillation
has been discovered in *Saccharomyces cerevisiae*

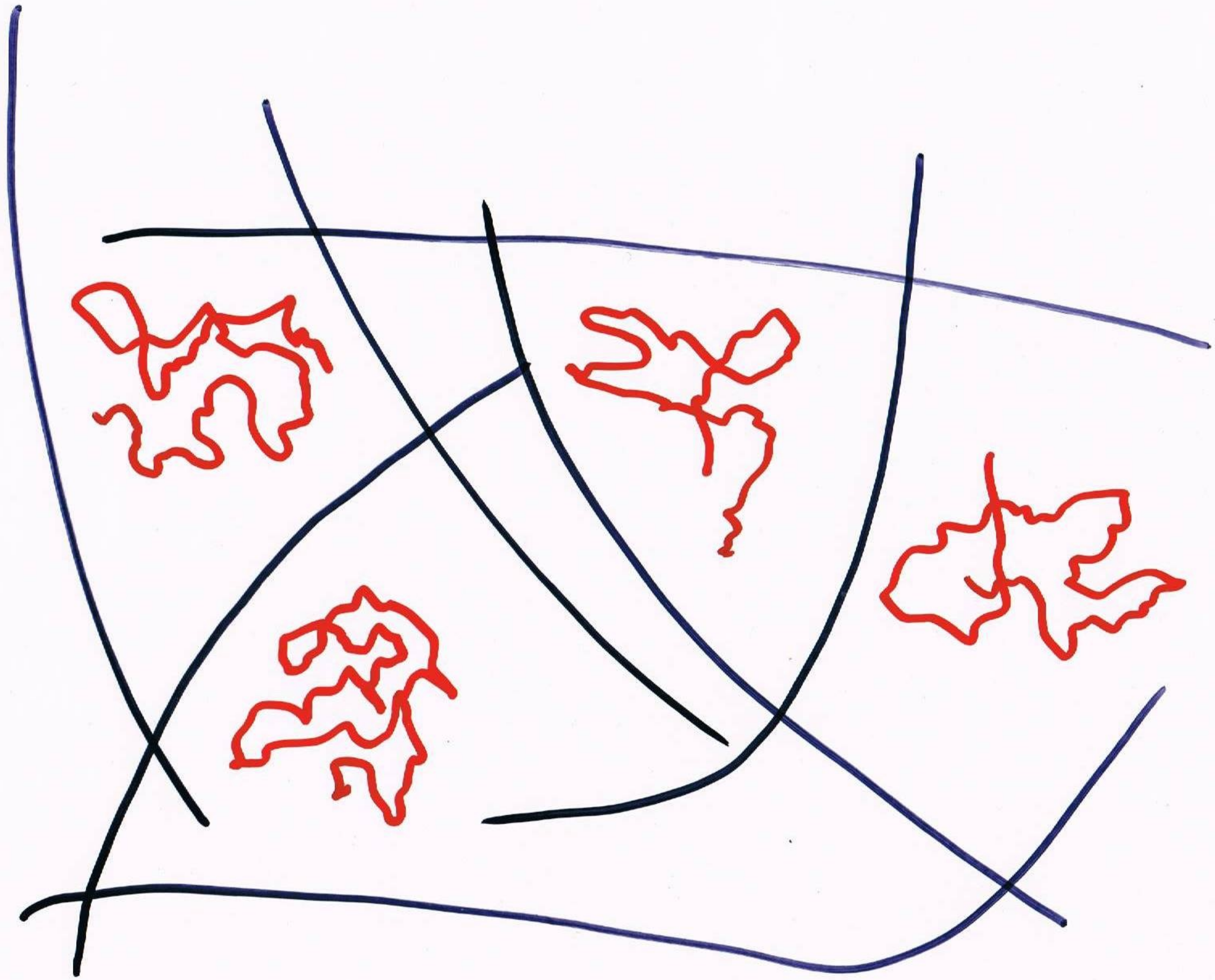


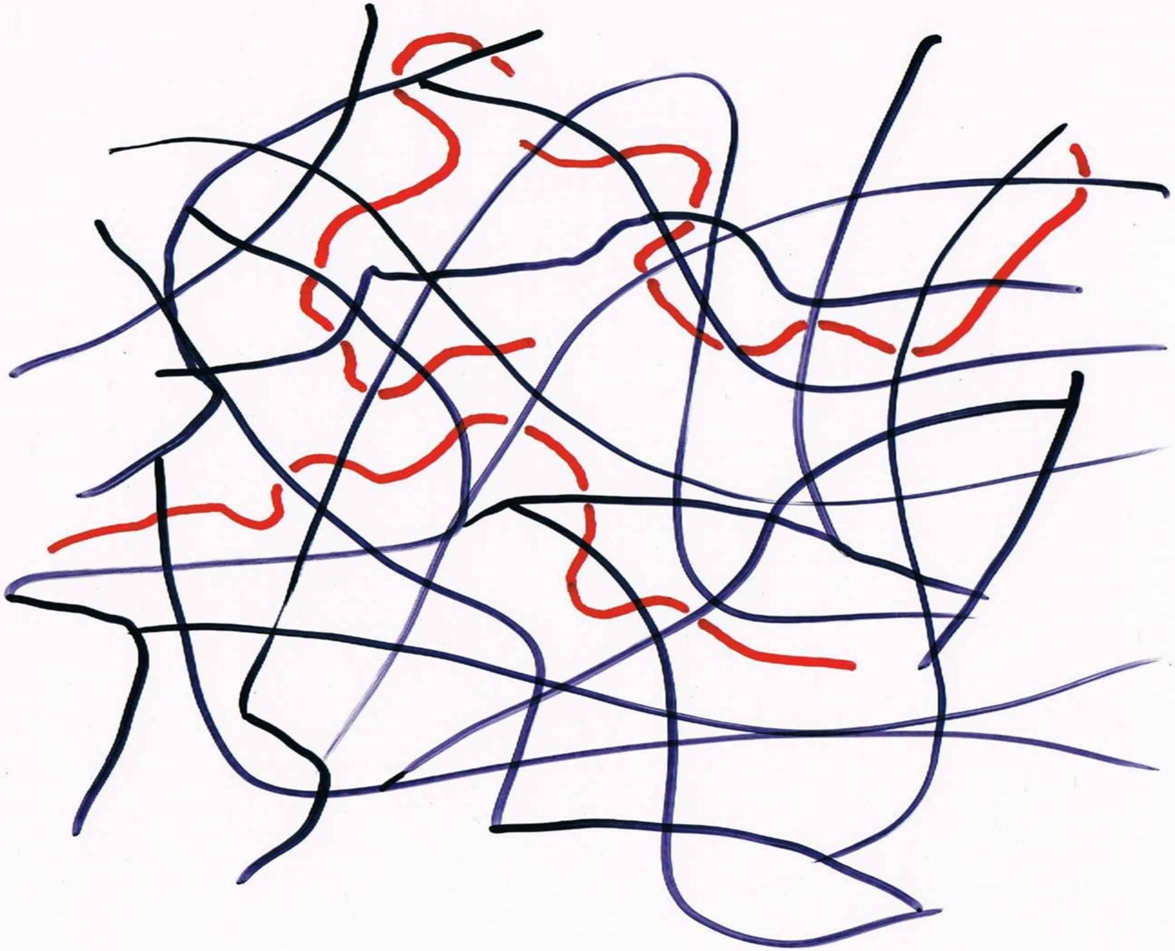
Yeast
Cohanim 2005

One way to experimentally observe DNA curvature is to watch DNA moving in gel electrophoresis

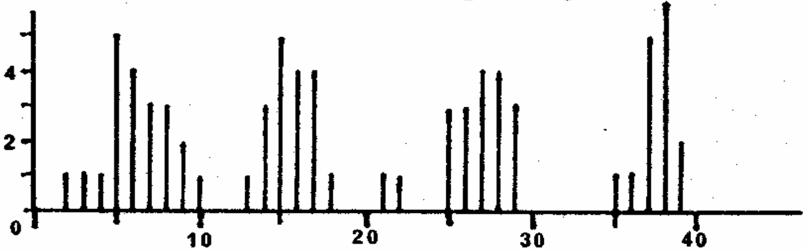
DNA moves head-on through the narrow pores of the polyacrylamide gel – reptation

The curvature is an obstacle, since the curved molecule keeps deflecting from the along field direction,
and it has to be made straight (force applied) to get through

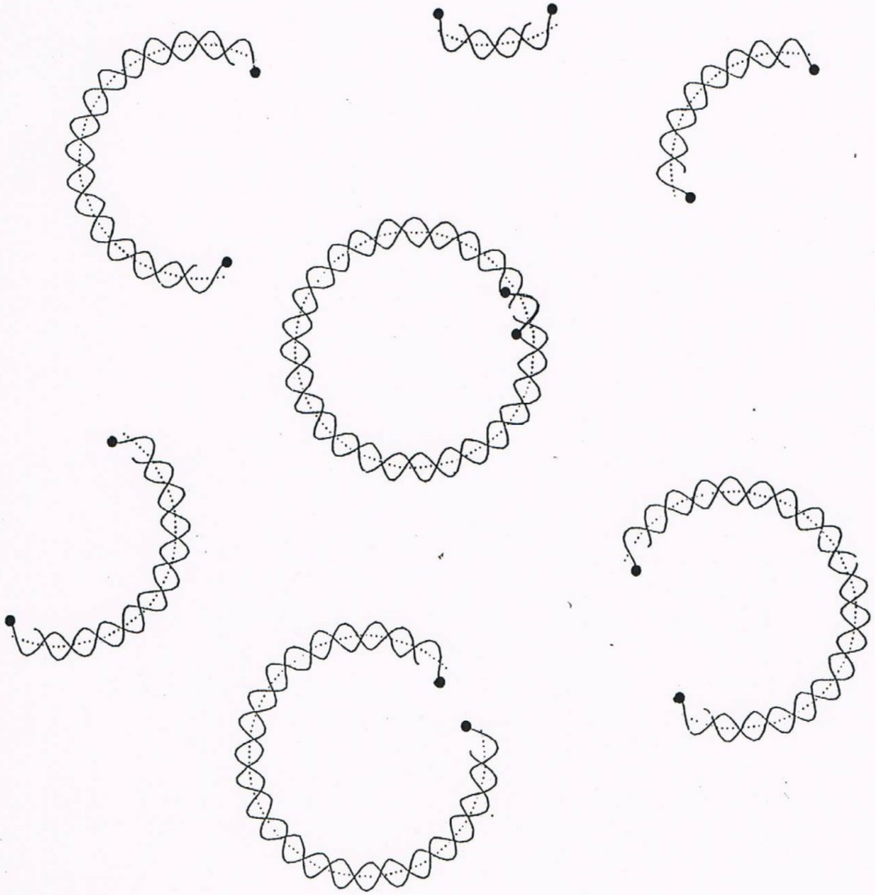


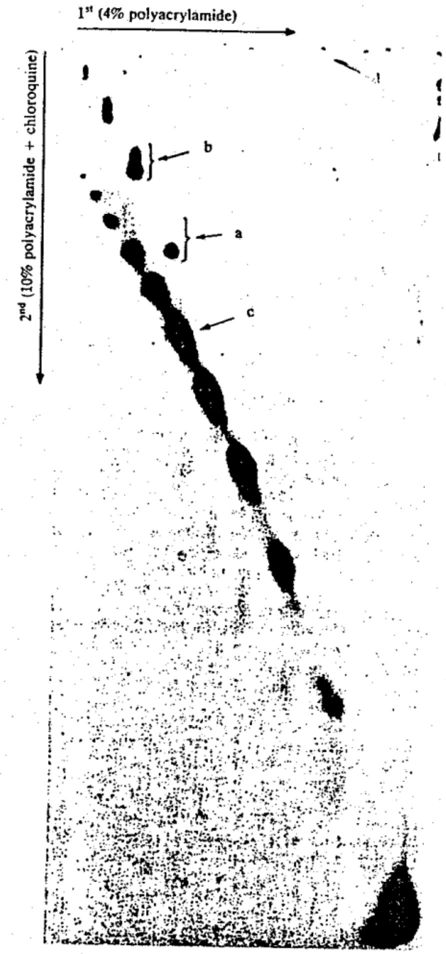


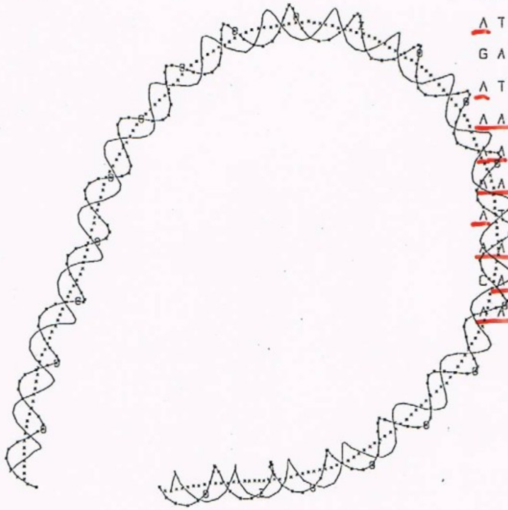
A	tcccAAAAAtgtcAAAAAAtaggcAAAAAAtgccAAAAAtccc	kDNA
B	gtatAAAAAAgctgAAcgagAAAcgtAAAAtgatatAAAtatc	attP
C	gatcgAAAAcAAAAAAtgctttAAAtagcattttAAAAcata	Ch. thummi th.
D	acacAAAAAActcatgAAAAtggtgctggAAAAcccattcAAggt	SV40 Hind F
E	cctcAAAAcgagggAAAAtcccctAAAAcgagggatAAAAcatccctcAAAttgg	ORI lambda
F	tgccAAttcatccattAActtctcagtAAcagatacAAActcatcacgAAcgtc	ORI PhiX174 (Hind R3)



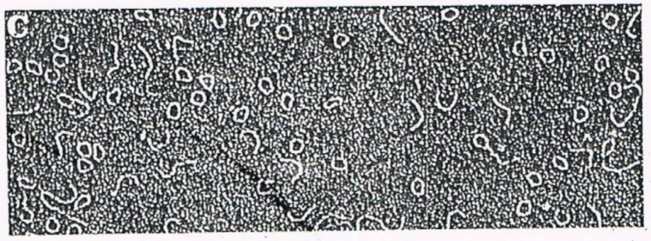
TCTCTAAAAAATATATAAAAA

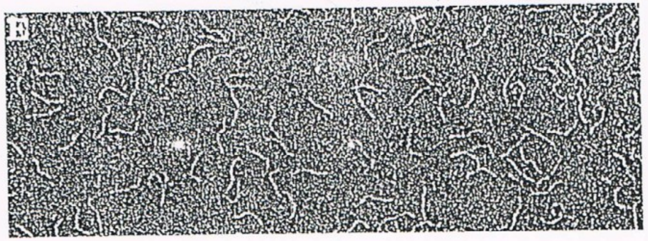



1st (4% polyacrylamide)
2nd (10% polyacrylamide + chloroquine)
b
a
c



```
S E Q :

C C C T A A A A T T C C A A C C G A A A
                  10                  20
A T C G C G A G G T T A C T T T T T T G
                  30                  40
G A G C C C G A A A A C C A C C C A A A
                  50                  60
A T C A A G G A A A A A T G G C C A A A
                  70                  80
A A A T G C C A A A A A A T A G C G A A
                  90                  100
A A T A C C C C G A A A A T T G G C A A
                  110                 120
A A A T T A A C A A A A A A T A G C G A
                  130                 140
A T T T C C C T G A A T T T T A G G C G
                  150                 160
A A A A A A C C C C C G A A A A T G G C
                  170                 180
C A A A A A C G C A C T G A A A A T C A
                  190                 200
A A A T C T G A A C G T C T G
                  210
```

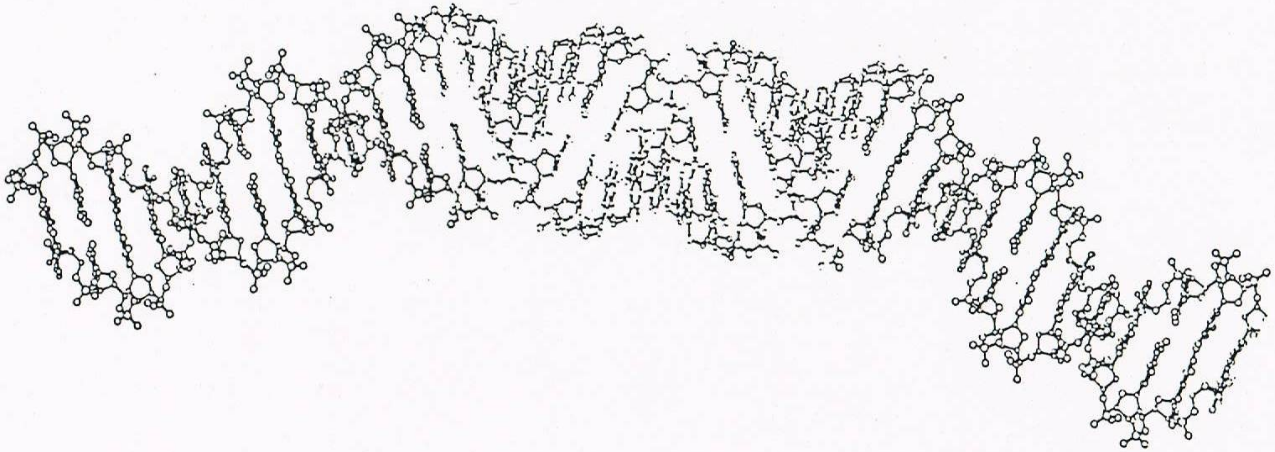




Griffith et al. Cell 46, 717-724 (1986)

II.1

JUNCTION MODEL
OF DON CROTHERS



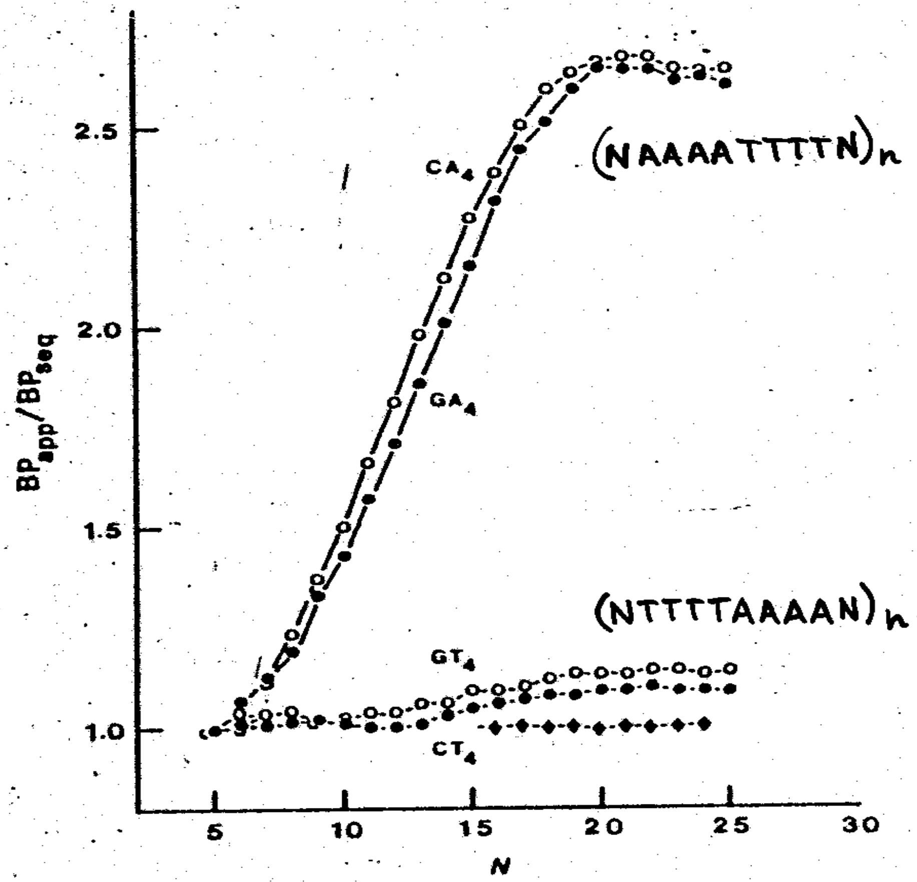
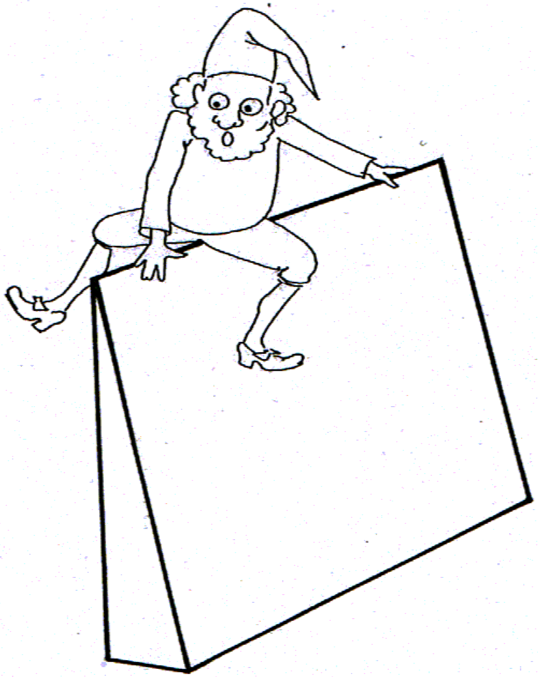


Fig. 2 Gel electrophoretic behaviours of duplex polymers having a repeating decamer motif. CA$_4$, $[CA_4T_4G]_N$; GA$_4$, $[GA_4T_4C]_N$; GT$_4$, $[GT_4A_4C]_N$; CT$_4$, $[CT_4A_4G]_N$. Mobilities of the various polymers, represented as the ratio of the apparent number of base pairs (BP$_{app}$) to the true number of base pairs (BP$_{seq}$), are plotted as a function of the degree of polymerization, *N*. The two curves plotted with solid circles represent sequence inversions of one another; the same applies to the two curves with open circles. ◆, $[G_3TCGAC_3]_N$ (lane *b* of Fig. 1, displaying a normal electrophoretic pattern for a decamer-based series).

Nature 321, 449, 1986

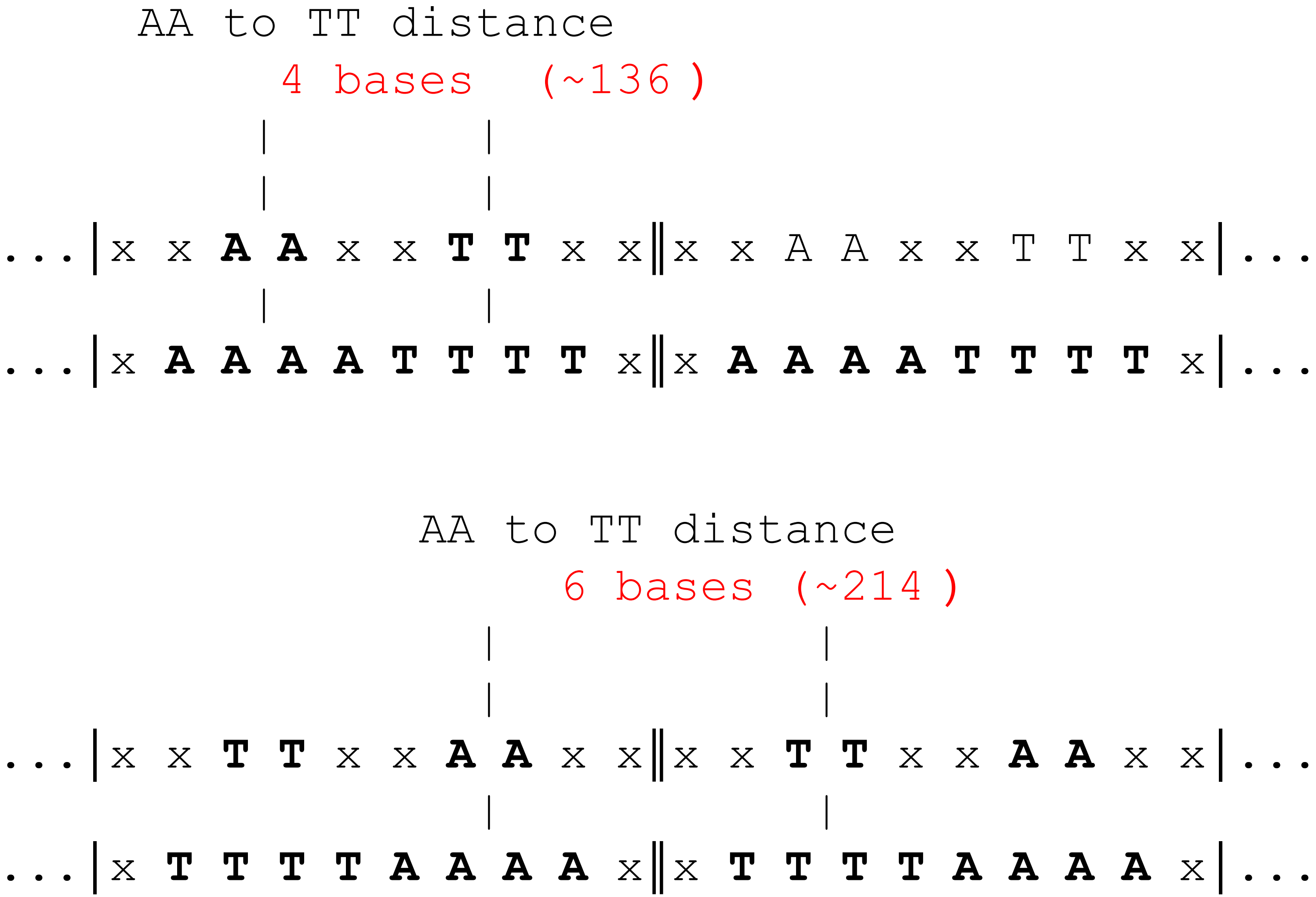


In the experiments of Hagerman he discovered that repeating GAAAATTTTC behaves in the gel like curved DNA
(slow migration)

While repeating GTTTTAAAAC behaves like straight DNA.

He concluded that since these are two identical wedges, AAAA and TTTT, their net influence on DNA curvature should be the same in two cases, like summing two weights (scalar summation). Hence – the wedge model is wrong.

But the wedges are not scalars!



AA to TT distance

4 bases (~136)

...|x x **A A** x x **T T** x x‖x x A A x x T T x x|...

...|x **A A A A T T T T** x‖x **A A A A T T T T** x|...

AA to TT distance

6 bases (~214)

...|x x **T T** x x **A A** x x‖x x **T T** x x **A A** x x|...

...|x **T T T T A A A A** x‖x **T T T T A A A A** x|...

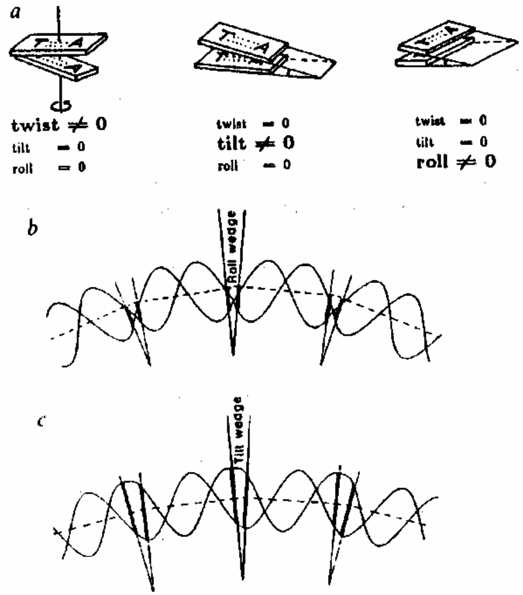


Fig. 1 Tilt and roll angles. *a*, Twist, tilt and roll angles formed by two adjacent base pairs. *b*, Curvature by roll components of the wedges, opening towards the major groove. *c*, Curvature by tilt components of the wedges, opening towards the backbone. Note that *b* and *c* show mutually perpendicular projections of the same DNA fragment containing three wedges separated by one helical turn (here 10 bp), thus causing unidirectional curvature of DNA. Tilts in *b* and rolls in *c* are not seen, being perpendicular to the plane of the paper.

late the previously unknown values of roll and tilt in the AA·TT wedge: $r = 8.4°$ and $t = 2.4°$. These two quantities are essential for computing the shape of any DNA fragment curved by AA·TT

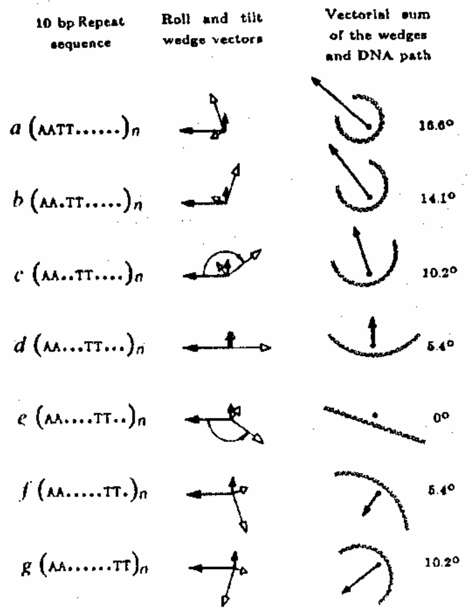
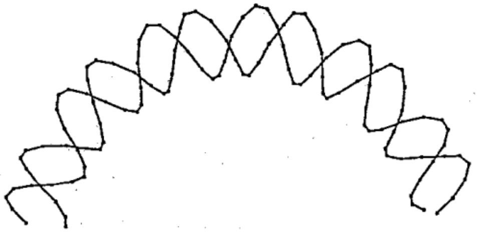


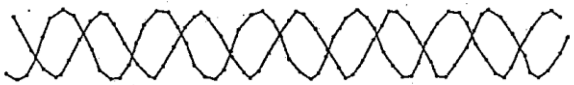
Fig. 2 Curvature caused by interplay of AA and TT wedges in a 10-bp repeat. Separating TT from AA by one more base results in a 36° rotation of TT versus AA wedge components denoted by unfilled (TT) and filled (AA) arrowheads in the central column, as viewed along the axis of the DNA. Each wedge component is shown as a vector pointing in the direction of its opening, the length of the vector being proportional to the opening angle. The long vectors are rolls, the short vectors are tilts. The numbers on the right are the magnitudes of the vectorial sum of AA and TT wedges of the central column, this sum being also the magnitude of the DNA axis deflection angle per 10 bp. In line *d*, the parallel and antiparallel orientations of tilts and rolls respectively, result from the 5-bp separation between AA and TT. The DNA pitch of

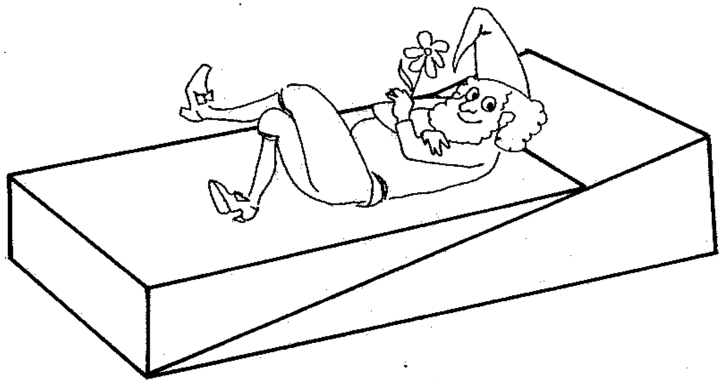
III 1

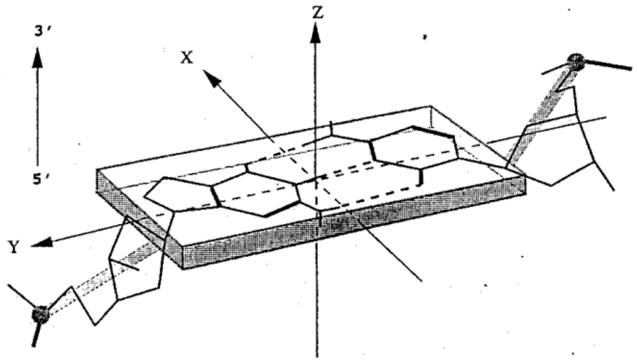
(5'-CAAAATTTTG-3')$_6$

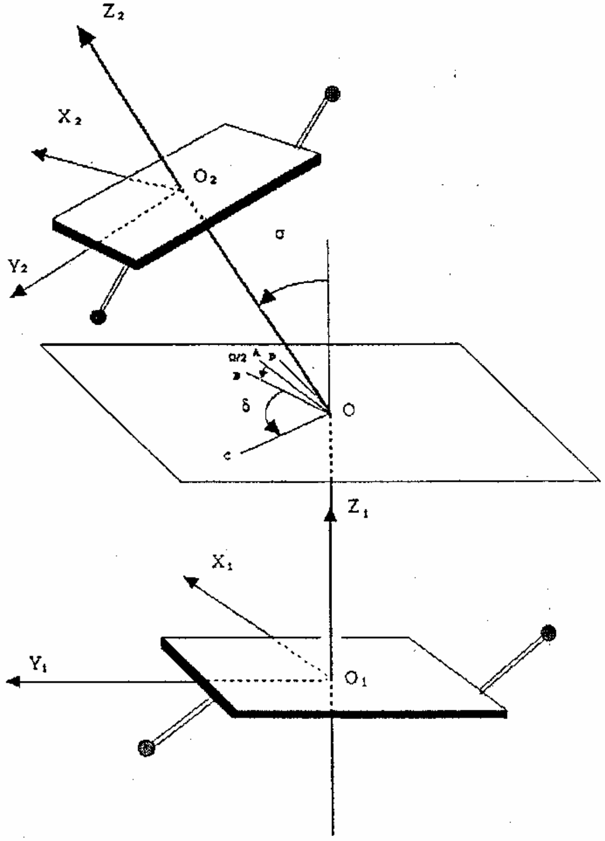


(5'-CTTTTAAAAG-3')$_6$

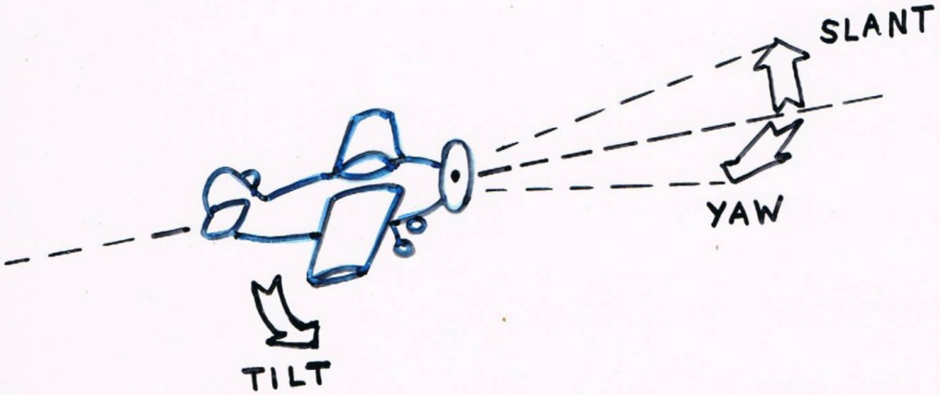


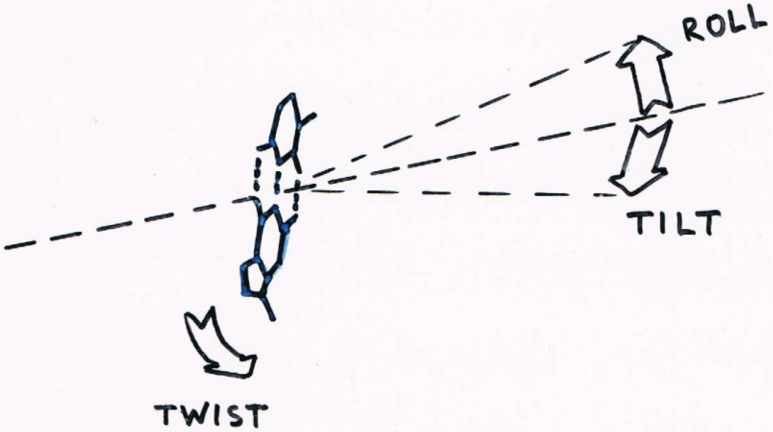



3′
5′
Z
X
Y



Z_2
X_2
O_2
Y_2
σ
Ω/2
A
P
B
δ
O
c
Z_1
X_1
Y_1
O_1


SLANT
YAW
TILT


ROLL
TILT
TWIST

The work described below has been given
to Alex Bolshoy, Ph D student at 1991,
as an excersise.
It turned out to become a whole project.
Only good mathematician could do that.

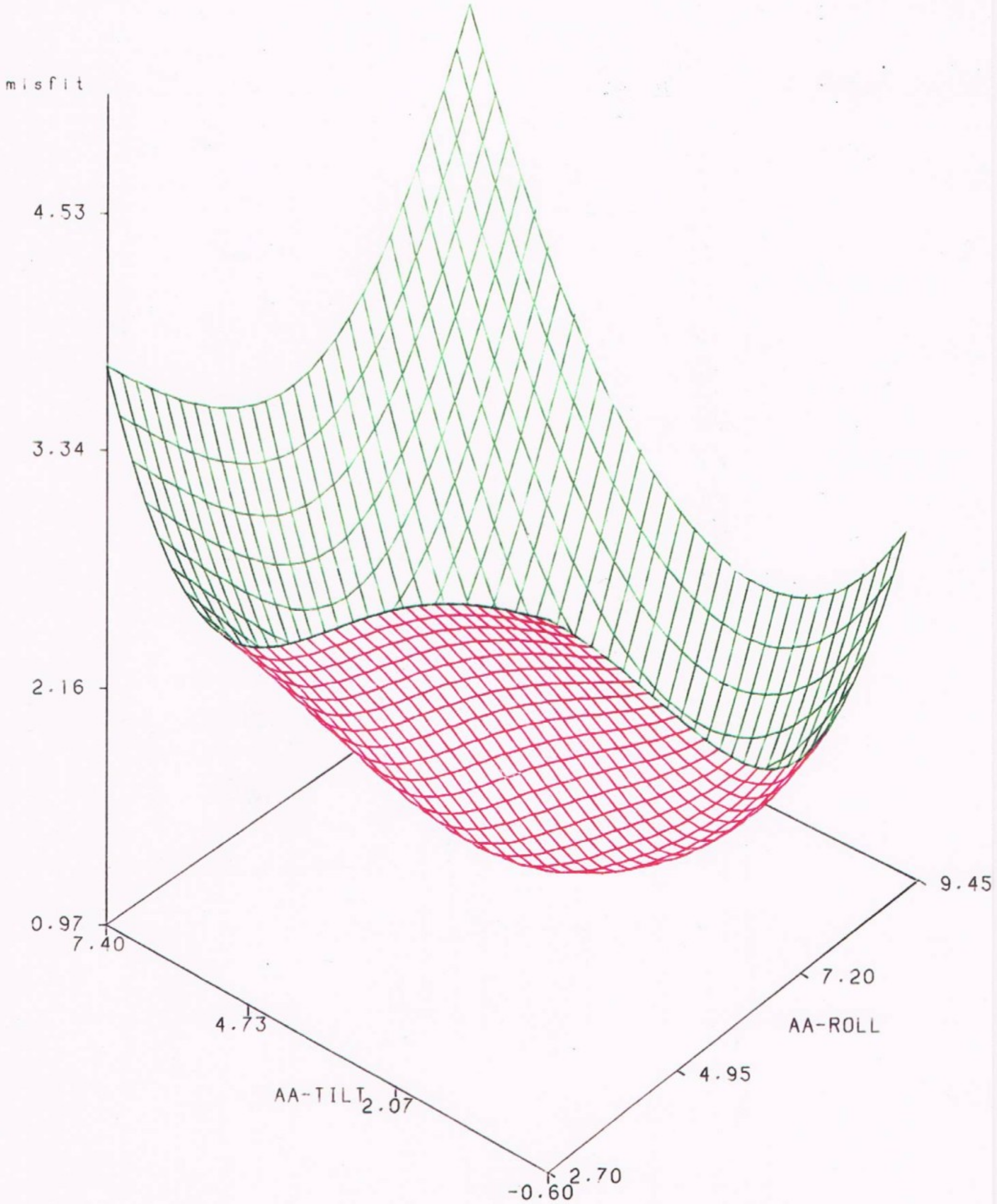
Today both Alex and myself are Professors
in the Institute of Evolution, Haifa.

To ne kazhdyi svladne

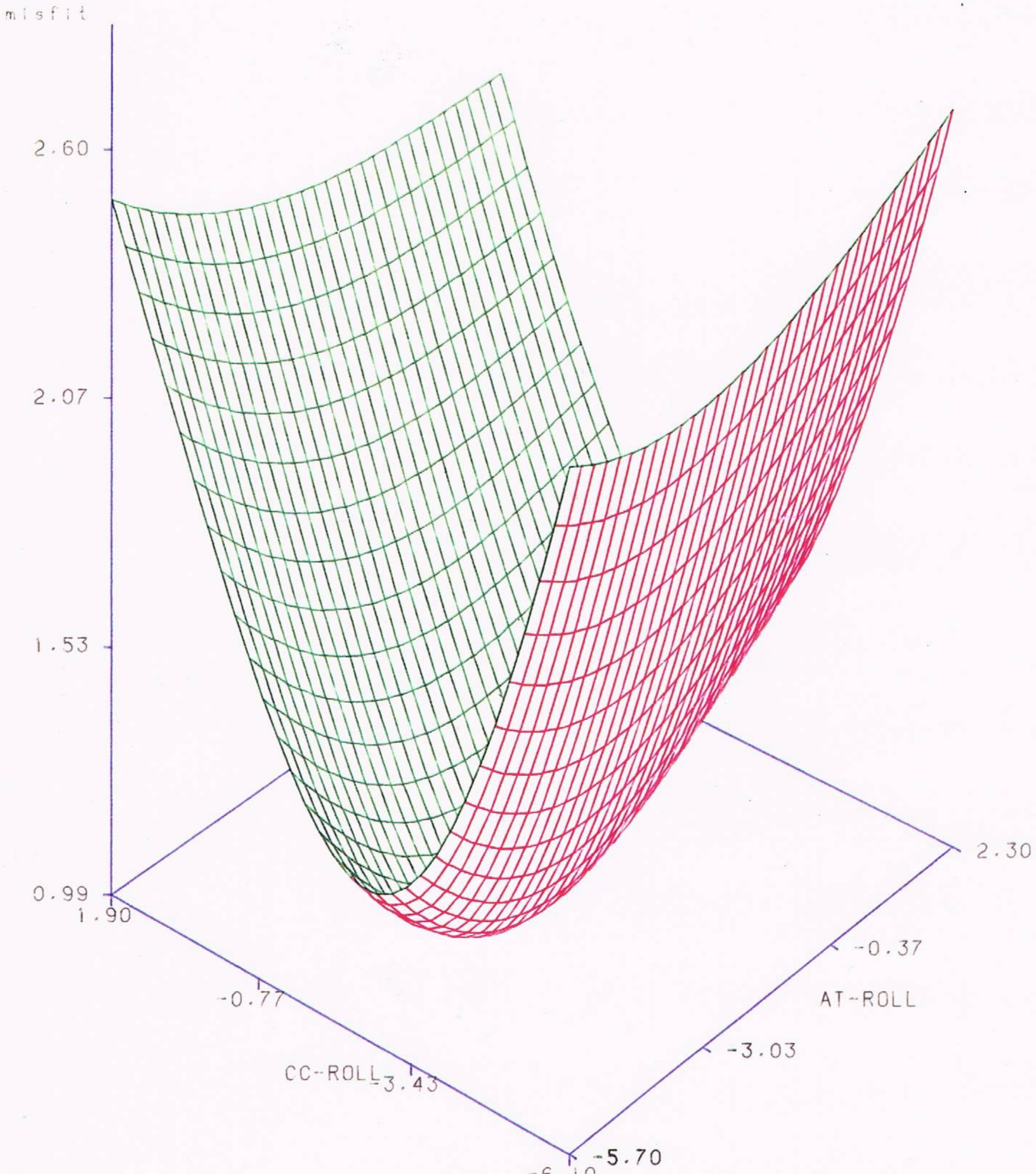
Table 1. Curved and straight synthetic DNA fragments.

	Repeat unit	Curvature (k-factor)		Misfit(std)
	Circles	Experimental curvature	Calculated curvature	
1	TCTCTAAAAAATATATAAAAA	0.59cu (0.06)	0.586	0.0
2	TCAAATTGGGGAAAGATCCC	0.51cu (0.05)	0.405	2.0
3	GGGCAAAAAACGGCAAAAAAC	0.52cu (0.05)	0.604	1.7
	AA- containing and control fragments	Experimental k-factor	Calculated k-factor	
4	CTTTTAAAAG	1.01 (0.03)	1.01	0.0
5	GTTTTAAAAC	1.01 (0.03)	1.01	0.0
6	GGGTCGACCC	1.00 (0.02)	1.03	1.5
7*	GGCAACAACG	1.01 (0.02)	1.08	3.4
8	GGCAAGAACG	1.04 (0.04)	1.05	0.3
9	GGCAATAACG	1.06 (0.04)	1.06	0.0
10	GGCCAAACCG	1.14 (0.06)	1.16	0.3
11	GGGCAAAAAACGGCAAAAAAC	1.43 (0.03)	1.42	0.2
12	GGCTGGCCAAAAAACGGGCAA AAAACGGCAAAAAACGGCTCC	1.26 (0.03)	1.21	1.5
13	GGCTGGGCAAAAAACGGCAAA AAACGGCTCC	1.19 (0.03)	1.21	0.7
14	GGCTGGCCAAAAAACGGCTCC	1.14 (0.03)	1.13	0.3
15	GGCACGGTCGGGCAAAAAACG GCTGGATCCC	1.07 (0.03)	1.02	1.6
16	GGCAGGGCGGTCGACGGGCAA AAAACGCCGTCGGGCGGATCC	1.06 (0.03)	1.05	0.3
17	GGGCAAAAACGCCAAAATTTT GCCGCGGGCC	1.11 (0.03)	1.16	1.5
18	GGGCAAAAACGGGCGGCCAAA ATTTTGCCGC	1.01 (0.02)	1.01	0.0
19	AAAAAAATTTTTTTTTAAA	1.00 (0.02)	1.03	1.5
20	AAAAAAAAAAAAAAAAAAA	0.98 (0.03)	1.01	1.0
21	TGTCCTTCTTGGTTCTCTTGTC	1.00 (0.02)	1.02	0.8
22	CCCCCGGGGG	1.05 (0.06)	1.01	0.7
23	GACAGGACTC	1.01 (0.03)	1.03	0.8
24	CCATCGATGG	0.98 (0.03)	1.02	1.4
25	CGGGATCCCG	1.00 (0.02)	1.02	1.0
26	GCGGGTAGTTTTTTCCTACAC	1.13 (0.02)	1.12	0.5
27	GCGCGATTTTTACGAAAAAAA	1.25 (0.02)	1.25	0.2
28	GGCTGGCCAAAAAACGGCTCC	1.14 (0.02)	1.13	0.4
29	ACCTGGGCAAAAAACGGCTCC	1.14 (0.02)	1.15	0.4
30	GGCTCACCAAAAAACGGCTCC	1.12 (0.02)	1.08	2.0
31	TCACTTATATAAAAAATATAT	1.13 (0.02)	1.14	0.5
32	TCGCTTATATAAAAAATATAT	1.13 (0.02)	1.12	0.3
33	GCCCCTAAAAAGCCCCTTTTA	1.12 (0.02)	1.13	0.4
34	GTGGGACAAAGTGCCCACAAA	1.06 (0.02)	1.06	0.1
35	CTGTGAAAAAACACACTTTTT	1.13 (0.02)	1.15	1.1
36	AAAAACACACAAAAAACACAC	1.29 (0.02)	1.30	0.4
37	TTTTAAAAAC	0.99 (0.04)	1.04	1.2
38	GGCCTTTTTAAAAACCGGGCC	1.03 (0.03)	1.02	0.2
39	GGCCTTTTTAAAAAAACCGCC	1.07 (0.03)	1.09	0.6
40	GGCCTTTTTAAAAAAAAACCG	1.15 (0.03)	1.12	0.9
41	GGCCTTTTTTTAAAAAAACCC	1.21 (0.03)	1.22	0.2
42	CGGAGCCGTTTTTTGCCCAGC	1.15 (0.03)	1.13	0.6
43	CCGGCCAAAAAAAACGCGGC	1.09 (0.03)	1.04	1.6
44	CCGGCCAAAAAAAAAACGCG	1.04 (0.03)	1.01	1.0
45	CCGGCCAAAAAAAAAAAACGC	1.01 (0.03)	1.02	0.3
46	GCCCCAAAAAAAAAAAAAACG	1.05 (0.03)	1.06	0.4
47	GCC[illegible]AAAAAAAAAAAAAAG	1.07 (0.03)	1.08	0.4
	non-AA fragments			
48	CATGTCACCGACGCATCACCG	1.07 (0.02)	1.02	2.3
49	TCCCCAGACCTCCCCACCACG	1.02 (0.02)	1.01	0.3
50	GCGAGACGGTACCGACGTCTC	1.10 (0.02)	1.06	2.0
51	TGTGAGAGGGGCATGAGATCA	1.11 (0.02)	1.11	0.2
52	TACGGATCTCGGCATGACTCTC	1.06 (0.02)	1.09	1.6
53	CGGAGCTATCCGGAGCCTATC	1.07 (0.02)	1.07	0.0
54*	GGAGAGCTCACACGACTAGTC	1.03 (0.02)	1.17	6.8

AA ROLL // AA TILT



Misfit Distribution Function near the MIN

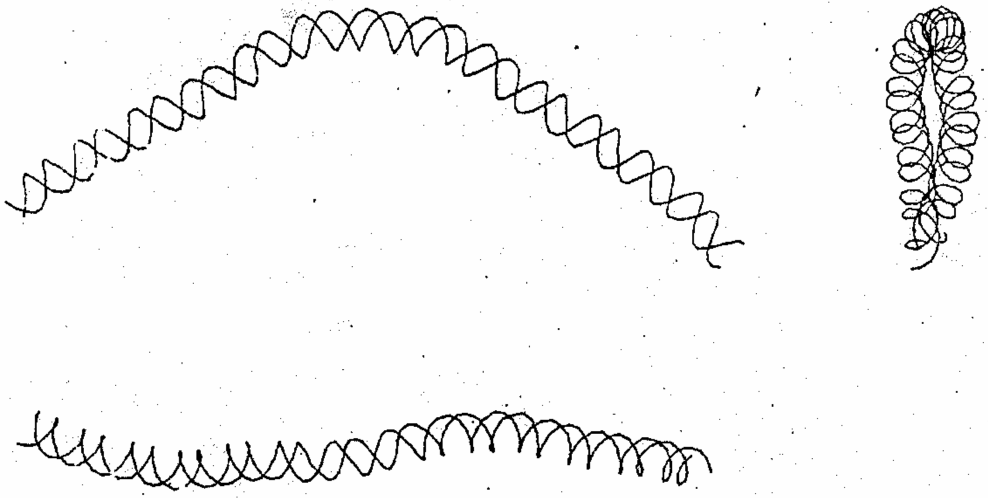


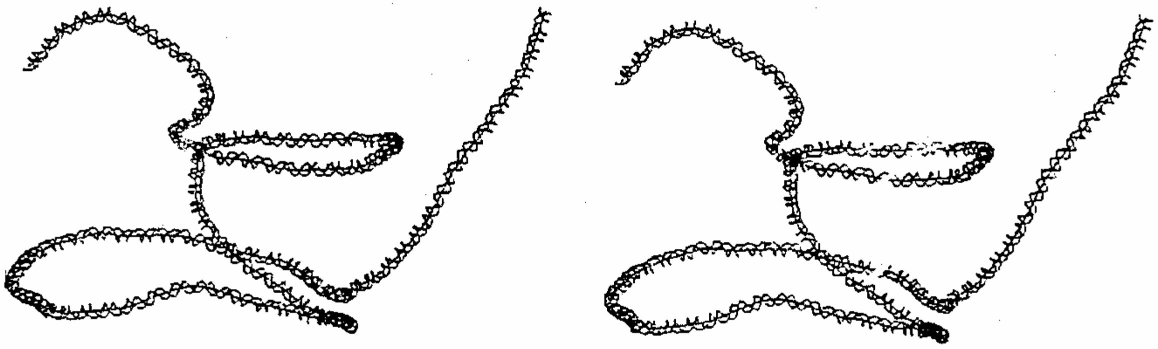
ANGLES DESCRIBING SHAPE OF DNA
(DNA SHAPE CODE)

	Roll	Tilt	Twist
AA	-6.5	3	35.6
AC	(-1)	(-1)	34
AG	8	(0)	28
AT	3		31.5
CA	2	3	34.5
CC	1	2	33.7
CG	7		30
GA	-3	-5	37
GC	-5		40
TA	1		36

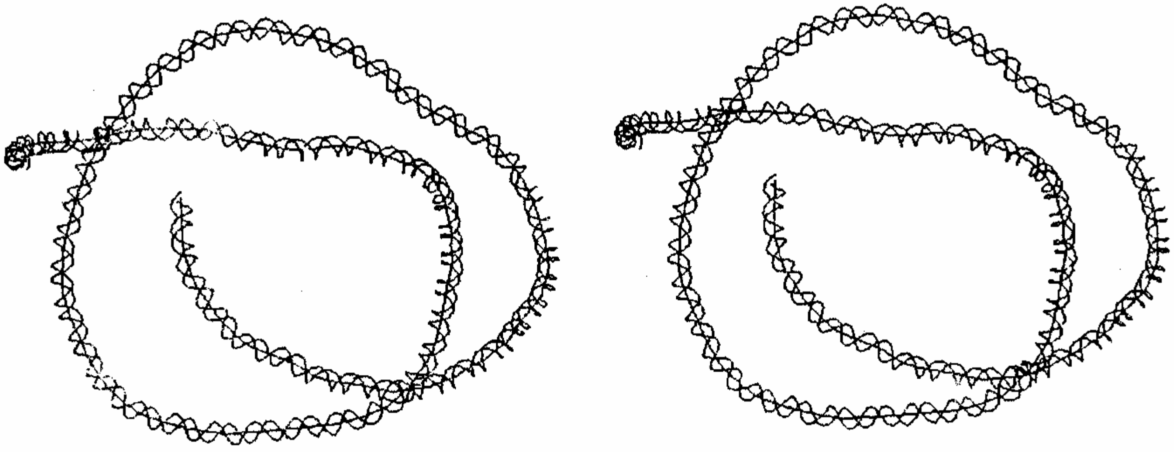
Positive Roll opens towards minor groove
Positive Tilt opens towards phosphates

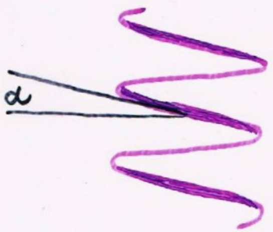
Bolshoy et al., 1991
Kabsch et al., 1982





DNA fragment from chicken chromosome W (stereo pair).
Computed by E. Shpigelman.





CRICK (1976):

$$\text{TWIST} = \underset{\substack{\text{NUMBER}\\ \text{OF TURNS}\\ \text{OF THE}\\ \text{SUPERHELIX}}}{N} \cdot \sin \underset{\substack{\text{ASCENDING}\\ \text{ANGLE}}}{\alpha}$$

THE TWIST RESULTS IN THE CHANGE OF DNA HELICAL REPEAT RELATIVE TO THE WINDING SURFACE

- FOR LEFT-HANDED SUPERHELIX $P < \underset{\substack{\text{HELICAL}\\ \text{REPEAT}\\ \text{OF}\\ \text{NON-CONSTRAINED}\\ \text{DNA}}}{P_0}$

- FOR RIGHT-HANDED SUPERHELIX $P > P_0$

TAKING KNOWN GEOMETRY OF THE NUCLEOSOME SUPERHELIX ONE GETS:

$$\underset{\text{NUCL.}}{P} = \underset{\text{FREE}}{P_0} - 0.15 \text{ BP}$$

$$10.39 = 10.55 - 0.15 \text{ BP} \ (\pm 0.01)$$

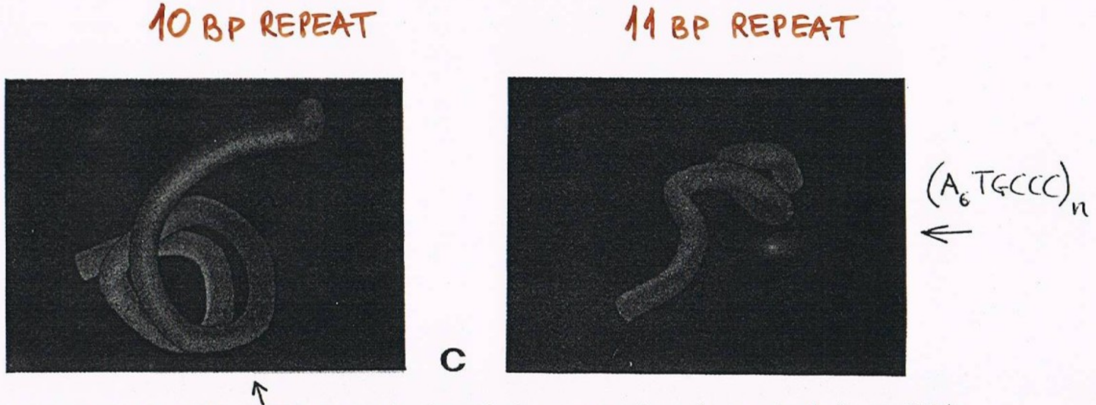
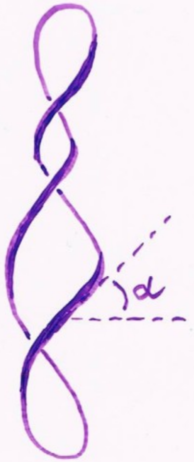


Fig. 2 Stereo micrographs of $[(A)_5TGCCC]_{54}$ DNA molecules and a 3D reconstructions of one molecule. For cryo-EM the DNA molecules are suspended in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH. 8.0)(refs 6,9). The molecules, in a thin vitrified layer of buffer are confined to a thickness of about 50 nm (ref. 9). As the axial length of the superhelices is greater than 50 nm, they adopt an overall orientation approximately parallel to the plane of the thin layer. They are thus seen in almost lateral projections. The large angular difference between stereo partners (+15° and -15° respectively) allows precise 3D reconstruction by a numerical method[7,9,10] but makes it difficult to perceive 3D by direct viewing of the stereopair (*a*). *b*, Some molecules are traced over for clarity. *c*, The 3D reconstruction of the superhelical path of one of the observed $[(A)_5TGCCC]_{54}$ DNA molecules (left). For comparison, a similar reconstruction obtained from $[(A)_6TGCCC]_n$ DNA molecules is presented (right). Scale bar = 100 nm. The DNA plasmid with the insert $[(A)_5TGCCC]_{54}$ was kindly provided by G.J. Brahms and the insert purified as described[8]. To obtain $[(A)_6TGCC]_n$ oligomers 22 bases long (2 times 11 bp), phosphorylated, custom synthesized and HPLC purified oligomers (Med-Probe) were used for thermal annealing and subsequent ligation. For the ligation 400 U of T4 DNA ligase (Biolabs), was used to ligate 0.5 µg of annealed 22-mers in 10 µl reaction volume, during 16 h at 18 °C.

J. Dubochet
J. Bednar
P. Furrer
A. Z. Stasiak
A. Stasiak
A. A. Bolshoy

NATURALLY SUPERCOILED PROKARYOTIC (EUBACTERIAL) DNA MAKES AN INTERWOUND RIGHTHANDED SUPERHELIX



AN ADDITIONAL TWIST IS INTRODUCED

$T = N \sin\alpha \cdot 360°$

DNA IN THE NUCLEOSOME ($\alpha < 0$): 10.39 BP/TURN

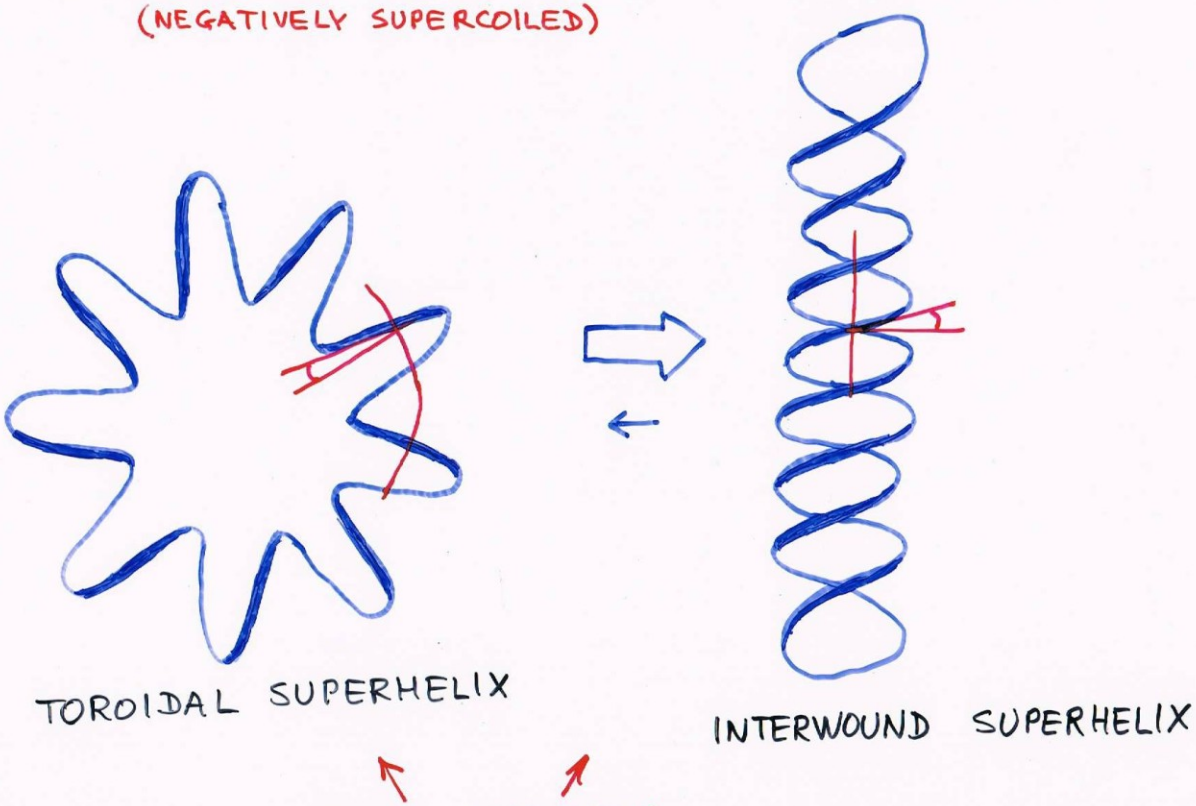
FREE DNA ($\alpha = 0$): 10.54 BP/TURN

EUBACTERIAL SUPERCOILED DNA ($\alpha > 0$): ~ 11.0 BP/TURN

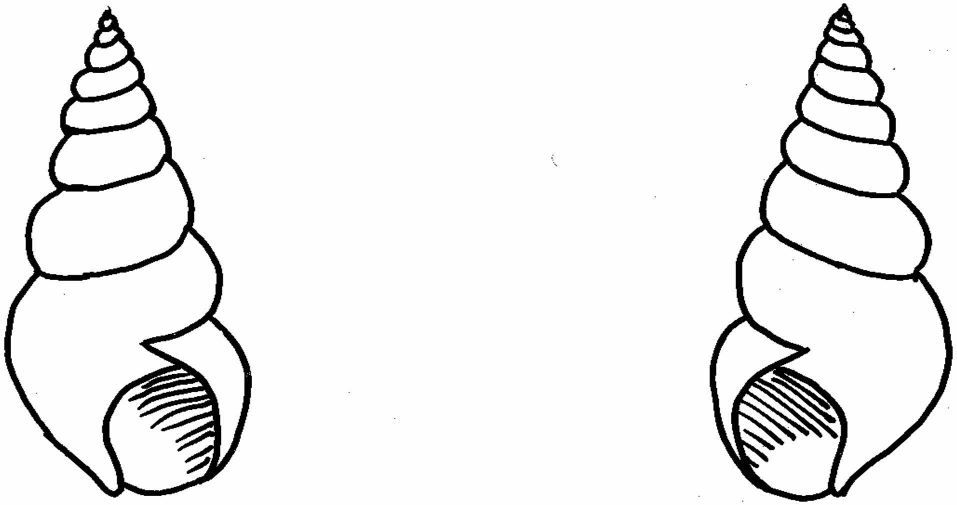
ARCHEBACTERIAL –"– ($\alpha < 0$): ~ 10.0 BP/TURN

TOPOLOGICALLY EQUIVALENT SUPERHELICAL STRUCTURES

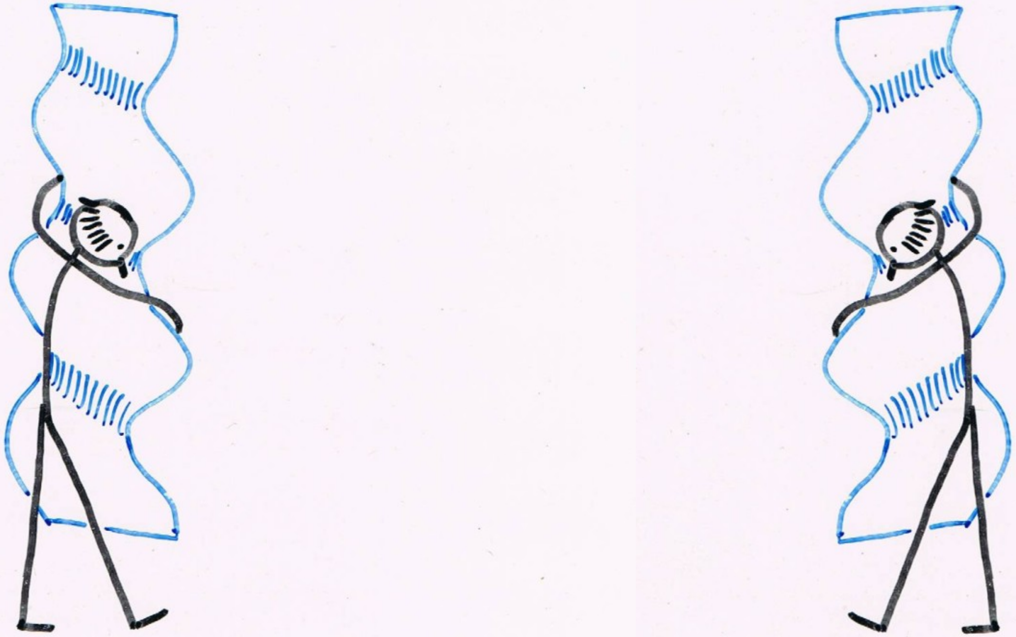
(NEGATIVELY SUPERCOILED)



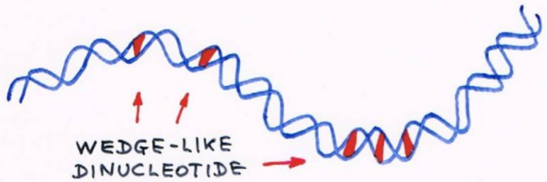
THESE HELICES ARE OF OPPOSITE HANDEDNESS
(AND YET EQUIVALENT !)



1 III



DNA SHAPE CODE



WEDGE-LIKE DINUCLEOTIDE STEPS

A. Bolshoy
I. Grosse
R. Harrington
H. Herzel
W. Kabsch
P. McNamara
C. Sander
J. Sussman
E. Trifonov
L. Ulanovsky
O. Weiss

	TWIST°	ROLL°	TILT°
AA•TT	35.7	−6.5 •	3.2
AC•GT	34.4	−0.9	−0.7
AG•CT	27.9 •	8.4 •	−0.3
AT•AT	31.2	2.6	
CA•TG	34.5	1.6	3.1
CC•GG	33.7	1.2	1.8
CG•CG	29.8	6.7 •	
GA•TC	36.9	−2.7	−4.6
GC•GC	40.1 •	−5.0	
TA•TA	36.0	0.9	

CURVATURE:

xxAGxxxxxxxxAGxxxxxxxxAGxx
xxxxxxxAAxxxxxxxxAAAxxxxxx
xxAGxxxAAAxxCGxxxGCxxxAGxx

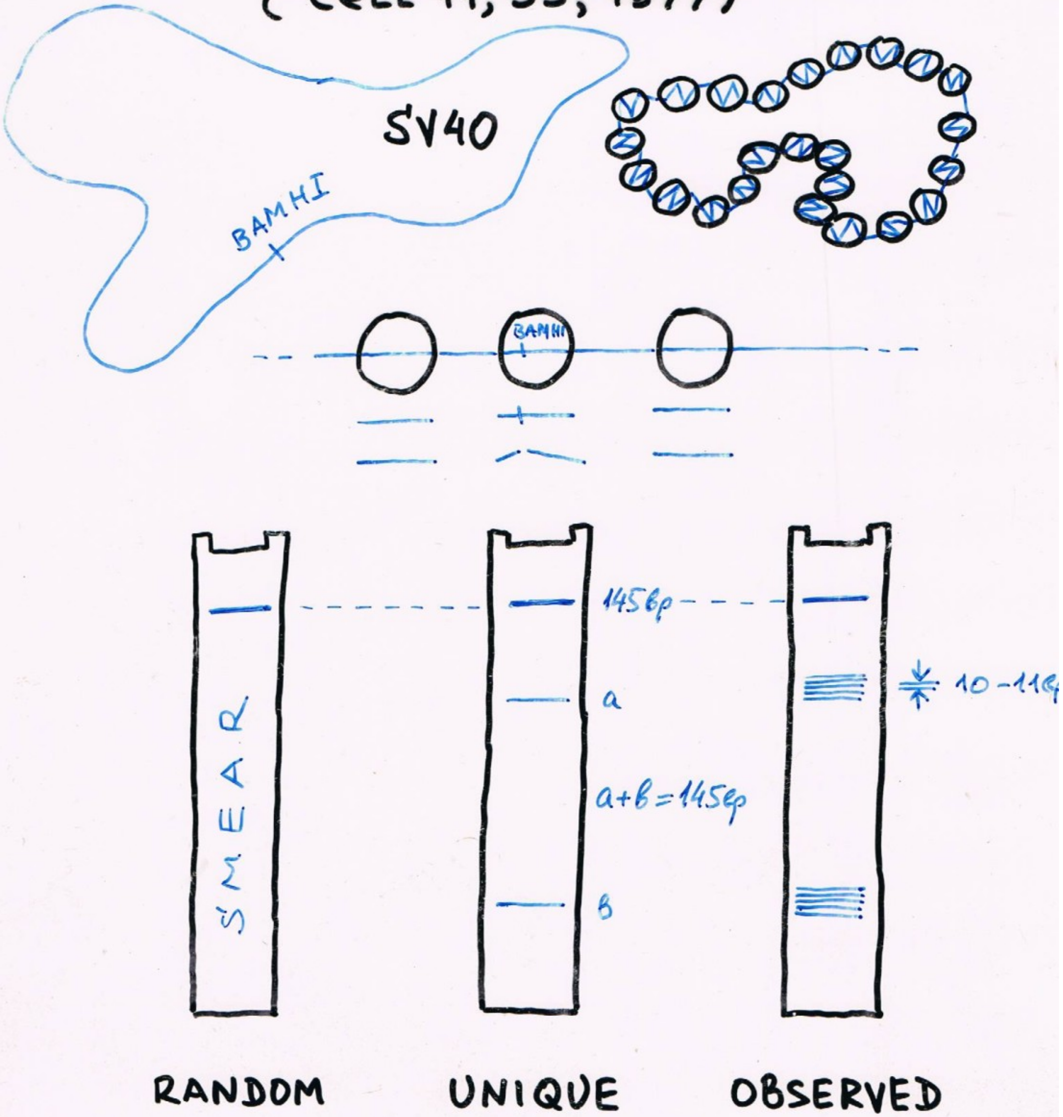
10.55 BASES

WRITHE:

SAME,
BUT DIFFERENT PERIOD
(11.2 BASES IN BACTERIA)

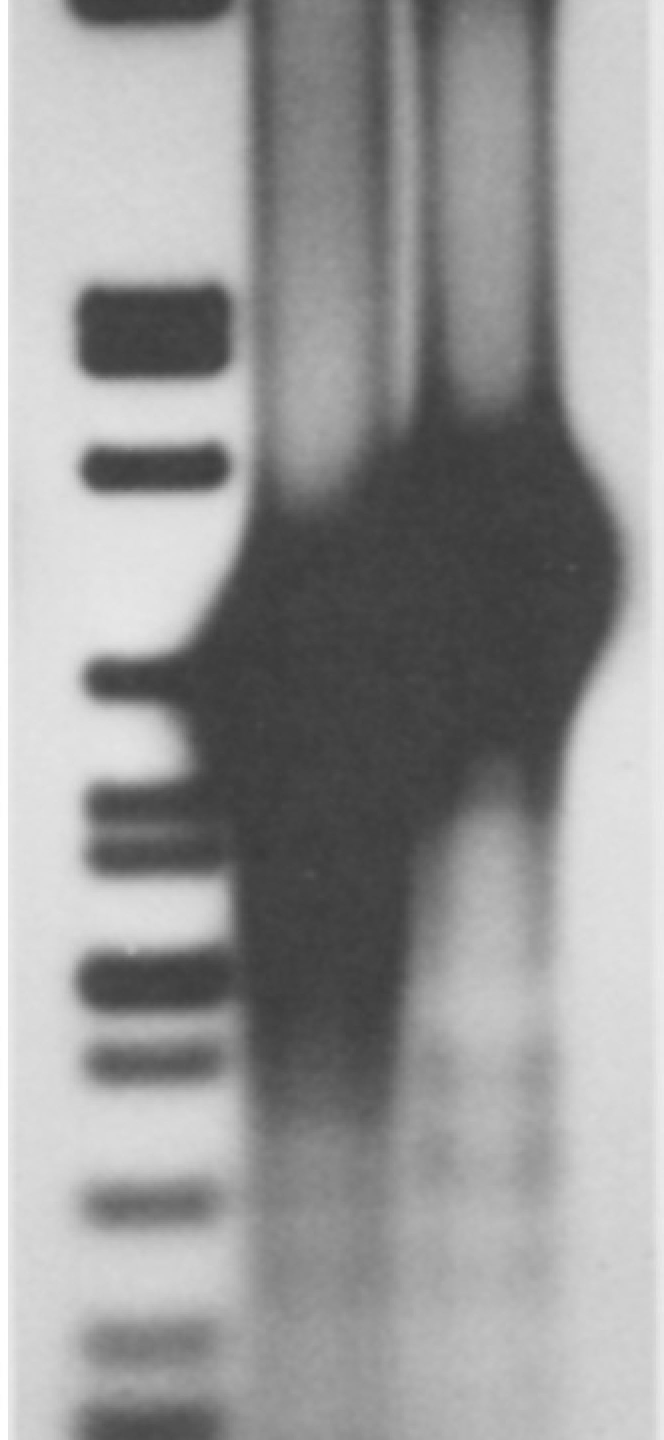
CHROMATIN CODE

EXPERIMENT OF B. PONDER AND L. CRAWFORD
(CELL 11, 35, 1977)



Digestion of BamHI nucleosome of SV40 by BamHI

Ponder BAJ, Crawford LV,
Cell 11, 35-49, 1977



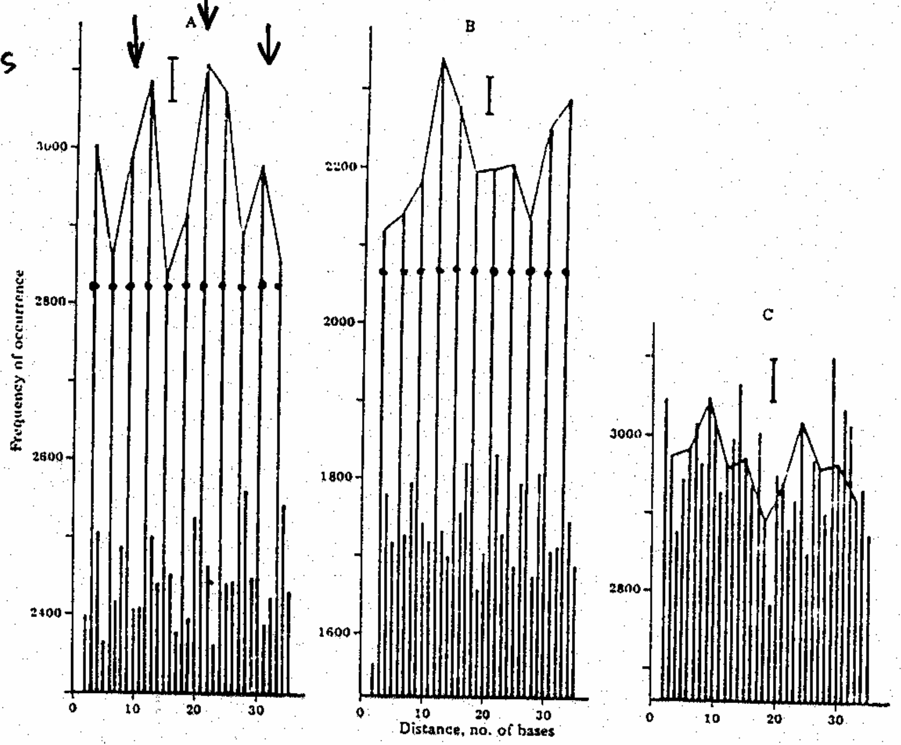
TRIFONOV, SUSSMAN, 1980

3818 Biochemistry: Trifonov and Sussman

Proc. Natl. Acad. Sci. USA 77 (1980)

~ 10.5 BASES

3 BASES



EUKARYOTES

PROKARYOTES

RANDOM

~30 000 BASES

Whole-genome periodicities (distance analysis)

	AA	TT	CG	GC	CA	TG	AG	CT	AT	GG	CC	GA	TC	AC	GT	TA
S. cerevisiae	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
C. elegans	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-
A. thaliana	+	+	-	+	+	+	-	-	+	+	-	-	-	-	-	-
D. rerio	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-
C. albicans	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
A. mellifera	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
D. melanogaster	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
A. gambiae	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. reinhardtii	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G. gallus	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
D. discoideum	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
H. sapiens	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
M. musculus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

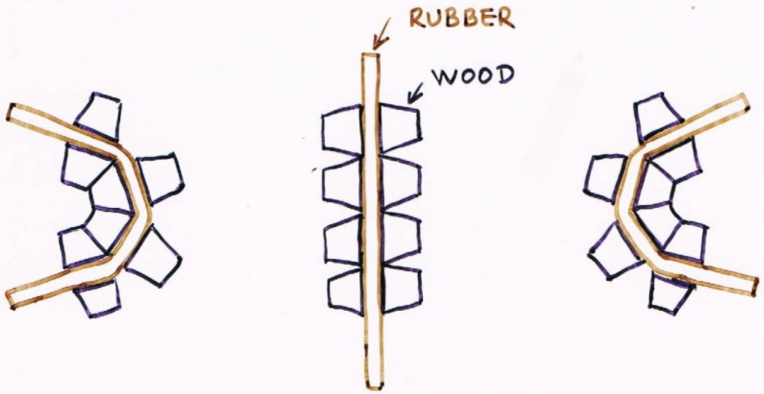
T.Bettecken, E.N.T., 2009

Although DNA curvature and DNA bending
are both reflected in the sequence
as 10-11 base periodicity of the dinucleotides,

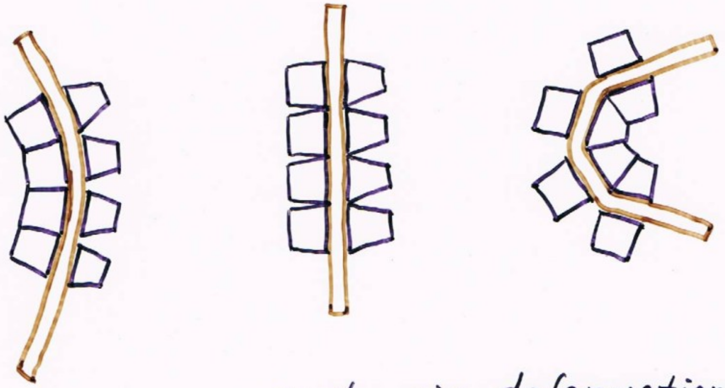
these are two different phenomena

and the corresponding sequence patterns are different

DEFORMATIONAL ANISOTROPY (IN 2D)

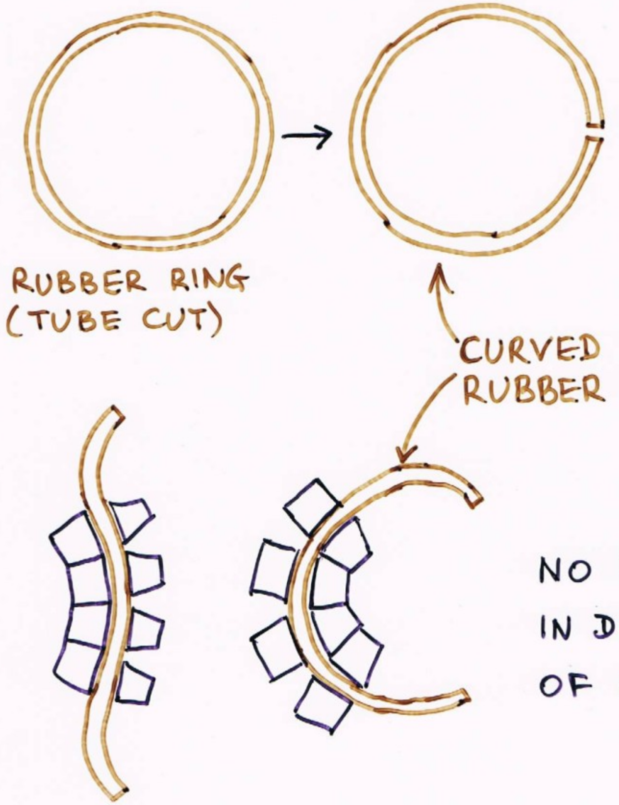


isotropic deformation



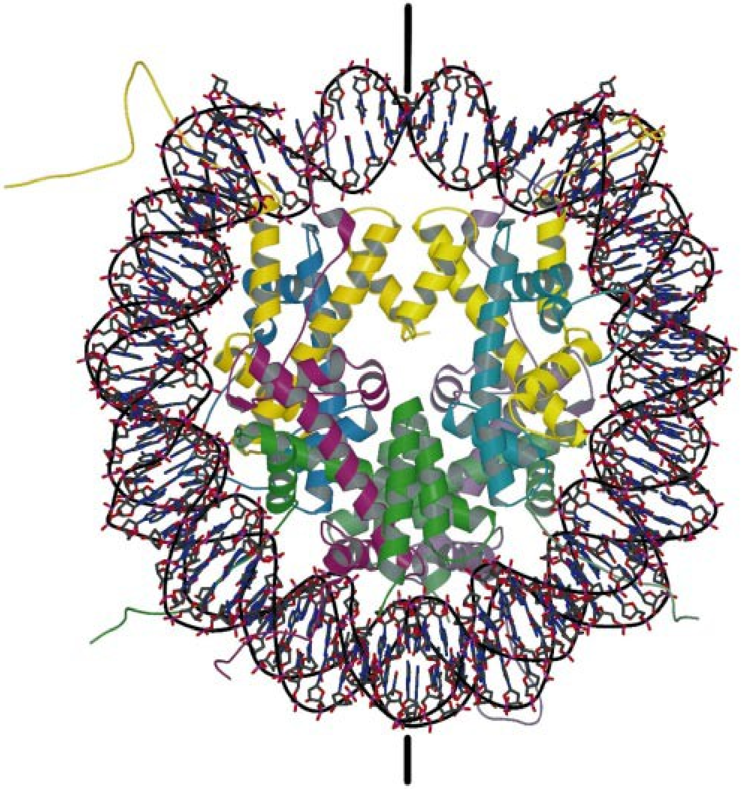
anisotropic deformation

DIRECTION OF BETTER BENDING
AND DIRECTION OF INTRINSIC CURVATURE
ARE NOT NECESSARILY THE SAME

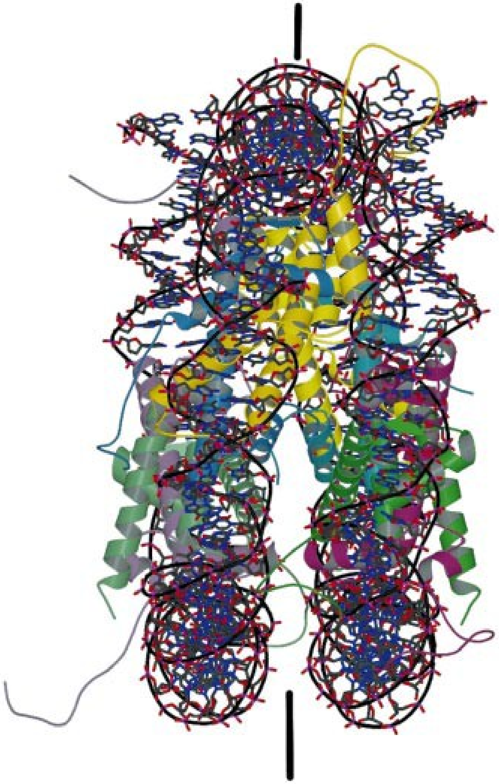


NO BENDING
IN DIRECTION
OF CURVATURE

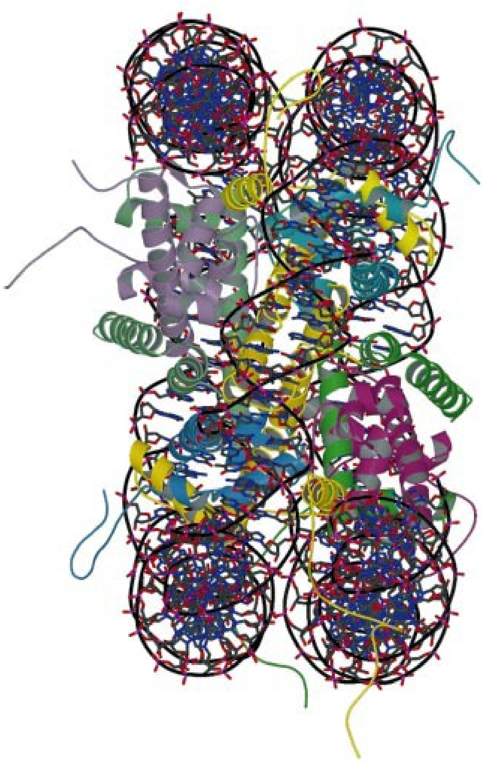
BETTER BENDING
AGAINST CURVATURE



Ventral

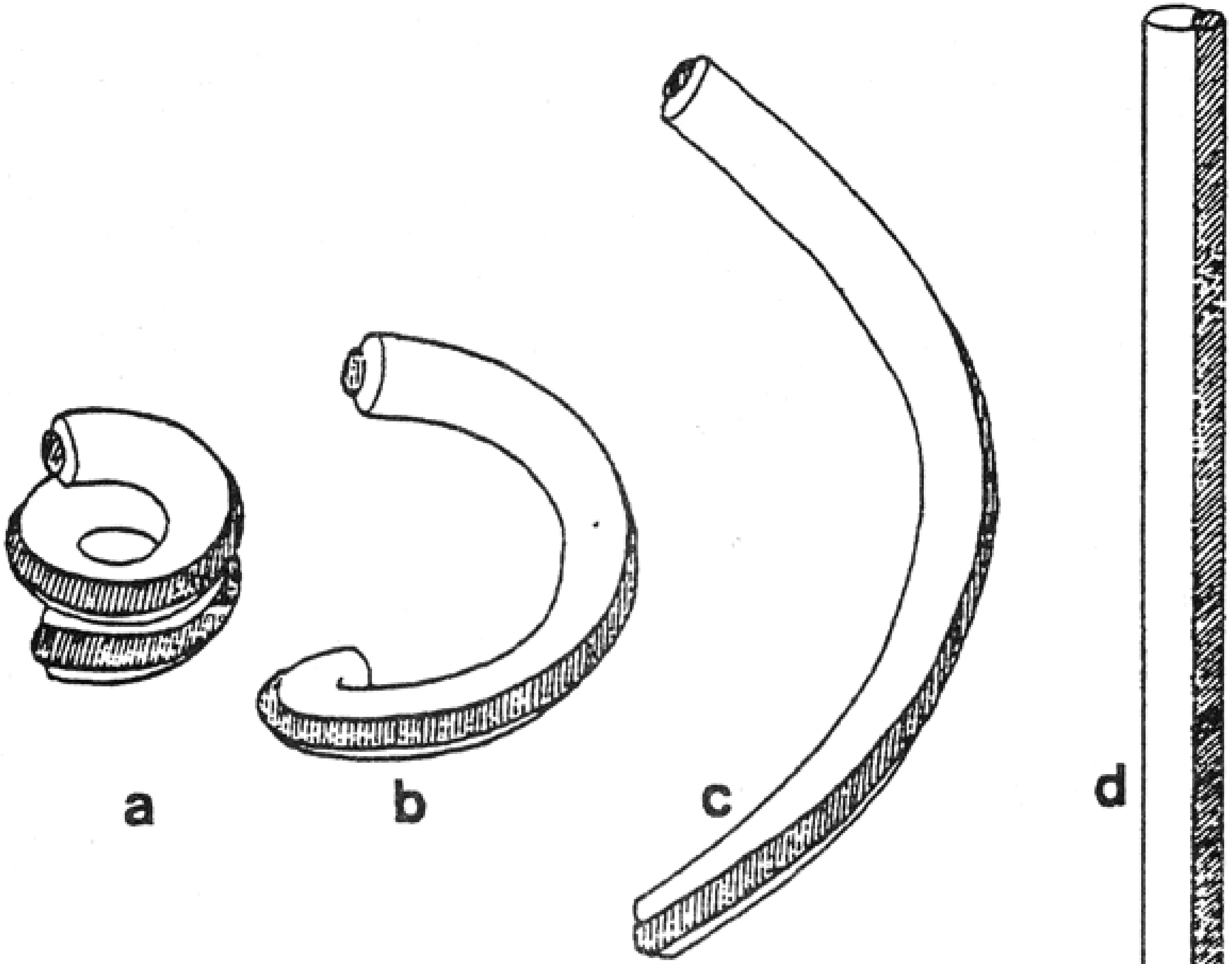


Side



Dyad

Lab of G. Bunick, 2000


a
b
c
d

Structural and sequence periodicity of nucleosome DNA

DNase I digestion of chromatin	10.30-10.40 bp	Prunell, Kornberg, Lutter, Klug, Levitt, Crick, **1979**
Beat effect, DNase I	10.33-10.40 bp	Bettecken, **1979**
Analytical geometry of nucl. DNA	10.30-10.50 bp	Ulanovsky, **1983**
DNA path in nucleosome crystals	10.36-10.44 bp	Cohanim, **2006**
DNase I digestion of chromatin	10.36-10.44 bp	Duke University, **2013**

Common range 10.36-10.40 bp

Although the DNAse I makes cuts in the nucleosome DNA
every 10.3 to 10.4 bases,
at the local dyads 1 and 4 periods from the central dyad in both directions
the cutting is less efficient, as if locally inhibited.

If the period would be integer,
the orientations of potential cut sutes on the surface would be identical,
resulting in equal efficiency of cutting.

The non-integer period would cause many different orientations,
of which some could be unfavorable.

The nucleosome DNA structural period is between 10.333 and 10.400

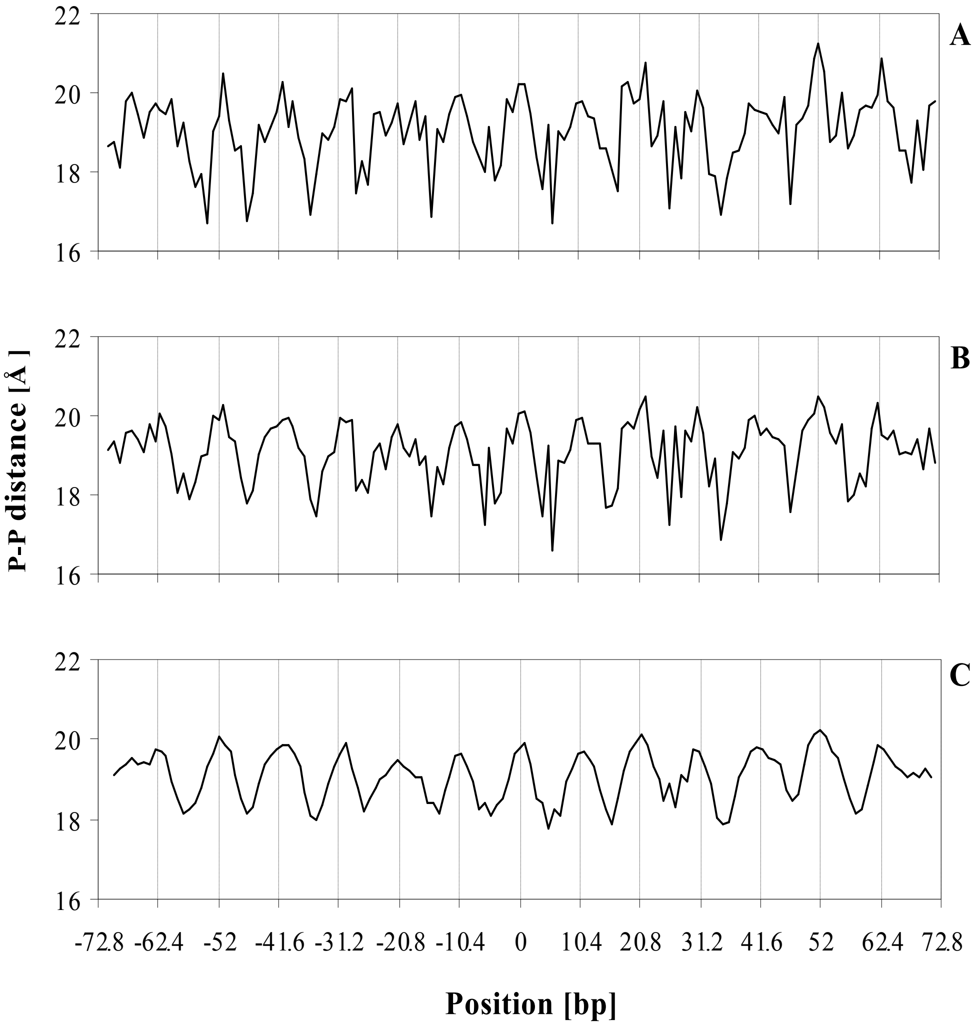
pitch of DNA						local dyads							
(base pairs)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
10.000-10.100	+	+										+	+
10.100-10.125		+	+								+	+	
10.125-10.167			+	+						+	+		
10.167-10.222				+	+				+	+			
10.222-10.273	+				+				+				+
10.273-10.333		+			+				+			+	
10.333-10.400													
10.400-10.444	+					+		+					+
10.444-10.556				+		+		+		+			
10.556-10.600	+					+		+					+
10.600-10.667													
10.667-10.727		+			+				+			+	
10.727-10.778	+				+				+				+
10.778-10.833				+	+				+	+			
10.833-10.875			+	+						+	+		
10.875-10.900		+	+								+	+	
10.900-11.000	+	+										+	+

Noninteger Pitch and Nuclease Sensitivity of Chromatin DNA
Edward N. Trifonov and Thomas Bettecken, Biochemistry, 1979

With the period 10.4 bases, and central position optimal for the cut:

Period No.	-5	**-4**	-3	-2	**-1**	0	**1**	2	3	**4**	5
Bases from Center	52	**41.6**	31.2	20.8	**10.4**	0	**10.4**	20.8	31.2	**41.6**	52
Off from Integer	0	**0.4**	0.2	0.2	**0.4**	0	**0.4**	0.2	0.2	**0.4**	0
	0	**13.6°**	6.8°	6.8°	**13.6°**	0	**13.6°**	6.8°	6.8°	**13.6°**	0

Nucleosome crystal data reveal the 10.4-base structural period of the nucleosome DNA (A. Cohanim et al., 2006)



1KX5

(C. Davey et al., 2002)

1AOI+1KX4

(K. Luger et al. 1997)

+1KX5

Same, smoothed

Nucleosome core -
particle built
of two side-by-side superhelices
(histones and DNA),
1.5 turns each

It contains ~125 bp of DNA
with structural period 10.4 bp

The topologically linear structure
suggests a simple mode
of nucleosome unfolding
during template processes

Prediction (1980):

In the fragments of DNA bent in the nucleosome the sequence should favor periodically positioned like-named elements, 10-11 bases apart.

Since ~70% of DNA is involved in the nucleosomes – any long sequence should also possess the periodicity.

(Since the nucleosomes generally are not phased, the periodicity would span only the nucleosome sequence size)

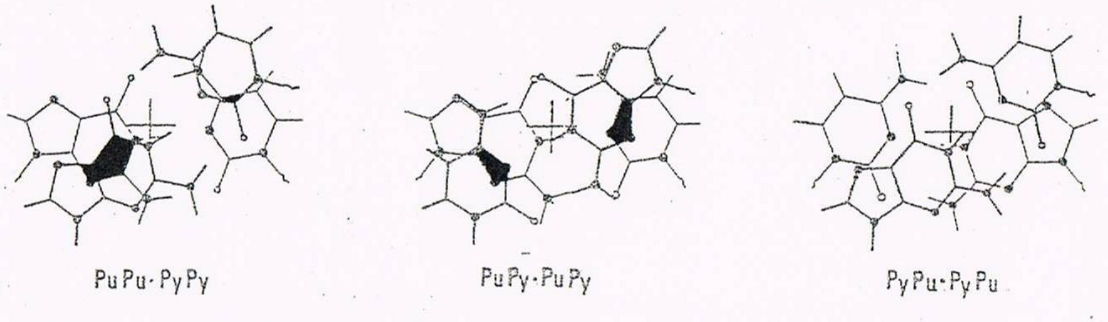
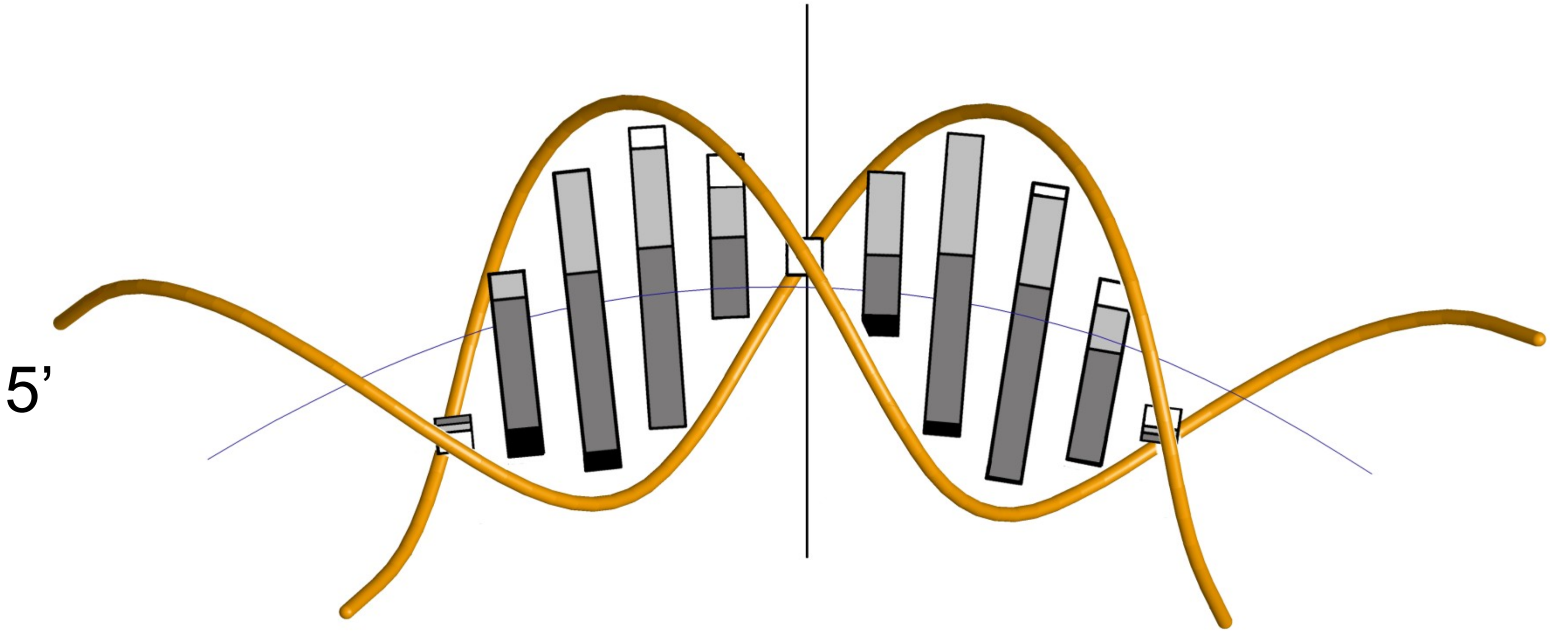


FIGURE 1. Projected views of two successive base-pairs of B DNA. Three possible cases of purine and pyrimidine base overlap are shown. Helix axes (perpendicular to the base-pairs) are indicated by crosses. Overlapping of the heterocyclic rings is shown in black. (From Arnott, S., Dover, S. D., and Wonacott, A. J., *Acta Crystallogr.*, B25, 2192, 1969. With permission.)

E.T.
CRC Crit. Rev. Bioch.
v. 19, 1985

Purine-purine (RR) stacks should be placed closer to the surface of histone octamer,

to minimize cost of deformation



5’…YYYRRRRRRYYYYYYRRR…

Second important prediction:

The deformation (bending) should follow the *dyad symmetry* of DNA molecule.

So should the dinucleotide elements (stacks).

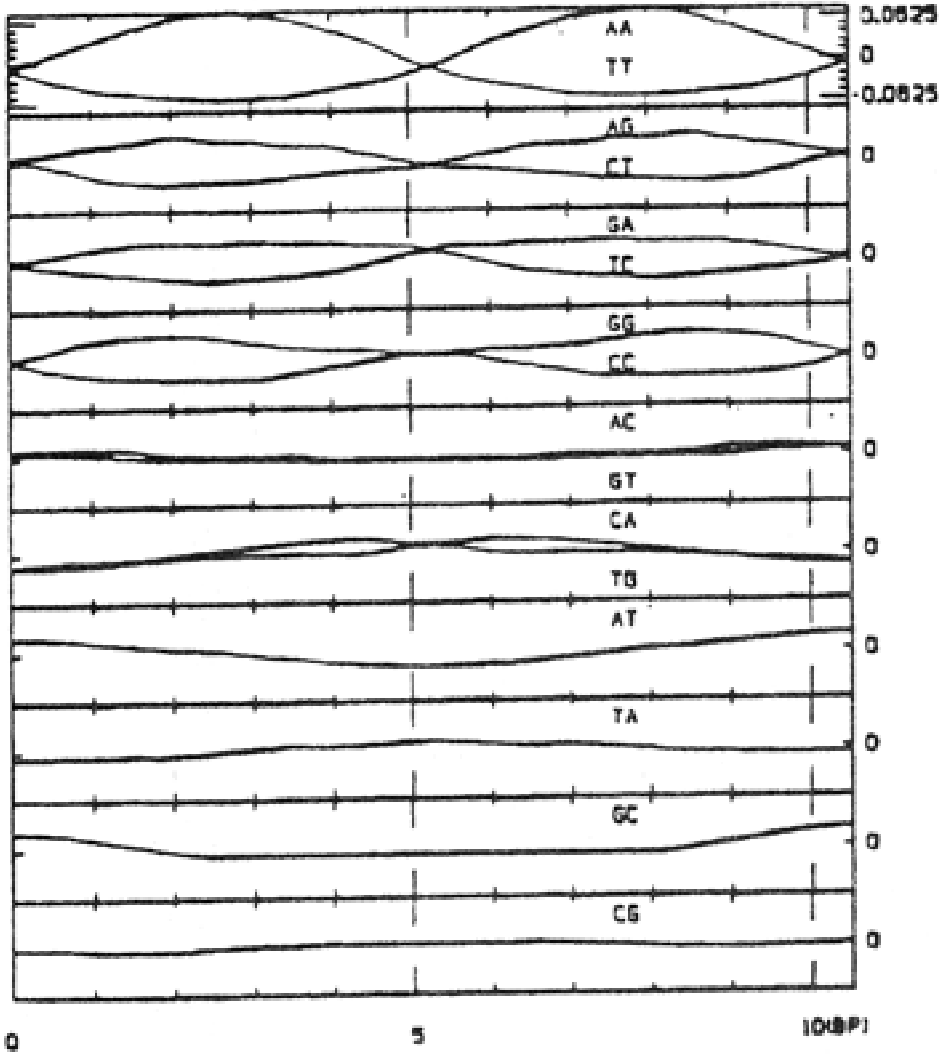
Thus, within the sequence period
AA and **TT** elements should be
on opposite sides from the axes, at the same distance

axis axis axis

↓ ↓ ↓

5'...TTTTAAAAATTTTTAAAA...

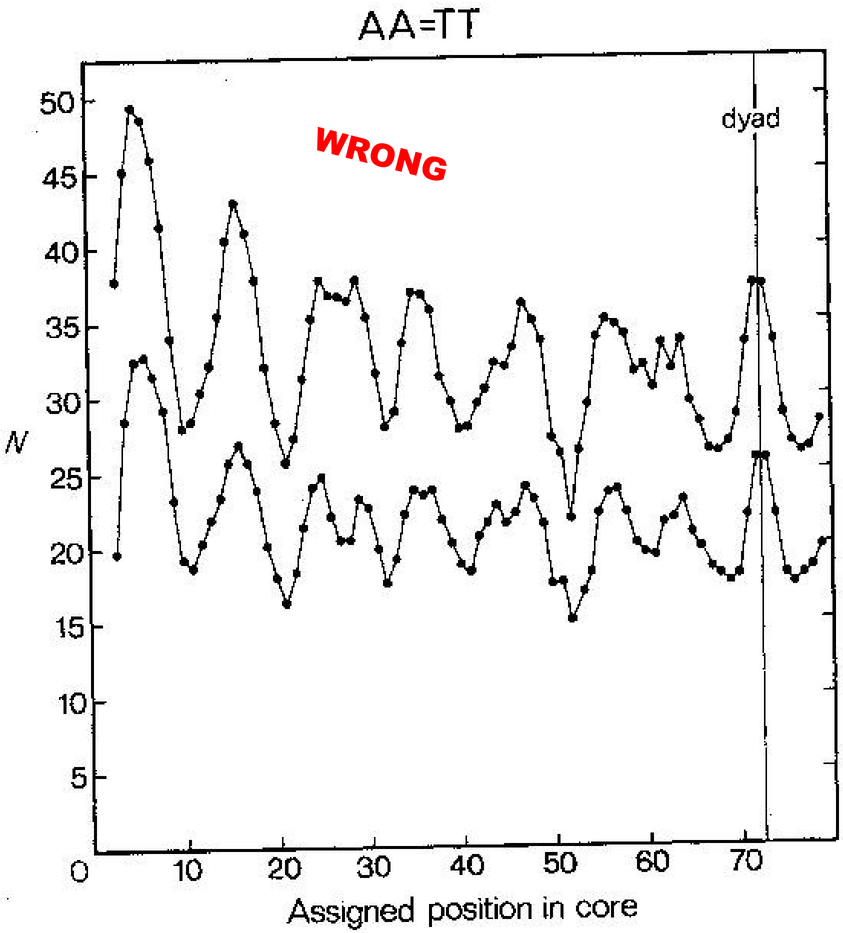
First matrix of nucleosome DNA bendability



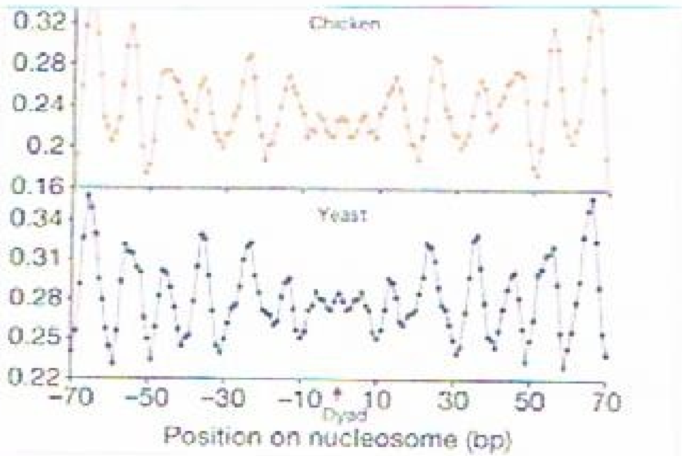
Mengeritsky and ENT, 1983

The *dyad symmetry* of the DNA in the nucleosome
has been mistakenly replaced in 1986 (Cambridge UK)
by *mirror symmetry*.

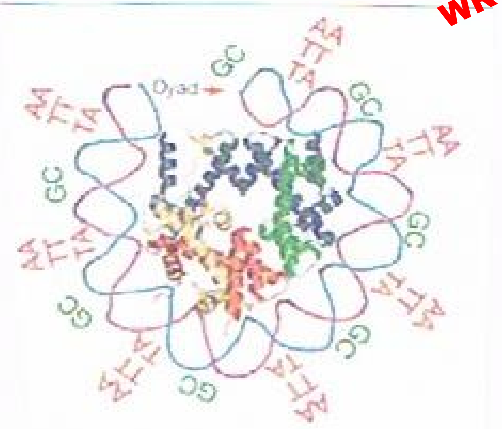
This had catastrophic consequences
for trustful naïve chromatin community (biologists)
(blind to the difference),
causing major confusion worldwide, still in effect



Satchwell SC, Drew H, Travers AA
J Mol Biol 1986



WRONG



Segal,…, Widom, Nature 2006

```
         minor
        groove
          out
            |
            |
n n n A A n n n T T n n n     our team
            |                     1980-1996
            |
A A A n n G G C n n A A A     Satchwell et al.
T T T     G C C     T T T         1986
A A T     A G C     A A T
A T T     G C T     A T T
            |
            |
 A A n n n G C n n n A A      Segal et al.
 T T        |        T T          2006
 T A        |        T A
            |
            |
 Y R R R R R Y Y Y Y Y R      our team
 T A       A T       T A          2009-2013
 C G       G C       C G
```

History of the chromatin code

~10.5 base periodicity of some dinucleotides Trifonov, Sussman (1980)

Pre-genomic studies

...T T A A A A A T T T T T A A A A A T T...	Mengeritsky, Trifonov (1983)
...Y Y R R R R R Y Y Y Y Y R R R R R Y Y...	Mengeritsky, Trifonov (1983)
...x Y R x x x R Y x x x Y R x x x R Y x...	Zhurkin (1983)
...S S S S x W W W W x S S S S x W W W W...	Satchwell *et al.* (1986)
...x S S S x x W W W x x S S S x x W W W...	Shrader, Crothers (1989),Tanaka et al.,(1992)
...C C x x x x x C C C C C x x x x x C C...	Bolshoy (1995)
...V W G x x x x x x x V W G x x x x x x...	Baldi *et al.* (1996)
...x x G G R x x x x x x x G G R x x x x...	Travers, Muyldermans (1996)
...A C G C C T A T A A A C G C C T A T A...	Widlund *et al.* (1997)
...C T A G x x x x x x C T A G x x x x x...	Lowary, Widom (1998)
...S S A A A A A S S S S S A A A A A S S...	Fitzgerald, Anderson (1998)
...C C G G G G G C C C C C G G G G G C C...	Kogan *et al.* (2006)

Genome-scale analyses

...T T A A A A A T T T T T A A A A A T T...	Cohanim et al. (2006)
...Y T A R A A A T T T Y T A R A A A T Y...	Salih et al. (2008)
...Y Y R R R R R Y Y Y Y Y R R R R R Y Y...	Salih et al. (2008)
...S S S S x W W W W x S S S S x W W W W...	Chung, Vingron (2009)

Whole-genome nucleosome databases

...C C G G A A A T T T C C G G A A A T T...	Gabdank et al. (2009)

Physics

...C C G G A A A T T T C C G G A A A T T...	Trifonov (2010)

Methods of sequence analysis used for detection of nucleosome pattern(s)

1. Distance analysis (positional correlation)
2. Iteration with random start
3. Multiple alignment
4. Regeneration of the signal from its parts
5. Shannon N-gram extension

Methods that failed:
Fourier transform
Hidden Markov model
Many more failures not publicized

Nucleosome positioning sequence pattern is very weak

(as the nucleosomes should be easy to unfold)

That is why it took so long to crack the code.

The weak pattern overlaps with other messages (“noise”).

That makes the signal/noise ratio very low.

VERY large

database of the nucleosome DNA sequences is needed,

to extract the signal and describe it in detail

It is easy, however, to detect the signal

Only few properly positioned dinucleotides per nucleosome
are sufficient to claim unique position for the nucleosome

Two good nucleosomes may have completely different sequence.

```
cacgaaagccacgccggaatc
gcgcggcttgtgtgaatccag

ccggaaatttccggaaatttc
```

These two sequences
have not a single common base.
But both are very good for nucleosome

The ideal sequence
to which they both match

Available databases

of natural nucleosome DNA sequences :

S. Satchwell et al., 1986	115	sequences (chicken)
I. Ioshikhes et al., 1996	~200	sequences (mixture)
M. Kato et al., 2003	~1,300	sequences (human)
S. Johnson et al., 2006	163,651	sequences (*C. elegans*)

Mavrich et al., 2008	$\sim 10^5$	sequences (yeast)
Schones et al., 2008	$\sim 10^6$	sequences (H. sapiens)
Mavrich et al., 2008	$\sim 10^6$	sequences (fruit fly)

Micrococcal nuclease (MNase)
is popular nuclease for digestion of chromatin.
It cuts preferentially at ↓WWWW (↓AATT)
sites
at the ends of the nucleosome DNA

All these databases contain nucleosomes with only marginal periodicity which may be detected, but very difficult to reveal details.

The maps derived by MNase digestion are especially inaccurate, providing rather diffuse nucleosome occupancies rather than positions.

Various signal extraction techniques have to be applied

Regeneration of signal from its incomplete versions:

AA

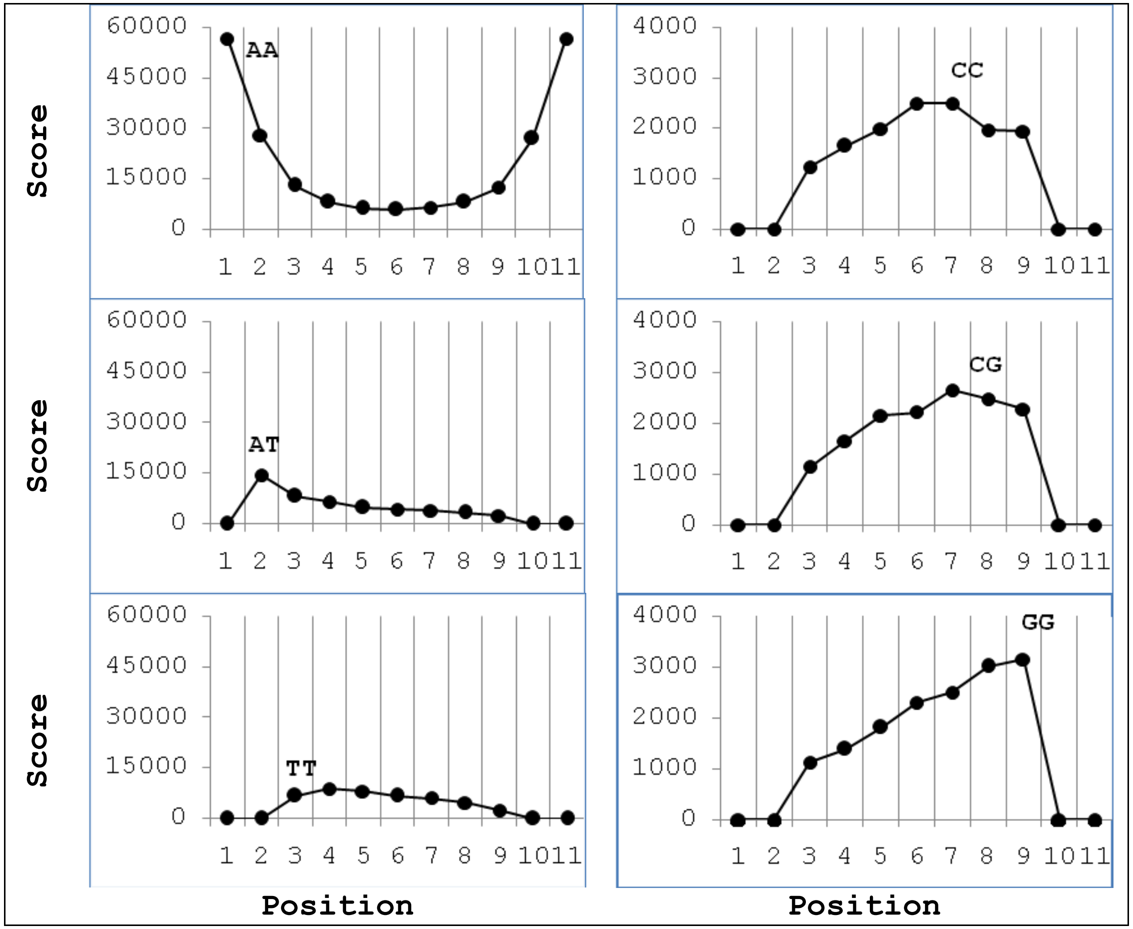
↓ positional autocorrelation

`AAnnnnnnnnAA`

↓ regeneration

`AAnnnCCnnnAA`

AAnnnnnnnAA repeat structure (*C. elegans*)



Regenerated pattern (AAATTTCCGG)(AAAT…
That is, repeating GGAAATTTCC = R5Y5

Several reasons for a given dinucleotide to occupy specific position within the repeat:

1. Physical (deformational) preference.

2. Sequence linkage (inclusion effect). Dinucleotide AB has to have neighbors NA and BN.

3. Exclusion effect. Less committed elements are pushed away from strong positions.

4. Compositional bias. Frequent dinucleotides contribute more to the periodicity.

5. Existence of many different codes overlapping on the same sequence (e. g. triplet code, framing code, splicing code, amphipatic helices)

Positional matrix of bendability

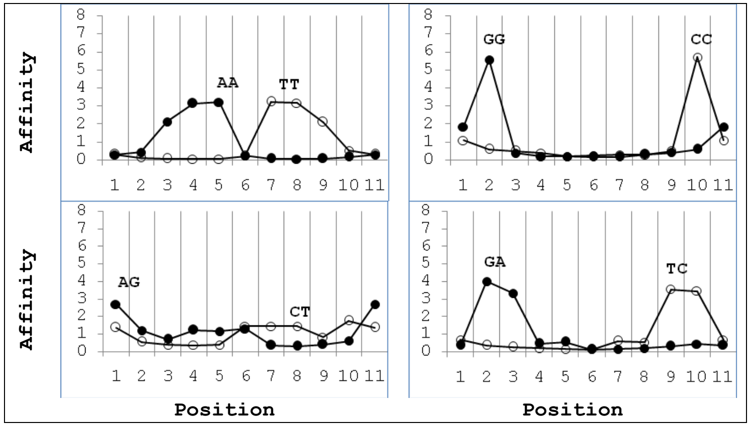
1	2	3	4	5	6	7	8	9	0	1	2
C	G									C	G
	G	G									
	G	A									
		G	A								
		A	A								
			A	A	A						
					A	T					
						T	T	T			
								T	T		
								T	C		
									T	C	
									C	C	
										C	G

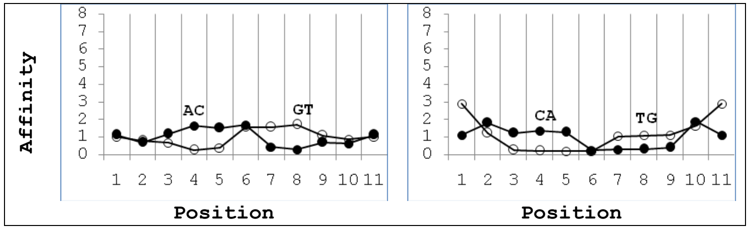
LINEAR FORM OF
THE POSITIONAL MATRIX OF BENDABILITY:

CGRAAATTTYCG

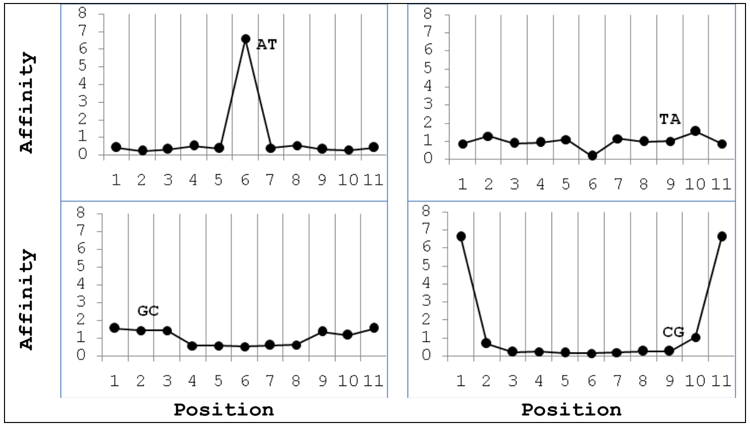
Matrix of bendability

for all 6 chromosomes
of *C. elegans*





Self-complementary elements
AT and CG are separated by
5 bases (half-period) and
positioned at the axes
of complementary symmetry



Shannon N-gram extension

Trinucleotides of C. elegans genome

		counts
1	AAA	4162266
2	TTT	4160750
3	ATT	2488998
4	AAT	2486813
5	GAA	1873844
6	TTC	1871673
7	CAA	1667120
8	TTG	1663842
9	TCA	1498069
10	TGA	1496493
....		....

Shannon N-gram extension

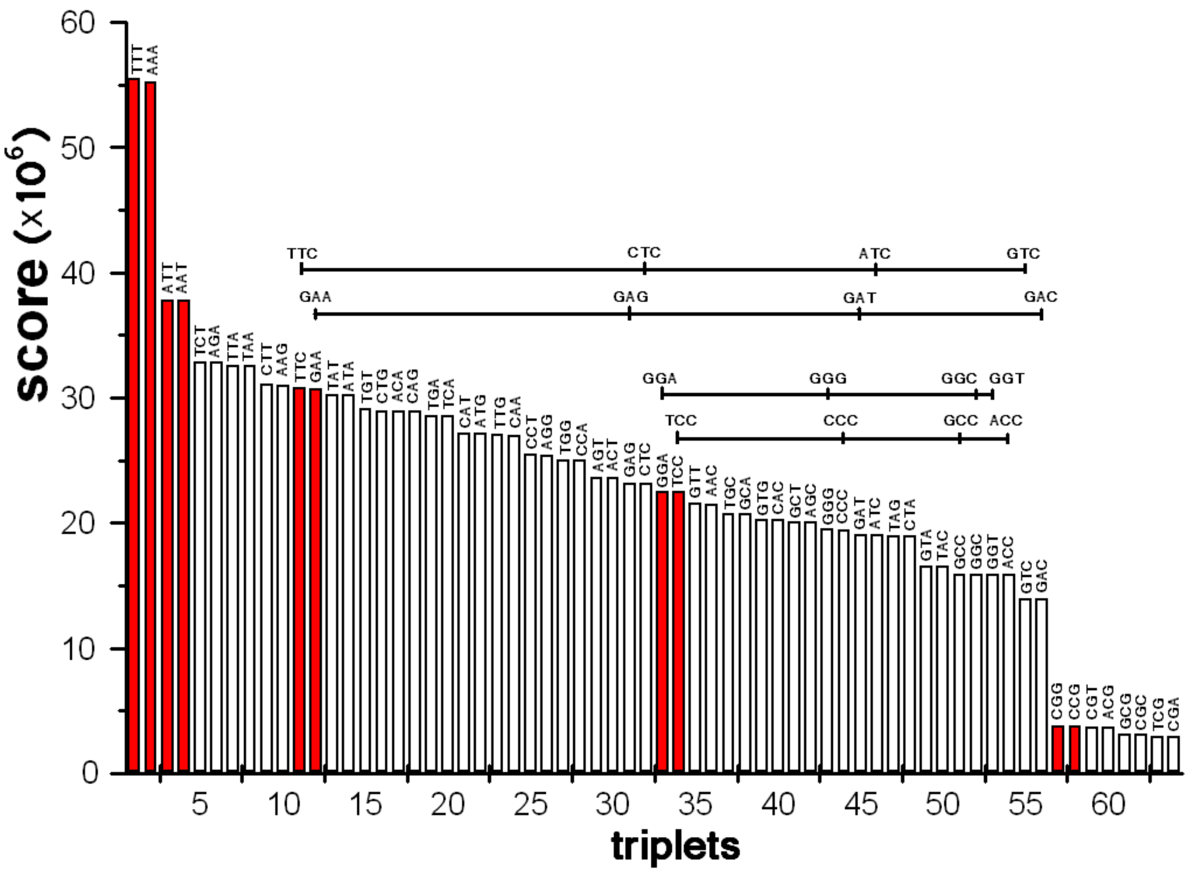
```
                     AAA
                    AAA        A. Rapoport,
                      AAT      Z. Frenkel,
                   GAA ATT     E.N.T., 2010
                  TGA   TTT
                 TTG     TTT
                TTT       TTC
               TTT         TCA
              ATT           CAA
             AAT             AAA
            AAA               AAA
           AAA                 AAT
          GAA                   ATT
         TGA                     TTT
        TTG                       TTT
       TTT                         TTC
      TTT                           TCA
   ...TTTTGAAAATTTTGAAAATTTTCAAAATTTTCA...

   ...AAA... : TTTtgAAAATTTTcaAAA
   ...CGA... : TTTcgAAAATTTTcgAAA
regeneration : TTYCGRAAATTTYCGRAA
```

TOPMOST TRINUCLEOTIDES MAKE TOGETHER THE DOMINANT PATTERN

GAAAATTTTC:

GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC

Trinucleotides of human genome fuse in the sequence CC GGAAA TTTCC GG



extention motifs	species	starting triplets
C AAAAA TTTTT G	A.gamb	TTT
T AAAAA TTTTT A	A.mell	TTT
AAAAA TTTTT	A.thali	AAA
TTTTC AAAAA TTTTT GAAAA	C.albic	AAA
GAAAA TTTTC	C.eleg	AAA
GG CC	C.reinh	GGC
AAAAA TTTTT	D.disc	AAA
C AAAAA TTTTT G	D.melan	AAA
AAAAA TTTTT	D.rerio	AAA
C AGAAA TTTCT G	G.gall	TTT
AAAAA TTTTT	H.sapi	TTT
GAAAA TTTTC	M.musc	TTT
GAAAA TTTTC	S.cerev	AAA

Fig. 3. N-gram Shannon extensions of the most frequent trinucleotides of various genomes, as indicated. Only the central parts of the extensions (underlined) are shown.

extention motifs	species	starting triplets
C AAAAA TTTTC GAAAA TTTTT G	A.gamb	TCG
AAAAA TTTTC GAAAA TTTTT	A.mell	CGA
AAAAA TTTTC GAAAA TTTTT	A.thali	TCG
AAAAA TTTTC GAAAA TTTTT	C.albic	TCG
GAAAA TTTTC GAAAA TTTTC	C.eleg	CGA
AAAAA TTTTC GAAAA TTTTT	D.disc	TCG
GC AAAAA TTTTC GAAAA TTTTT GC	D.melan	TCG
AAAAA TTTCC GGAAA TTTTT	H.sapi	CGG
GAAAA TTTTC GAAAA TTTTC	S.cerev	CGA
GGC GCC	C.reinh	CGC
TTTT AAAAC GTTTT AAAA	D.rerio	ACG
A GAAAC GTTTC T	G.gall	CGT
AC GT	M.musc	CGT

Fig. 4. Extensions of the topmost CG-containing trinucleotides of various genomes, as indicated. Only the central parts of the extensions (underlined) are shown. Four genomes with extensions that do not conform to others, are separated.

Rapoport et al., 2010

CHROMATIN CODE:

C G R A A A T T T Y C G

Y R R R R R Y Y Y Y Y R

as derived by 3 independent methods:

1. From physics of DNA deformation
2. From nucleosome database of C. elegans
3. By Shannon N-gram extension

The hidden chromatin code is described by the motif:

CGRAAATTTYCG

○ ○ ○

An ideal nucleosome DNA in simple sequence form
is periodical repetition of this motif:

CGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCG

...TTTCCGGAAATTTCCGGAAA...

...ATTCGTTCCATTGAAGGCCG...

...CGAACGCTTGGTTAGCGATT...

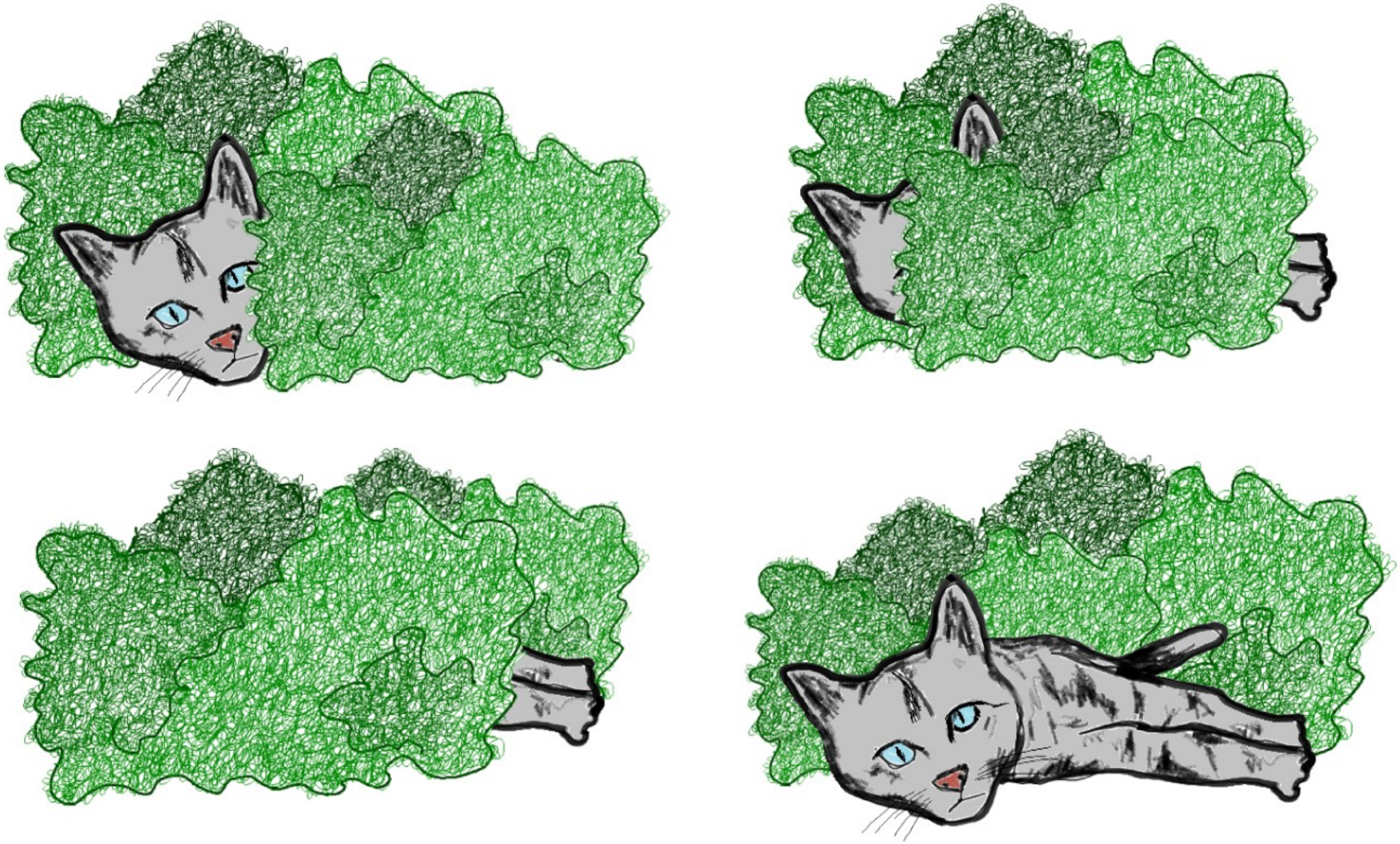
...CCAGAATAAATACAGTCCAA...

...AATCGCCTTTAAAGGGGTTT...

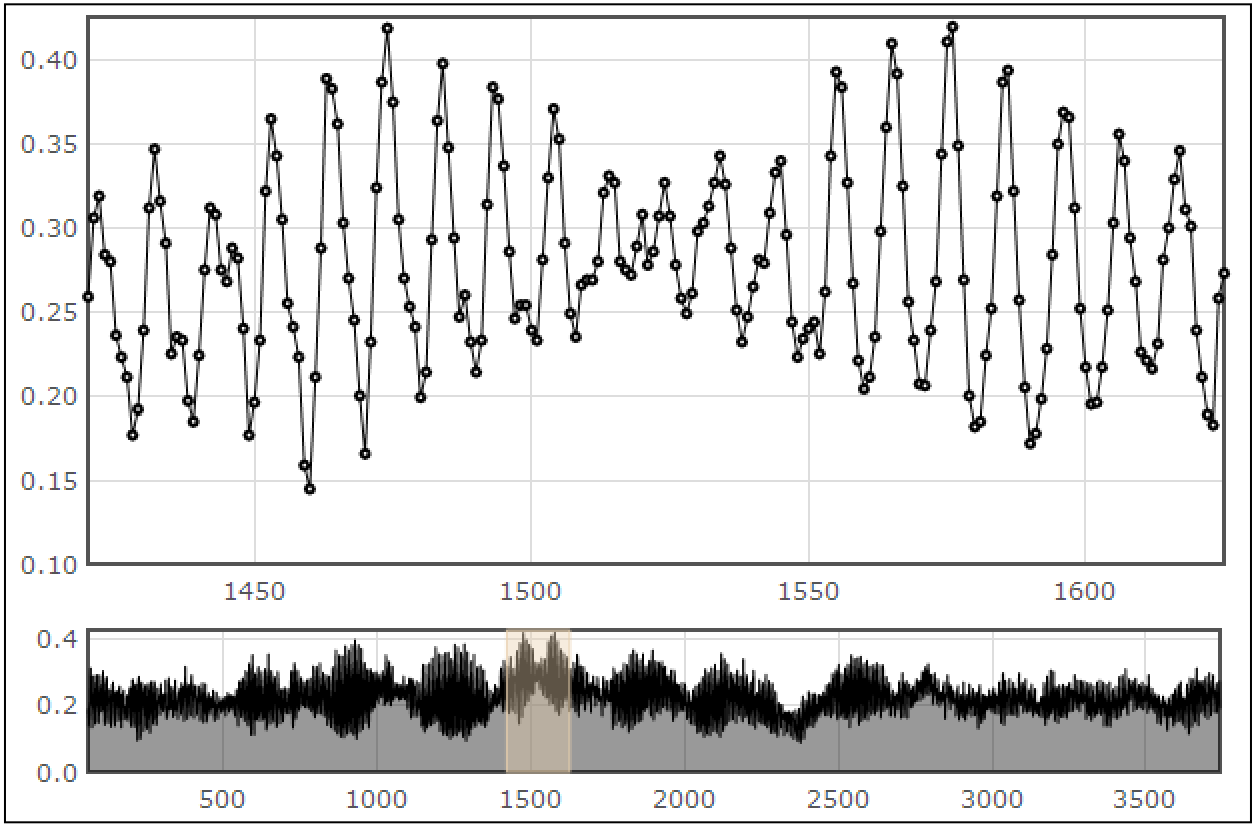
...GAGTTCGACTCCAATCAGGG...

...CGGTACCCTCAGACCCATTC...

...CATCTATTCCAAATTTTCGC...

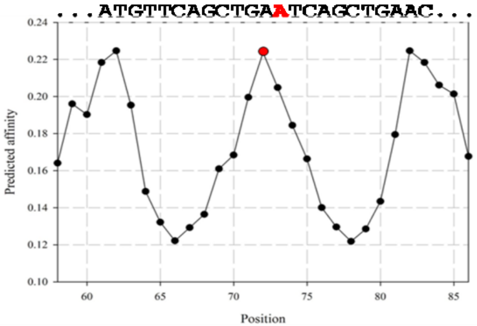


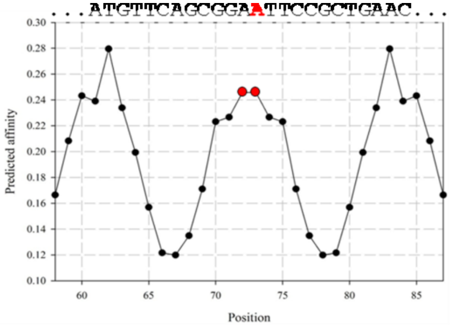
Cat in bushes. Courtesy of I. Gabdank

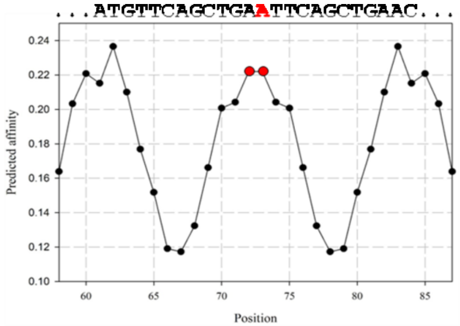


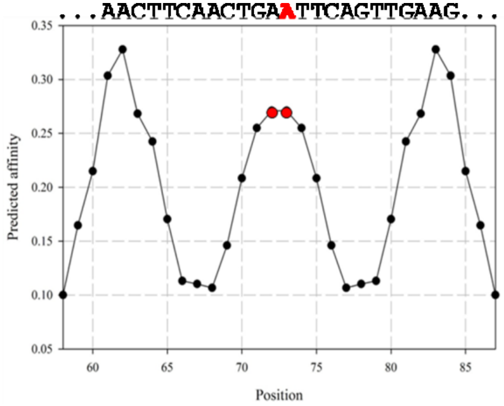
Example of the output from the nucleosome mapping server
http://www.cs.bgu.ac.il/~nucleom

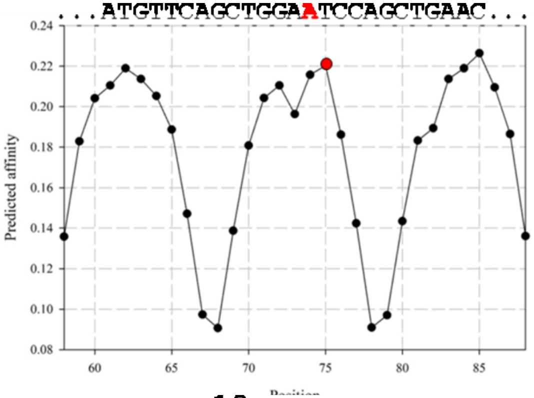
Examples of mapping of sharply positioned nucleosomes

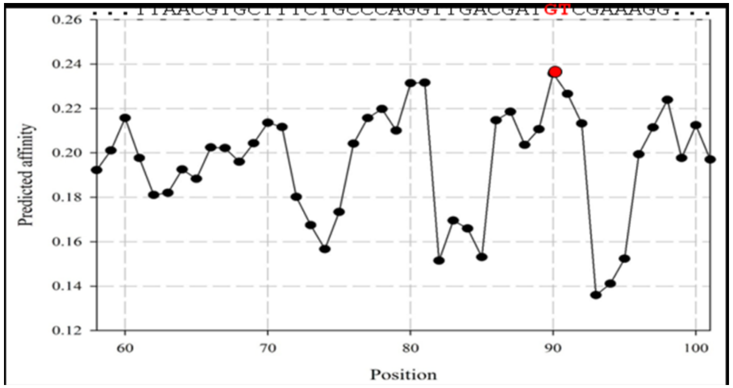


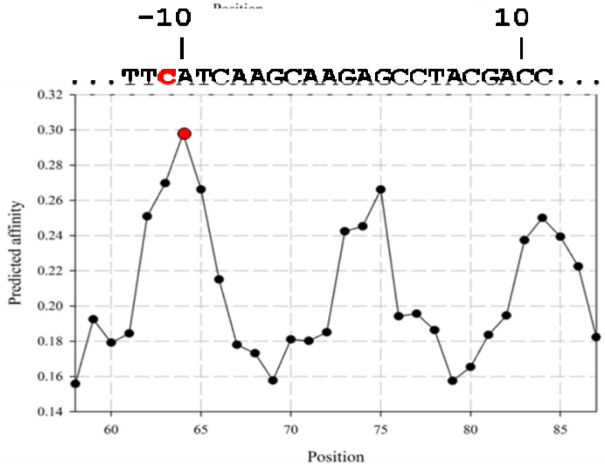


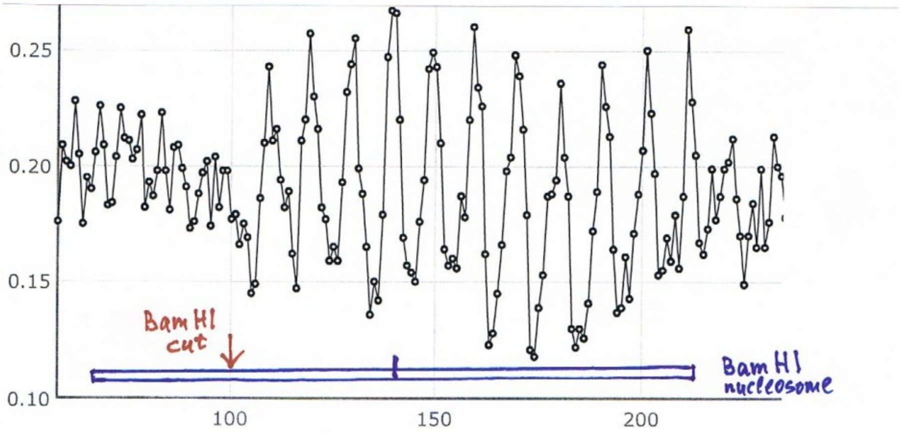












BamHI nucleosome of Ponder and Crawford, 1977

Match of the BamHI nucleosome (typical semistable nucleosome) to the standard nucleosome probes (GAAAATTTTC)n and (RRRRRYYYYY)n

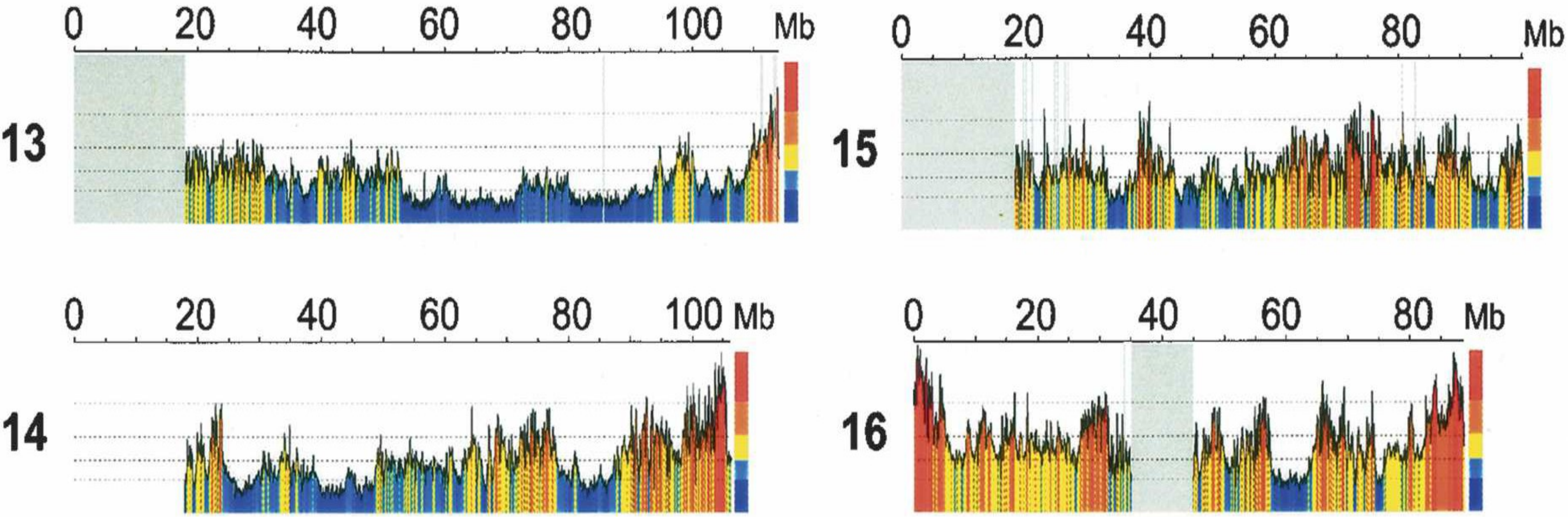
CGGAAATTTTCCGGAAATTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTCCGGAAATTTTCCGGAAATTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTCCGGAAATTTTCC
Cagaggagcttcctgggga**TCC**a**GA**c**AT**gataagataca**TT**gat**GA**gt**TT**ggaca**AA**ccacaactag**AAT**gcagt**GAAA**aaaatgcttt**ATTT**gtga**AA**t**TT**gtgatgcta**TT**gct
YRRRRRag**YYYY**ct**RRR**ga**YYYRRR**c**RY**gata**RRR**taca**YY**gat**RRR**t**YY**ggac**RRR**ccacaact**RRRRY**gcagt**RRRR**aaaa**YR**cttt**RYYY**gt**RRRR**t**YY**gtgatgcta**YY**g**YY**

The RR/YY dinucleotide match is 41/116, between 29/116 (random) and 116/116 (strongest)

BamHI fragments of BamHI nucleosome DNA

Calculated	Observable in the gel	
24		
34		
43		
54	~53	
64	~63	misfit
	(~73)	1 base
82	~83	
92	~93	
103		
112		
122		

Sequences with different G+C composition utilize different RR and YY dinucleotides for nucleosome positioning



Human isochores

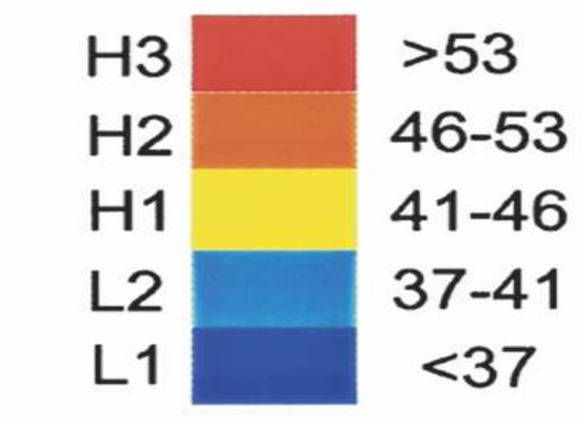
Lab of G. Bernardi, 2006

Nucleosome positioning patterns of various isochores (Frenkel et al., 2011)

by N-gram extension

```
C AGGGG CCCCT G
C GGGGA TCCCC G
C AGAAA TTTCT G
T AAAAA TTTTT A
T AAAAA TTTTT A

Y RRRRR YYYYY R
```

isochores G+C %



```
R Y Y Y Y Y R R R R R Y Y Y Y Y R R R R R Y
 |         |         |         |         |
A|T T T T T|A A A A A|T T T T T|A A A A A|T
 |         |         |         |         |
 |        T|G        |        T|G        |        isochores L1
A|T T T T  |  A A A A|T T T T  |  A A A A|T
 |        C|A        |        C|A        |
 |         |         |         |         |
A|T T T T C|G A A A A|T T T T C|G A A A A|T
A|T T T C C|G G A A A|T T T C C|G G A A A|T        most
A|T T C C C|G G G A A|T T C C C|G G G A A|T        frequent
A|T C C C C|G G G G A|T C C C C|G G G G A|T        patterns
 |         |         |         |         |
A|C        |        A|C        |        A|C
 |  C C C C|G G G G  |  C C C C|G G G G  |
G|T        |        G|T        |        G|T        isochores H3
 |         |         |         |         |
G|C C C C C|G G G G G|C C C C C|G G G G G|C
```

10-11 base periodicity
in prokaryotes

Original calculations on a small sequence ensemble (30 000 bases only) indicated that the sequence periodicity of 10-11 bases is characteristic of only eukaryotic sequences

Later on it turned out that
prokaryotic genomes are periodical as well,
apparently to maintain DNA superhelicity

In prokaryotes where 85% of genome are protein-coding
the DNA curvature signal (10-11 base period) massively overlaps
with the protein-coding signal (3 base period)

Triplet extension (Shannon) patterns for A+T rich prokaryotic genomes

species	G+C content %	extension motif
F. nucleatum	27.2	[(a)t]**(A)(T)**[(a)t]
N. equitans	31.6	(ta)t**(A) t**(at)
- " -		(at)**a (T)**a(ta)
S. solfataricus	35.8	[(t)a]ttt**(A)(T)**[(a)(t)]
T. denicola	37.9	[(a)t]**(A)(T)**[a(t)]
C. pneumoniae	40.0	[g(a)]**G(A)**[g(a)
- " -		[(t)c]**(T)C**[(t)c]
M. acetivorans	42.7	[g(a)]**G(A)(T)C**[(t)c]
A. aeolicus	43.3	[gg(a)]**gG(A)**[gg(a)]
- " -		[(t)cc]**(T)C**c[(t)cc]
B. subtilis	43.5	[g(a)(t)]**G(A)(T)C**[(a)(t)c]
T. maritima	46.2	(gaa)**G(A)**[g(a)]
- " -		[(t)c]**(T)C**(ttc)
D. ethenogenes	48.9	(cggc)cggc**(T)C**agccg(gccg)
consensus		**G(A)(T)C**

CGAAAATTTTCG

same as in eukaryotes!:

CGRAAATTTYCG

α-helices

10-15 aa long

(30-45 bases in DNA)

often **amphipatic**

(alternating hydrophobic/hydrophilic aa)

Period ~3.5 residues

(~10.5 bases in DNA)

Leu (L) - TTx in DNA

Lys (K) - AAx in DNA

What this periodical motif codes for in prokaryotes?

(GAAAATTTTC)(GAAAATTTTC)(GAAAATTTTC)....

GAA	AAT	TTT	CGA	AAA	TTT	TCG	AAA	ATT	TTC
glu	**asn**	**phe**	**arg**	**lys**	**phe**	**ser**	**lys**	**ile**	**phe**

non-polar
amino acids

ala
gly
ile
leu
met
phe
pro
val

polar
amino acids

arg
asn
asp
cys
glu
gln
his
lys
ser
thr
trp
tyr

Alu NUCLEOSOMES

Alu sequence (consensus)

```
                 ggccgggcgcggtgg  15
ctcacgcctgtaatcccagcactttgggaggc  47
CGaggcgggCGgatcacctgaggtcaggagtt  79
CGagaccagcctggc-caacatggtgaaaccc 110
CGtctctactaaaaatacaaaaattagccggg 142
CGtggtggcgCGcgcctgtaatcccagctact 174
CGggaggctgaggcaggagaatCGcttgaacc 206
CGggaggcggaggttgcagtgagccgagatcg 238
CGccactgcactccagcctgggCGacagagcg 270
agactccgtctcaaaaaaaa
```

Alu, hidden 8-base repeat

ggccggg **cg**c**gg**tgg 15
ctc**a**cgcc **tg**t**aa**tcc **cag**c**a**ctt **tggga**ggc 47
CGaggcgg gc**gga**tca **c**ct**ga**ggt **cagga**gtt 79
CGagacca gcctggc- **caa**c**a**tgg **tgaaa**ccc 110
CGtctcta **c**t**aaa**aat ac**aaa**aat **tag**ccggg 142
CGt**gg**tgg **cg**c**g**cgcc **tg**t**aa**tcc **cag**ctact 174
CGggaggc **tgagg**cag g**agaa**tcg **c**tt**ga**acc 206
CGggaggc g**gagg**ttg **cag**t**g**agc **cgaga**tcg 238
CGcc**a**ctg **ca**ct-cca -gcctggg **cga**c**a**gag 268
CGagactc **cg**tctcaa a**aaaa**a
Yrrrrxxx **Yrrrr**xxx **Yrrrr**xxx **Yrrrr**xxx

that is, the Alu repeat is itself a degenerate simple tandem repeat

Two halves of Alu

```
                   ggccggg cgcggtgg  15
ctcacgcc tgtaatcc cagcactt tgggaggc  47
CGaggcgg gcggatca cctgaggt caggagtt  79
CGagacca -gcctggc caacatgg tgaaaccc 110
CGtctcta ctaaaaat acaaaaa           133
                t tagccggg CGtggtgg 150   (15)
cgcgcgcc tgtaatcc cagctact CGggaggc 182   (47)
tgaggcag gagaatcg cttgaacc CGggaggc 214   (79)
ggagg
     ttg cagtgagc cgagatcg CGccactg 246   31 base
cact                                       insert
    -cca -gcctggg cgacagag CGagactc 276 (110)
cgtctcaa aaaaaa                     290 (133)
```

The insert is of very proper size, apparently, to maintain/improve the $(31\text{-}32)_n$ pattern

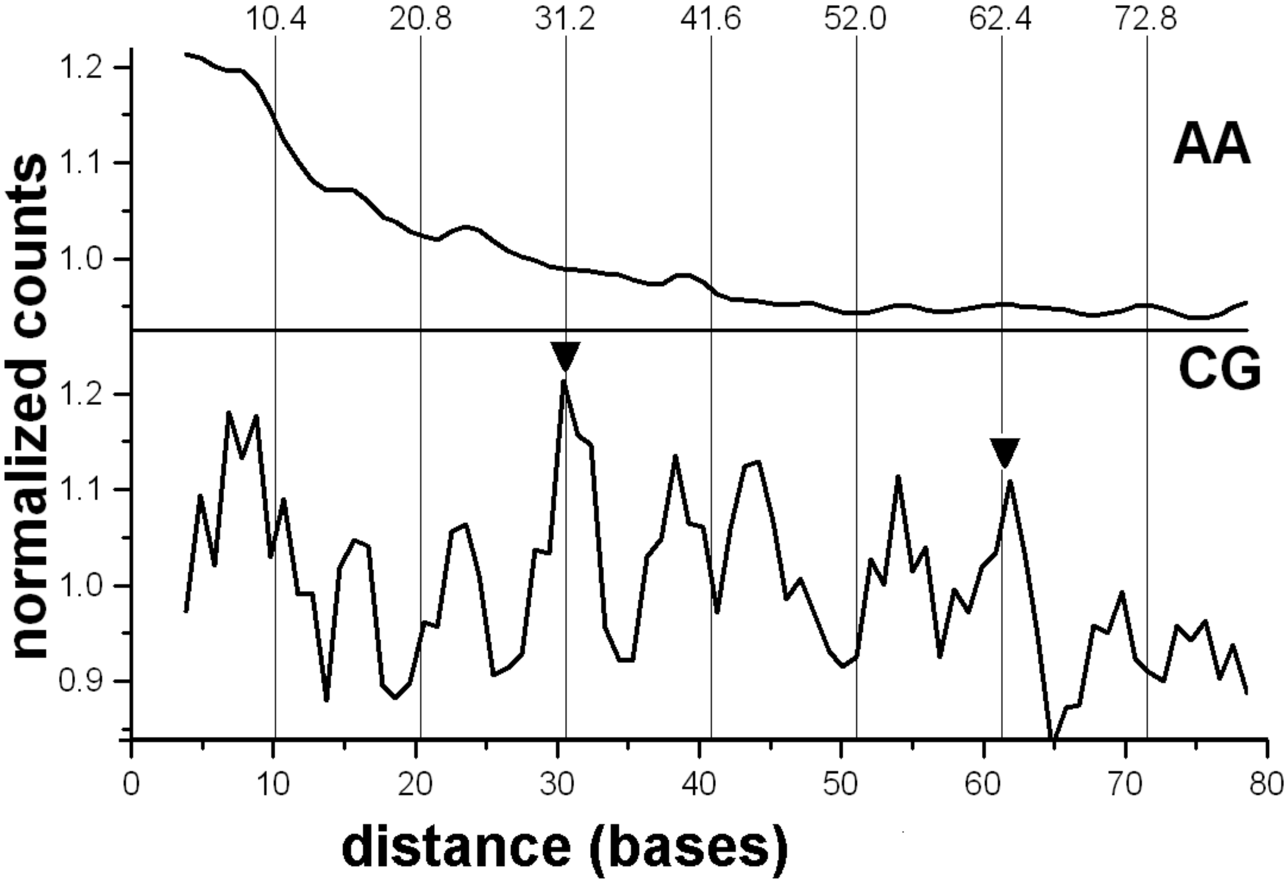
Alu is made of two repeating pieces of 7S RNA

```
                 ggccgggcgcggtgg  15
                  ==============
ctcacgcctgtaatcccagcactttgggaggc  47
=G=GT=======G=======TAC=C========       7S RNA
CGaggcgggcggatcacctgaggtcaggagtt  79
T====T===A=====G=T====TC========
CGagaccagcctggc-caacatggtgaaaccc 110
=TG=G=TGTAG==CG-=T=T
CGtctctactaaaaatacaaaaattagccggg 142
                          ======
CGtggtggcgcgcgcctgtaatcccagctact 174
==C=========T=======G===========      7S RNA
CGggaggctgaggcaggagaatcgcttgaacc 206
==============T====G=========GT=
CGggaggcggaggttgcagtgagccgagatcg 238
=A====TTCTG==C==T====C==TAT
CGccactgcact-cca-gcctgggcgacagag 268
CGagactccgtctcaaaaaaaa
```

All major types of the Alu repeats have regularly positioned CG

```
                                                                               97
nucleosome 1 bends:                                                              ↓
AluJ   agcactttgggaggcCGaggcgggaggatcacttgagcccaggagttCGagaccagcctgggcaacatagtgaaacccCGtctctacaaaaaatacaaaaattagccgggCGtggtggcgcgcgcct
AluSx  agcactttgggaggcCGaggcgggcggatcacctgaggtcaggagttCGagaccagcctggccaacatggtgaaacccCGtctctactaaaaatacaaaaattagccgggCGtggtggcgcgcgcct
AluSq  agcactttgggaggcCGaggcgggtggatcacctgaggtcaggagttCGagaccagcctggccaacatggtgaaacccCGtctctactaaaaatacaaaaattagccgggCGtggtggcgggcgcct
AluSp  agcactttgggaggcCGaggcgggcggatcacctgaggtcgggagttCGagaccagcctgaccaacatggagaaacccCGtctctactaaaaatacaaaaattagccgggCGtggtggcgcatgcct
AluSc  ccagcactttgggaggcCGaggcgggcggatcacgaggtcaagagatCGagaccatcctggccaacatggtgaaacccCGtctctactaaaaatacaaaaattagctgggCGtggtggcgcgcgcct
AluY   cagcactttgggaggcCGaggcgggcggatcacgaggtcaggagatCGagaccatcctggctaacacggtgaaacccCGtctctactaaaaatacaaaaaattagccgggCGtggtggcgggcgcct
AluYa5 cagcactttgggaggcCGaggcgggcggatcacgaggtcaggagatCGagaccatcccggctaaaacggtgaaacccCGtctctactaaaaatacaaaaaattagccgggCGtagtggcgggcgcct
AluYa8 ccagcactttgggaggcCGaggcgggcggatcacgaggtcaggagatCGagaccatcccggctaaaacggtgaaacccCGtctctactaaaactacaaaaaatagccgggCGtagtggcgggcgcct
AluYb8 cagcactttgggaggcCGaggcgggtggatcatgaggtcaggagatCGagaccatcctggctaacaaggtgaaacccCGtctctactaaaaatacaaaaaattagccgggCGcggtggcgggcgcct

                                                                              223
nucleosome 2 bends:                                                              ↓
AluJ   gtagtcccagctactCGggaggctgaggcaggagaatcgcttgaaccCGggaggcggaggttgcagtgagccgtgatCGCGccactgcactccagcctgggcgacagagCGagaccctgtctcaaa
AluSx  gtaatcccagctactCGggaggctgaggcaggagaatcgcttgaaccCGggaggcggaggttgcagtgagccgagatCGCGccactgcactccagcctgggcgacagagCGagactccgtctcaaa
AluSq  gtaatcccagctactCGggaggctgaggcaggagaatcgcttgaaccCGggaggcggaggttgcagtgagccgagatCGCGccactgcactccagcctgggcaacaagagCGaaactccgtctcaa
AluSp  gtaatcccagctactCGggaggctgaggcaggagaatcgcttgaaccCGggaggcggaggttgcggtgagccgagatCGCGccattgcactccagcctgggcaacaagagCGaaactccgtctcaa
AluSc  tgtagtcccagctactCGggaggctgaggcaggagaatcgcttgaaccCGggaggcggaggttgcagtgagccgagatCGcgccactgcactccagcctggcgacagagCGagactccgtctcaaa
AluY   tgtagtcccagctactCGggaggctgaggcaggagaatggcgtgaaccCGggaggcgcaggttgcagtgagccgagatCGcgccactgcactccagcctgggcgacagagCGagactccgtctcaa
AluYa5 gtagtcccagctacttgggaggctgaggcaggagaatggcgtgaaccCGggaggcgcaggttgcagtgagccgagatccCGccactgcactccagcctgggcgacagagCGagactccgtctcaaa
AluYa8 gtagtcctagctacttgggaggctgaggcaggagaatggcgtgaaccCGggaggcgcaggttgcagtgagccgagatccCGccactgcactccagcctgggcgacagagCGagactccgtctcaaa
AluYb8 gtagtcccagctactCGggaggctgaggcaggagaatggcgtgaaccCGggaagcgcaggttgcagtgagccgagattgCGccactgcagtccagcagtccggcctgggCGacagagcgagactcc
```

Whole genome (human) shows only 31n periodicity

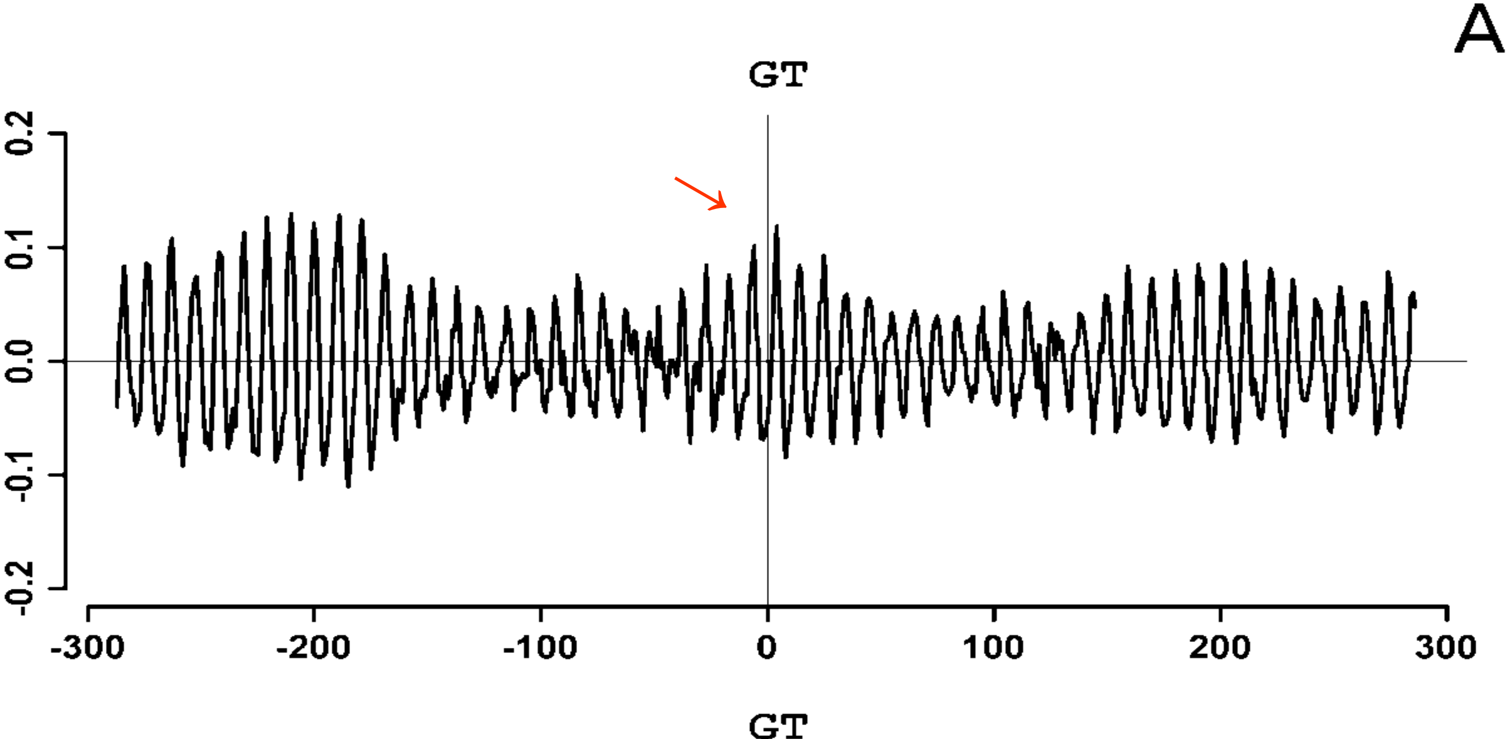


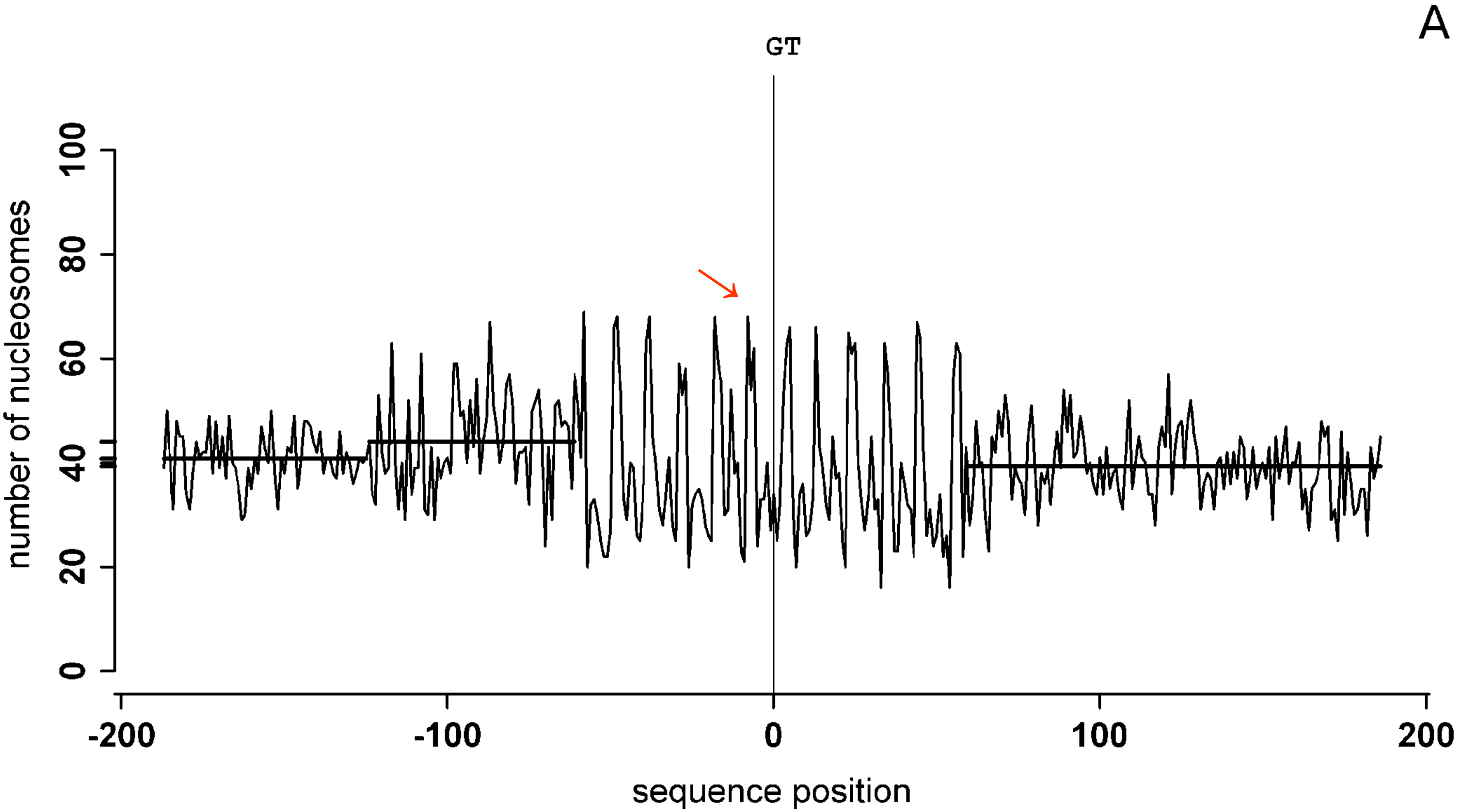
Methylation/demethylation of properly positioned CG
in the nucleosome DNA
leads to weakening/strengthening
of the nucleosome,
which is, thus, an epigenetic nucleosome

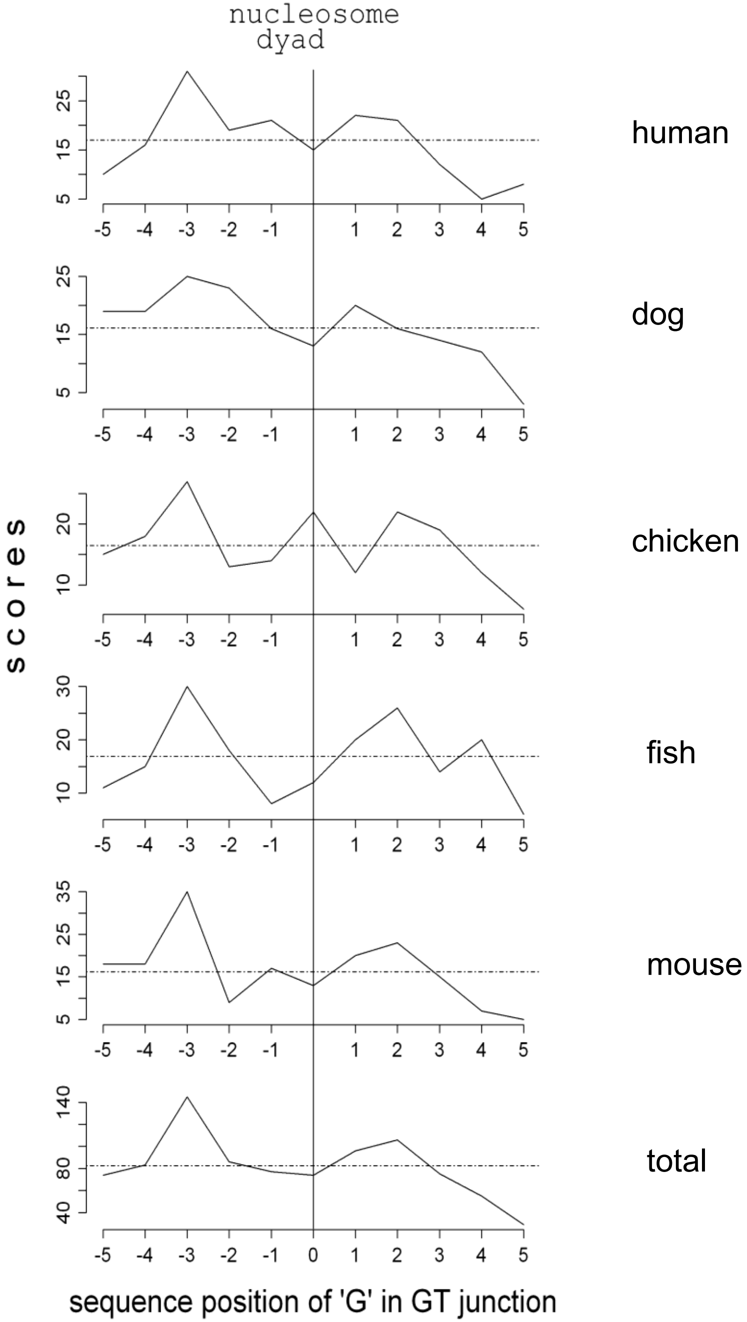
Applications of single-base resolution nucleosome mapping

Example of the nucleosomes at and around GT splice junction

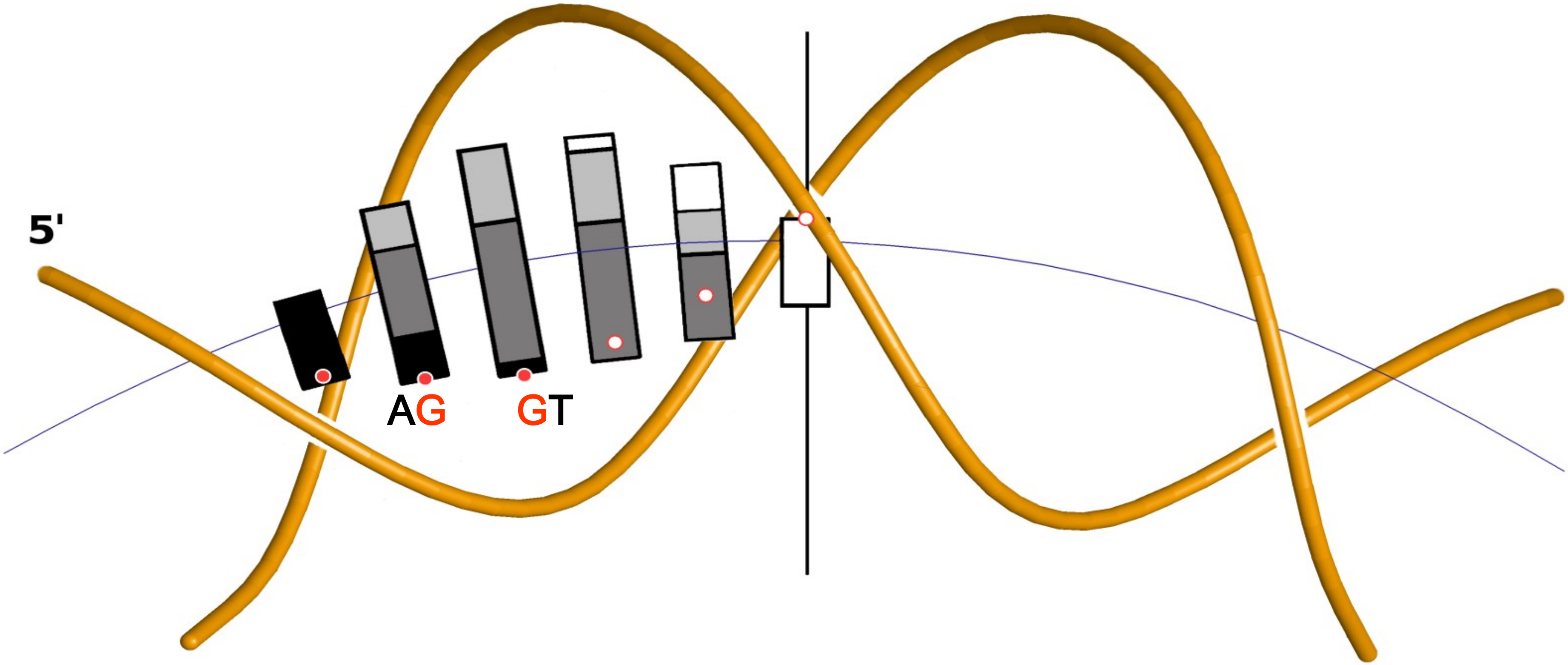
Hapala, 2011




A
GT
number of nucleosomes
0
20
40
60
80
100
-200
-100
0
100
200
sequence position



Position -3
preferred


5'
AG
GT

Guanines of GT- and AG-ends of introns are oriented towards the surface of the histone octamer, away from exterior.

Such orientation protects guanines from spontaneous depurination and oxidation

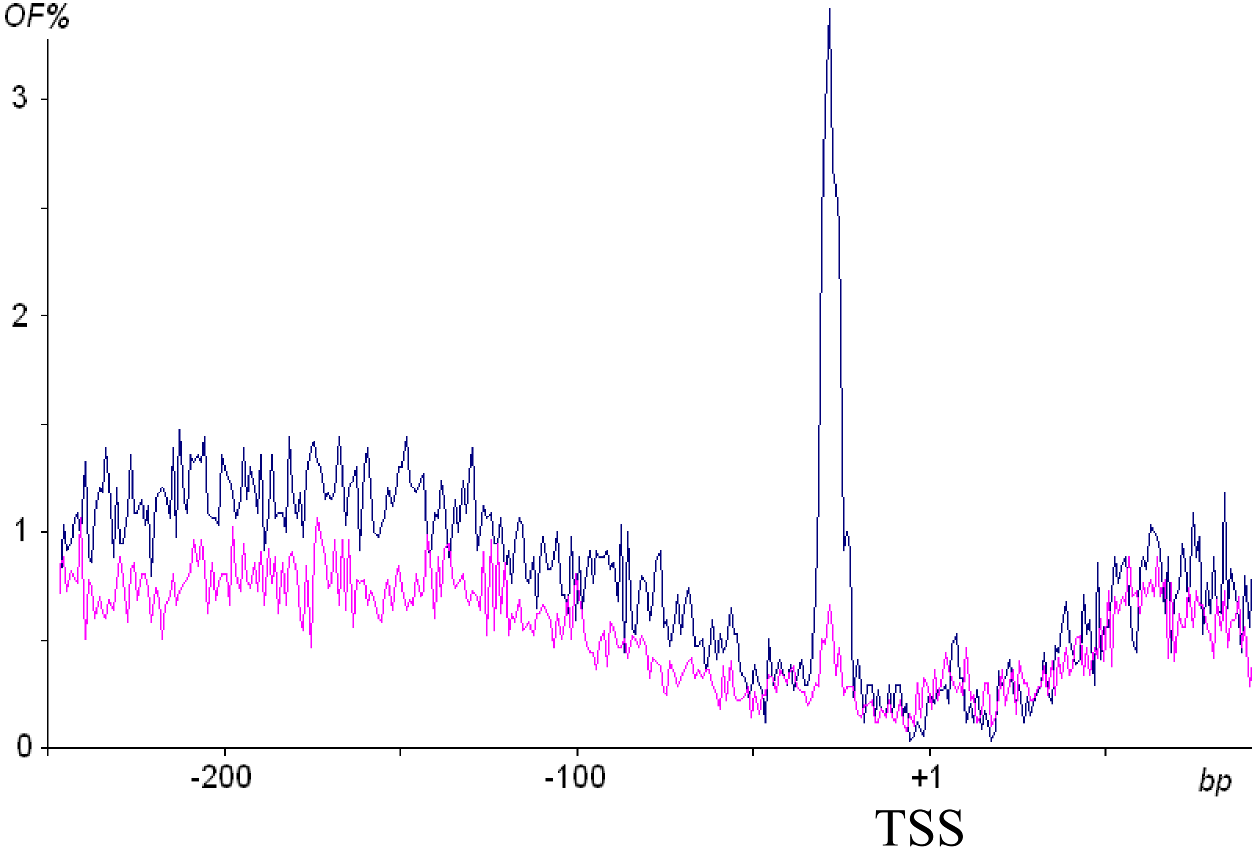
The most frequent spontaneous damages to DNA bases:

depurination of G

oxidation of G

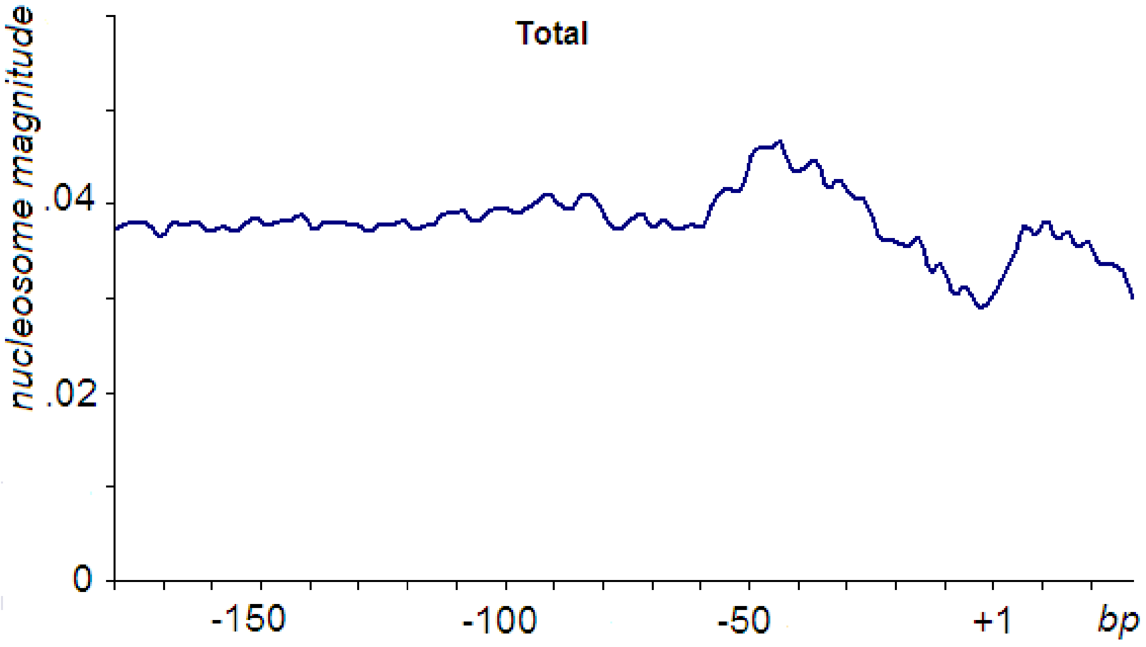
deamination of C

TATA-box

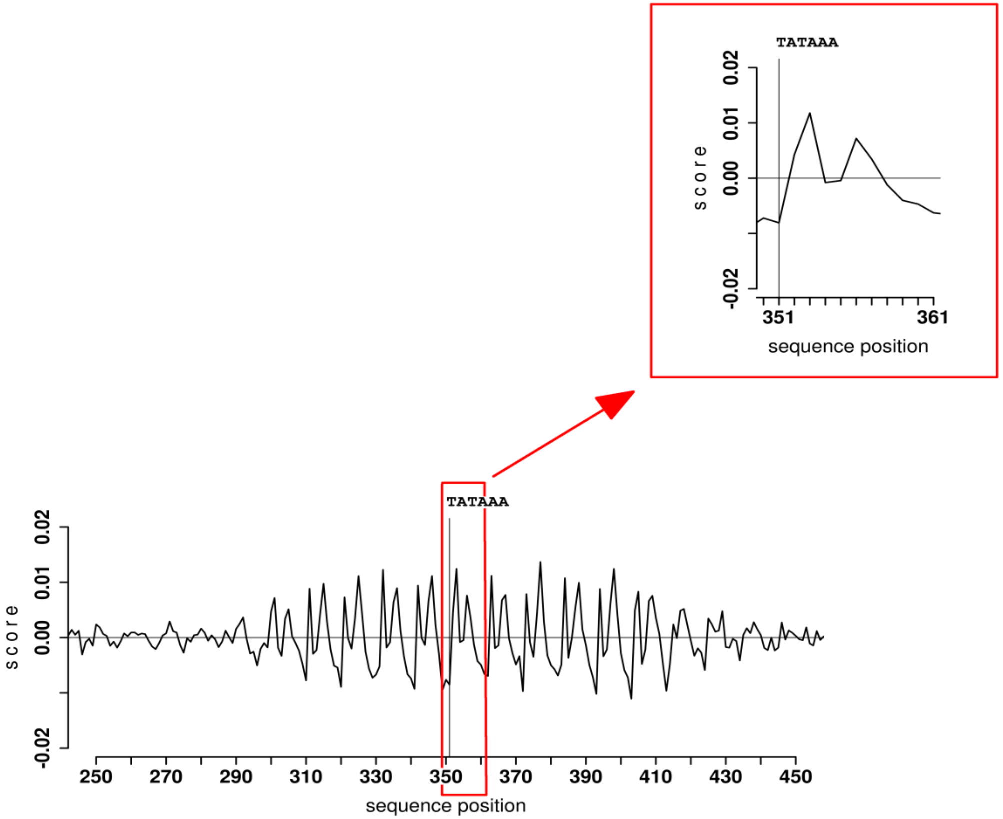


Gershenzon, Drosophila, 2006

Nucleosomes around transcription start sites (Drosophila)



TSS



Nucleosome DNA which carries promoter TATAAA box has two rotational settings encoded in the sequence (two peaks within one period).

Jan Hapala & ET, 2013

TATA-switch

Two alternative positions of TATAAA box
in the promoter nucleosomes
are separated by 140 (220) degrees,
which corresponds to exposed and inaccessible
orientations of the box.

By shifting the DNA along its path by 4(6) bases,
the promoter is switched **ON** or **OFF.**

The switch (shift) may be triggered by remodelers or transcription factors.

Plenty of various other nucleosome positioning patterns have been suggested during 30 years since the first observation of sequence periodicity.
At the best they provide occupancy maps (resolution of ~15 bases).

The (GRAAATTTYC)n and (RRRRRYYYYY)n are the only patterns that generate maps with single-base resolution, verified by crystal data.

The future of the chromatin structure/function is with the high resolution studies.

Deciphering of the chromatin code opens a new era of high resolution chromatin studies

One can now obtain accurate information on translational and rotational positioning of DNA in the nucleosomes,

for any sequence,
in no time

Nucleosome mapping in no time, with 1 base resolution:

http://www.cs.bgu.ac.il/~nucleom/

Gabdank et al., 2010

Higher order structure of chromatin

Nucleosomes are organized in 3D space in an unknown way – higher order chromatin structure

Important element of the higher order structure is dinucleosome (1981, laboratories of L. Burgoyne and of V. Vorobiev)

BURGOYNE & SKINNER

99, 893, 1981

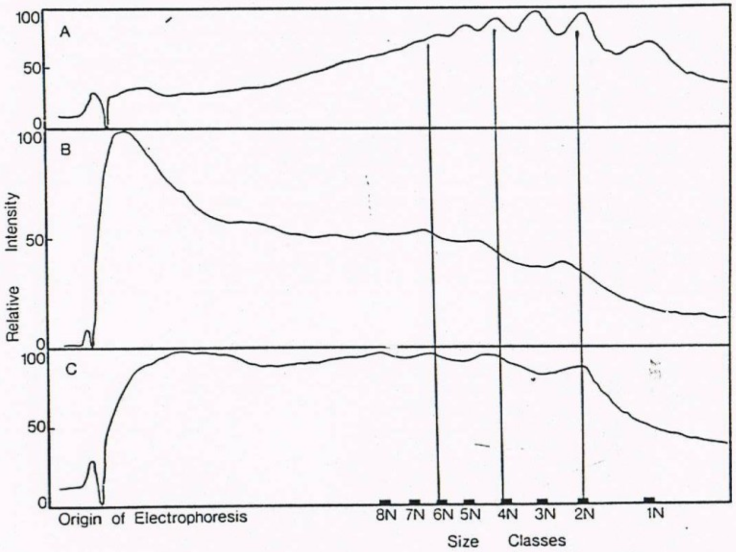
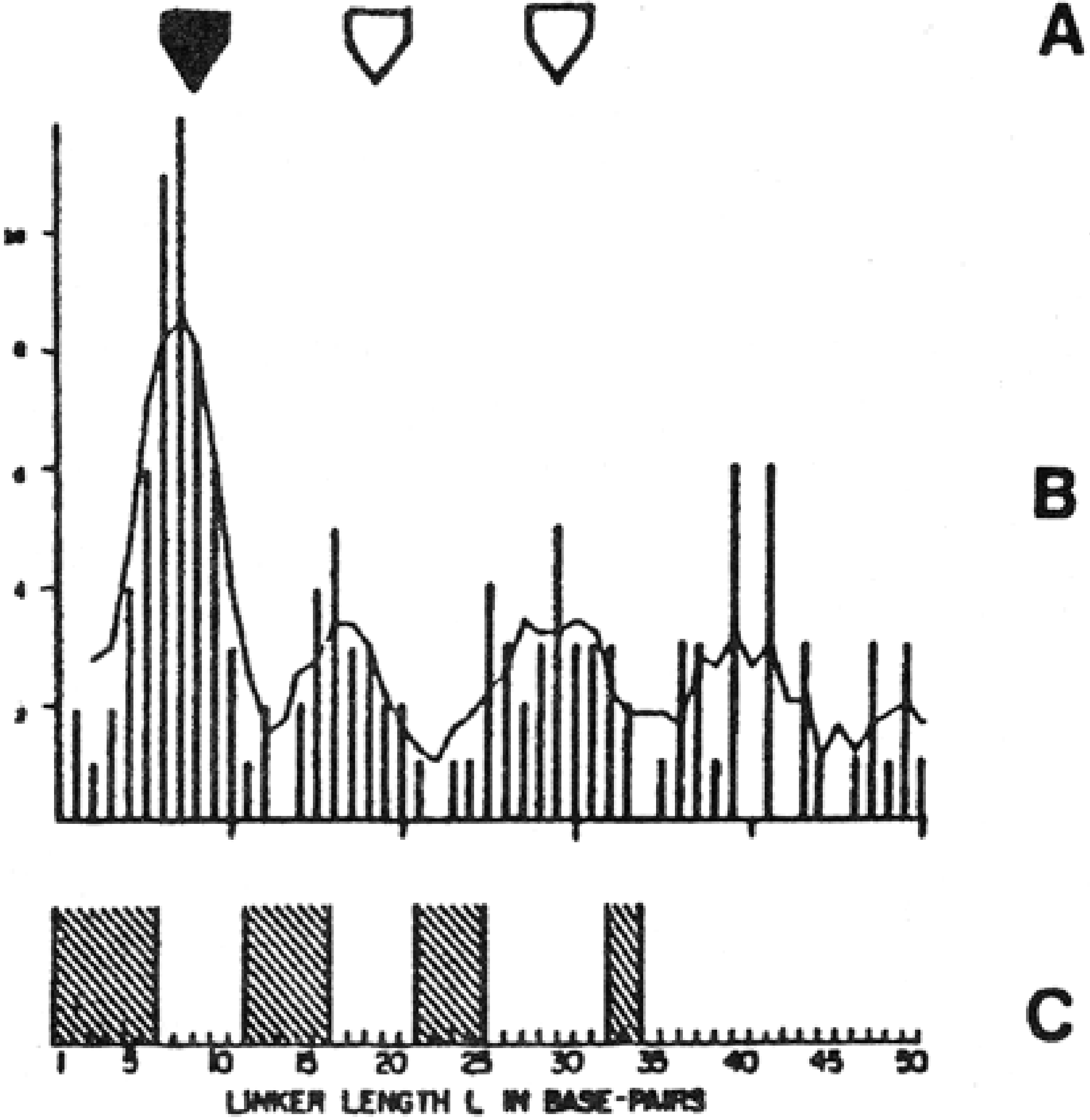
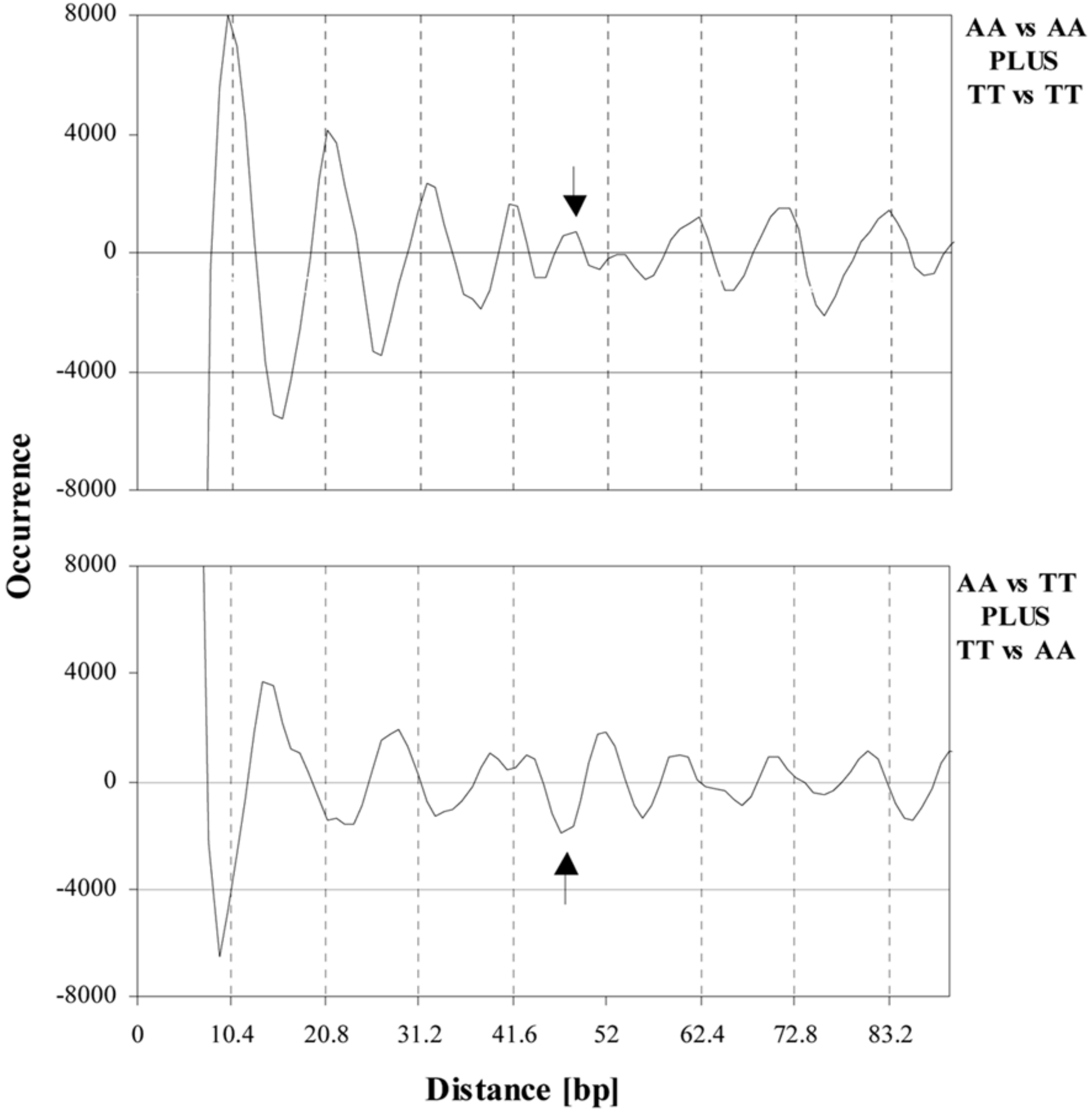


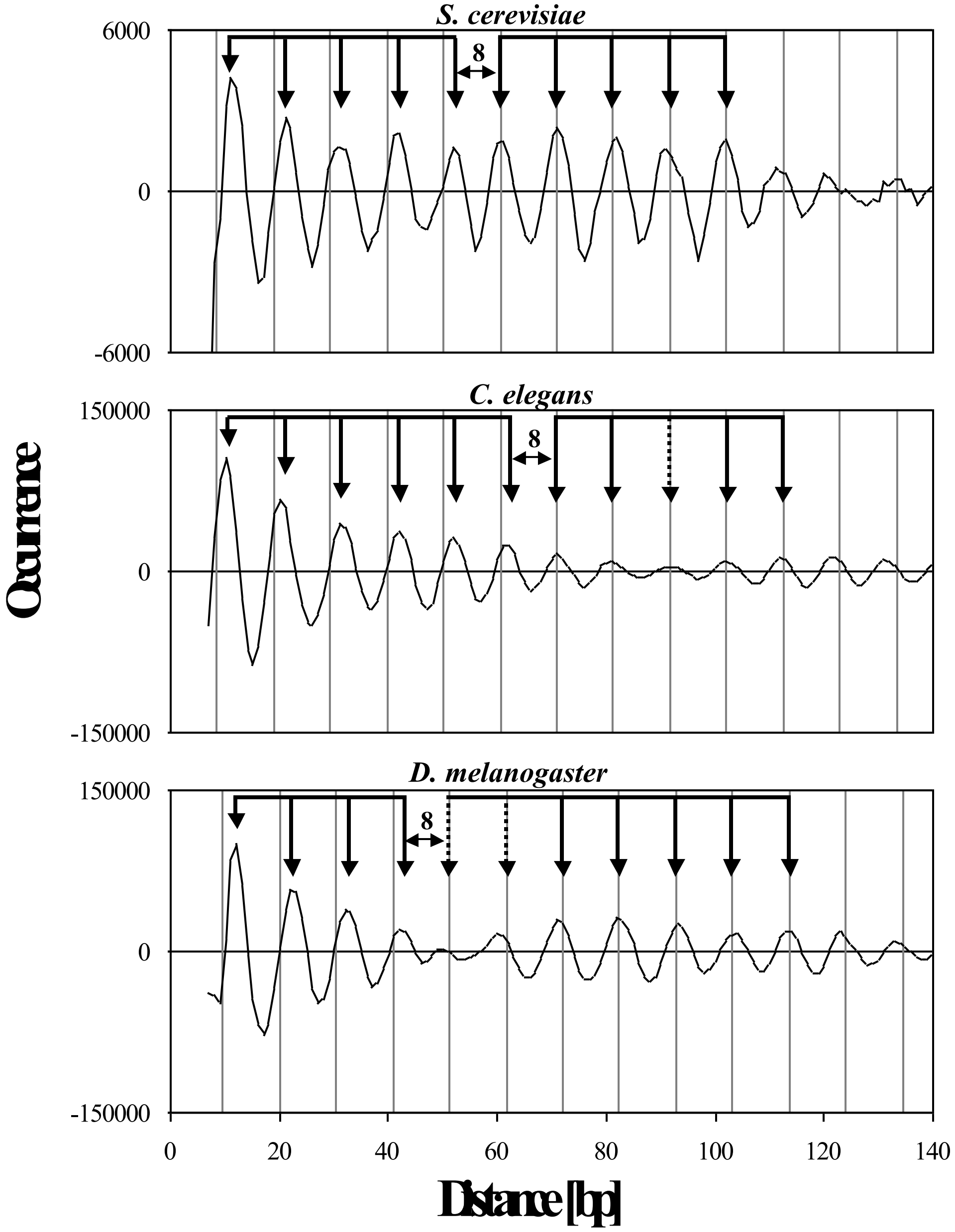
FIGURE 3 Ferritin based, DNAase-I armed probe attack on rat liver nuclei. All conditions as for the experiment shown in Figure 2. 25 mins digestion time. Curve A - Standard 1N, 2N, etc. series producted by autolysis of rat liver nuclei by their intrinsic Ca-Mg nuclease. Curve B - Rat liver nuclei digested with Ferritin-DNAase-I as in Fig. 2. 15 mins digestion. Curve C - As for Curve B, 30 mins digestion.

The deformational properties of DNA is not the only sequence-dependent factor of nucleosome positioning.

The second factor is the steric exclusion rules, imposing limitations to the linker lengths.


A
B
C
10
8
6
4
2
1
5
10
15
20
25
30
35
40
45
50
LINKER LENGTH L IN BASE-PAIRS


8000
4000
0
-4000
-8000
AA vs AA
PLUS
TT vs TT
8000
4000
0
-4000
-8000
AA vs TT
PLUS
TT vs AA
Occurrence
0
10.4
20.8
31.2
41.6
52
62.4
72.8
83.2
Distance [bp]


S. cerevisiae
6000
0
-6000
8
C. elegans
150000
0
-150000
8
D. melanogaster
150000
0
-150000
8
Occurrence
0
20
40
60
80
100
120
140
Distance [bp]

STRONG NUCLEOSOMES

The periodic signal in the nucleosome DNA sequence
is very weak, and it is rather hard task to find out
what would be the true nucleosome positioning sequence.

Actually, none of the experimentally extracted
nucleosome DNA sequences shows any visible periodicity.

The periodic hidden signal could be only revealed
by one or another signal processing procedure
applied to large amount of sequences.

Lowary and Widom (1998) took
large ensemble of synthetic DNA fragments
with random sequences,
and selected those of them
which formed **strong nucleosomes**

The sequences demonstrated very strong periodicity of TA dinucleotides

Clone 601,

from collection of Lowary and Widom (1998):

...CAGCGCG**TA**CGTGCGTT**TA**AGCGGTGC**TA**GAGCTGTC**TA**C...

TACGTGCGTT**TA**
TAAGCGGTGC**TA**
TAGAGCTGTC**TA**

We took all **TA**nnnnnnnn**TA** segments from the collection of Lowary/Widom, and analysed which dinucleotides are most frequently located in the interval between **TA**, and in which positions

Regeneration of signal from its incomplete versions:

AA

↘ positional autocorrelation

AAnnnnnnnnAA

regeneration

↓ all occurrences of AAnnnnnnnnAA are aligned, and other dinucleotides counted within the period

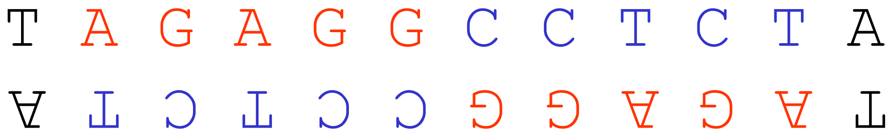
AAnnnnCCnnAA

Gabdank, 2009

Bendability matrix for strong nucleosome DNAs of Lowary and Widom collection

	0	1	2	3	4	5	6	7	8	9	0
AA	0	16	3	0	0	1	0	0	0	0	0
AC	0	5	2	5	2	3	5	3	1	0	0
AG	0	**25**	11	**9**	2	4	1	1	1	0	0
AT	0	2	0	3	1	1	3	1	2	0	0
CA	0	0	1	0	2	4	3	1	0	0	0
CC	0	0	0	0	5	4	7	3	6	0	0
CG	0	0	4	4	4	4	4	5	3	0	0
CT	0	0	0	2	1	2	1	9	11	**22**	0
GA	0	0	**12**	4	3	3	0	0	0	0	0
GC	0	0	4	7	6	7	5	10	5	0	0
GG	0	0	7	4	3	3	7	0	1	0	0
GT	0	0	2	7	6	4	5	6	2	6	0
TA	**48**	0	1	1	4	1	2	3	0	0	**48**
TC	0	0	0	0	1	1	1	4	10	0	0
TG	0	0	0	1	8	6	4	2	1	0	0
TT	0	0	1	1	0	0	0	0	5	20	0

T A G A G x x x x **C T A** – manually
T A G A G G C C T C T A – by dynamic programming
Y R R R R R Y Y Y Y Y R



The periodical pattern hidden in the sequences of Lowary and Widom is selfcomplementary, and manifests alternation of RRRRR and YYYYY

Taking the elegant idea of Lowary and Widom as a lead

we extracted *natural* strong nucleosomes

from whole genomes *computationally.*

We looked for periodical sequences in genomes

Magic distances, 10.4•n bases

	nearest integers
10.4	10
20.8	21
31.2	31
41.6	42
52.0	52
62.4	62
72.8	73
83.2	83
93.6	94
104.0	104
114.4	114

The ideal nucleosome positioning sequence
would contain some periodically repeating motif,
and all the distances between the same dinucleotides
would be magic distances.
Strong nucleosome DNA would show many magic distances.

The strongest nucleosomes of *A. thaliana* display very clear though still imperfect periodicity

```
TAAACTCTTTAAAAATCTTTTAAAAACCCTTGTACATATCTTAAAACCCTTTTAAAATCTCTTGTAAATCTTTAAAACCCTTTTAAAATCCCTTGTAAATCTTTTAAAACCCTTT
AAATATTTTAAAACACTTTTCAAACAATTTTGAACCCTTTAAAAATCTTTATAAAACCTTTGTAAATCTTTTAAAGCCCTTTAAAATCTCTTATAAATCTTTTAAAACCCTTTTA
CCCTGTAAAACTTTTAAAACCCTTTTAAAATCCCTTGTAAATCTTTTTAAACCCTTTTAAAATCCTTGTAAATATTTTAAAATCCCGTGTAATTCTTTTAAAACTCTTTTAAAAT
AAATTTTAAAAAGGTTTTATAAGATTTGCAAGGGATTTTAAAGGGATTTAAAAGATTTACAAAAGTTTTTTAAAGGTTTAAAATTGTTTTAAAAGGATTTTAAAATATTTACAAG
TTTTAAAAGGGTTTTAAAATATTTACATATGTTTTTTAAAGTTTTTTAAAGGGTTTAAAAGTGTTTTGCAAGATTTACAAGAGATTTTAAAAGGGTTTTAAGAGATTTACAAGAG
ATCCTTTAAAAAATCATGTAAATCTTTTTAAAACCTTTTAAAATCCCTTGTAAATCTTTTAAAATCCTTTTAAAATCTCTTGTAAATGTTTAAAAACCCTTTTAAAATCTCTTGT
AAGGGTTTTAAAATATTTACAAGGGATTTTAAAAGGGTTTTAAAAAATTTACAAGTGATTTTAAAAGATTTACAAGGGATTTTAAAAGGTTTTAAAAAAATTTACAAAAGTTTAT
AAATCTTTTAAAACCCTTTTAAAATCCCTTGTAAATCTTTTAAAACACTTTTAAACCCTTTAAAAATCTTTAAAAAAACCTTTATAAATCTTTTAAAACTCTTTAAAATCTCTTG
AAATGTTTTAAAACCTTTTTAAAATAATTTTAAACCCTTTAAAAATCGTTAAAAAACTTTTGTAAATCTTTTAAAGCCCTTTAAAATCCCTTGTAAATATTATAAAACCCTTTTA
TGATTTTAAAAGGGTTTAAAAAGATTTACAAGGGATTTTAAAAGGGTTTTAAAAAATTTACAAGAGATTTTAAAAGGTTTTAAAAAGATTTACAAGAGTTTTAAAGGGTCTTCTT
ATCTTTTAAAAATCCTTGTACATCTTTTAAAACCCTTTCAAACCCTTTAAAAATCTCTTGTAAATCTTTTAAAACCCTTTTAAAATCCCTTGTAAATCTTTCAAAACACTTTAAA
CCTTTAAAATCCCTTGTAAATCTTTTAAAACCCTTTTCAAATCCCTTGTAAATGTTTTAAAACCCTTTTAGAACAATTTTAAACCCTTTAAAAATCTTTAAAAACCCTTTGTAAA
TTTACAAAGGTTTTTAAAAGATTTTGAAAGGGTTTAAAAGTGTTTTAAAAGATTTACAAGGGATTTTAAAAGGGTTTTAAAGATTTACAAGAGATTTTAAAAGGGTTTTAAAAGA
CTTGTAAATCTTTTAAAACCCTTTTAAAATCCTTTGTAAATATTTTAAAAGCCTTTTAAAATCCATTGTAAATCTTTTAAAATCCTTTGTAAATCTTTTAAAACCCTTTTAAAAT
AGGATTTTAAAAATGTTTTAAAAGATTTACAATGGATTTTAAAAGGGTTTAAAATATTTATAAGGGATTTTGAAGGGCTTTCAAAGATTTATAAAGGTTTTTTAAAAATTTTTAA
TTGTAAATTATTTAAAAATCTTTTAAAACTCCTTGTACATCTTTTAAAACTCTTTTAAAATTTCTTGTAAATCTTTAAAACCCTTTAAAATCCCTTGTAAATCTTTTAAAATACT
ACCCTTTAAAAATCTTTTAAAAATCTTTGTAAATCTTTTAAAGCCCTTTGAAATCCCTTGTAAATATTTTAAAATCTTTTAAAATTCCTTGTAAATGTTTTAAAACCCTTTTAAA
GATTTGCAAAAGATTTTAAAAGATTTACAAAGGATTTTAAAAGATTTACAATGGATTTTAAAGGGGTTTAAAAGATTTACAAAGGTTTTTTAAAGATTTTTAAAGGGTTTTAAAT
```

The ideal pattern for *A.thaliana*
is repetition of TAAAAATTTTTA,
again, alternation of RRRRR and YYYYY,
and complementary symmetry

Before this picture was generated

(Dec. 2012) nobody ever had seen

that the nucleosome sequences

look, indeed, periodical

From the bendability matrices

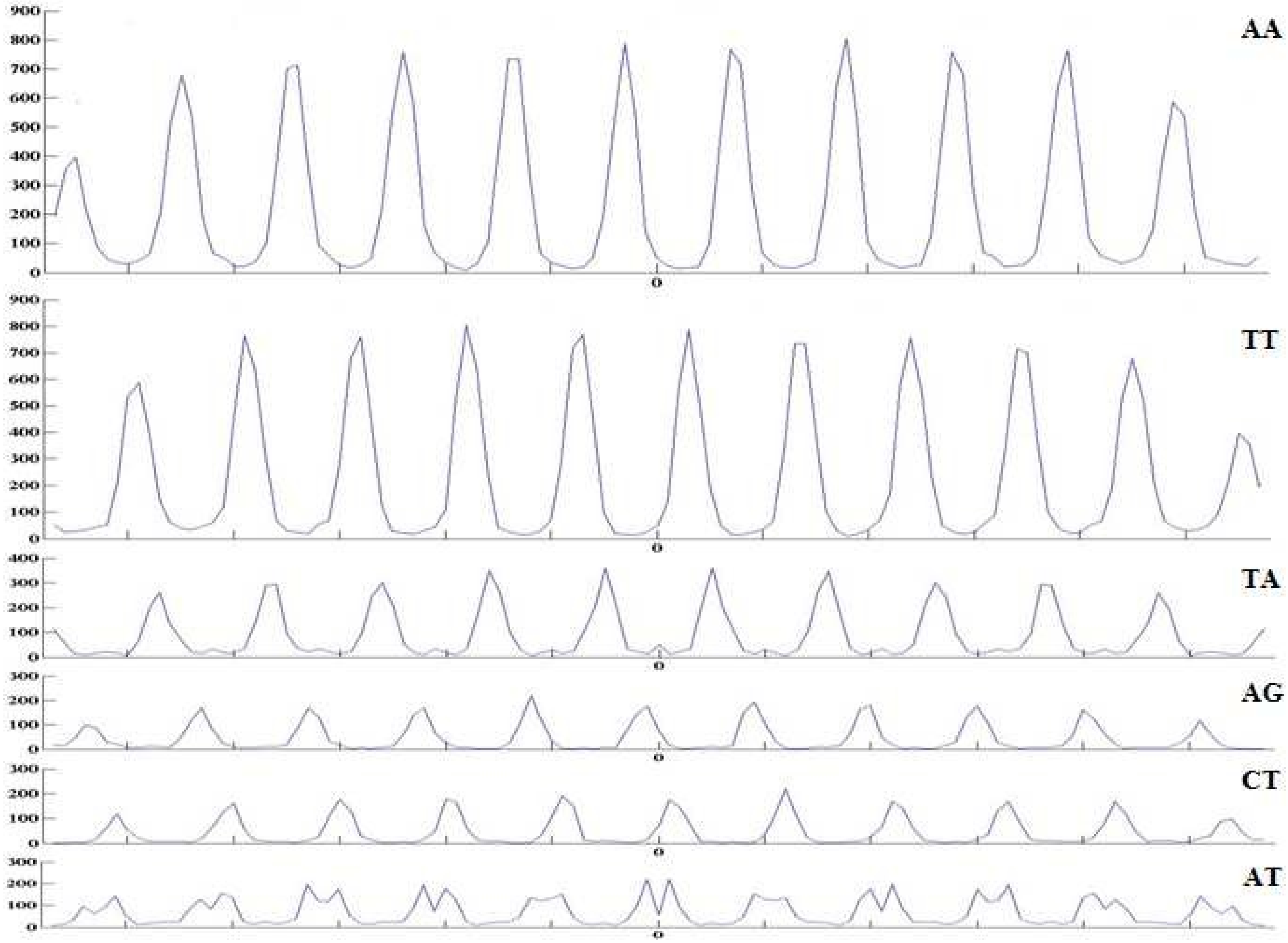
for the strong nucleosomes:

T AGAGG CCTCT A	Lowary and Widom
T AAAAA TTTTT A	A.thaliana
T AAAAA TTTTT A	C.elegans
T AAAAA TTTTT A	H.sapiens
T AAAAA TTTTT A	isochores L1, L2, H1 and H2
C GGGGG CCCCC G	isochores H3
Y RRRRR YYYYY R	common for all

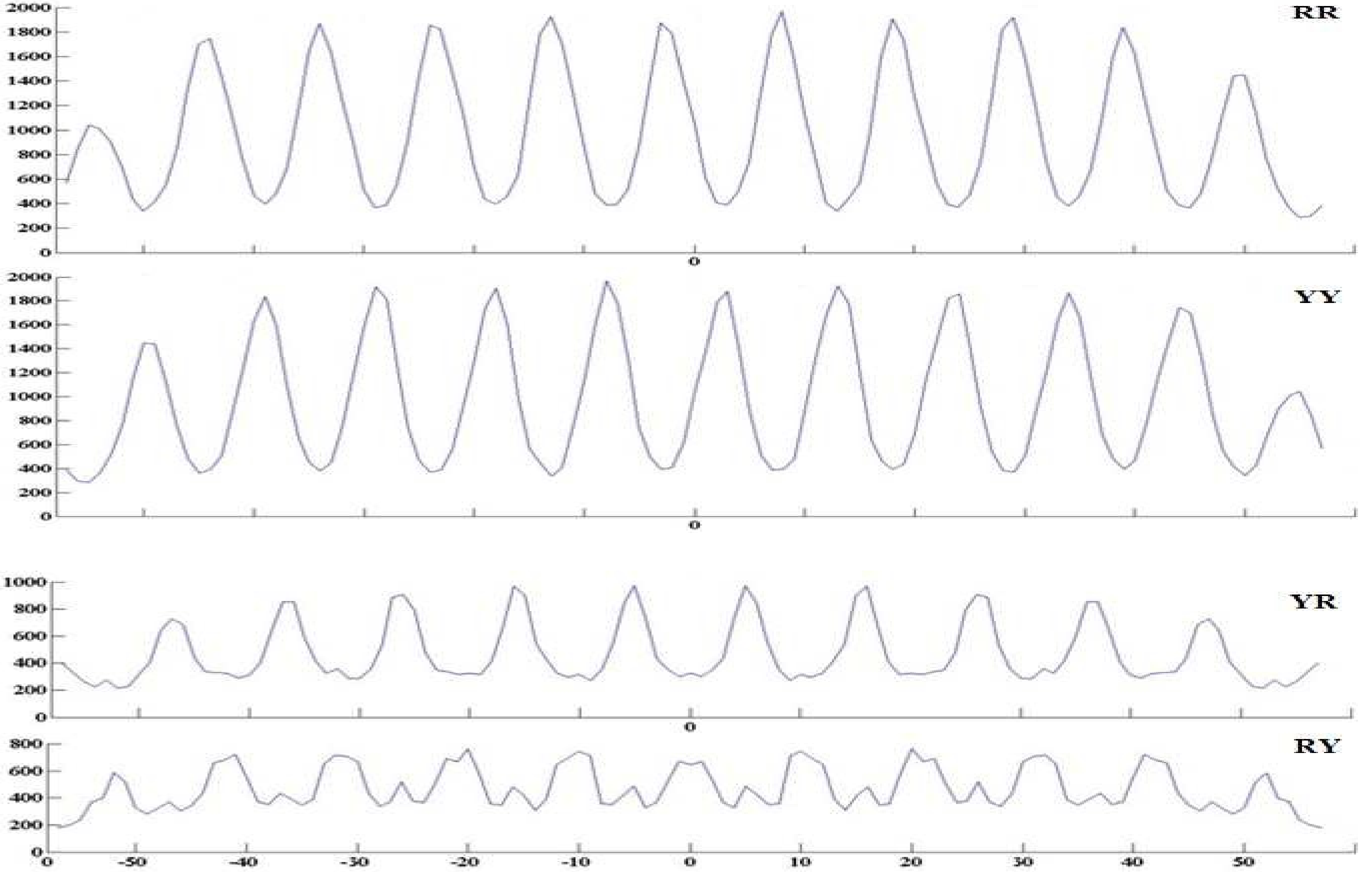
A. thaliana	T AAAAA TTTTT A	strong nucleosomes
	T AAAAA TTTTT A	Shannon extension
C. elegans	T AAAAA TTTTT A	strong nucleosomes
	c **gr**AAA TTT**yc** **g**	signal regeneration
isochores L1, L2	T AAAAA TTTTT A	strong nucleosomes
	T AAAAA TTTTT A	Shannon extension
isochores H1	T AAAAA TTTTT A	strong nucleosomes
	c A**g**AAA TTT**c**T **g**	Shannon extension
isochores H2	T AAAAA TTTTT A	strong nucleosomes
	c **gggg**A T**cccc** **g**	Shannon extension
isochores H3	C GGGGG CCCCC G	strong nucleosomes
	C **a**GGGG CCCC**t** G	Shannon extension

Y RRRRR YYYYY R – all,
and all with complementary symmetry

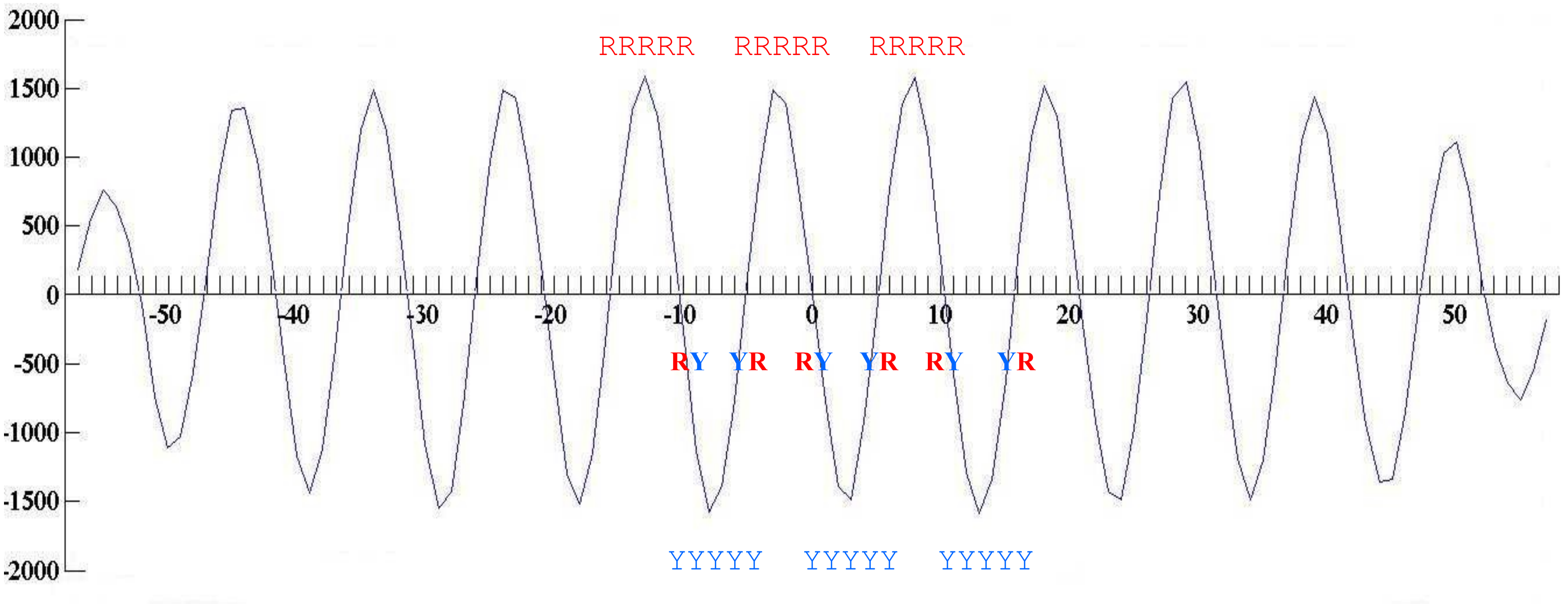
Full size nucleosome DNA bendability matrix (A. thaliana, strong nucleosomes)



Bendability matrix for [R,Y] dinucleotides



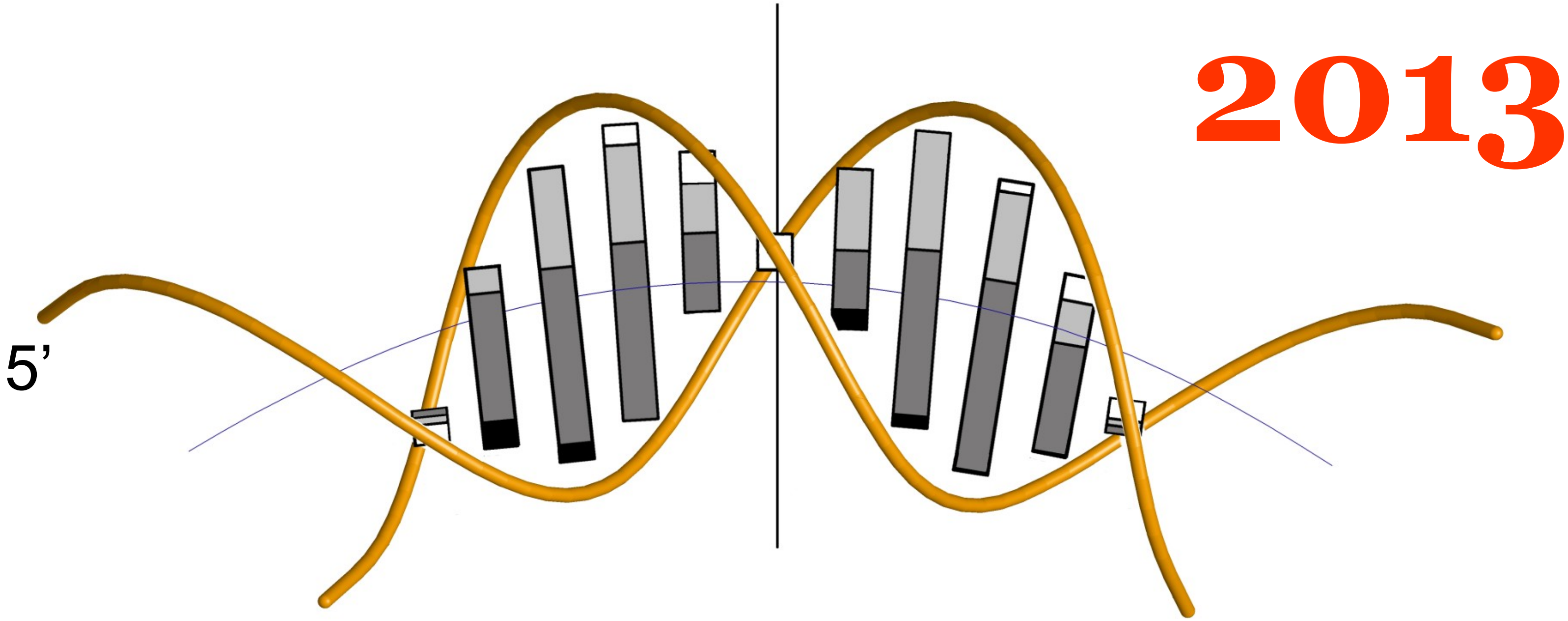
Full length [R,Y] nucleosome mapping consensus sequence probe (from RR-YY distribution)



5`-**YR**RRRRYYYYYRRRRR**YYYYYY**RRRRRYYYYYRRRRRYYYYY**RRRRRR**YYYYYRRRRR
YYYYYRRRRR**YYYYYY**RRRRRYYYYYRRRRRYYYYY**RRRRRR**YYYYYRRRRRYYYY**YR**- 3`

Nucleosome positioning pattern

2013

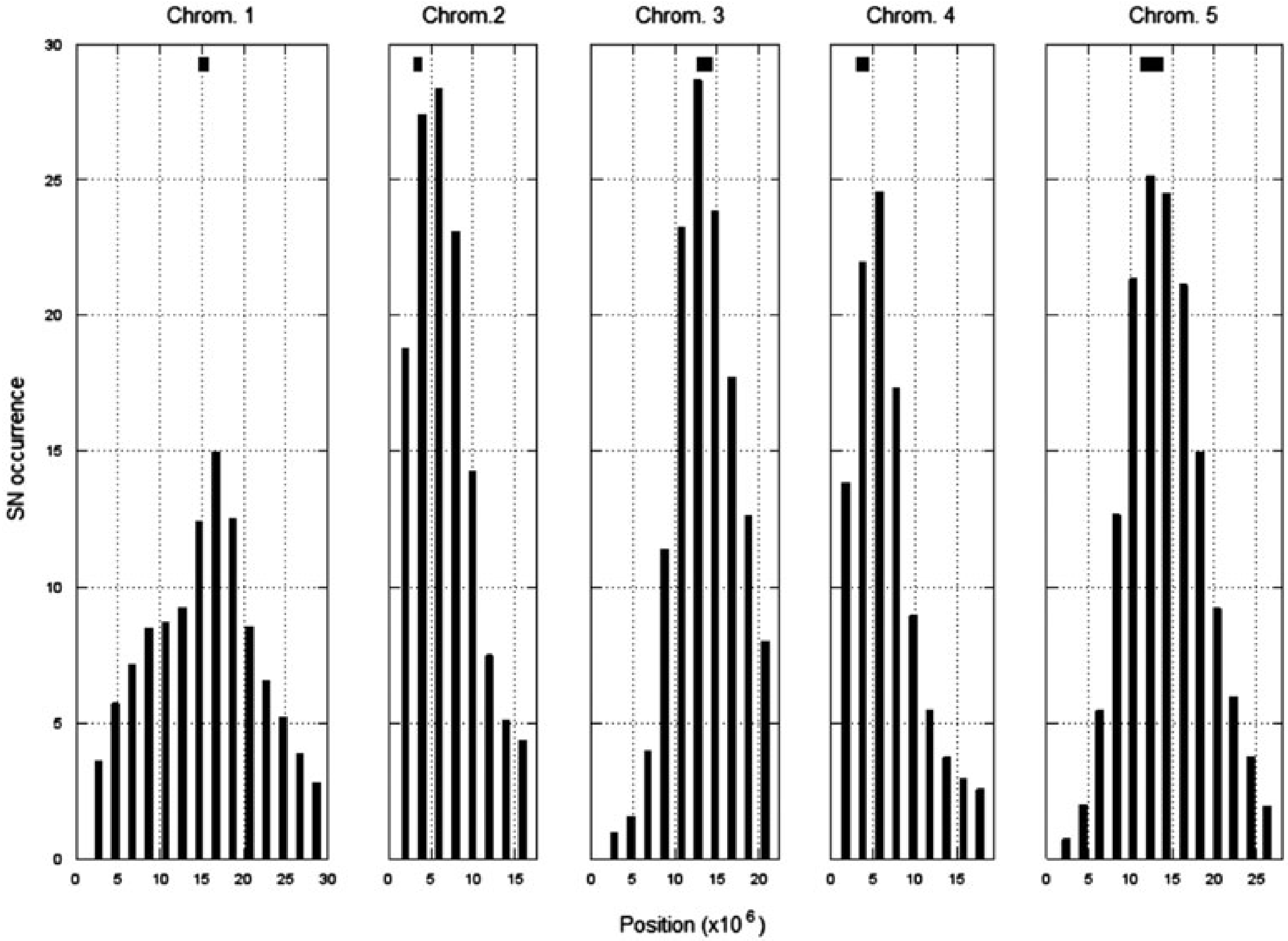


5’…YYYRRRRRYYYYYRRR…

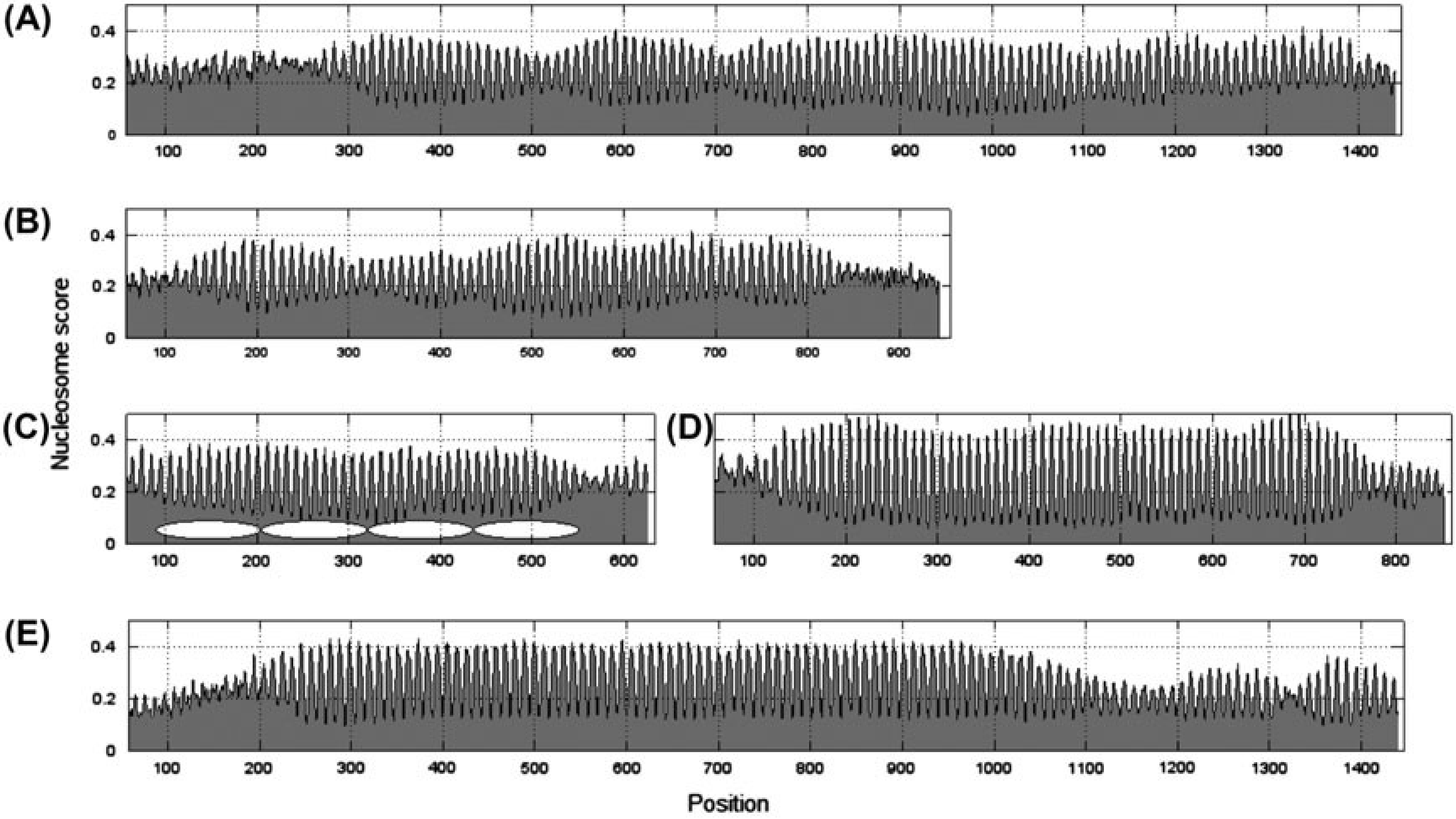
TA	**AT**
CG	**GC**
TG	AC
CA	GT
Contact with arginines	Exposed

The rest of the period is occupied by RR (AA,AG,GA,GG) and YY (TT, TC, CT, CC) dinucleotides, in their optimal partial unstacking positions

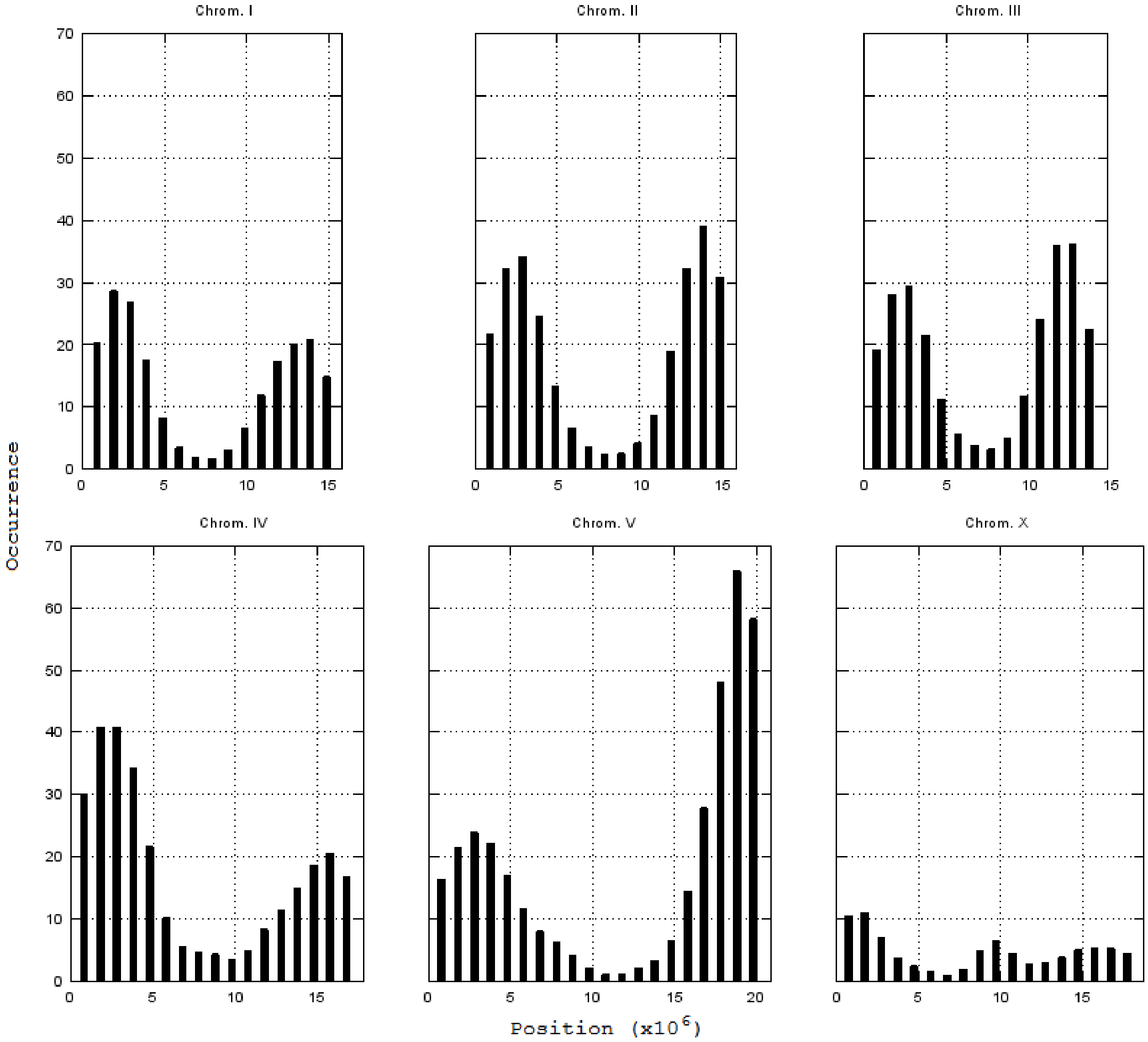
Strong nucleosomes (SNs) concentrate in centromere regions (*A .thaliana*)



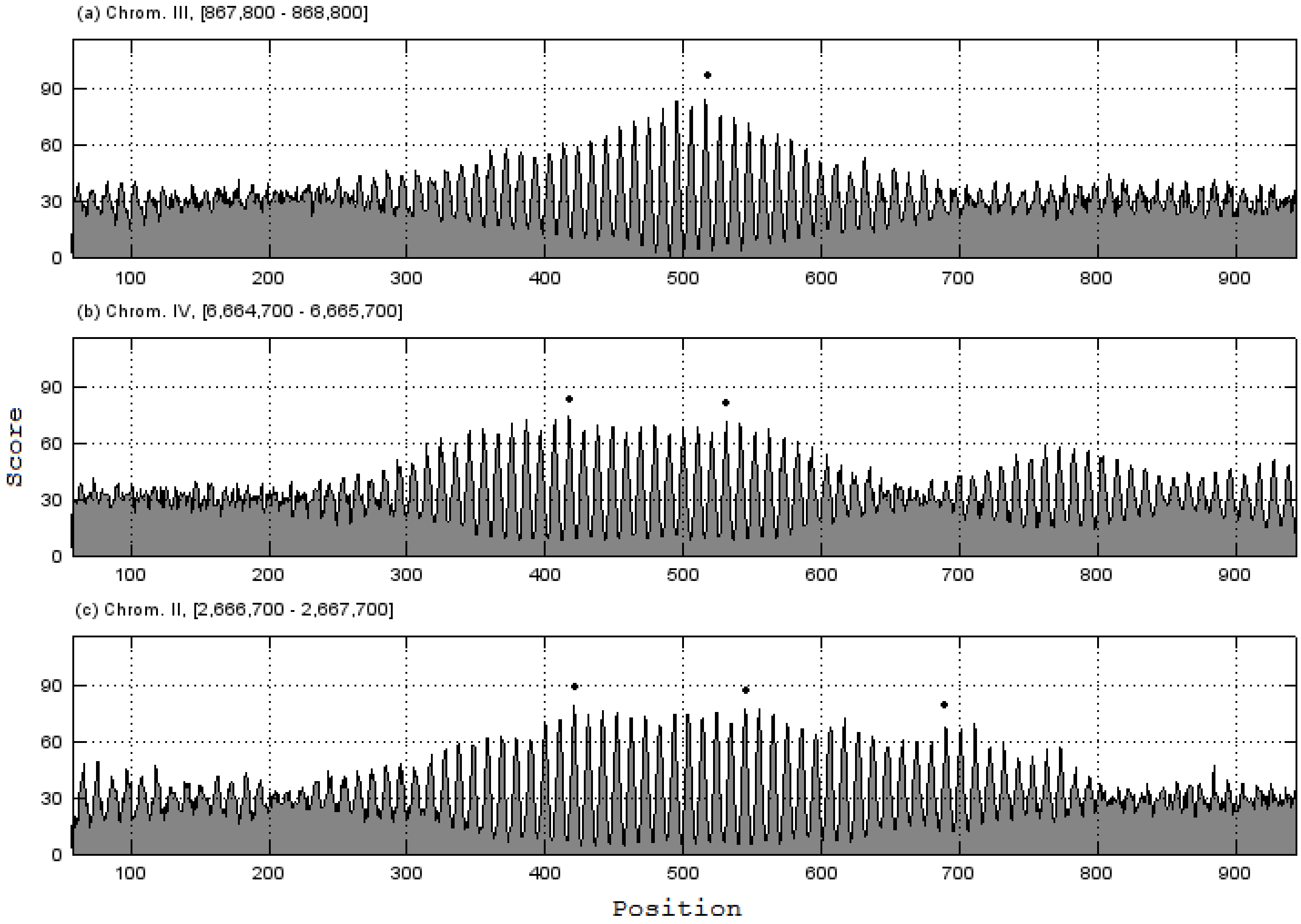
Maps of columnar chromatin structures



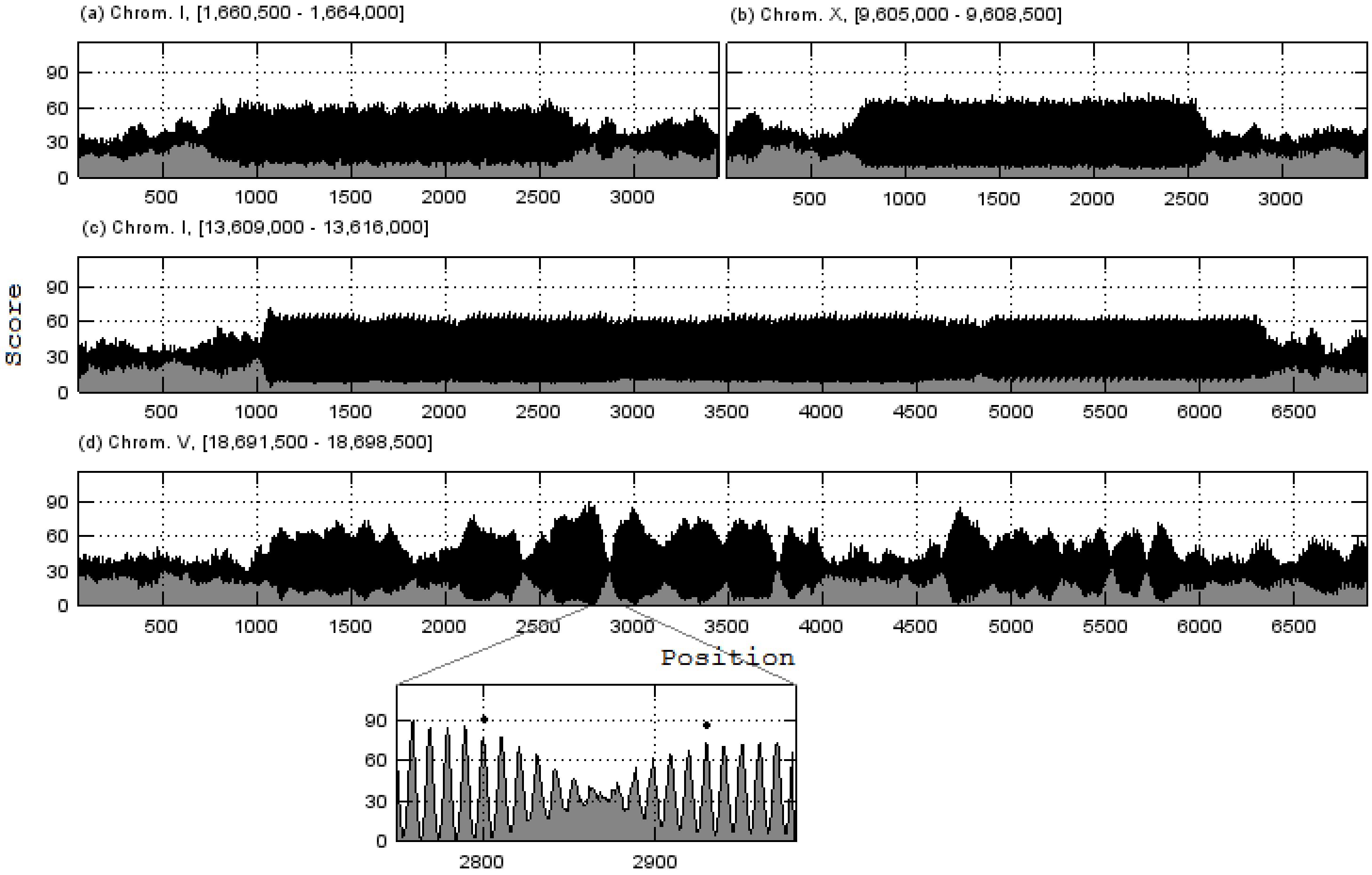
SNs in C. elegans



Mononucleosomes and short columns



SN columns and clusters



The dinucleotide stacks are placed in such positions within the nucleosome DNA period to ensure best possible bending.

The better the bending – the stronger the nucleosome.

But the bulk of the nucleosomes are only marginally stable.

Only a fraction of properly positioned dinucleotides
is present in any given nucleosome DNA sequence.

In average 40 bases in each nucleosome DNA contribute to the nucleosome positioning message. This amounts to
~20% of genome occupied by the chromatin code

Triplet code takes ~3% of genome

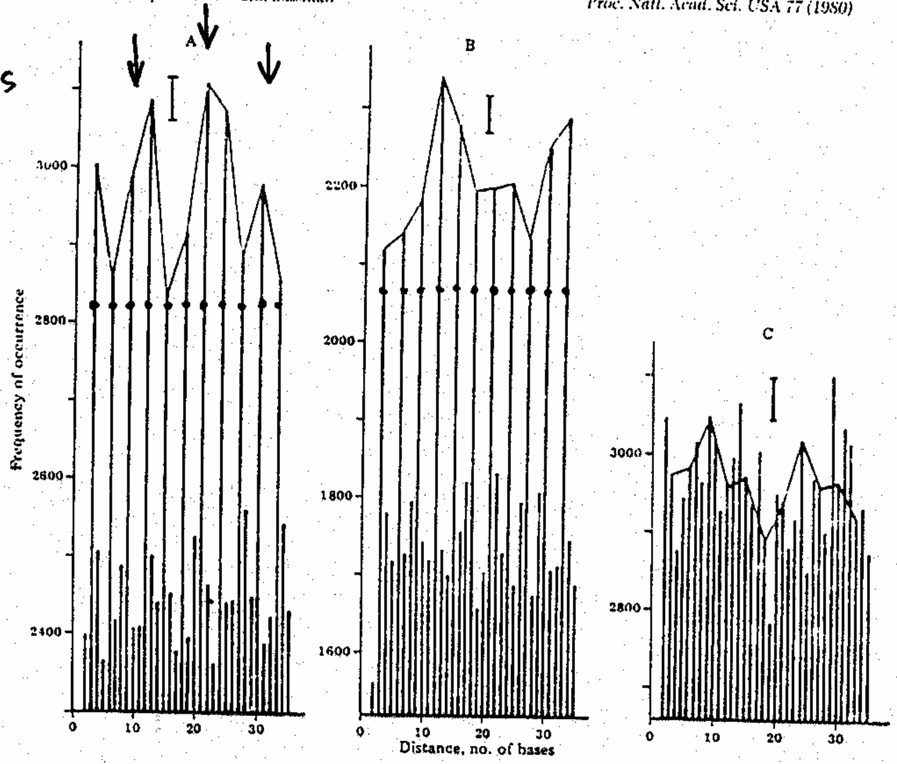
These are two major codes in the genomic sequences, and they do interact as they also overlap

Interaction between translation triplet code and chromatin code

TRIFONOV, SUSSMAN, 1980

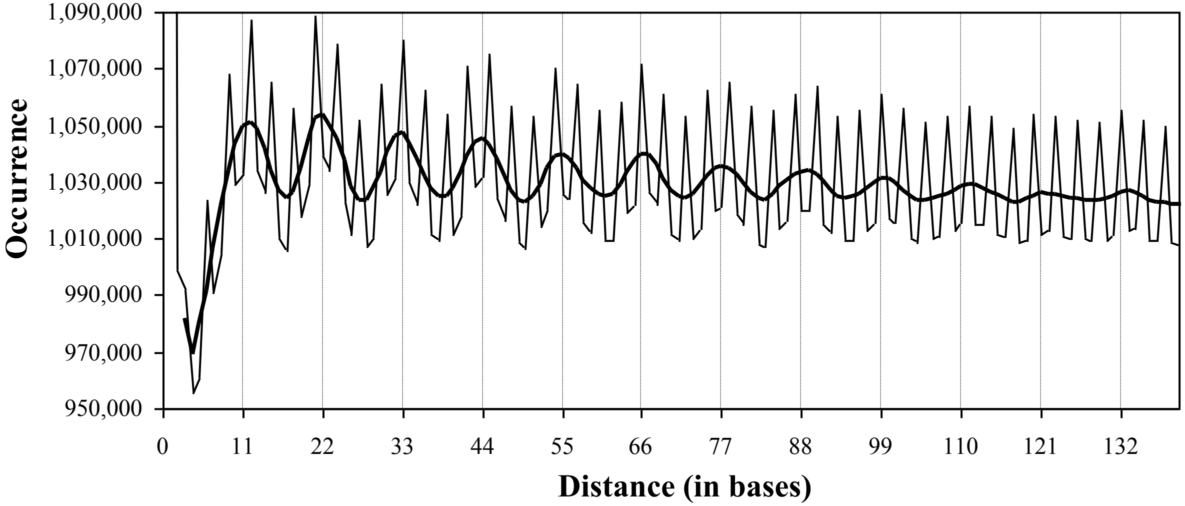
~ 10.5 BASES

3 BASES



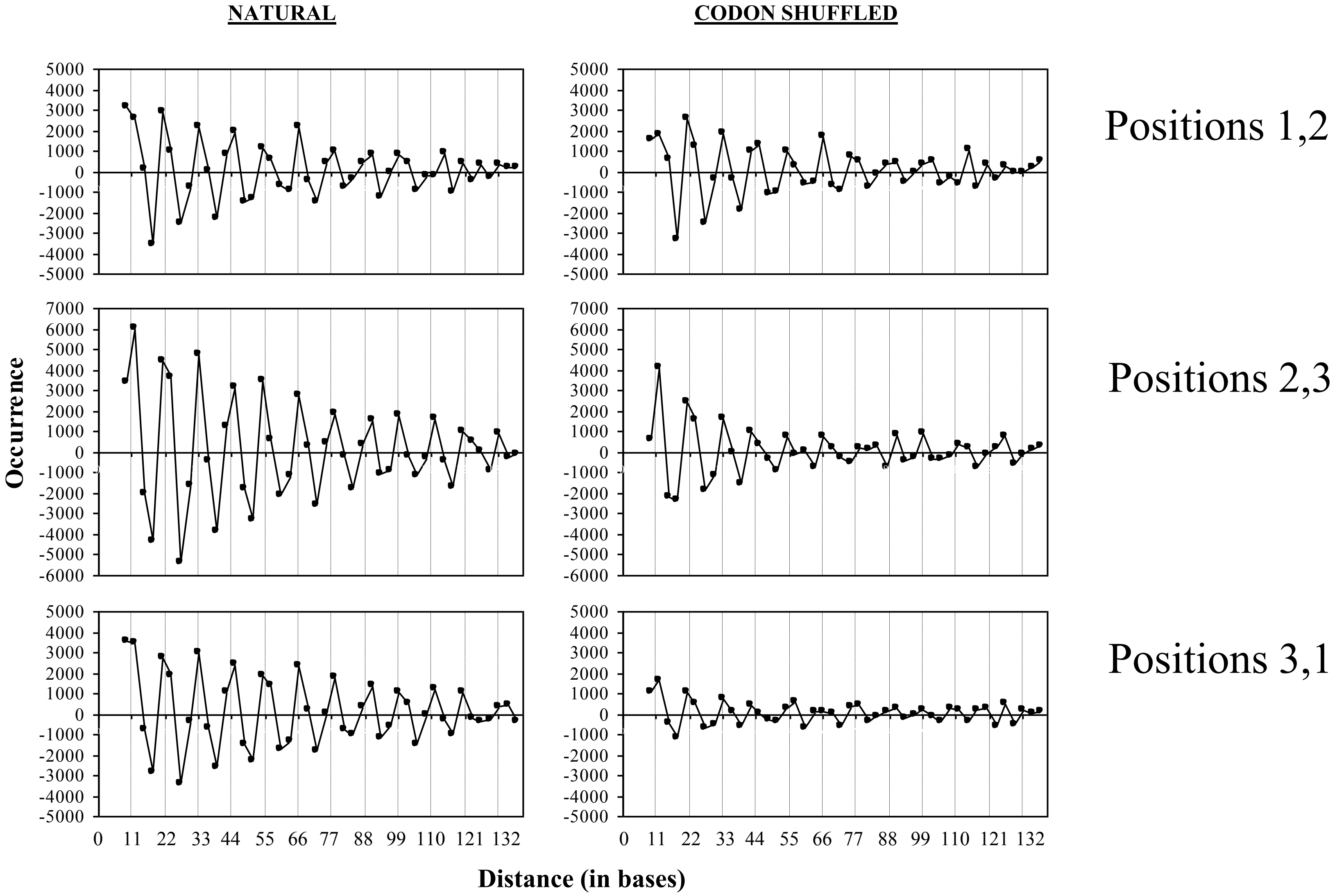
EUKARYOTES PROKARYOTES RANDOM

~30 000 BASES



Cohanim, 2006
Eubacteria

Randomizing third positions brings the oscillations down



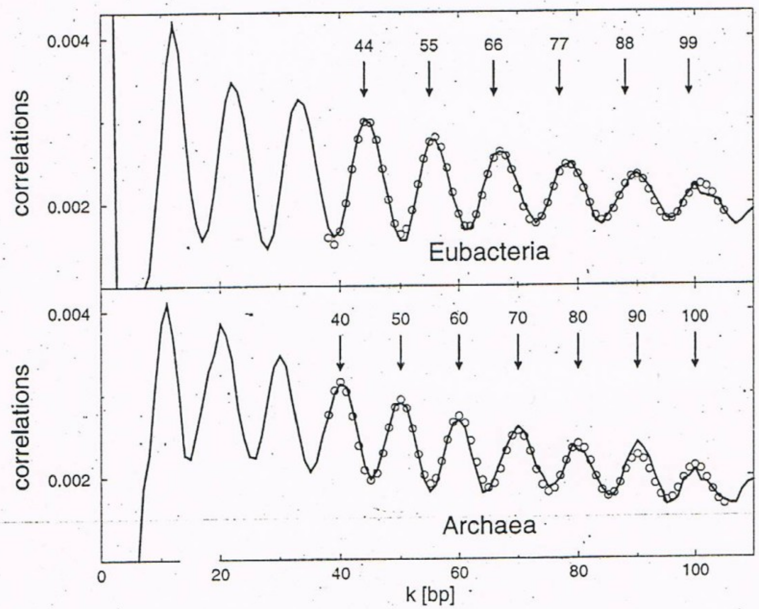


Fig. 2 Comparison of correlation functions from Eubacteria and Archaea. The functions represent the arithmetic means of WW-correlation functions from 8 eubacterial genomes and 3 archaeal genomes (listed in Table 1). The circles are obtained by nonlinear curve fitting. In order to highlight the difference in the periodicities, arrows are drawn at distances of 11 bp (upper graph) and 10 bp (lower graph).

H. HERZEL,
O. WEISS, E.T., 1998 III 1

Table 1: Periodicities of genomic DNA

	genome length	nucleotides	dinucleotides
Escherichia coli	4.6 M	11.0	11.0
Bacillus subtilis	4.2 M	11.2	11.2
Synechocystis sp. PCC6803	3.5 M	11.5	11.6
Haemophilus influenzae	1.8 M	11.2	11.0
Helicobacter pylori	1.7 M	11.2	11.2
Borrelia burgdorferi	1.0 M	10.9	–
Mycoplasma pneumoniae	0.8 M	11.3	11.4
Mycoplasma genitalium	0.6 M	11.5	11.5
Archaeoglobus fulgidus	2.2 M	10.0	10.0
Methanococcus jannaschii	1.8 M	10.0	10.0
Methanobacterium thermo.	1.8 M	10.1	–

Caption We estimate the periods from the correlation functions in the range from 38 to 105 bp via nonlinear curve fitting described in the Methods. We exclude distances below 38 bp to avoid dominance of protein correlations. The middle column presents the periods of correlations of weakly binding nucleotides (A or T) whereas the right column gives the periods of correlations of AA or TT dinucleotides. In two cases (B. b. and M. t.) the dinucleotide correlation functions exhibit no clear periodicities.

H. HERZEL,
O. WEISS,
E.T. (1998)

III 1

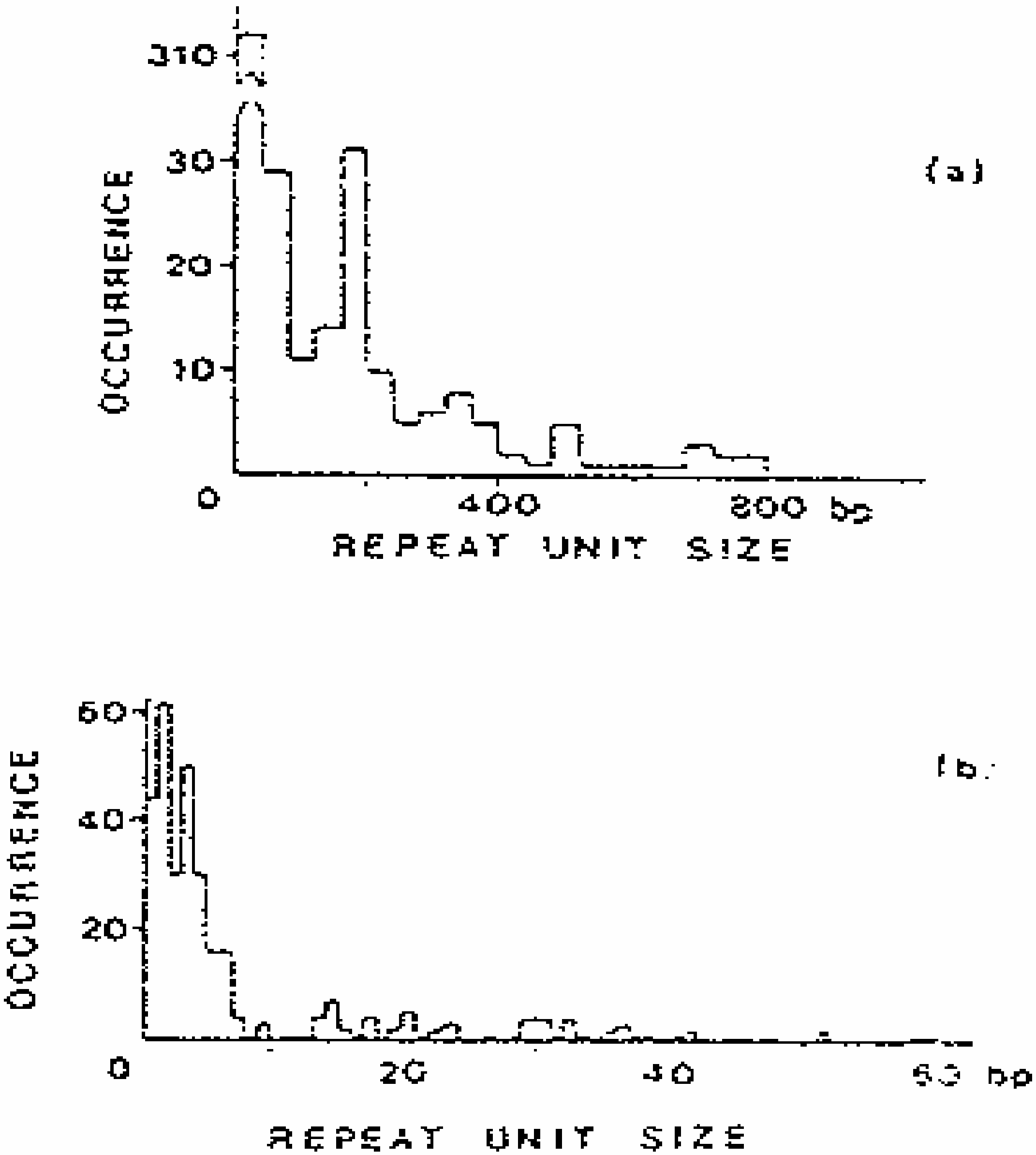
THE COLLEAGUES WITH WHOM WE AGONIZED TOGETHER ALL THESE YEARS (1978-2010) TO FINALLY REACH THE GOAL:

Joel Sussman (1978)
Thomas Bettecken (1979)
Galina Mengeritsky (1983)
Levy Ulanovsky (1983)
Roni Wartenfeld (1984)
Jacqui Beckmann (1991)
Ilya Ioshikhes (1992)
Alex Bolshoy (1992)
Kostya Derenshtein (1996)
Mark Borodovsky (1996)
Dmitry Denisov (1997)
Edward Shpigelman (1997)
Kevin Shapiro (1997)

Hanspeter Herzel (1998)
Ivo Grosse (1998)
Olaf Weiss (1998)
Yuko Wada-Kiyama (1999)
Kentaro Kuwabara (1999)
Yasuo Sakuma (1999)
Ryoiti Kiyama (1999)
Yoshiaki Ohnishi (1999)
Michael Zhang (1999)
Jiri Fajkus (2001)
Toshimichi Ikemura (2003)
Takashi Abe (2003)
Simon Kogan (2003)

M.Kato (2003)
Amir Cohanim (2005)
Yehezkiel Kashi (2005)
Fadil Salih (2007)
Bilal Salih (2007-2014)
Idan Gabdank (2009)
Danny Barash (2009)
Zakharia Frenkel (2009)
Alexandra Rapoport (2010)
Jan Hapala (2010-2014)
Vijay Tripathi (2013)
Reshma Nebhani (2014)

Modulation
(fast adaptation)
code


310
30
20
10
0
400
800 bp
OCCURRENCE
REPEAT UNIT SIZE
(a)
60
40
20
0
20
40
60 bp
OCCURRENCE
REPEAT UNIT SIZE
(b)

MODULATION OF TRANSCRIPTION

Unit / No. of repeats / location / reference

A 20-55 upstream of *ADR2* gene of *S. cerevisiae* Nature 304, 652, 1983

T 11-45 upstream of *Dictyostellium* actin genes NAR 22, 5099, 1994

T 9-42 Gcn4-activated transcription, *his3* gene, yeast EMBO J 14, 2570, 1995

T 10-80 upstream, vaccinia virus late promoters JMB 210, 771, 1989

GT 30-130 *CAT* constructs, monkey, human cells MCB 4, 2622, 1984

RY 94,144 mouse *ADH1* gene, first intron Gene 57, 27, 1987

ACCGA 5-12 UAS1 site of yeast *CYC1* gene MCB 6, 4690, 1986

CTTCC 2,3 upstream activator of yeast *PGK* gene NAR 16, 8245, 1988

AARKGA 2-8 human IFN beta gene, PRDI element Science 236, 1237, 1987; EMBO J 8, 101, 1989

ATCTTTC 15-28 Between promoters P2 and P1 of adhesin genes of *H. influenzae,* PNAS 96, 1077, 1999

AGGGCAGAGC 1-3 mouse •DRE element, •-globin promoter MCB 10, 972, 1990

GGGGCGGGGC 1,2 Sp1 sites, adenovirus early promoter JBC 266, 20406, 1991

CAAAAATGCC 9-35 transient expression of galactokinase BBRC 180, 1273, 1991

11 bp 1-4 mouse metallothionein I gene, MREa element, MCB 5, 1480, 1985

12 bp 1,3 bovine papilloma virus, E2 site EMBO J 7, 525, 1988

12 bp 1-4 human IFN beta gene, PRDII element EMBO J 8, 101, 1989

12 bp 1-6 MRE element of mouse metallothionein-I promoter, Nature 317, 828, 1985

14 bp 1-4 soybean heat shock promoter element JMB 199, 549, 1988

14 bp 1-4 C. elegans HS element in mouse cells MCB 6, 3134, 1986

14 bp 1-4 Drosophila HS element in yeast cells NAR 14, 8183, 1986

14 bp 1-5 cell-cycle dependent transcription of the yeast *HO* gene, Cell 42, 225, 1985

16 bp 1,5 human oligoA synthetase gene EMBO J 7, 411, 1988

17 bp 1,3 yeast allantoate permease gene, GATAA containing element, MCB 9, 602, 1989

17 bp 1-8 SV40-rat construct, preproinsulin gene MCB 8, 2737, 1988

17 bp 1,5 yeast allantoate permease gene MCB 9, 602, 1989

18 bp 1-5 immediately early genes, human cytomegalovirus, JV 63, 1435, 1989

31 bp 1-8 NF-•B factor binding site upstream of mouse beta-globin gene, JMB 214, 373, 1990

32 bp 1,2 yeast allantoate permease gene MCB 9, 602, 1989

32 bp 1,2 immediately early genes, human cytomegalovirus, JV 63, 1435, 1989

32 bp 1-4 upstream of the *SUC2* gene of *S. cerevisiae,* MCB 6, 2324, 1986

39 bp 1,2 copper-induced transcription of yeast copper-metallothionein gene, MCB 6, 1158, 1986

57 bp 1-4 H element, Ty1 transposon, yeast *CYC7* MCB 8, 5299, 1988

60 bp 1-3 cauliflower mosaic virus activator EMBO J 7, 1589, 1988

113 bp n expression of a reporter gene Gene 189, 13, 1997

122 bp 1-4 maize streak virus activator element EMBO J 7, 1589, 1988

240 bp n rDNA spacer in Drosophila NAR 10, 7017, 1982; PNAS 85, 5508, 1988; MCB 10, 4667, 1990

ENHANCERS

Unit / No. of repeats / location / reference

12 bp 1-3 SV40 constructs expressing E2 peptide of bovine papilloma virus, EMBO J 7, 525, 1988

12 bp 2-6 ftz-dependent enhancer, Drosophila Nature 336, 744, 1988

14 bp 1,2 phorbol ester induction, HIV, R region MCB 7, 3994, 1987

16 bp 1,5 interferon-responsive, *tk* gene constructs, transfected monkey cells, EMBO J 7, 1411, 1988

17 bp 1,2 yeast upstream activator sequence, in HeLa cells, Cell 52, 169, 1988

17 bp 1,4 CRE enhancer of human vasoactive intestinal peptide gene, PNAS 85, 6662, 1988

18 bp 1,2 cAMP responsive, human glycoprotein hormone, MCB 7, 3759, 1987

20 bp 4,8 core of SV40 enhancer, constructs JMB 201, 81, 1988

30 bp 11-21 EBV transcription and replication MCB 6, 3838, 1986

50 bp 1-6 herpes virus saimiri JMB 201, 81, 1988

57 bp 1-4 H element of Ty1 transposon, *CYC7* gene MCB 8, 5299, 1988

60 bp n rDNA spacer, *X. laevis* Cell 35, 449, 1983

68 bp 1-3 BKV transcription Science 222, 749, 1983

72 bp 1-3 SV40, constructs JV 55, 823, 1981

81 bp n rDNA spacer, *X. laevis* Cell 35, 449, 1983

99 bp 1,2 murine Akv retrovirus JV 64, 3185, 1990

109 bp 1,2 MCF virus, oncogenicity JV 63, 1284, 1989

140 bp 1-13 mouse rRNA gene spacer PNAS 87, 7527, 1990

OTHER ACTIVITIES

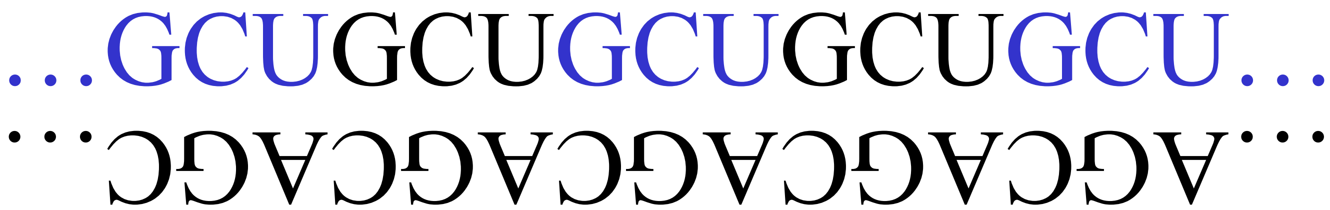
Unit / No. of repeats / location / reference

A 17-20 promoter region, *Mycoplasma* surface antigen variation, EMBO J 10, 4069, 1991
C 8-44 5'-UTR, virulence of mengovirus JV 70, 2027, 1996
GT n recombination, mouse somatic cells MCB 6, 3948, 1986
GT n recombination, Rec A binding JMB 273, 105, 1997
GT n meiosis, yeast MCB 6, 3934, 1986
CG n recombination, mouse somatic cells MCB 6, 3948, 1986
AAG 2-8 exon M2 of mouse IG• gene, enhancement of splicing, MCB 14, 1347, 1994
GACA 22-35 phenotypic switching of a lypopolysaccharide epitope, PNAS 93, 11121, 1996
AAGTGA 4-8 upstream inducible element, human beta interferon gene, JV 64, 3063, 1990
GAAAGT 2,4 mediates virus-inducible transcription of human interferon genes, PNAS 88, 1369, 1991
ATAGTAAA 13,17 iteron in plasmid pAD1 of *E. faecalis*, mating response to sex pheromone, J Bact 177, 5453, 1995
CTGAGGTCAA 1-5 F2 half-element of chicken lysozyme silencer S-2.4 kb, Cell 61, 505, 1990
14 bp 1-5 3'-terminal UTR, tobacco vein mottling virus, disease symptom severity, PNAS 88, 9863, 1991
17 bp 1-8 modulation of translation, rat preproinsulin, MCB 8, 2737, 1988
31 bp 1-6 packaging of Adenovirus Type 5 DNA JV 64, 2047, 1990
40 bp 1,2 polyoma virus expression JV 62, 3896, 1988
46 bp 1-4 virus-responsive element of IFN•1 promoter, induced expression, Cell 50, 1057, 1987
48 bp 2,5 transforming activity of a retrovirus NAR 26, 4868, 1998
68 bp 1-3 BK virus, transforming activity JV 55, 867 & 823, 1985
240 bp 13-350 modulation of meiotic drive, Rsp of SD system of *Drosophila* Nature 332, 394, 1988; Cell 54, 179, 1988
TG 20-30 regulation of period in circadian rhythm Science 278, 2117, 1997
SKQPFRK 2-7 chloroplast ribosomal protein S18 FEBS Let 279, 190, 1991
YSPTSPS 9-26 yeast RNApolII, modulation, response to enhancer signals Nature 347, 491, 1990; MCB 8, 321, 1988
YSPTSPS 3-78 mouse RNApolII, modulation MCB 8, 330, 1988
12 aa 7-11 Mycoplasma surface antigen variation EMBO J 10, 4069, 1991
31 aa 3,4 stage- and tissue specificity of human microtubule-associated protein tau, EMBO J 8, 393, 1989
34 aa 0-17 plant resistance to bacterial spot disease, Nature 356, 172, 1992
42 aa 3-13 segment polarity armadillo gene, *Drosophila*, phenotypic series, Cell 63, 1167, 1990
53 aa 11-50 kringle IV, processing and secretion of apolipoprotein (a), JBC 271, 32403, 1996
82 aa 1-9 alpha C protein, *Streptococci*, modulation of host immunity, PNAS 93, 4131, 1996

Diseases with repeats in non-coding regions

		Triplet	**n** in norm/pathology
FRAXA	(fragile X syndrome)	CGG	6-53/230+
FXTAS	(FRAXA associated tremor/ataxia syndrome)	CGG	6-53/55-200
FRAXE	(fragile XE mental retardation)	GCC	6-35/200+
FRDA	(Friedreich's ataxia)	GAA	7-34/100+
DM	(myotonic dystrophy)	CTG	5-37/50+
SCA8	(spinocerebellar ataxia Type 8)	CTG	16-37/110-250

from Wikipedia



...GCUGCUGCUGCUGCU...

...AGCAGCAGCAGCAGC...

this is
GCU repeat,
but also CUG repeat,
UGC repeat,
AGC repeat,
GCA repeat,
and CAG repeat

Diseases with repeats in non-coding regions

	Triplet	n in norm/pathology
FRAXA (fragile X syndrome)	CGG GCC	6-53/230+
FXTAS (FRAXA associated tremor/ataxia syndrome)	CGG GCC	6-53/55-200
FRAXE (fragile XE mental retardation)	GCC GCC	6-35/200+
FRDA (Friedreich's ataxia)	GAA GAA	7-34/100+
DM (myotonic dystrophy)	CTG GCU	5-37/50+
SCA8 (spinocerebellar ataxia Type 8)	CTG GCU	16-37/110-250

Polyglutamine diseases (polyCAG = polyGCU)

		n in norm/pathology
DRPLA	(dentatorubropallidoluysian atrophy)	6-35/49-88
HD	(Huntington's disease	10-35/35+
SBMA	(spinobulbar muscular atrophy)	9-36/38-62
SCA1	(spinocerebellar ataxia Type 1)	6-35/49-88
SCA2		14-32/33-77
SCA3		12-40/55-86
SCA6		4-18/21-30
SCA7		7-17/38-120
SCA17		25-42/47-63

from Wikipedia

Tandem repeat expansion diseases and disorders

Repeat/Copy number ***n*** range/Location/Disease or disorder/References

(3 bp/1 aa) *n* 5 to over 200 5′-, 3′- and over coding regions 15 different neurodegenerative and other diseases Usdin and Grabczyk, 2000 Brais et al., 1998 Delot et al., 1999

(4 bp) ***n*** 75 to 11.000 intron 1 of *ZNF9* myotonic dystrophy gene type 2 Liquori et al., 2001

(5 bp) ***n*** 10 to 4.500 intron 9 of *SCA10* gene type 10 spinocerebellar ataxia Matsuura et al., 2000

(12 bp) ***n*** 2 to over 60 5′ from cystatin B gene progressive myoclonus epilepsy Lalioti et al., 1997

(14 bp) ***n*** 40 to 150 5′ from insulin gene type 1 susceptibility to diabetes Bennett et al., 1995, Kennedy et al., 1995

(15 bp) and (18 bp) ***n*** few to 90 5′ from cystatin B gene progressive myoclonus epilepsy Virtaneva et al., 1997

(24 bp/8 aa) ***n*** 5 to 34 coding region of the prion protein gene Creutzfeldt-Jakob disease Cochran et al., 1996

(28 bp) ***n*** 30 to 100 3′ from *HRAS1* proto-oncogene ovarian cancer risk Phelan et al., 1996

(342 bp/114 aa) ***n*** 15 to 37 apo(a) coding region Lp(a) level, susceptibility to atherosclerosis and thrombosis, Lindahl et al., 1990, Koschinsky et al., 1990

(3200 bp)***n*** 2 to 100 *FSHD* gene region FSHD muscular dystrophy van Deutekom et al., 1993

There is only few percent difference between genomes of human and chimpanzee.

Mostly in copy numbers of simple repeats.

Humans are retuned monkeys

PROTEOMIC CODE

(PROTEIN SEQUENCE MODULES)

Two related sequences, aligned

33% match

```
Q816J5
DVNLPKFDGFYWCRQIRHESTCPIIFISARAGEMEQIMAIESGADDYITKPFHYDVVMAKIKGQLRR
|||||-|||----|--|--|----------------------||||---|||------|-----|||
DVNLPGIDGWDLLRRLRERSSARVMMLTGHGRLTDKVRGLDLGADDFMVKPFQFPELLARVRSLLRR
Q7DCC5
```

```
CPIIFISARAGEMEQIMAIE Q816J5 Two-component response regulator B. cereus
 ||||||||   | | ||||
VPIIFISARDSDMDQVMAIE Q97IX4 Response regulator                C. acetobutylicum
|| ||||||| | | |   |
VPVIFISARDADIDRVLGLE O32192 Transcr. regulatory protein cssR B. subtilis
||  | ||||  ||||||||
VPILFLSARDEEIDRVLGLE Q89D26 Two-component response regulator B. japonicum
 ||  | || || | |||||
IPIIMLTARSEEFDKVLGLE Q8R9H7 Response regulators               Th. tengcongensis
  | ||||||   ||| |||
SRIMMLTARSRLADKVRGLE Q88RT2 heavy metal response regulator   Ps. Putida
 | ||||   || ||||||
ARVMMLTGHGRLTDKVRGLD Q7DCC5 Two-component response regulator Ps. Aeruginosa
```

```
Q816J5 Two-component response regulator
DVNLPKFDGFYWCRQIRHESTCPIIFISARAGEMEQIMAIESGADDYITKPFHYDVVMAKIKGQLRR
|||||-|||----|--|--|----------------------||||---|||------|-----|||
DVNLPGIDGWDLLRRLRERSSARVMMLTGHGRLTDKVRGLDLGADDFMVKPFQFPELLARVRSLLRR
Q7DCC5 Probable two-component response regulator
```

No-match relatives

```
LEVALALSQADIIVRDALVS Q8UBQ7 Uroporphyrin-III C-methyltransferase         A. tumefaciens
|  | || ||| || ||||
LHAANALRQADVIVHDALVN Q92P47 probable Uroporphyrin-III C-methyltransferase   Rh. meliloti
| |   |  ||||||||||
LRAQRVLMEADVIVHDALVP Q8YEV9 Uroporphyrin-III C-methyltransferase         B. melitensis
||| | ||||||||||||||
LRAHRLLMEADVIVHDALVP Q98GP6 Siroheme synthase (precorrin methyltransferase) Rh. loti
|   ||| |||||
LKGQRLLQEADVILYADSLV Q8DLD2 Precorrin-4 C11-methyltransferase            S. elongatus
 ||||   ||||| ||  |||
IKGQRIVKEADVIIYAGSLV Q8REX7 Precorrin-4 C11-methyltransferase            F. nucleatum
 ||||      |||||||||
VKGQRLIRQCPVIIYAGSLV Q88HF0 Precorrin-4 C11-methyltransferase            Ps. putida
| |  ||  |||  ||||||
VRGRDLIAACPVCLYAGSLV Q8UBQ5 Precorrin-4 C11-methyltransferase            A. tumefaciens
```

```
Q8UBQ7 methyltransferase
HVWLAGAGPGDVRYLTLEVALALSQADIIVRDALVS
-|---|||||-----|--------------------
TVHFIGAGPGAADLITVRGRDLIAACPVCLYAGSLV
Q8UBQ5 methyltransferase
```

No-match relatives

Methyltransferases

```
LEVALALSQADIIVRDALVS Q8UBQ7
|  | || ||| || ||||
LHAANALRQADVIVHDALVN Q92P47
| |   |  ||||||||||
LRAQRVLMEADVIVHDALVP Q8YEV9
||| | ||||||||||||||
LRAHRLLMEADVIVHDALVP Q98GP6
|   ||| |||||
LKGQRLLQEADVILYADSLV Q8DLD2
 ||||   ||||| || |||
IKGQRIVKEADVIIYAGSLV Q8REX7
 ||||      |||||||||
VKGQRLIRQCPVIIYAGSLV Q88HF0
| |  ||  |||  ||||||
VRGRDLIAACPVCLYAGSLV Q8UBQ5
```

No-match relatives

LEVALALSQADIIVRDALVS	Q8UBQ7
VRGRDLIAACPVCLYAGSLV	Q8UBQ5

To be related

the sequences

do not have to be similar

(upto even complete mismatch)

Existing most advanced sequence alignment techniques (e. g. BLAST) would not be able to qualify such fully dissimilar sequences as relatives

unless many intermediate sequences are analyzed (that amounts to a whole research project)

One can make long

walks

from fragment to fragment in the

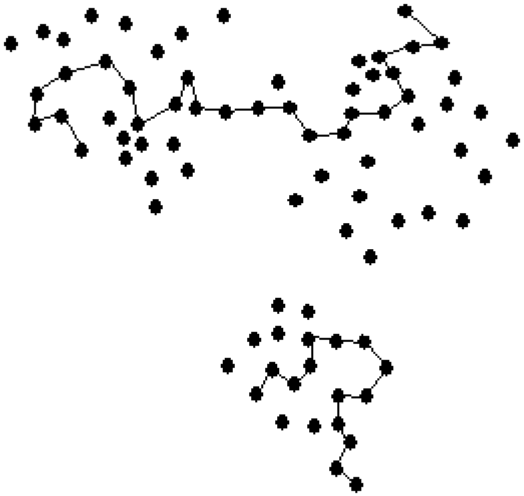
formatted protein sequence space

(sequence fragments of the same length, 20 residues,
gathered from all or many proteomes)

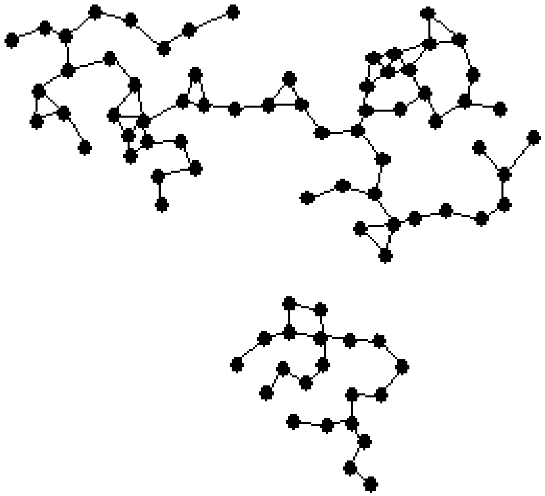
Pair-wise connected matching fragments make also

networks

WALK



NETWORK



Frenkel, 2006

60% match threshold networks:

320,000 proteins from 120 prokaryotes, ~100,000,000 fragments

The largest (monster) network 9,368,905 sequence fragments (~10% of all)

Next largest 2,535 fragments

Networks of sizes 120 to 2,535 fragments (several thousand, 3.8% of all fragments)

Small networks cover 86% of the space

35% of fragments are single, no relatives

Number of different fragments in complete (random) space:

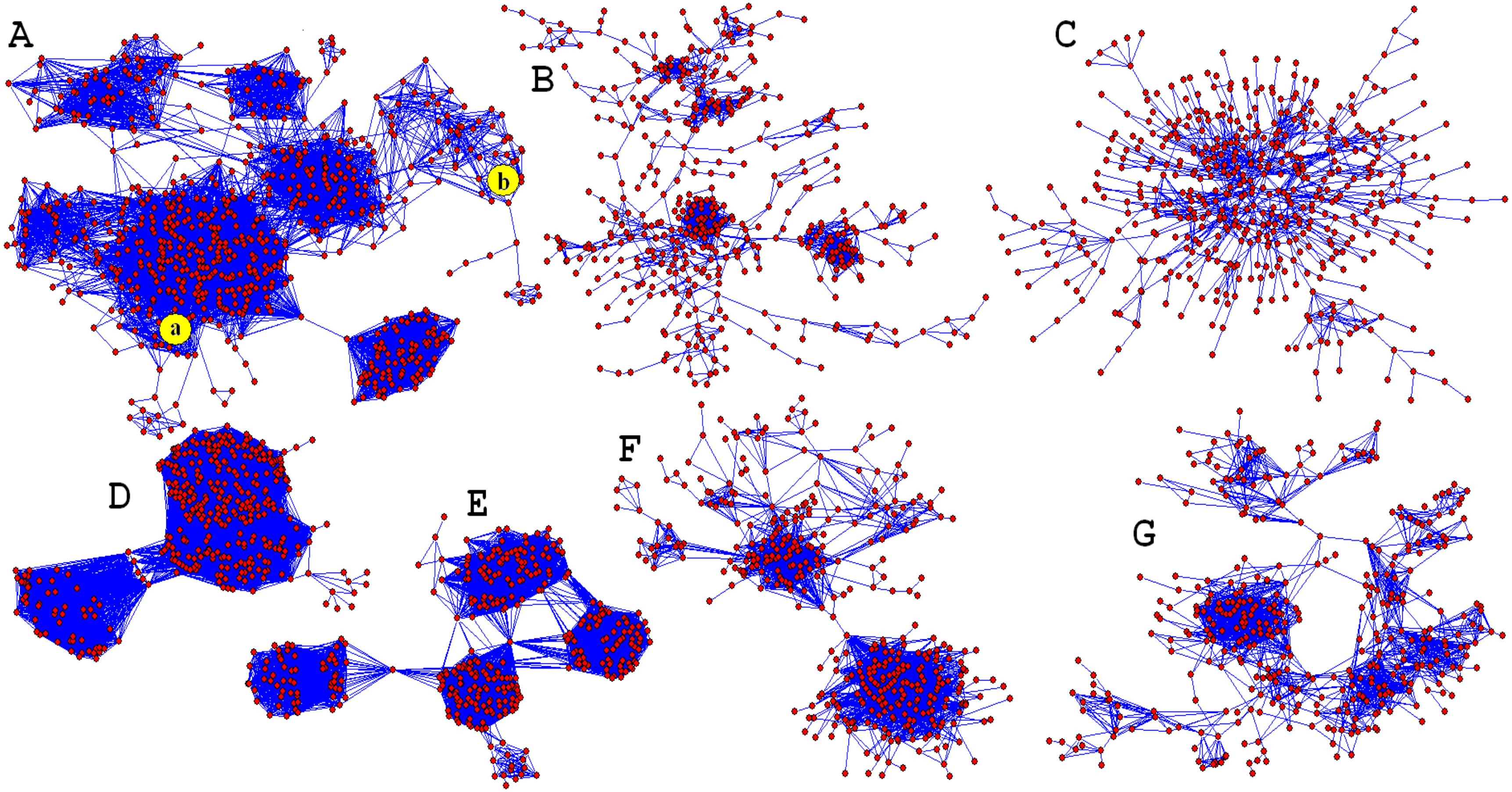
$20^{20} \sim 10^{26}$

Number of fragments in complete natural space:

$10^{7} \bullet 3 \bullet 10^{4} \bullet 300 \sim 10^{14}$

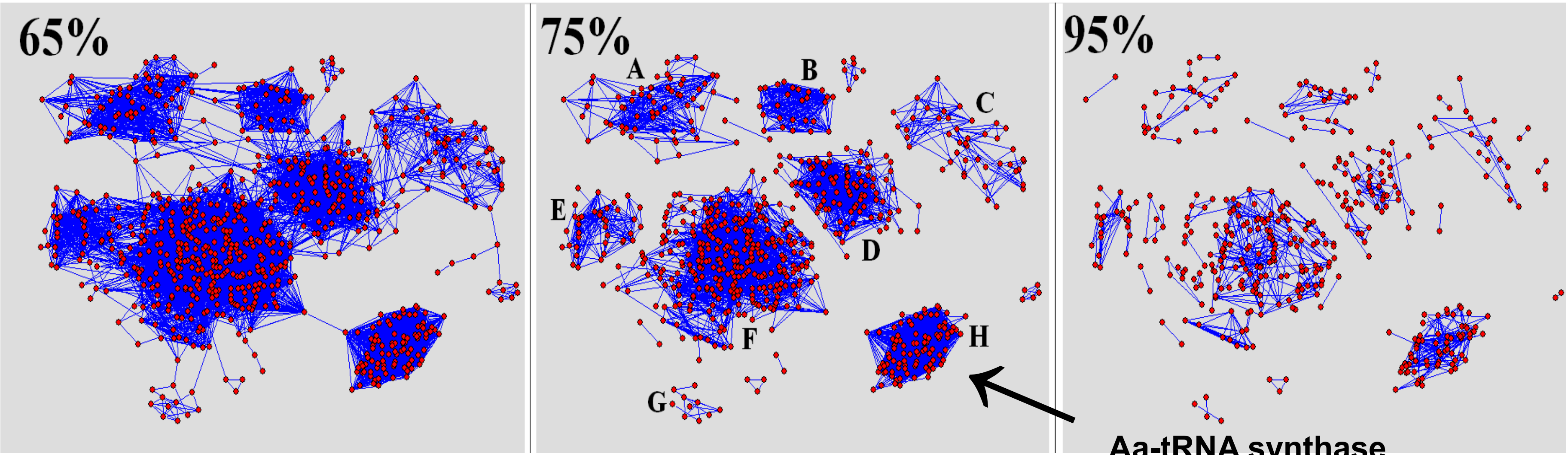
Probability that a given fragment in natural space

is randomly generated is 10^{-12}


A
a
b
B
C
D
E
F
G

Networks of fragments of aa-tRNA synthetases

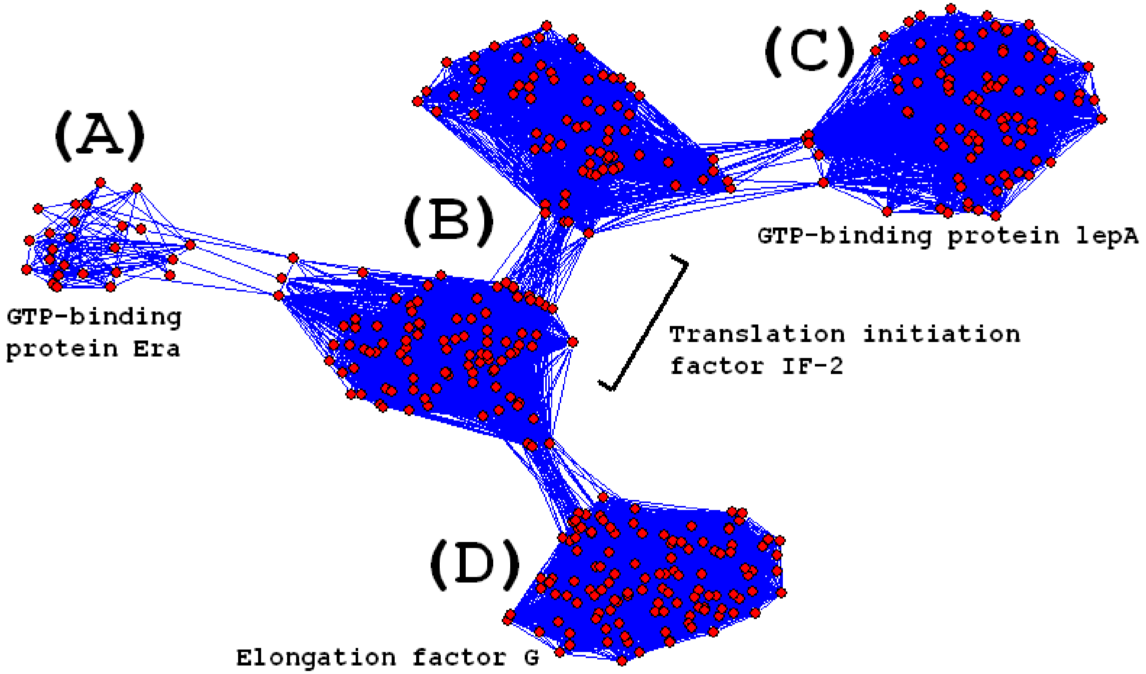
at various thresholds of sequence match



Aa-tRNA synthase module of lepA

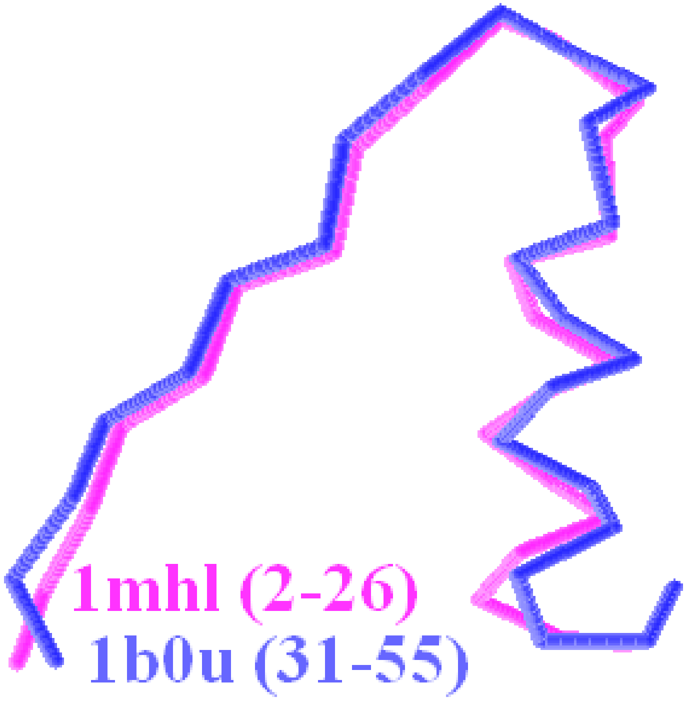
A tyr trp B met C arg trp D cys
E leu F met leu ile val G ile H lepA

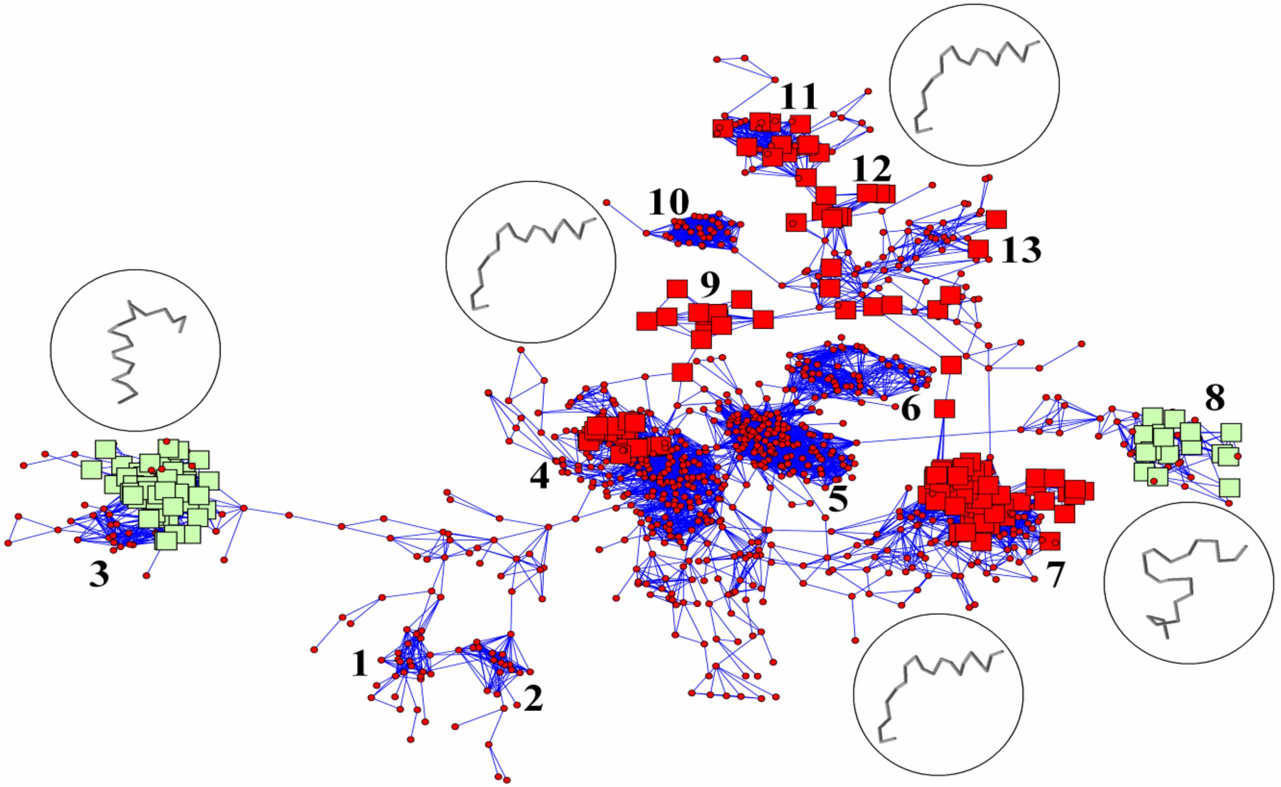
Network of GTP binding proteins



Sequence fragments with the same function
are found in the same network

```
1mh1_ c.37.1.8 Rac (GTP-
binding)
{Human (Homo sapiens)}
2                       26
QAIKCVVVGDGAVGKTCLLISYTTN
        |    ||   |
AGDVISIIGSSGSGKSTFLRCINFL
31                      55
1b0ua_ c.37.1.12 (A:) ATP-
binding subunit
of the histidine permease
{Salmonella typhimurium}
```



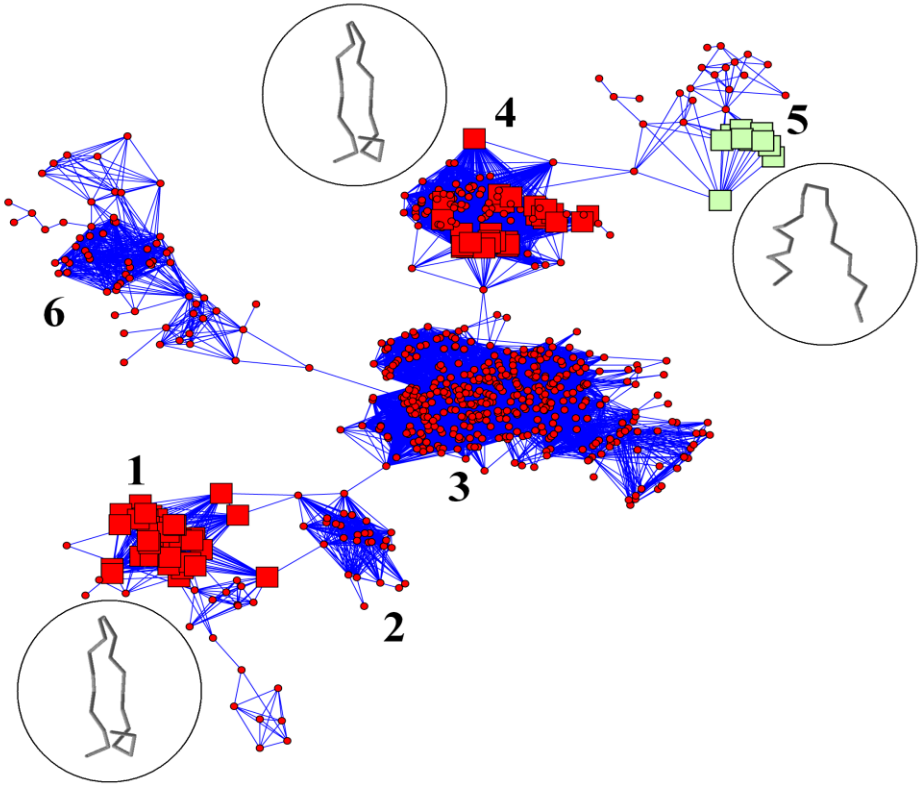


1 Putative peptidoglycan bound protein
2 Collagen adhesion protein
3 Ribosomal protein L11
4 Penicillin-binding protein 2x
5 Penicillin-binding protein 1
6 Penicillin binding protein 2A
7 D-alanyl-D-alanine carboxypeptidase

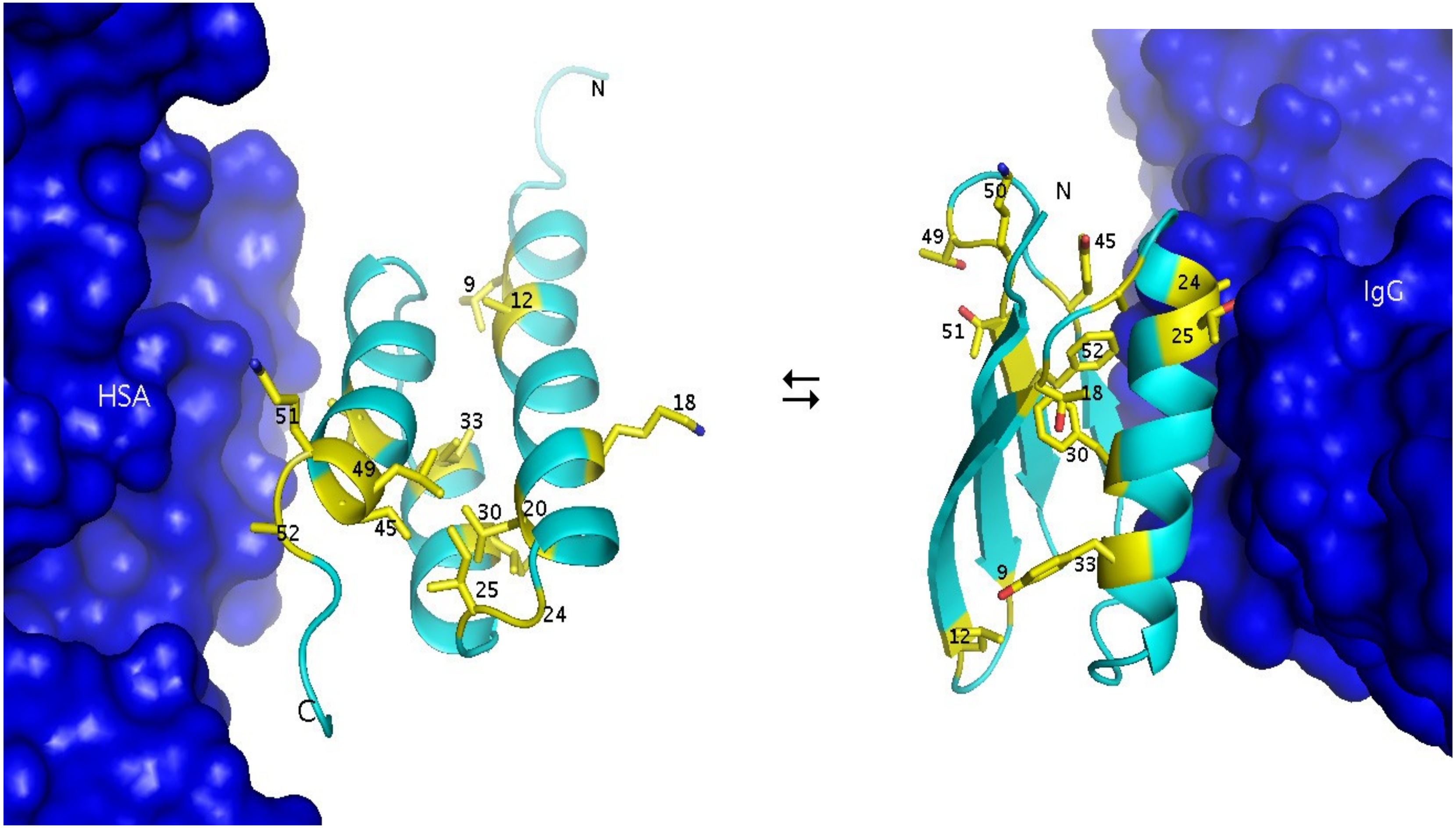
8 cytochrome

9 Beta-Lactamase
10 Mannitol-1-phosphate 5-dehydrogenase
11 glutaminase
12 Beta-lactamase
13 Esterase EstB

Fragments of the same network
have, essentially, the same structure.
Periferal fragments may be different

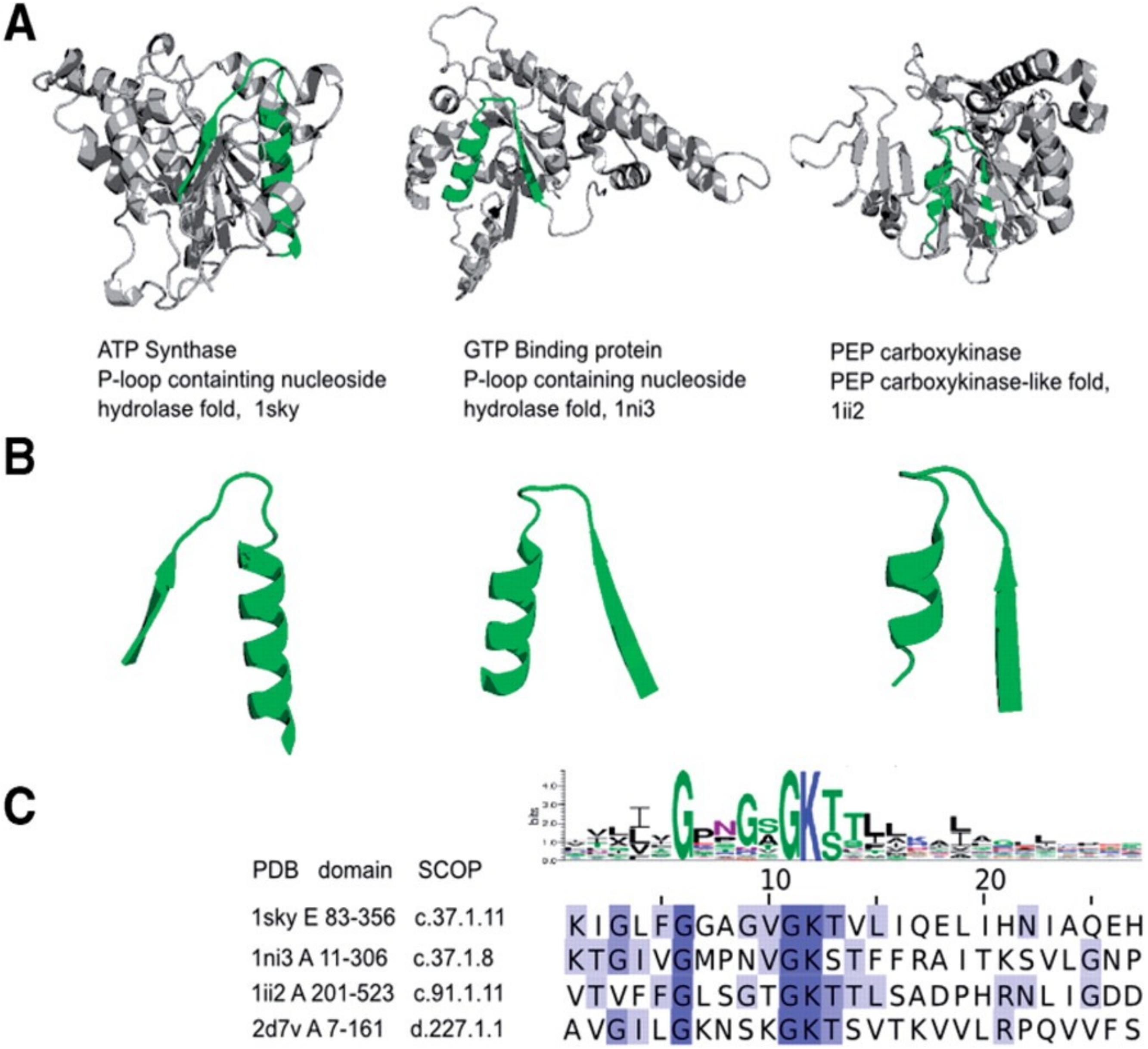

4
5
6
3
1
2

Two alternative structures with the same sequence



Lab of P. N. Bryan, 2009

Matches of the nucleotide–triphosphate-binding (p-loop) prototype in crystal structures.



ATP Synthase
P-loop containting nucleoside
hydrolase fold, 1sky

GTP Binding protein
P-loop containing nucleoside
hydrolase fold, 1ni3

PEP carboxykinase
PEP carboxykinase-like fold,
1ii2

Vibrio cholerae
unknown protein
OsmC-like fold, 2d7v

PDB	domain	SCOP	
1sky	E 83-356	c.37.1.11	KIGLFGGAGVGKTVLIQELIHNIAQEH
1ni3	A 11-306	c.37.1.8	KTGIVGMPNVGKSTFFRAITKSVLGNP
1ii2	A 201-523	c.91.1.11	VTVFFGLSGTGKTTLSADPHRNLIGDD
2d7v	A 7-161	d.227.1.1	AVGILGKNSKGKTSVTKVVLRPQVVFS

**Goncearenco A , Berezovsky I N Bioinformatics
2010;26:i497-i503**

New definition of sequence relatedness:

fragments of the same network
are relatives

	Decay of the initial sequence pattern (bottom up)	Decay of the final sequence pattern (bottom up)	Every two nearest neighbors share at least 60% identity
1	LED**A**IKA**A**KAGA**D**IIMLDNM	**LEDAIKAAKAGADIIMLDNM**	LEDAIKAAKAGADIIMLDNM
2	PED**A**PRA**A**DAGA**D**IV**L**LDNM	P**EDA**PR**AA**D**AGADI**VL**LDNM**	PEDAPRAADAGADIVLLDNM
3	PEA**A**ERA**A**ATGA**DG**VGLLRM	P**E**A**A**ER**AA**AT**GAD**GVG**L**LR**M**	PEAAERAAATGADGVGLLRM
4	PEA**A**RKA**A**ATGA**DG**VGLLRT	P**E**A**A**R**KAA**AT**GAD**GVG**L**LRT	PEAARKAAATGADGVGLLRT
5	PAD**A**RAARAFGAE**G**IGLCRT	PA**DA**RA**A**RAF**GA**EG**I**G**L**CRT	PADARAARAFGAEGIGLCRT
6	PTDFKKALLFGAE**G**VGLCRT	PT**D**FK**KA**LLF**GA**EGVG**L**CRT	PTDFKKALLFGAEGVGLCRT
7	PLDIIKALVLGAKAVGLSRT	PL**D**I**IKA**LVL**GA**KAVG**L**SRT	PLDIIKALVLGAKAVGLSRT
8	GTDIIKALAIGANLVGL**G**RM	GT**D**I**IKA**LAI**GA**NLVG**L**GR**M**	GTDIIKALAIGANLVGLGRM
9	GTDIVKAIAAGA**D**LVGI**G**RL	GT**D**IV**KA**IA**AGAD**LVGIGRL	GTDIVKAIAAGADLVGIGRL
10	**S**GDIAKAIAAGA**D**AVML**G**SL	SG**D**IA**KA**IA**AGAD**AV**ML**GSL	SGDIAKAIAAGADAVMLGSL
11	IGLIEKAKAEGA**D**AVIL**G**CT	IGLIE**KA**KAE**GAD**AVI**L**GCT	IGLIEKAKAEGADAVILGCT
12	KRLVEIAKLEGA**D**AICH**G**CT	KRLVEI**A**KLE**GAD**A**I**CHGCT	KRLVEIAKLEGADAICHGCT
13	ARIVEIAKACGA**D**AIHP**G**YG	ARIVEI**A**KAC**GAD**A**I**HPGYG	ARIVEIAKACGADAIHPGYG
14	EKIIAAAKASGAEAIHP**G**YG	EKIIAA**A**KAS**GA**EA**I**HPGYG	EKIIAAAKASGAEAIHPGYG
15	EKLLAVAKRSGA**D**AVHP**G**YG	EKLLAV**A**KRS**GAD**AVHPGYG	EKLLAVAKRSGADAVHPGYG
16	EK**A**LAALESSGA**D**AVMI**G**RG	EKALAALESS**GAD**AV**M**IGRG	EKALAALESSGADAVMIGRG
17	LK**A**RAVLDYTGA**D**ALMI**G**R**A**	LKARAVLDYT**GAD**AL**M**IGRA	LKARAVLDYTGADALMIGRA
18	KK**A**F**E**VLQITQA**DG**LMI**G**R**A**	KKAFEVLQITQ**AD**GL**M**IGRA	KKAFEVLQITQADGLMIGRA
19	Q**NA**K**E**VYKITKC**DG**LMI**G**R**A**	QNAKEVYKITKC**D**GL**M**IGRA	QNAKEVYKITKCDGLMIGRA
20	Q**NA**K**E**ILGIDSV**DG**L**L**I**GSA**	QNAKEILGIDSV**D**GLLIGSA	QNAKEILGIDSVDGLLIGSA
21	**SNA**K**EL**MGVANV**DGAL**I**GGA**	SNAKELMGV**A**NV**D**GALIGGA	SNAKELMGVANVDGALIGGA
	SNAAELFAQPDIDGALVGGA	SNA**A**ELF**A**QPDI**D**GALVGGA	SNAAELFAQPDIDGALVGGA

Careful with consensus!

The words

COOKY

MANGO

MELON

HONEY

SWEET

all suggest something sweet or sweet-sour
and could be considered, thus, as recognition sequences for the 'sweet' quality. Their consensus sequence, however, conveys a rather different message:

MONEY

```
prima
prime      flack
pride      flock                          crate is cage
bride      frock                          crave is desire
bribe      crock                          craze is obsession
tribe      crack                          crock is drunk
trice      track      probe               flack is press agent
trace------trace      prone------prone   flock is web browser
trade      truce      prune      phone   grate is grid
grade      truck      prunk               graze is scratch
graze      trunk------trunk               prunk is preppy punk
grape      drunk      trank               trank is relax
grace                 trans
grate
grave
crave
crate
crane
craze
```

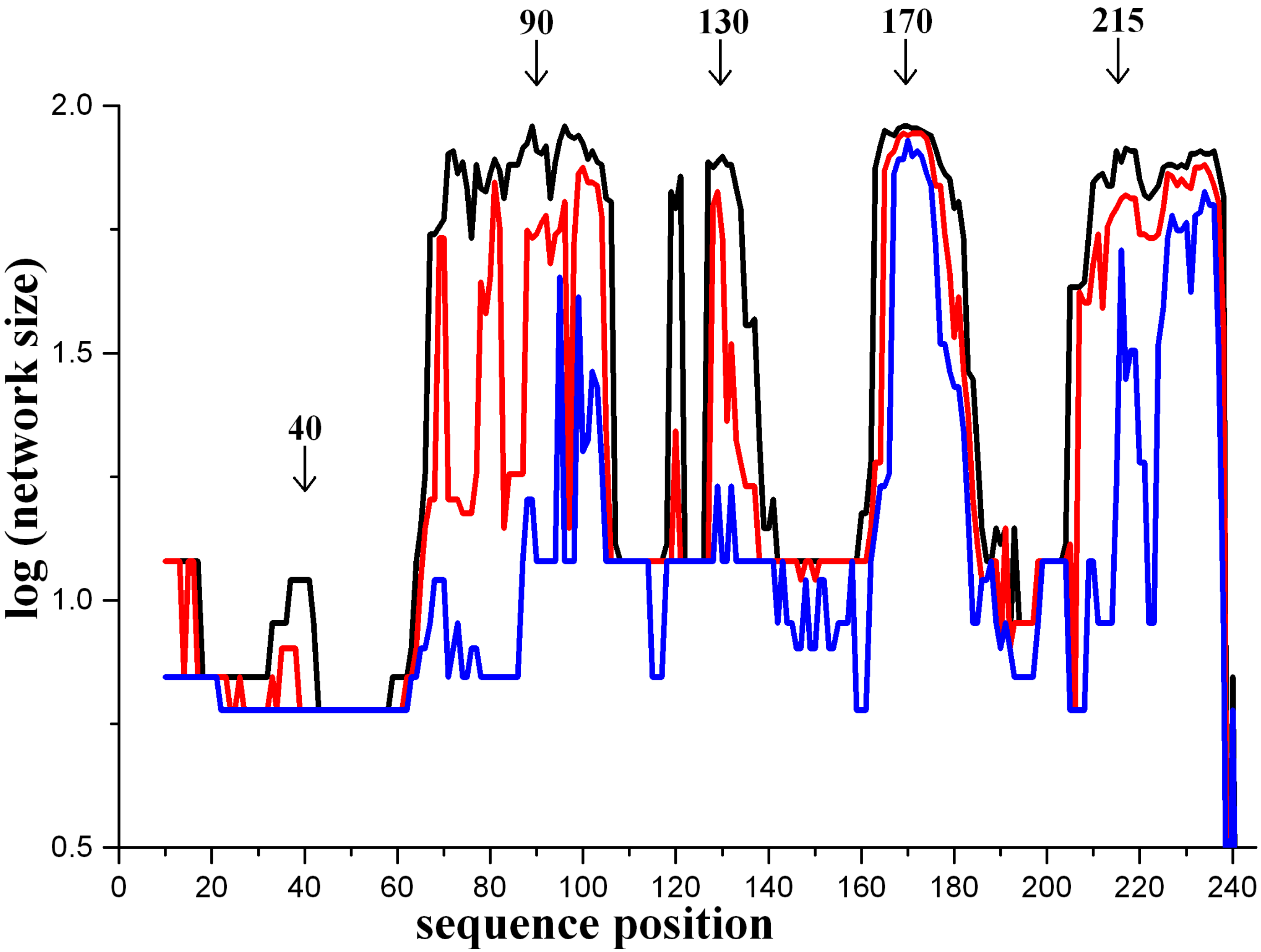
Every fragment
of the precalculated space
is tagged (protein, species)

It is also uniquely located in it s family network.

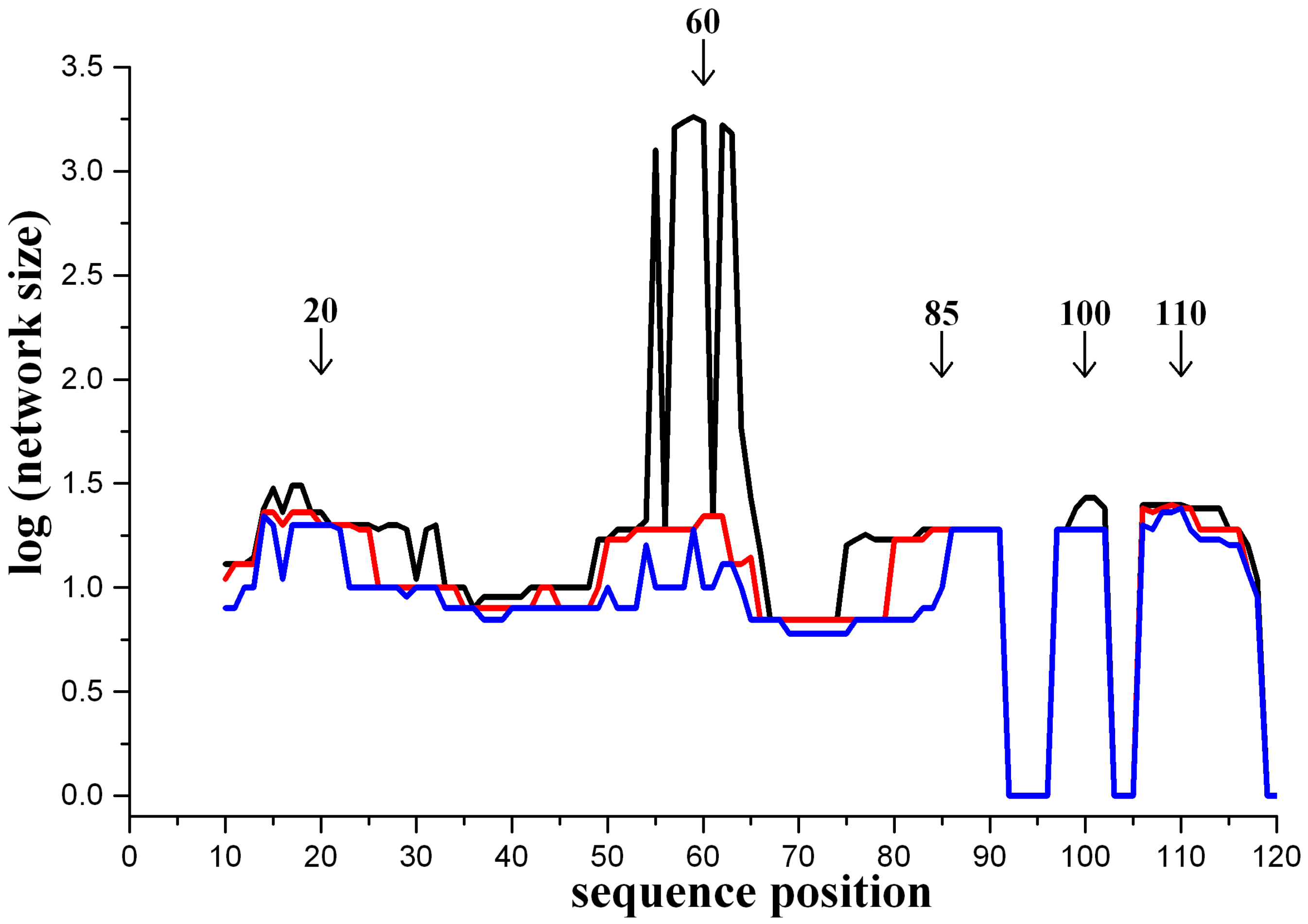
The size of the network says
how many relatives the fragment has

Thus, one can take a sequence
and for all fragments of it
find their networks and plot the sizes

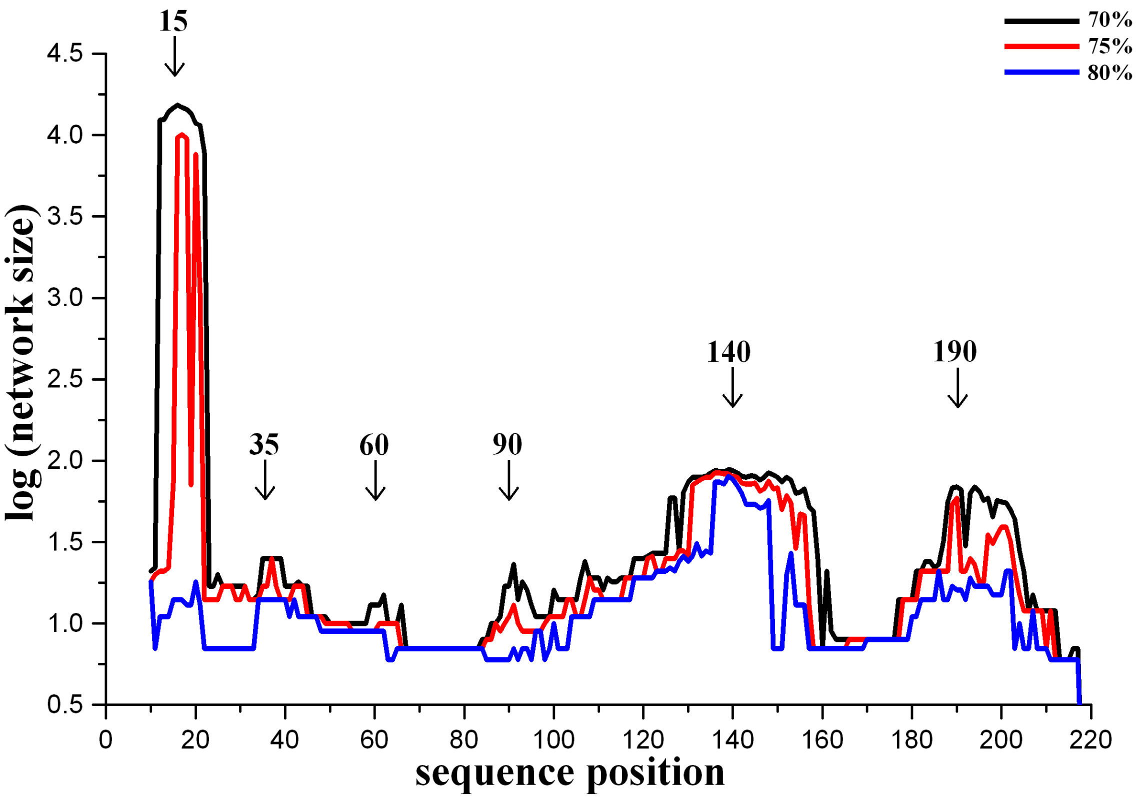
Modules of TIM-barrell protein



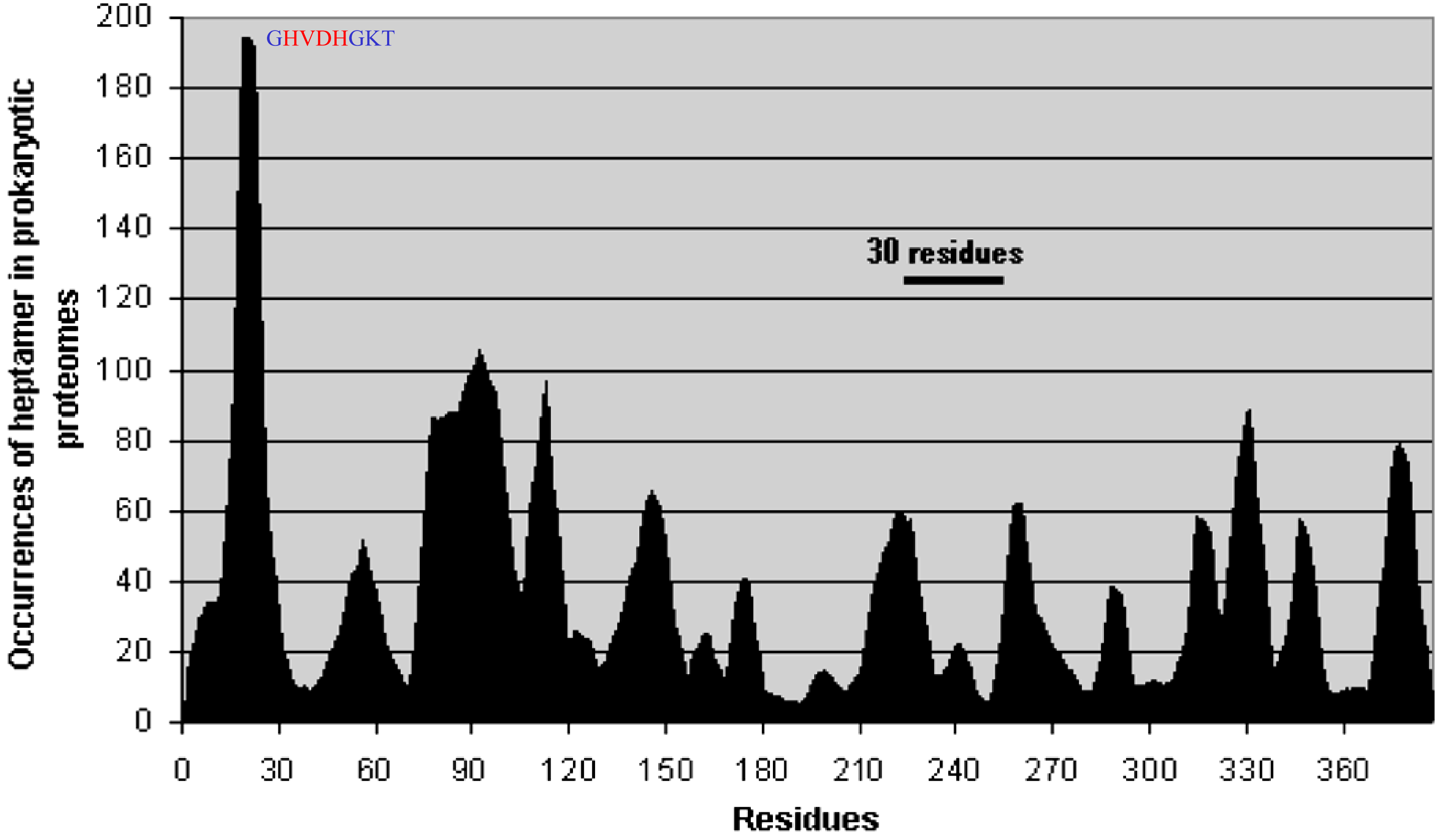
Modules of chemotaxis protein cheY



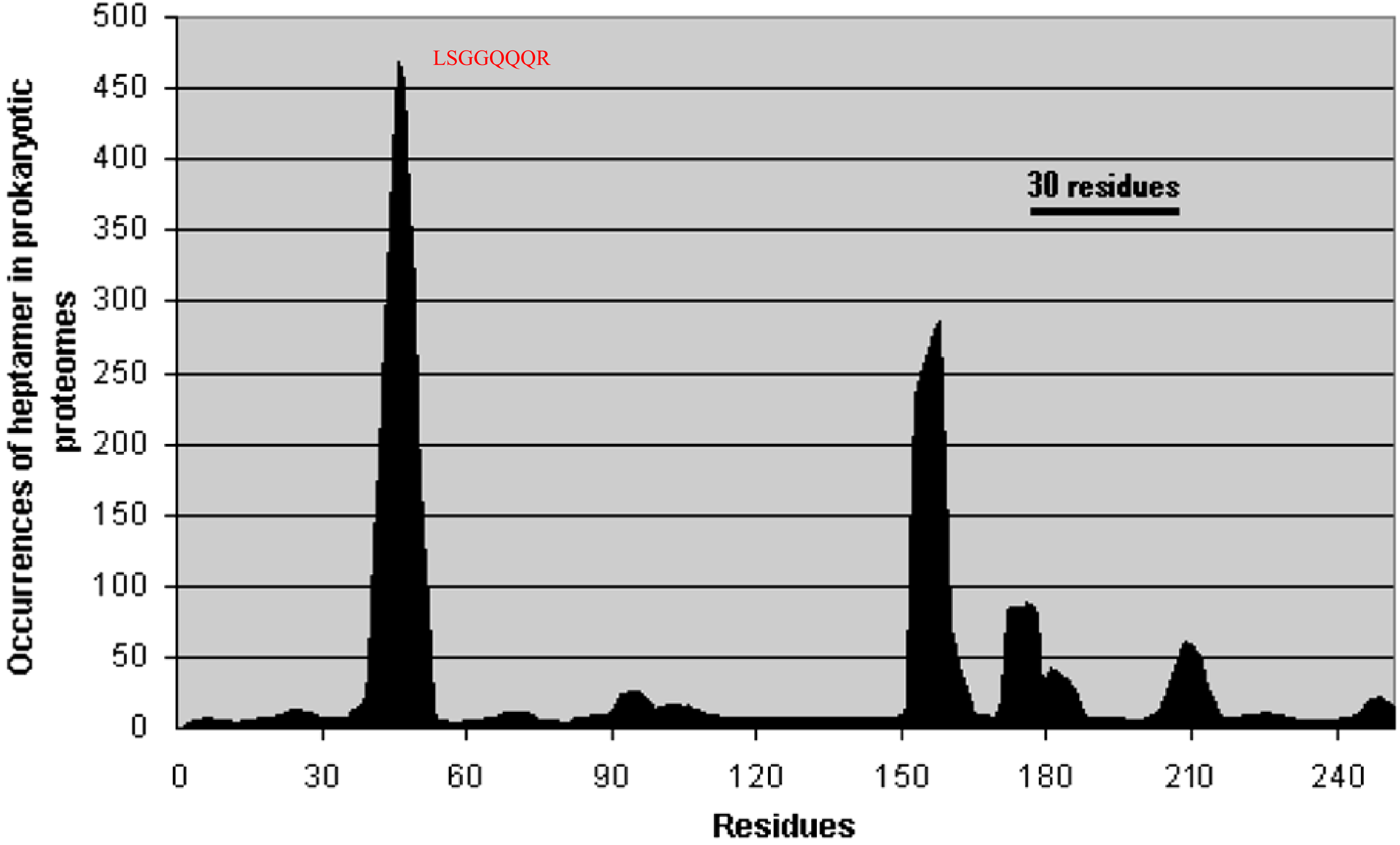
Modules of cytidylate kinase



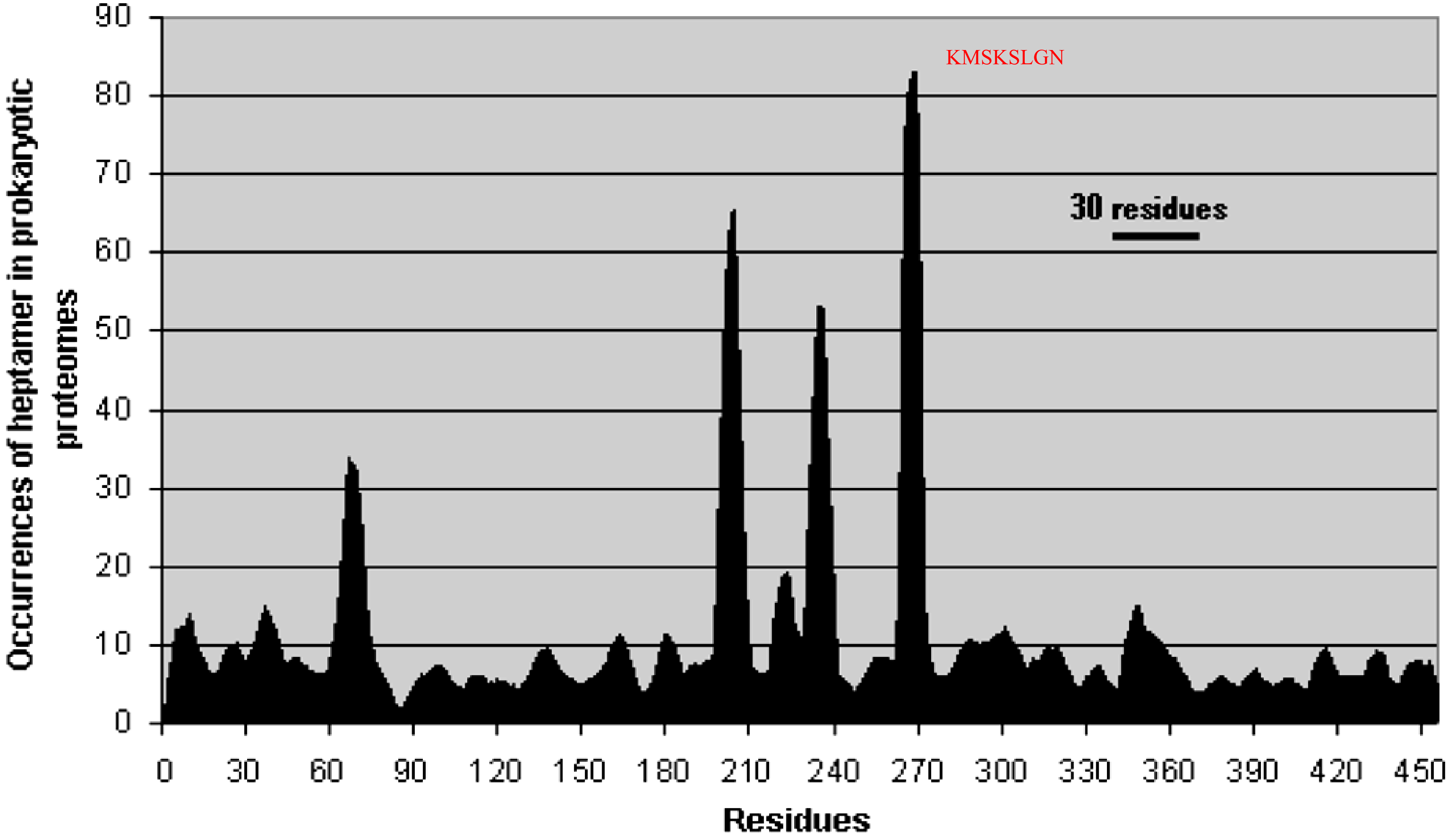
Intact elongation factor, Chain A, *E. Coli*



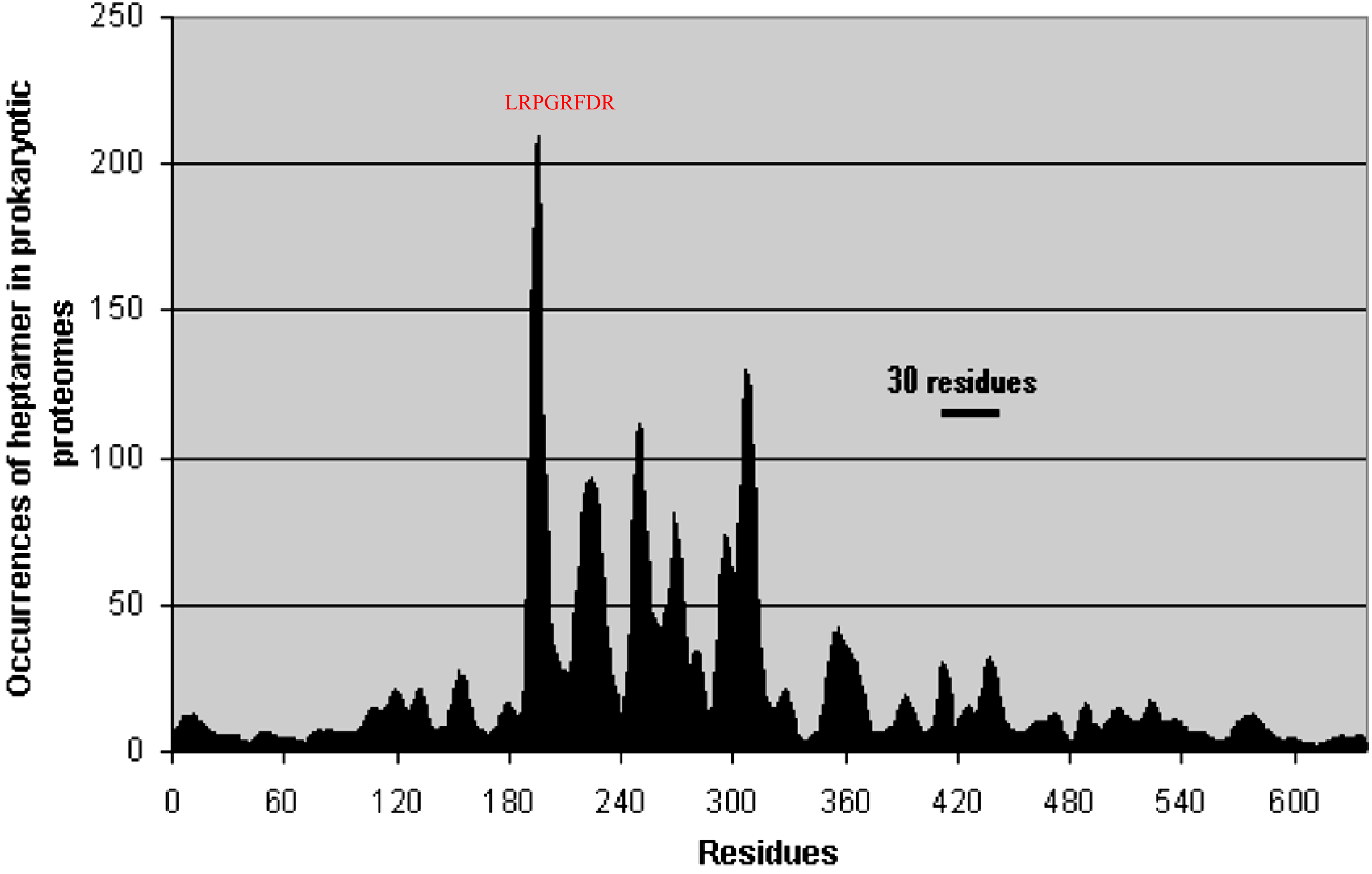
ATP-binding component of high-affinity phosphate-specific transport system, *E. Coli*



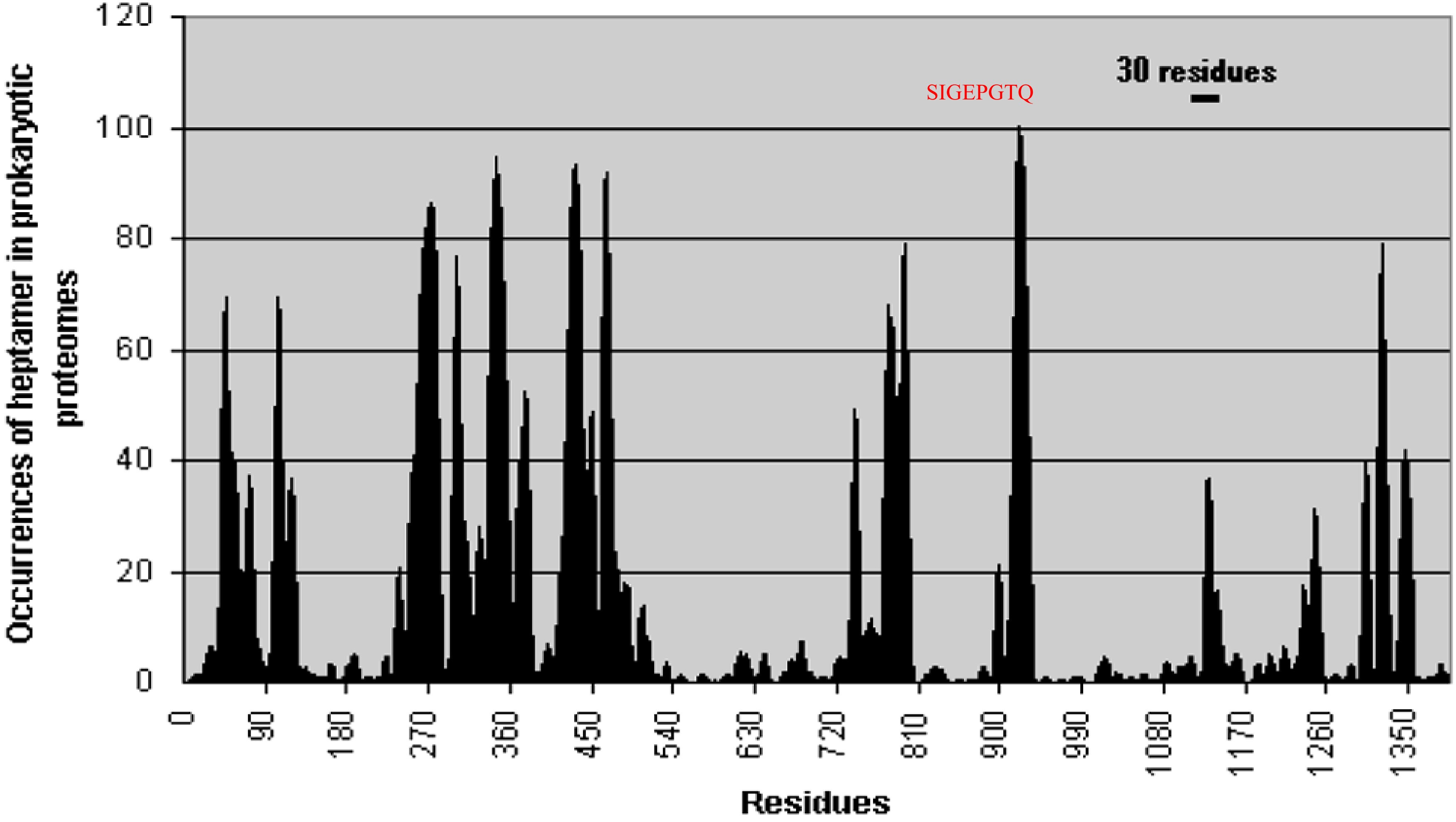
cysteine tRNA synthetase, *E. Coli* K12



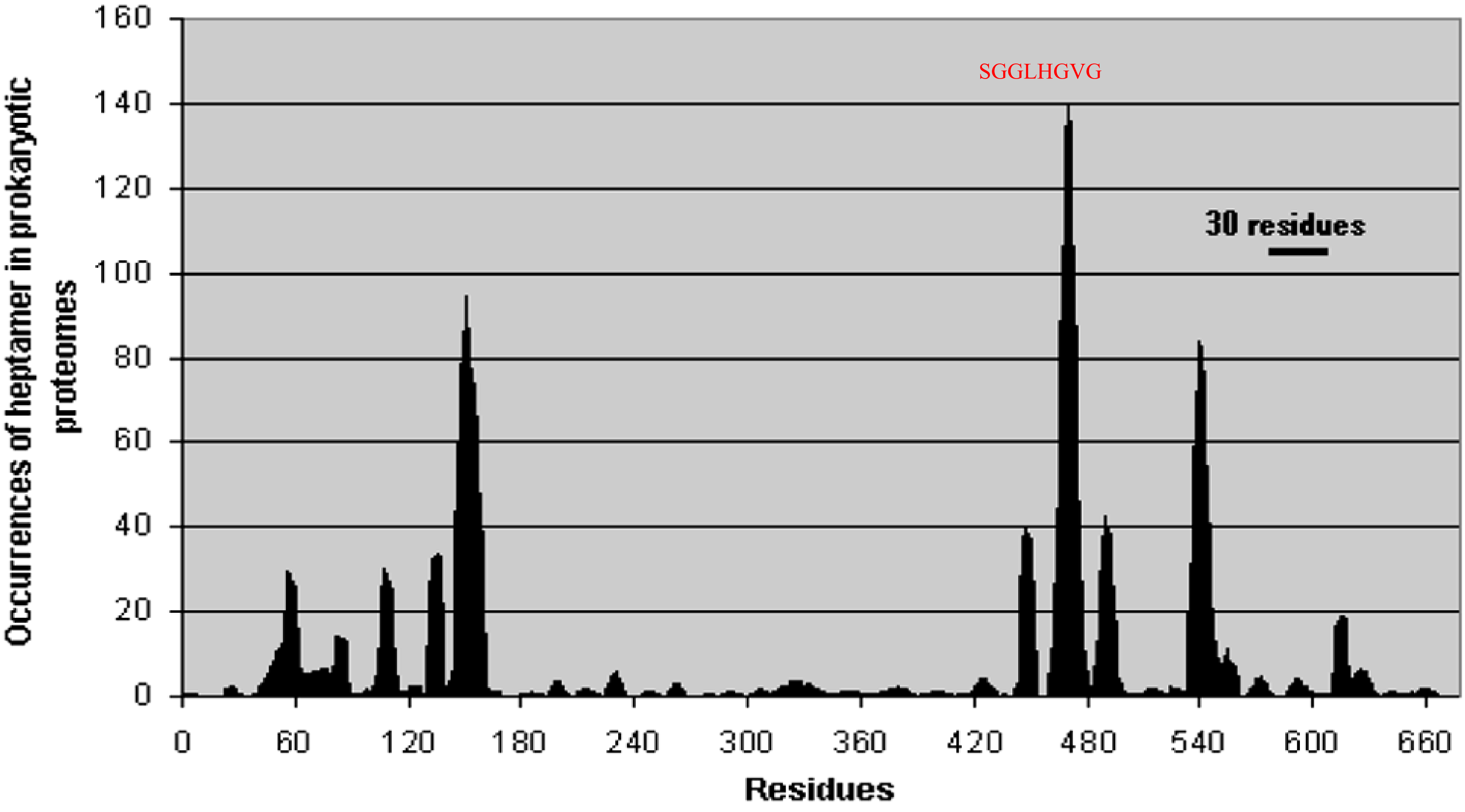
Cell division protein ftsH, *E. Coli*



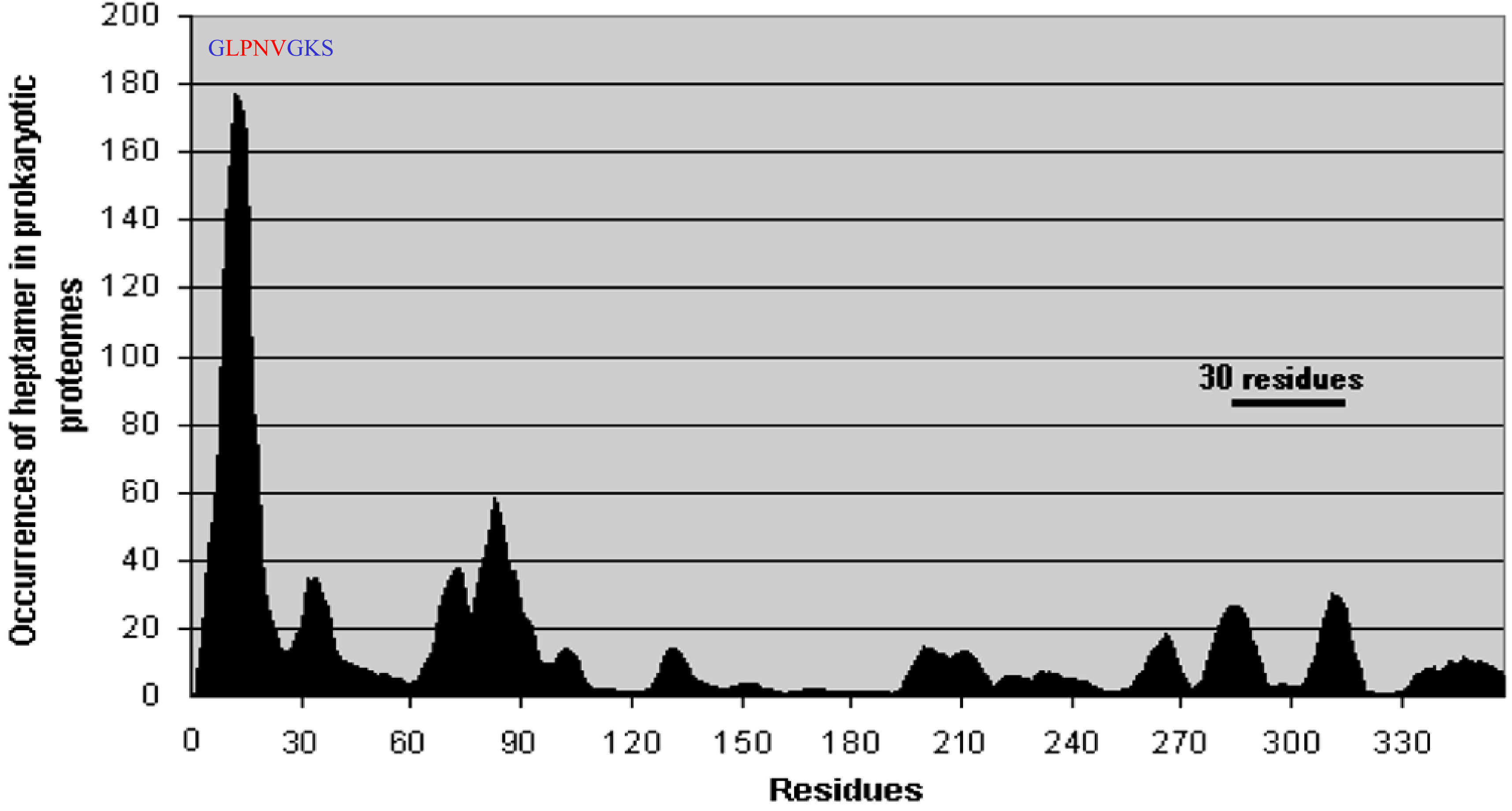
RNA polymerase beta subunit, *Rhodopseudomonas palustris CGA009*



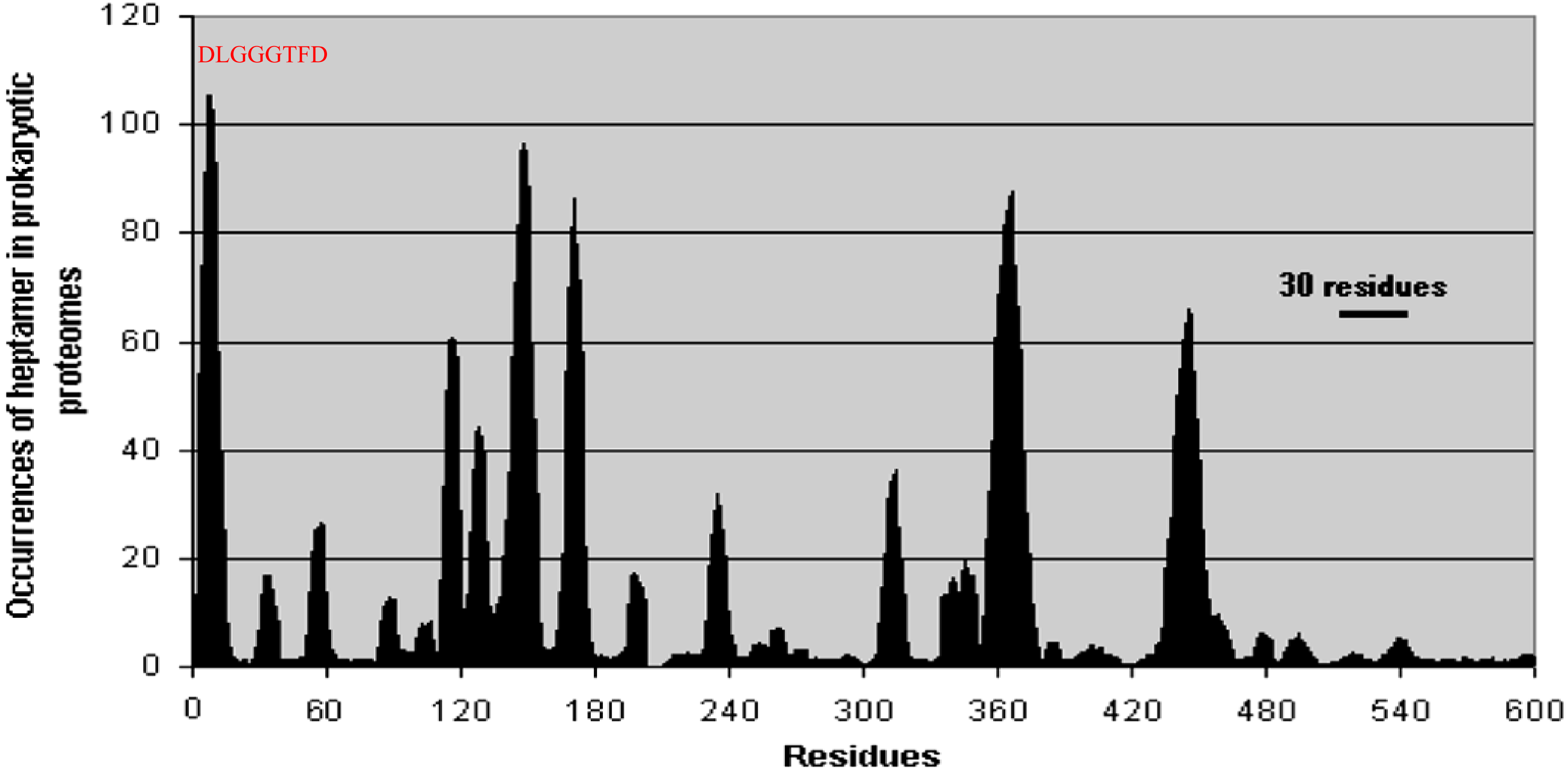
DNA topoisomerase, *Rhodopseudomonas palustris CGA009*



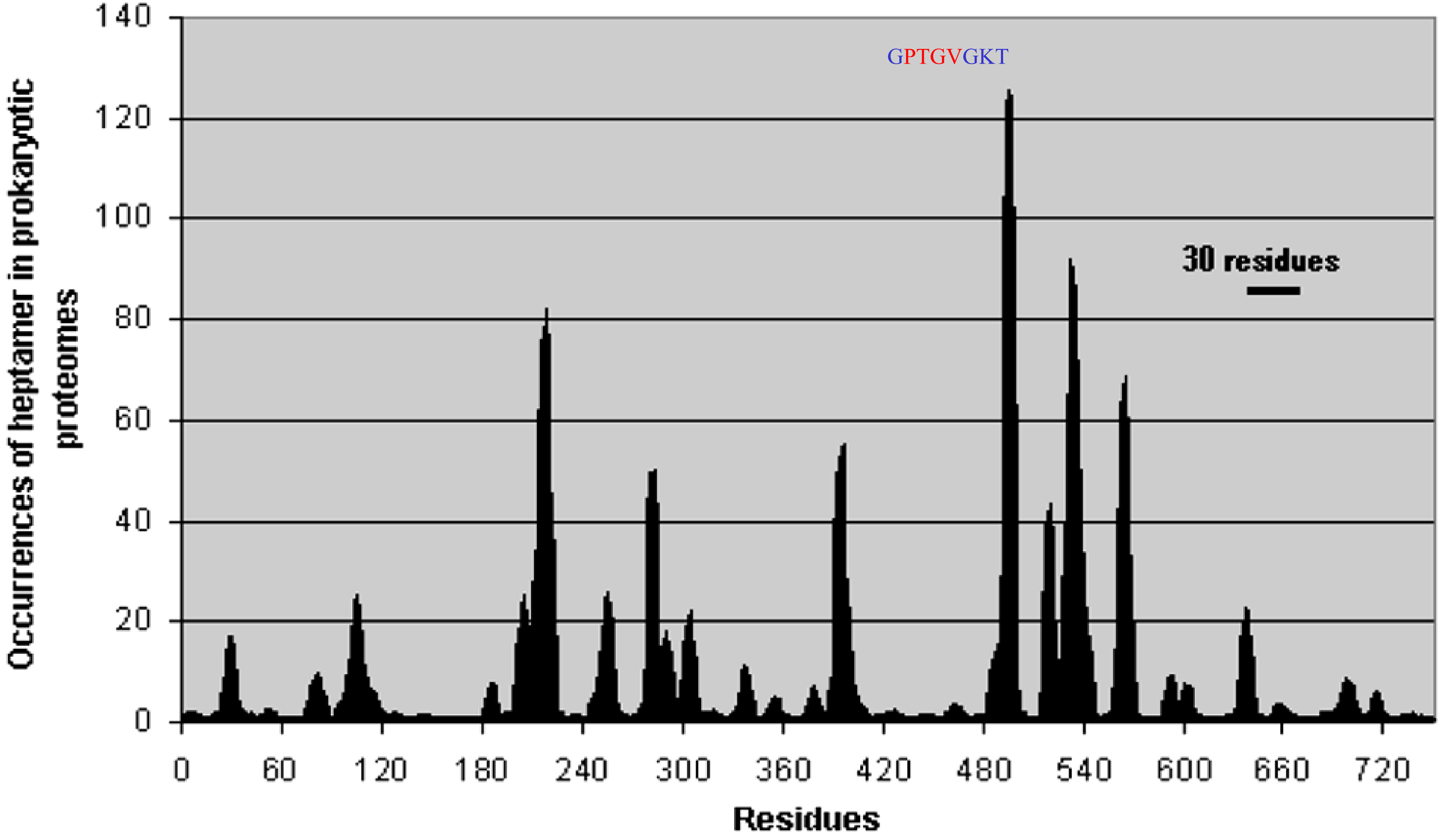
GTP-binding protein,
Hæmophilus influenzæ Rd KW20



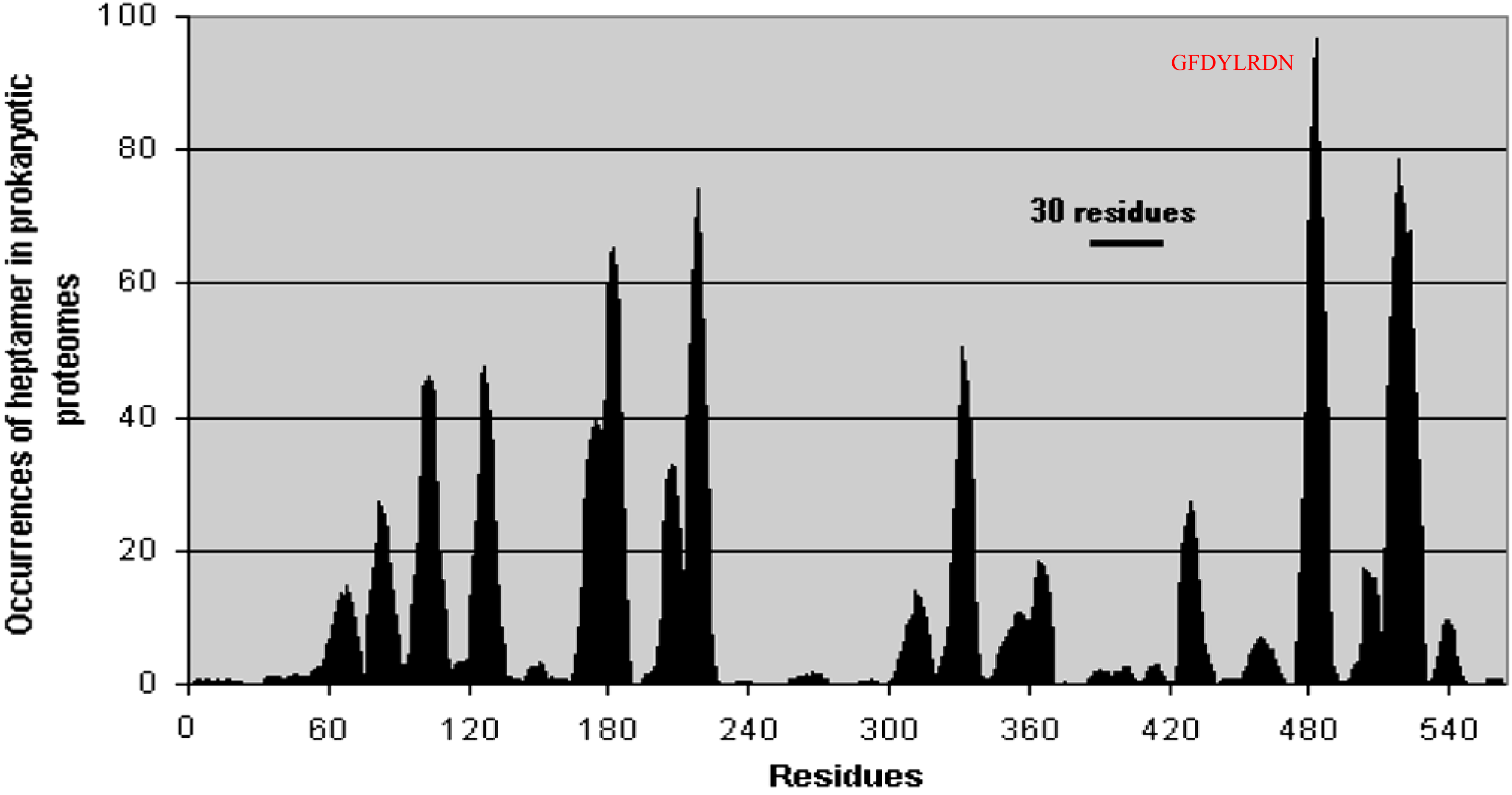
Heat shock protein DnaK
Fusobacterium nucleatum subsp. polymorphum

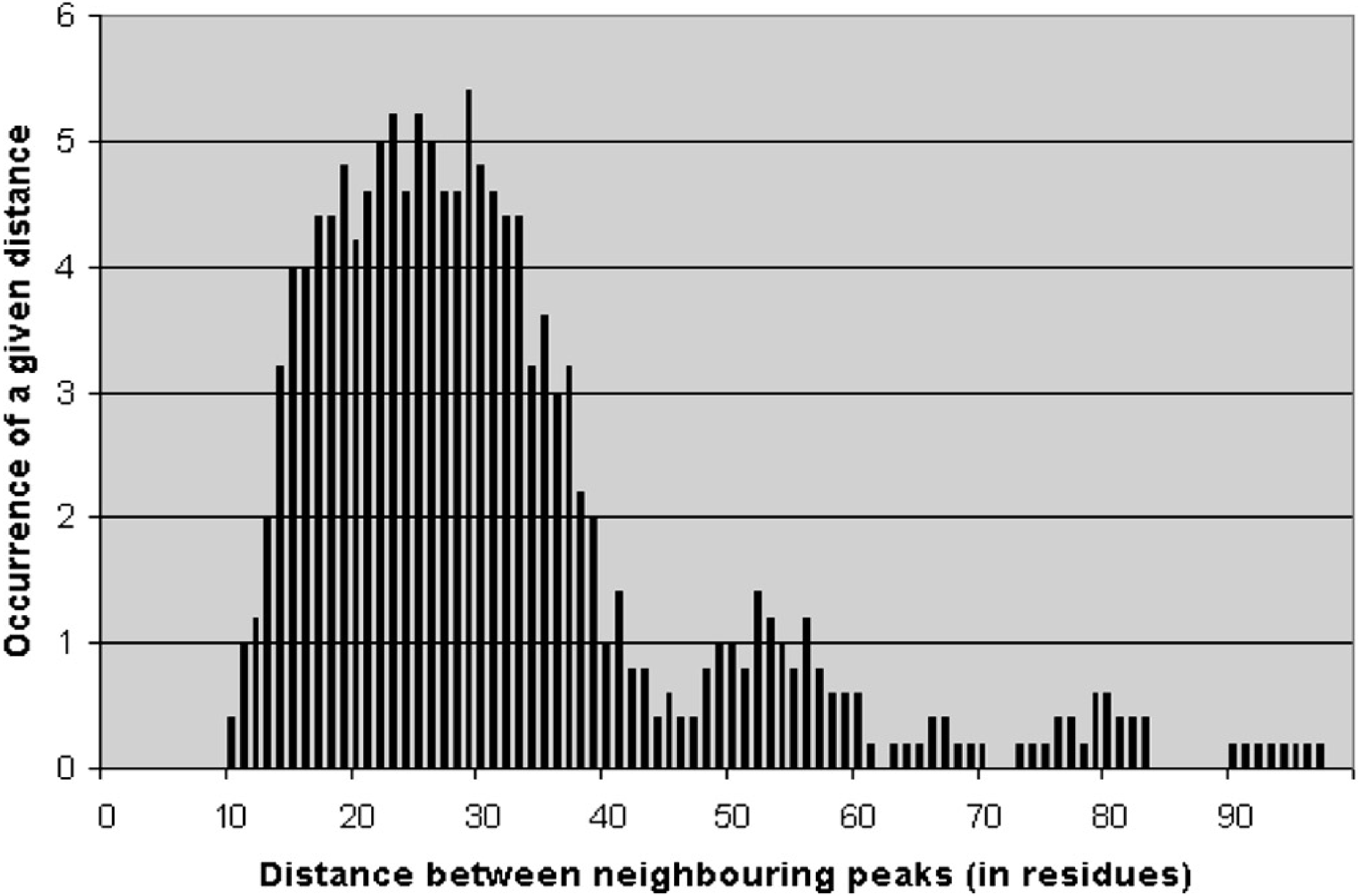


ClpA, ATP dependent protease, chaperonin
Nitrosomonas europæa ATCC 19718



protein translocase subunit SecA
Heliobacillus mobilis

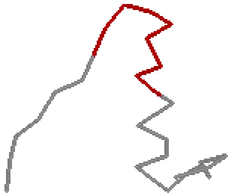


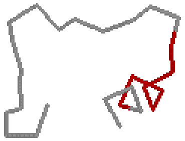

Occurrence of a given distance
6
5
4
3
2
1
0
0
10
20
30
40
50
60
70
80
90
Distance between neighbouring peaks (in residues)

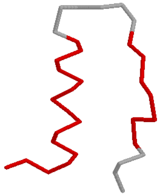
ABC transporters

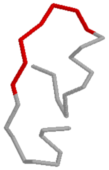
(… GPS S LTA S LSG S IYV …)

GPS (Aleph) **LTA (Dalet)** **LSG, LAD (Beth)** **IYV (Zayin)**









(36) GPSGSGKsTmL (38) fVFQqfnLiPlLTALENV (40) QLSGGQQQRVAIARAL(6)iLADEPTgALD (22) vvVTHDi (30) 1F3O

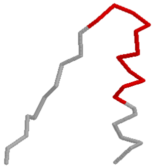
(32-72)**GPSGSGKTTLL**(29-41)MVFQNYALFPHLTALENV(31-42)Q**LSGGQQQRVAIARAL**(6 L**LADEPT**SALD(21-22)IYVTHDQ(28-263) **consensus**

The consensus sequences of the modules are built from overlapping motifs that appear in at least half of the 15 representative species. There are representatives of the above cassette in every species. Thus the ABC cassette as outlined above is OMNIPRESENT

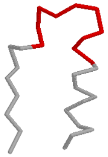
Proteases (cell division proteins FtsH)

(… GPP FVE FID DER RPG …)

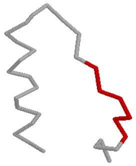
GPP (Aleph)



FVE

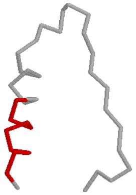


FID

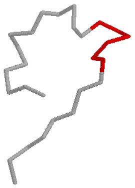


```
(197) LLVGPPGTGKTLLARAVAGEA(7)SGSDFVELFVGVGAARVRD(9)PCIVFIDEIDAVGR (10)    2CEA

(146-463)LLVGPPGTGKTLLARAVAGEA(7)SGSDFVEMFVGVGASRVRD(9)PCIIFIDEIDAVGR(7-11)   consensus
```

DER



RPG



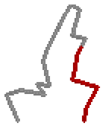
```
DEREQTLNQLLVEMDGF(8)MAATNRPDILDPALLRPGRFDKK  (297)      2CEA

DEREQTLNQLLVEMDGF(8)IAATNRPDxLDPALLRPGRFDRQ (95-415)   consensus
```

- another example of the omnipresent cassette

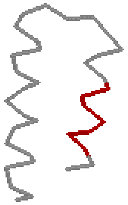
Omnipresent cassette of RNA polymerases

(... FAT NEK S NLL S S VLL NAD ...)

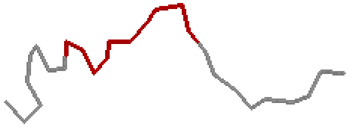
FAT



NEK



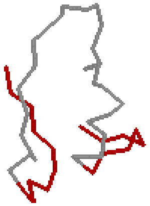
NLL



(529) VDGGRFATSDLNDLYRRLINRNNRLK (12) RNEKRMLQEAVDAL (27) GKQGRFRQNLLGKRVDYSGRSVIVVGP 2A6E

(224-518)LDGGRFATSDLNDLYRRVINRNNRLK (12) RNEKRMLQEAVDAL(25-27)GKQGRFRQNLLGKRVDYSGRSVIVVGP consensus

VLL **NAD**



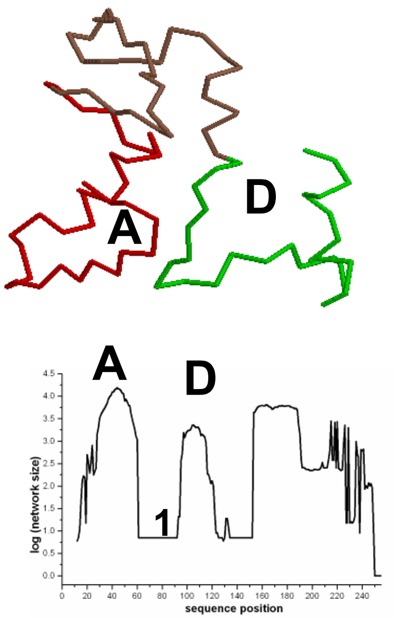
(62) KVVLLNRAPTLHRLGIQAF (18) AFNADFDGDQMAVH (776) 2A6E

(59-84)HPVLLNRAPTLHRLGIQAF (18) AF**NADFDGD**QMAVH (131-961) consensus

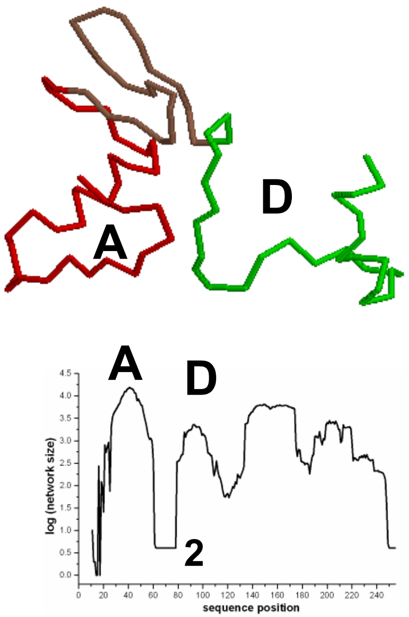
The maps of the modules show as well
the “silent” regions
– least conserved, least related to anything
and, perhaps, not very much loaded functionally.

These would be of not much interest
for the sequence alignment community

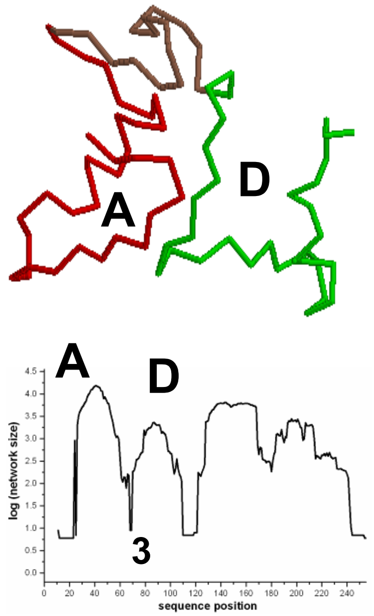
silent module 1



silent module 2



silent module 3



```
             A                silent modules 1-3                   D
IVLLVGPSGSGKTTLLRALAGLLGPDGG                              RRGIGMVFQEYALFPHLTVLENVALGL
     | ||||| | ||    |  |  |                              |    ||||   |  | ||||||
VISIIGSSGSGKSTFLRCINFLEKPSEGSIVVNGQTINLVRDKDGQLKVADKNQLRLLRTRLTMVFQHFNLWSHMTVLENVMEAP 1
     | ||||| | || |  || || |          || |      |  |           ||||   |  ||||  |
FMILLGPSGCGKTTTLRMIAGLEEPSRG---QIYIGDRLVADPEKGIFVPPK------DRDIAMVFQSYALYPHMTVYDNIAFPL 2
|    ||||||| | ||||||||    |          |          ||        |   |||||||||||| |  |  | |
FVVFVGPSGCGKSTLLRMIAGLETITSG---------DLFIGEKRMNDTPPA------ERGVGMVFQSYALYPHLSVAENMSFGL 3
```

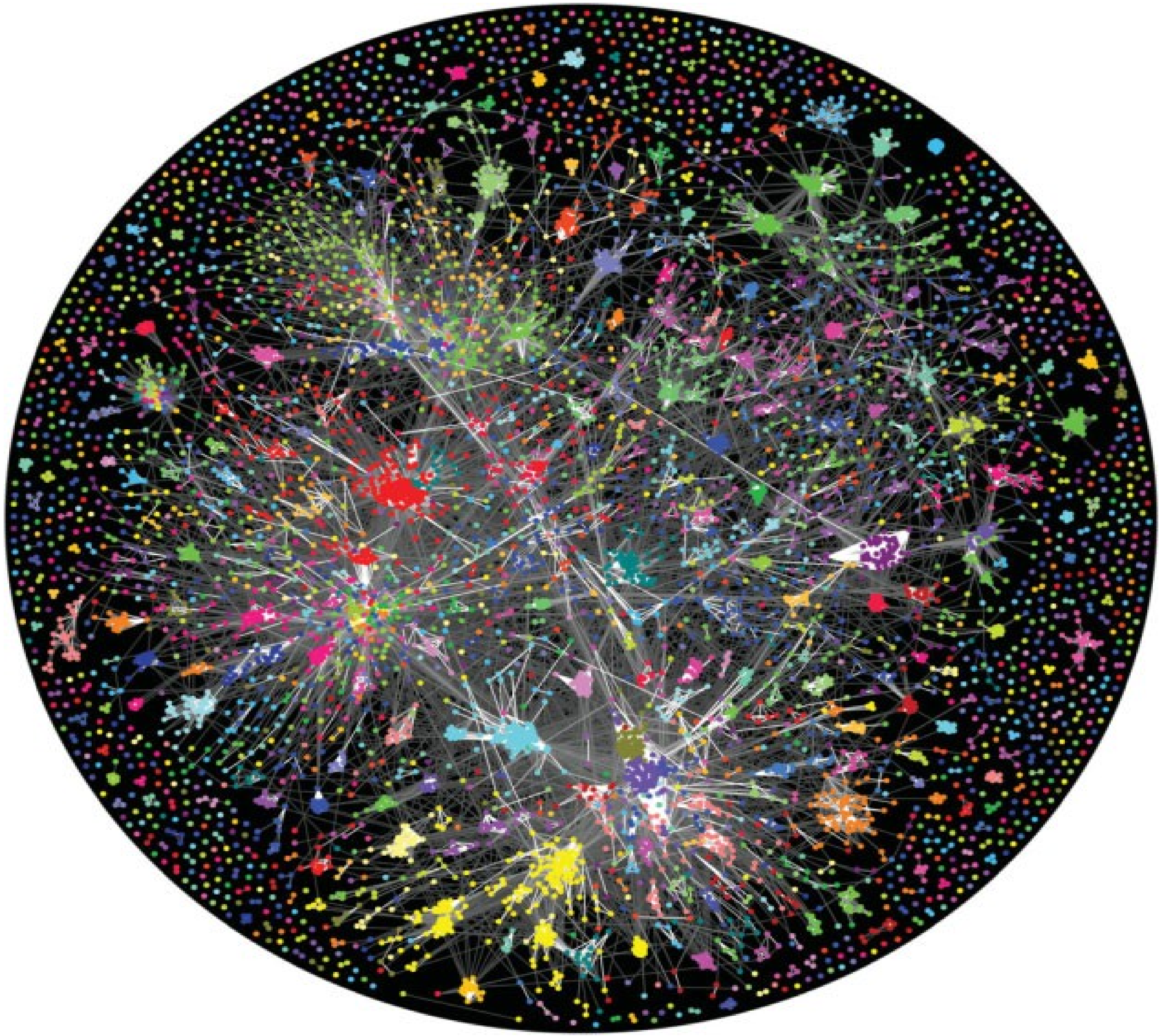
The silent modules appear to maintain
3D structural relationships between functionall modules

When long sequences are compared
it is worth first to identify
which segments are more
informative.

This is done by
mapping of the modules.

The list of modules revealed in the map
for a given protein sequence,
with reference to corresponding
(characterized) networks
of the precalculated sequence space

provides full annotation of the protein



V. Alva et al., PROTEIN SCIENCE 19 , 124-130, 2010

"…modular peptide fragments of between 20 and 40 residues
that co-occur in the connected folds
in disparate structural contexts.
These may be
descendants of an ancestral pool of peptide modules…"

V. Alva et al., PROTEIN SCIENCE 19 , 124-130, 2010

What are the protein modules:

Their sequences are represented by networks
in the protein sequence space -
separate network (or group of related networks) for each module.

Each module has its own unique structure.
Typically, these are closed loops of the contour length 25-30 residues.

Apart from general activity ascribed to the protein that harbors given module,
each module type has its own specific function.

Individual modules even of the same type are sequence-wise often different.
Their evolution from ancestral prototypes
may be traced along walks and networks in the sequence space.

Proteins are made
from standard size modules
of many types.

Each type has its unique structure and function,
but highly variable sequence

All current protein science turns inside out:

Protein world is world of modules

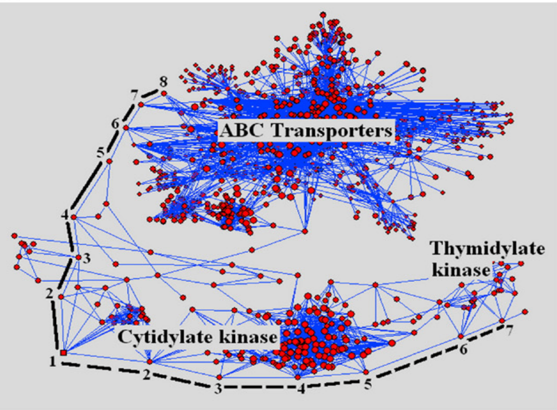
Every breakthrough that opens new vistas
also removes the ground
from under the feet of other scientists.

The scientific joy of those who have seen the new light
is accompanied by the dismay
of those whose way of life has been changed for ever.

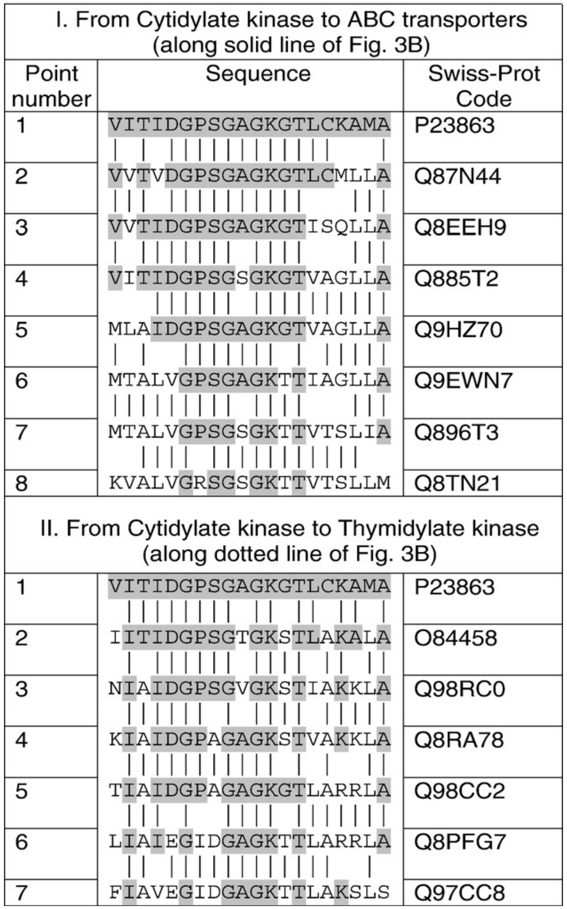
Fersht A, Nature Rev Mol Cell Biol, 2008

Examples of evolutionary paths

B



C



I. From Cytidylate kinase to ABC transporters (along solid line of Fig. 3B)

Point number	Sequence	Swiss-Prot Code
1	VITIDGPSGAGKGTLCKAMA	P23863
2	VVTVDGPSGAGKGTLCMLLA	Q87N44
3	VVTIDGPSGAGKGTISQLLA	Q8EEH9
4	VITIDGPSGSGKGTVAGLLA	Q885T2
5	MLAIDGPSGAGKGTVAGLLA	Q9HZ70
6	MTALVGPSGAGKTTIAGLLA	Q9EWN7
7	MTALVGPSGSGKTTVTSLIA	Q896T3
8	KVALVGRSGSGKTTVTSLLM	Q8TN21

II. From Cytidylate kinase to Thymidylate kinase (along dotted line of Fig. 3B)

Point number	Sequence	Swiss-Prot Code
1	VITIDGPSGAGKGTLCKAMA	P23863
2	IITIDGPSGTGKSTLAKALA	O84458
3	NIAIDGPSGVGKSTIAKKLA	Q98RC0
4	KIAIDGPAGAGKSTVAKKLA	Q8RA78
5	TIAIDGPAGAGKGTLARRLA	Q98CC2
6	LIAIEGIDGAGKTTLARRLA	Q8PFG7
7	FIAVEGIDGAGKTTLAKSLS	Q97CC8

MOST COMMON
PROTEIN SEQUENCE MODULES (PROTOTYPES)

Aleph	**GEIVLLVGPSGSGKTTLLRALAGLLGPDGG**
Beth	**LSGGQRQRVAIARALALEPKLLLLDEPTSALD**
Gimel	DVVVIGAGGAGLAAALALARAGAKVVVVE
Dalet	RRGIGMVFQEYALFPHLTVLENVALGL
Heh	PVIMLTARGDEEDRVEALLEAGADDYLTKPF
Vav	LLGLSKKEARERALELLELVGLEEKADRYP
Zayin	LLLKLLKELGLTVLLVTHDLEEA

Berezovsky et al. 2000-2003

The underlined motifs are omnipresent

```
KVALVGRSGSGKTTVTSLLM
FIAVEGIDGAGKTTLAKSLS
     GxxxxGKT   -   Walker A motif
                    (NTP binding)
```

Omnipresent 6-9 mers of 15 prokaryotes from different phyla

ALEPH ATP/GTP binding

```
 1      HVDHGKTTL
 2     GPPGTGKT
 3     GHVDHGKT
 4        GSGKTTLL
 5 IDTPGHV
 6     GPSGSGK
 7      PTGSGKT
 8       NGSGKTT
 9          GKSTLLN
10       SGSGKT
11       TGSGKS
12       PGVGKT
13       PNVGKS
14        GVGKTT
15        GTGKTT
16        DHGKST
17          GKTTLA
18          GKTTLV
19           KSTLLK
```

BETH ATPases of ABC transporters

```
20         QRVAIARAL
21   LSGGQQQRV
22                        LADEPT
23  TLSGGE
```

Other omni:

```
24  FIDEID
25  KMSKSL
26  WTTTPWT
27  NADFDGD
```

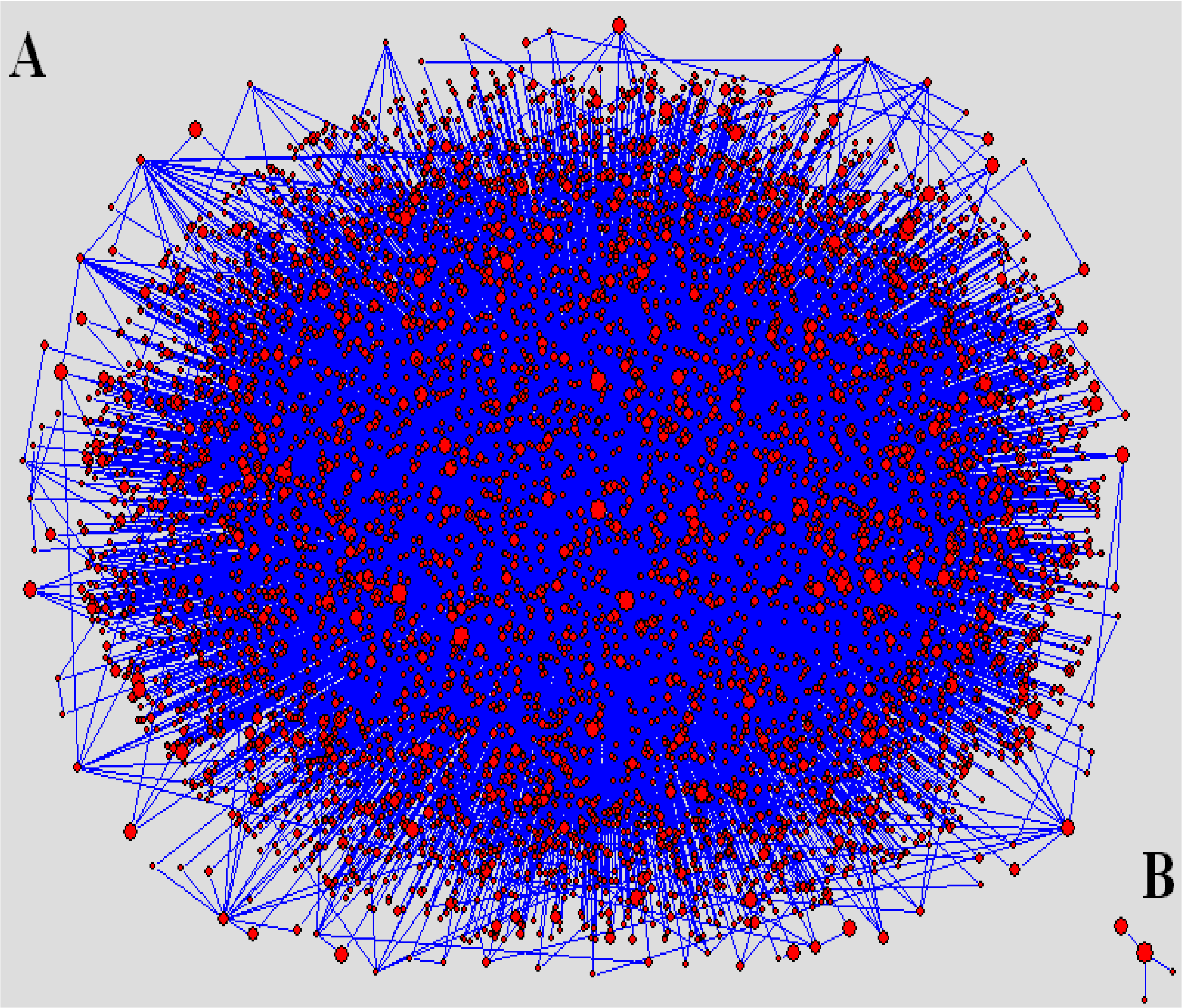
Omnipresence is a new measure of sequence conservation.
These elements are the most conserved ones,
coming, presumably from last common ancestor

ALEPH and BETH reconstructed from overlapping omnipresent motifs turn out to be relatives, though they do not match:

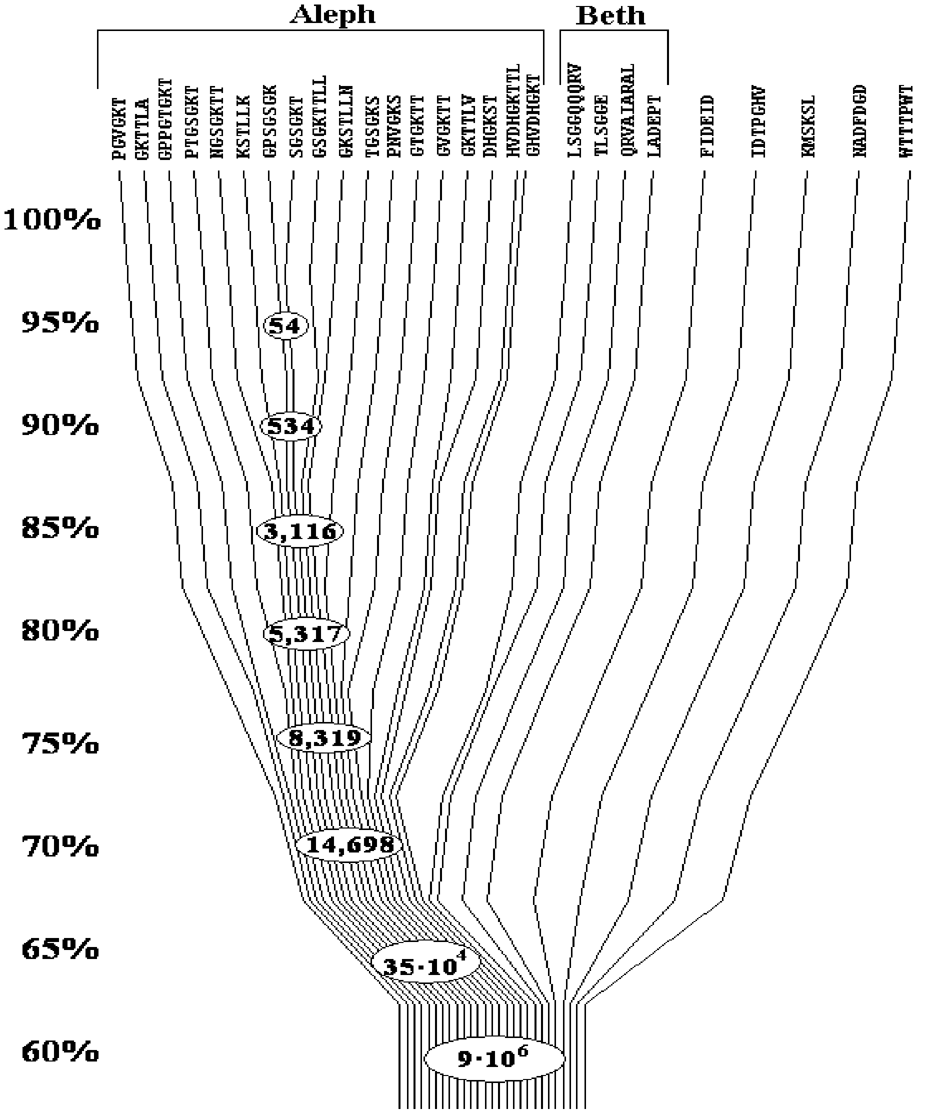
```
IDTPGHVDHGKTTLLN   ALEPH
  |
  TLSGGQQQRVAIARAL BETH
```

They both belong to 10% monster network.

All 27 omnipresent elements belong to the same network



10% MONSTER network (10^7 fragments)



Sequence space based
evolutionary tree of omnipresent elements

TO CONCLUDE THE CHAPTER ON NETWORKS:

I. Protein sequence characterization via networks in the sequence space does not require

gap penalties,
nor substitution matrices,
nor statistics of alignment

II. The networks in the sequence space represent protein modules. Each sequence fragment belongs to only one specific network, and, thus, is given an unequivocal annotation.

III. Each protein can be described as linear combination of several different modules, and presented as word in the alphabet of the modules – the proteomic code

Paths from Aleph to Beth and back

	A		B	
1	GEFVAIVGPSGCGKSTLLRL	Q825G5	GEFVAIVGPSGCGKSTLLRL	Q825G5
2	**GESLALTGESGSGKSTLLHL**	**Q7CP38**	**GEVVVIIGPSGSGKSTLLRS**	**Q97RJ0**
3	AQTI**ALIGESGSGKSTLLGI**	**Q8ZCB4**	**QVVVVGAGPSGSTVSALLKS**	**Q87R97**
4	**ATLAALIGAGGLGKLILLGI**	**Q813M6**	**DVVVVGAGPSGSSAARYLSE**	**O66509**
5	**AVIAALIGAGGFGALVFQGL**	**Q8X670**	**DVVVIGAGPGGYVAAIRASQ**	**Q9A7J2**
6	V**VLAGLVGAGGLGAEVTRGL**	**Q8U8Y4**	**DAVIIGGGPGGYVCAIKLAQ**	**Q9WYL2**
7	**VVGGGVVGAGTALDAVTRGL**	**Q82DH4**	**FAVITGGGPGAMEAANKGAQ**	**Q8KC62**
8	**VVGGGSTGAGVARDLAMRGL**	**Q9HNS4**	**LTVATGGGPGAMEAANLGAY**	**O86748**
9	**VVGGGFTGQSAALHLAEGGL**	**Q8UCD8**	**LDVGTGSGVLAMAAAKLGAA**	**Q9RU72**
10	LC**GGGFTGQSQALRLAIARA**	**Q8A0Z5**	**LDLGTGSGALAVHAARLGAR**	**Q826J9**
11	**LSGGERIALSIALRLAIAKA**	**Q97WH0**	**LDTGIMSGADIVAAIALGAR**	**Q9CBF2**
12	**LSGGQRRALGIALALASNPE**	**Q9YBQ1**	**MDGGIRSGQDVLKAVALGAR**	**Q8UD10**
13	**LSGGQRQRVAIARALALDPD**	**Q82BU6**	**VSGGIRSGADVAKALALGAD**	**Q8U870**
14	A**SGGMRDGVMMAKALAMGAS**	**O58893**		
15	L**SGGMRQRVMIAIALACGPD**	**Q89KL2**		
16	**LSGGQRQRVAIARALALDPD**	**Q82BU6**		

	C		D	
1	GEFVAIVGPSGCGKSTLLRL	Q825G5	GEFVAIVGPSGCGKSTLLRL	Q825G5
2	**GQVVVVLGPSGSGKSTLCRT**	**Q8RQL7**	**GKLVALLGPSGSGKSTLLRL**	**Q8Z0H0**
3	**GQVVMVTGAGGSIGSELCRQ**	**Q9HZ86**	**NKLVLLTGPSGSGKSTLALD**	**Q9KEY5**
4	RK**VAFVTGGAGGIGSETCRQ**	**Q9KCM1**	**IHLVNLSGPAGSGKTILALA**	**Q887P5**
5	GR**VAFVTGGAGGIGRATAER**	**Q8UA89**	**GHLQSASGPLGLMKTILALR**	**O50436**
6	**GKTAFITGGGQGIGLACAEA**	**Q89QA5**	**GHMDAAAGIGGLIKTVLALR**	**Q8U9Q4**
7	LV**TGANTGLGQGIALALAEA**	**Q8PE31**	**GHTGGAAGIAGLLKAVLAIE**	**O06586**
8	**LVTGANKGIGLAIARQLGAA**	**Q7CP30**	**GRTGGWAAIAGLLAAIGATV**	**Q98BE5**
9	**LVTGSSQGIGAAIAAGLARA**	**Q9RK29**	**GSRGIGAAIARRLAADGAHV**	**Q8XT12**
10	SAC**GSSSGSGAAVAAGLAPL**	**Q9A5H4**	**ASRGIGKAIAEVAARDGAPV**	**Q92PY2**
11	LPG**GSSSGAGVVVAAGLVPV**	**Q8UAX4**	**SSGKMGYAIAEVAANLGADV**	**Q819T8**
12	IS**GGSSGGSAVAVALGLVDV**	**Q975D0**	**SSGKMGYAVAQVARELGATV**	**Q88WL5**
13	**LSGGESFMAALALALGLSDV**	**Q87HE3**	**SSGNHAQAVALAARELGTTA**	**Q9XAA4**
14	**LSGGESFIAALALALSLAEV**	**Q830T3**	**SSGNHAQGVALAARLHGIPA**	**Q8UBW5**
15	**LSGGMIKRAALARALSLDPD**	**Q8UEV8**	**VSGGQAQRVALALALAGTPA**	**Q9EWP7**
16	**LSGGQRQRVAIARALALDPD**	**Q82BU6**	**LSGGQRQRVAIARALALDPD**	**Q82BU6**

GENOME SEGMENTATION CODE

“The proteins… can, with regard to molecular weight, be divided into four subgroups… The molecular masses characteristic of the three higher subgroups are – as a first approximation – derived from the molecular mass of the first subgroup by multiplying by the integers…”

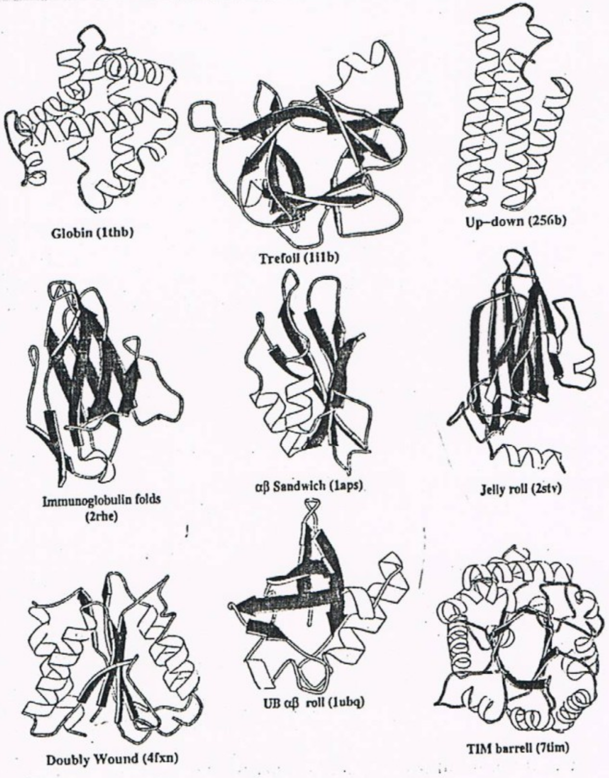
The Svedberg
Mass and size of protein molecules
Nature 123, 871 (1929)

~ 160 aa unit (Svedberg, 1937)

"…proteins of molecular weight greater than about 20 000 are often built up not as a single unit but by a combination of two or three large substructures. This finding suggests that a 3D structure based on the principle of a polar exterior surrounding a hydrophobic core can be conveniently achieved with a polypeptide molecular weight of about 10 000 – 16 000."

B. W. Matthews et al. (P. Sigler)
Nature New Biology
238, 37, 1972

TYPICAL FOLDS


Globin (1thb)

Trefoil (1i1b)

Up–down (256b)

Immunoglobulin folds (2rhe)

αβ Sandwich (1aps)

Jelly roll (2stv)

Doubly Wound (4fxn)

UB αβ roll (1ubq)

TIM barrell (7tim)

C.A.Orengo, D.T.Jones, J.M.Thornton
Nature 372, 631, 1994

R.B.Russel, G.J.Barton
JMB 244, 332, 1994

av. size 124aa
(90 – 160aa)

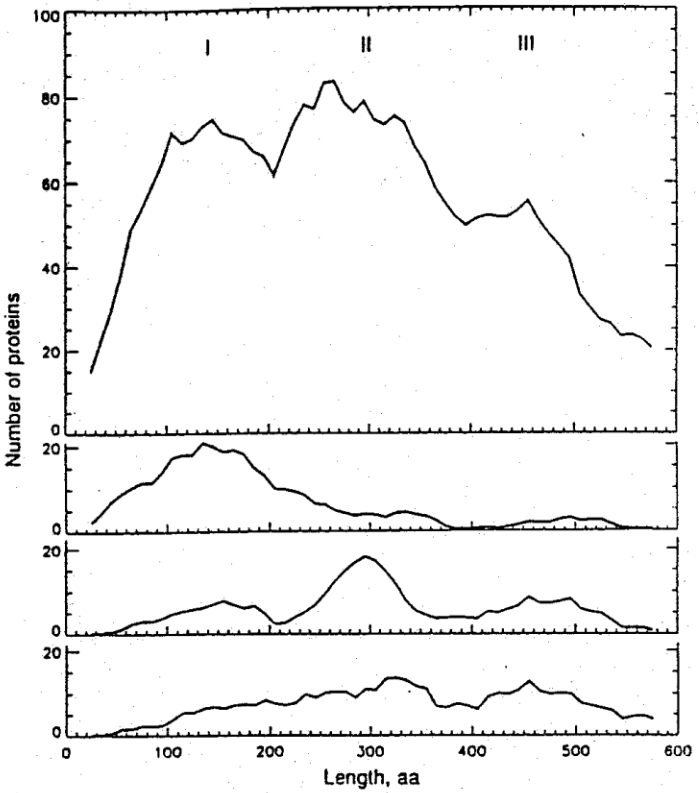


FIG. 4. Components of prokaryotic protein length distribution. Smoothed distributions (running window of 50 aa) are shown for groups of proteins that are major contributors to the peaks indicated (I–III).

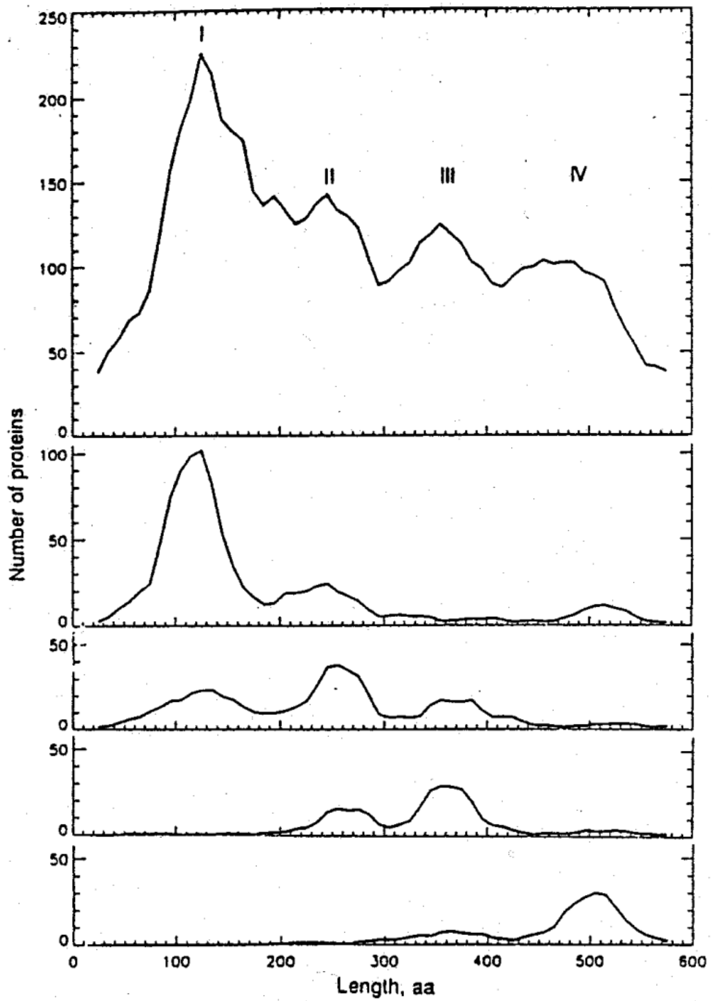
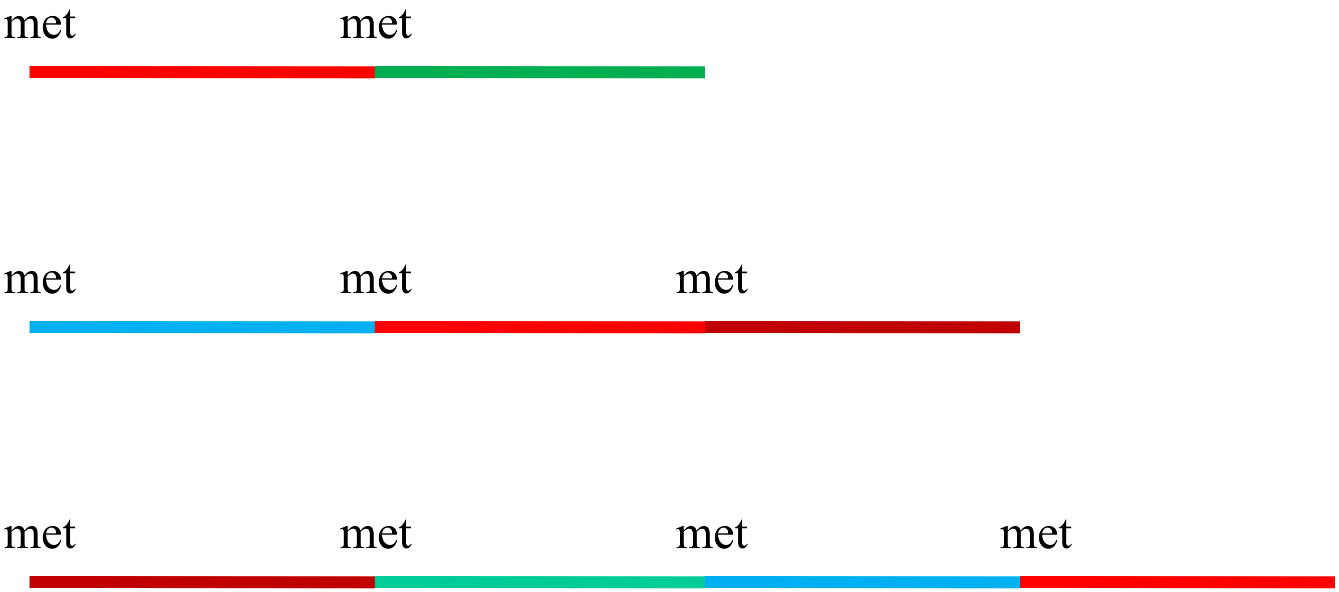


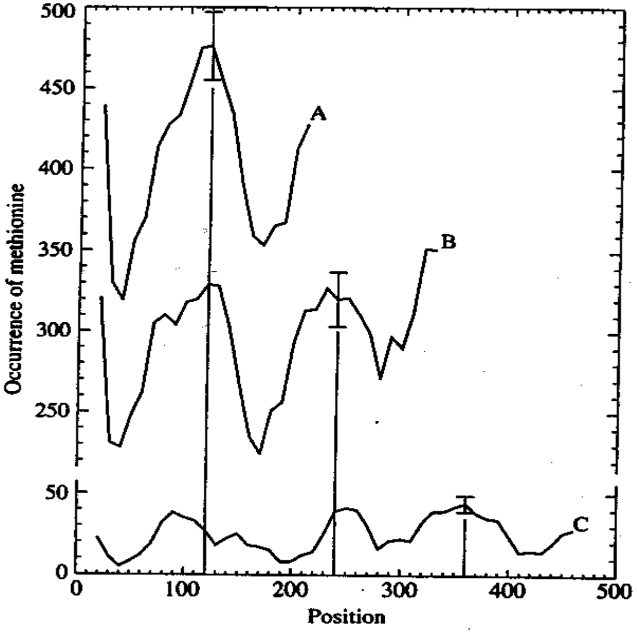
FIG. 2. Components of eukaryotic protein length distribution. Smoothed distributions (running window of 50 aa) are shown for groups of proteins that are major contributors to the peaks indicated (I–IV).

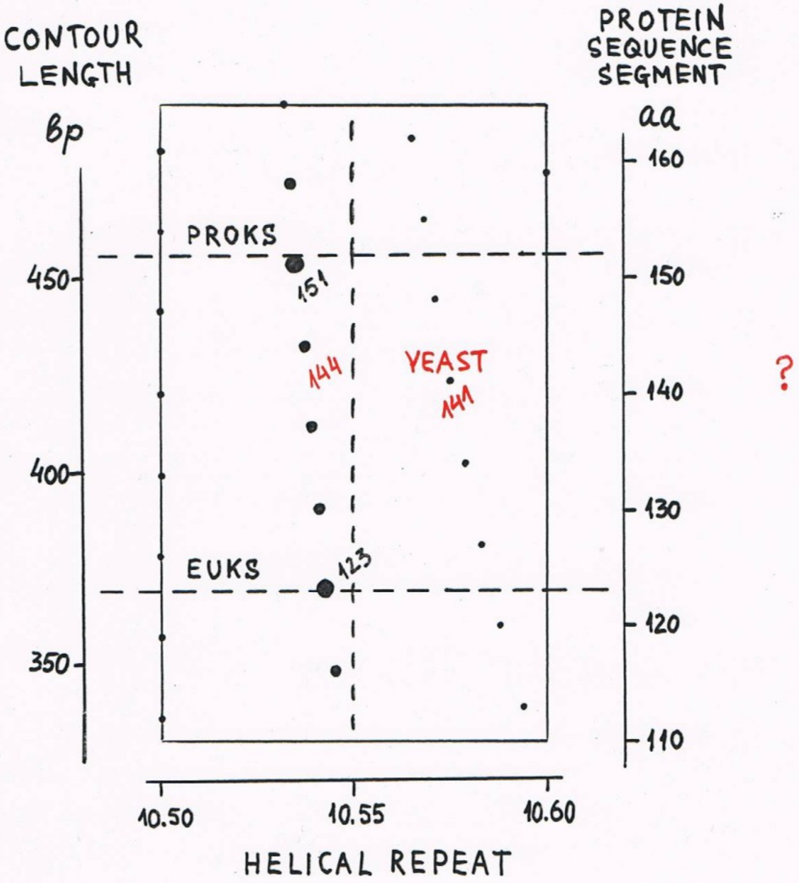


met met

met met met

met met met met


500
450
400
350
300
250
50
0
Occurrence of methionine
A
B
C
0
100
200
300
400
500
Position

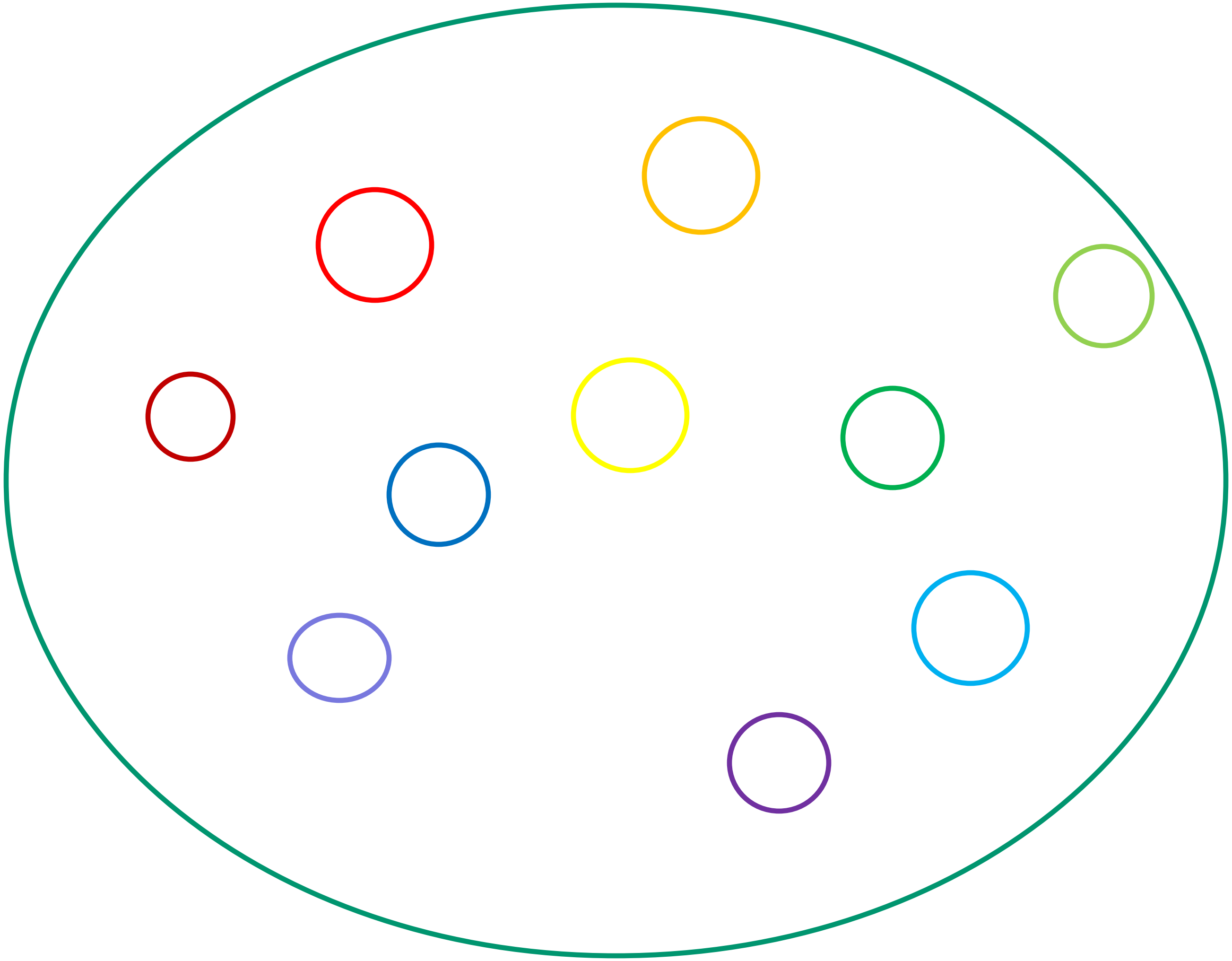

CONTOUR LENGTH
bp
PROTEIN SEQUENCE SEGMENT
aa
PROKS
EUKS
151
144
123
YEAST
141
?
450
400
350
160
150
140
130
120
110
10.50
10.55
10.60
HELICAL REPEAT

The Lord Of The Rings

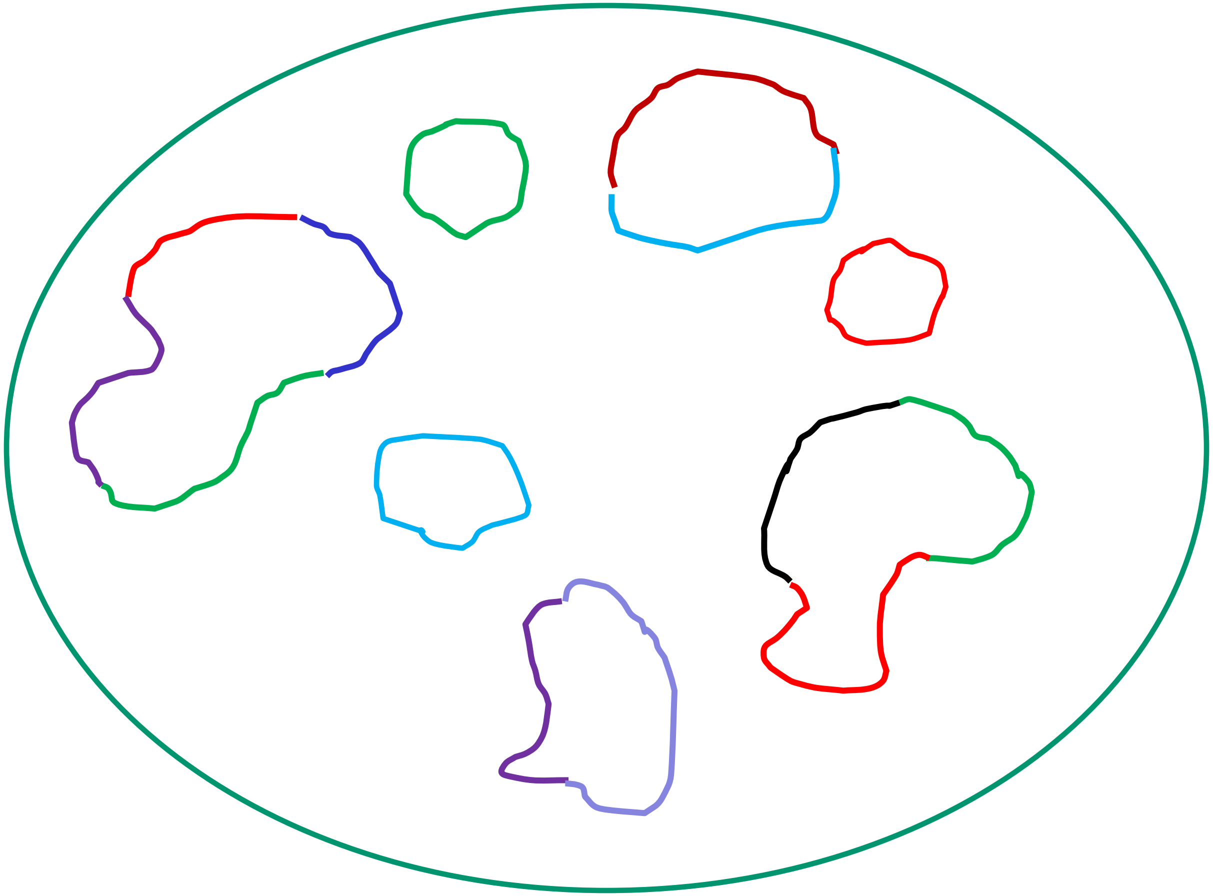
Three rings for the Elven-kings under the sky,
Seven for the Dwarf-lords in their halls of stone,
Nine for Mortal Men doomed to die,
One for the Dark Lord on his dark throne.

J. R. R. Tolkien

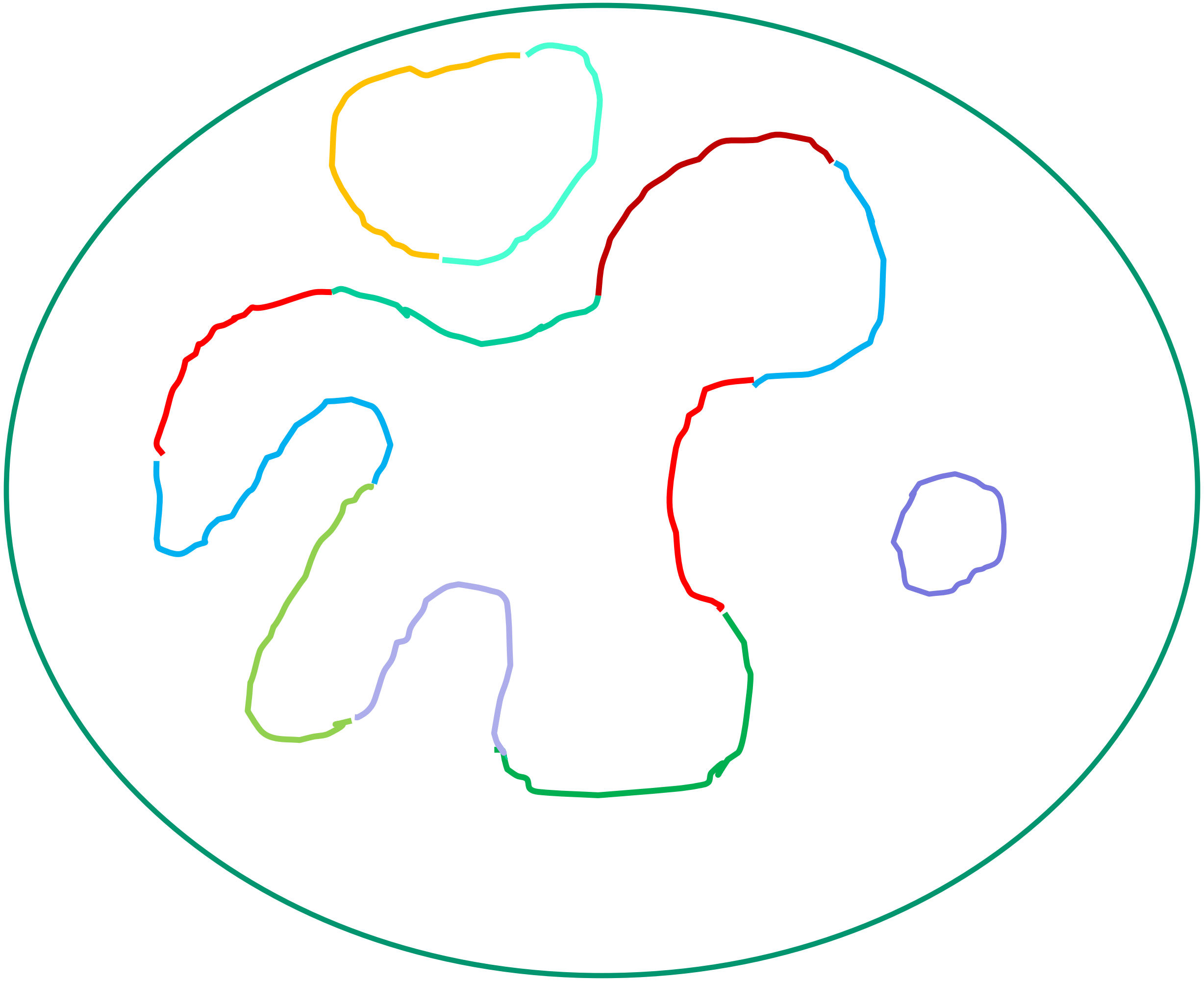
Pre-genomic, pre-recombination stage



Pre-genomic, recombination stage

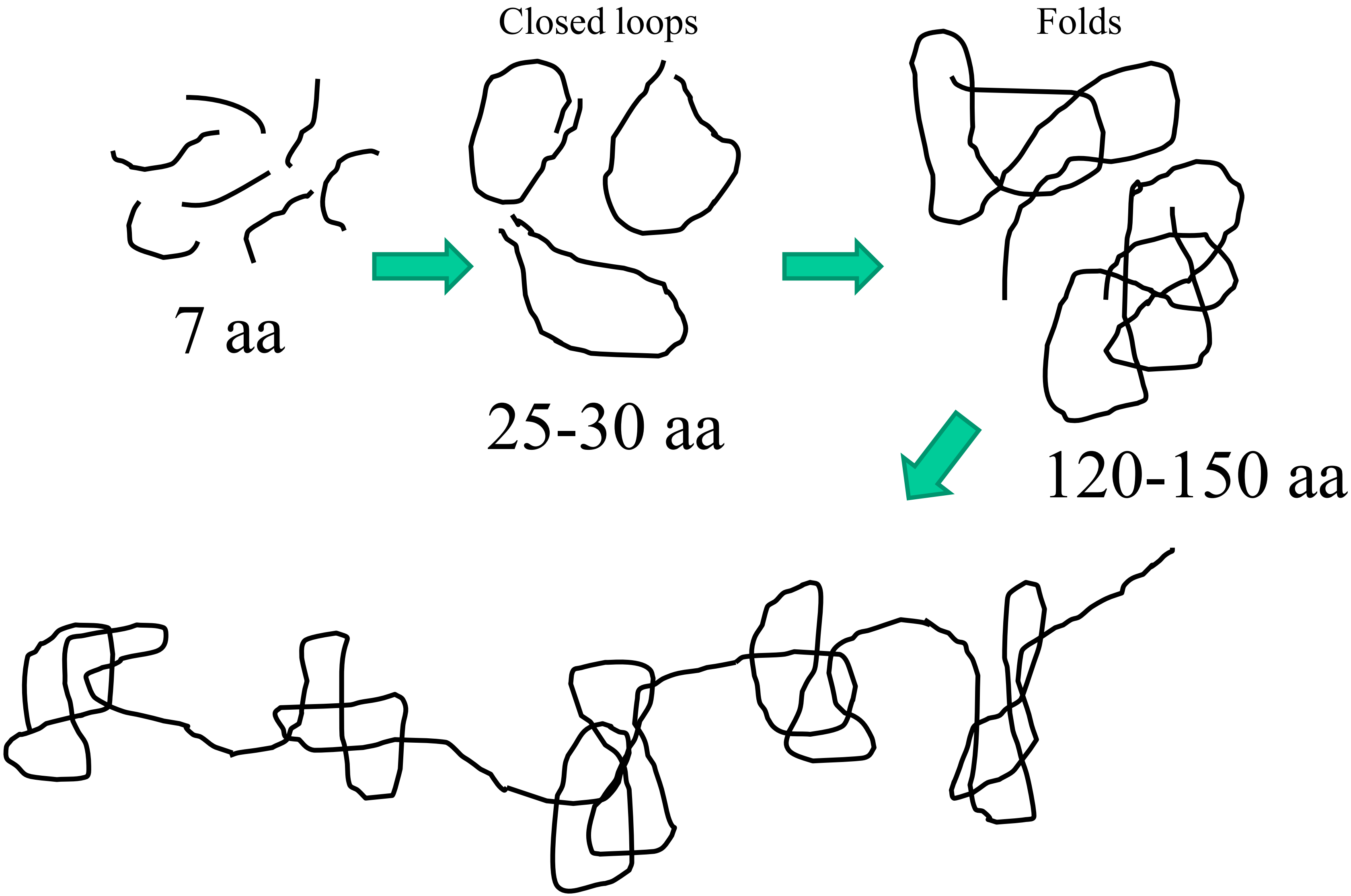


Early genomic stage



“Evolution may have proceeded largely, rather than periferally, through extrachromosomal elements”

D. Reanney
Bact. Rev. 40, 552, 1976



Closed loops

Folds

7 aa

25-30 aa

120-150 aa

Multifold proteins

One striking case of overlapping codes

Triplet extension patterns
for A+T rich prokaryotic genomes

species	G+C content %	extension motif
F. nucleatum	27.2	[(a)t]**(A)(T)**[(a)t]
N. equitans	31.6	(ta)t**(A)** **t**(at)
- " -		(at)**a** **(T)**a(ta)
S. solfataricus	35.8	[(t)a]ttt**(A)(T)**[(a)(t)]
T. denicola	37.9	[(a)t]**(A)(T)**[a(t)]
C. pneumoniae	40.0	[g(a)]**G(A)**[g(a)
- " -		[(t)c]**(T)C**[(t)c]
M. acetivorans	42.7	[g(a)]**G(A)(T)C**[(t)c]
A. aeolicus	43.3	[gg(a)]**gG(A)**[gg(a)]
- " -		[(t)cc]**(T)C**c[(t)cc]
B. subtilis	43.5	[g(a)(t)]**G(A)(T)C**[(a)(t)c]
T. maritima	46.2	(gaa)**G(A)**[g(a)]
- " -		[(t)c]**(T)C**(ttc)
D. ethenogenes	48.9	(cggc)cggc**(T)C**agccg(gccg)
consensus		**G(A)(T)C**

CGAAAATTTTCG

same as in eukaryotes!:

CGRAAATTTYCG

What this periodical motif codes for in prokaryotes?

```
(GAAAATTTTC)(GAAAATTTTC)....
   AAAATTTTC)(GAAAATTTTC)(G....
    AAATTTTC)(GAAAATTTTC)(GA....
```

GAA AAT TTT CGA AAA TTT TCG AAA ATT TTC
glu asn phe arg lys phe ser lys ile phe

AAA ATT TTC GAA AAT TTT CGA AAA TTT TCG
lys ile phe glu asn phe arg lys phe ser

AAA TTT TCG AAA ATT TTC GAA AAT TTT CGA
lys phe ser lys ile phe glu asn phe arg

non-polar
amino acids

ala
gly
ile
leu
met
phe
pro
val

polar
amino acids

arg
asn
asp
cys
glu
gln
his
lys
ser
thr
trp
tyr

Our pattern shows alternation of polar and non-polar residues, with the period 3.5 residues

NF kappaB recognition sequences
(NF kappaB is the heaviest duty transcription factor)

IL-1β-κB	GGGAAAA	TCC	T	
TNFα	GGGAAAG	CCC		C
Urokinase	GGGAAAG	TAC		C
E-selectin (PD3)	GGGAAAG	TTT		C
Ifn-B	GGGAAA	TTCC		C
Lymphotoxin	GGGAAG	CCCC		C
TCR-β	GGGAGA	TTCC		C
PRDII	GGGAAA	TTCCT	T	
GCR	GGGGGG	CACC	T	
ICAM1	TGGAAA	TTCC	H	
κB-33	TGGAAA	TTTC	H	
IL-2	AAGAA	TTTCC	H	
GM-CSF CK1	AGAAA	TTCC		C
G-CSF CK1	AGAAA	TTCC		C
IL-2 CD28RE	AGAAA	TTCC		C
IL-8 CD28RE	GGAAA	TTCC		C
GM-CSF	GGGAA	CTACC		C
TNFα (-655)	GGGAA	TTCAC		C
IL-2R	GGGAA	TTCCC		C
H2	GGGGA	TTCCC		C
E-selectin	GGGGA	TTTCC		C
LCAM	GGGGA	TTTCC		C
Lymphotoxin	GGGGG	CTTCC		C
GMCSF	TAGAA	TCTCC		C
IL-3 CD28RE	TGAGA	TTCC		C
IL-8	TGGAA	TTCCC	H	
Human P sequence	AAAA	TTTCC		C
TF	GGAG	TTTCC		C
Igκ	GGGA	CTTTCC		C
IL-2	GGGA	TTTCAC		C
IL-6	GGGA	TTTCC		C
Angiotensinogen	GGGA	TTTCCC		C
TNFα	GGGG	CTTTCC		C
VCAM	GGGG	TTTCCC		C
Mouse P sequence	AAA	TTTTCC		C
IFNγ	GAA	TTTTCC		C
6-16 ISRE	TCA	TTTTCC		C

GGRAA TTYCC

DNA curvature	**GAAAATTTTC**
Chromatin code	**GRAAATTTYC**
Amphipathic helices	**GAAAATTTTC**
NF kappaB	**GGRAATTYCC**
They all	**GRRAATTYYC**

Reading only one message, one gets three more, practically GRATIS !

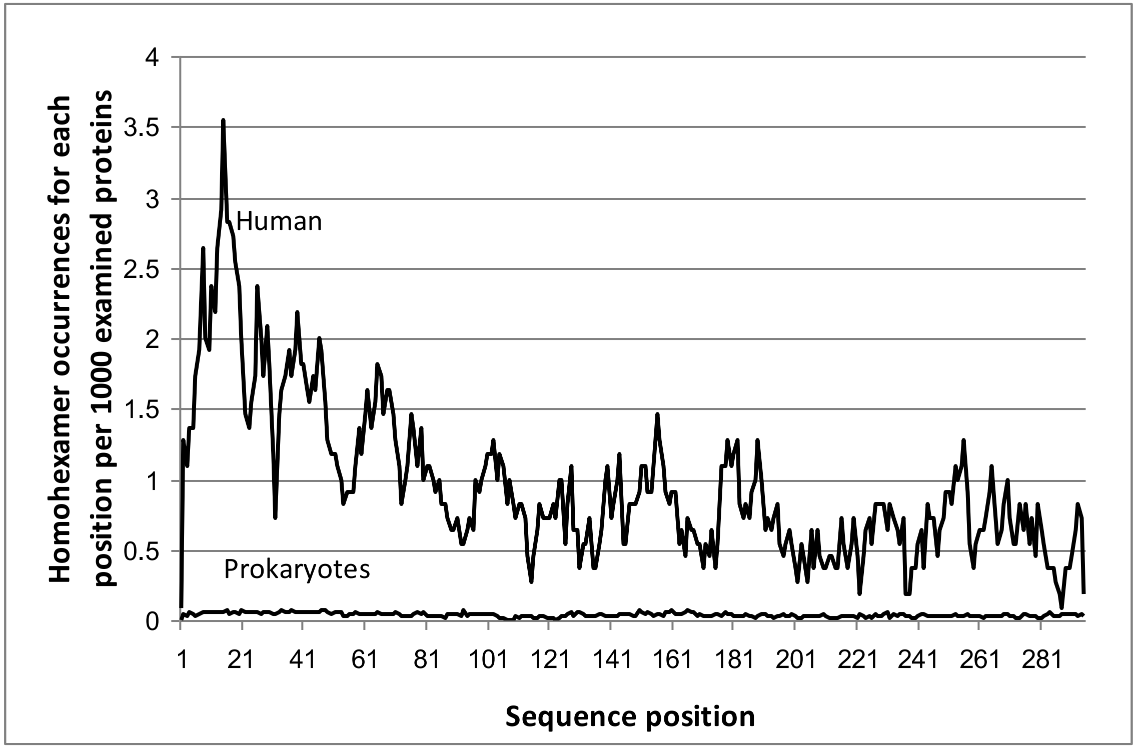
Not only there are many different codes
in the sequences,

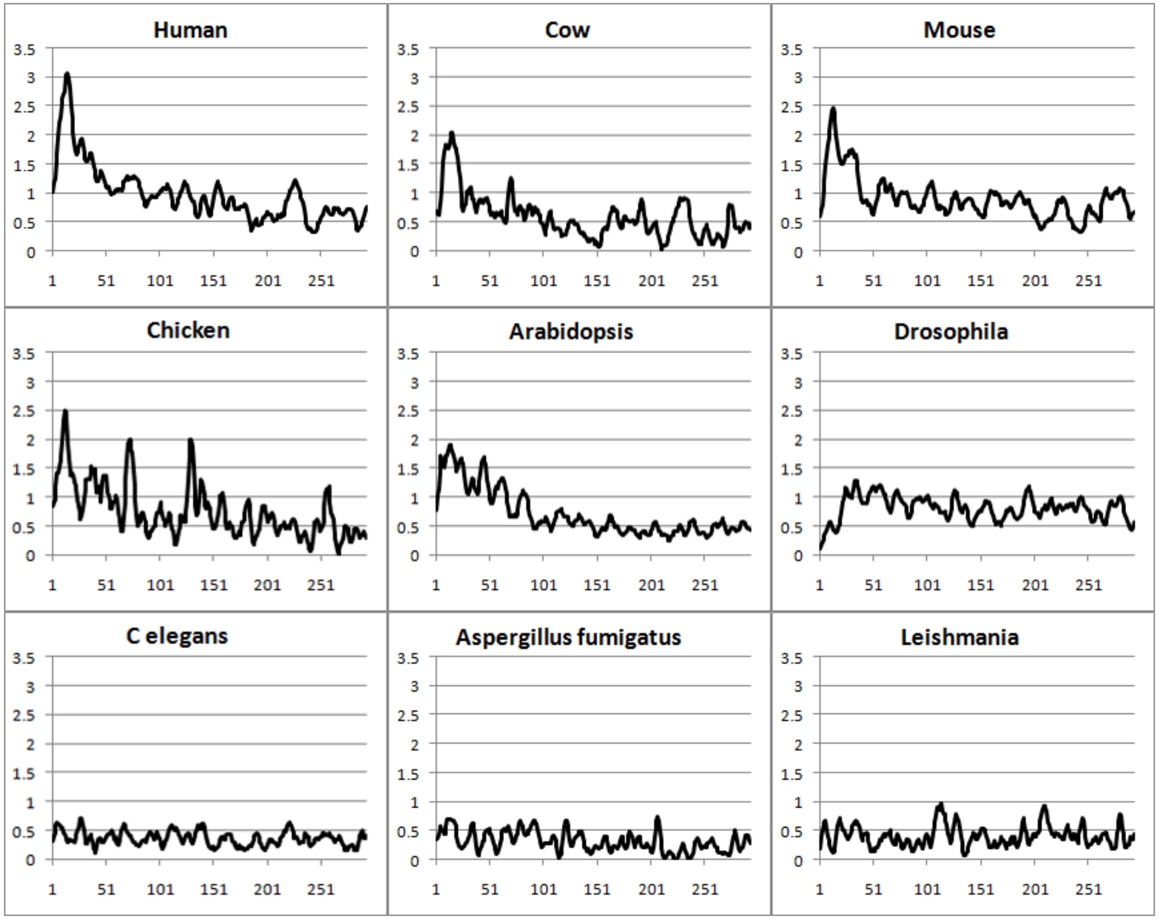
but also they overlap,

so that the same letters in a sequence
may take part simultaneously
in several different messages

Genome inflation code

Occurrence of homopeptides in protein sequences




Human
Cow
Mouse
Chicken
Arabidopsis
Drosophila
C elegans
Aspergillus fumigatus
Leishmania
3.5
3
2.5
2
1.5
1
0.5
0
1
51
101
151
201
251

Three known pathologically expanding ("aggressive") classes of triplets

GCU (GCU, CUG, UGC, AGC, GCA, CAG) ,

GCC (GCC, CCG, CGC, GGC, GCG, CGG) and

GAA (AAG, AGA, GAA, CTT, TTC, TCT).

They cause neurodegenerative diseases and chromosome fragility

Aggressive amino acids encoded by expanding triplets

L is encoded by **CTG** (GCT group) and **CTT** (AAG group),
A – by **GCT, GCA** (both GCT group), **GCC and GCG** (GCC group),
G – by **GGC** (GCC group),
P – by **CCG** (GCC group),
S – by **AGC** (GCT group) and **TCT** (AAG group),
E – by **GAA** (AAG group),
R – by **CGG, CGC** (both GCC group) and **AGA** (AAG group),
Q – by **CAG** (GCT group), and
K – by **AAG** (AAG group),
F – by UUC (AAG group),
C – by UGC (GCU group).

Majority of homopeptides are built from aggressive amino acids

human tripeptides 1st exons	Score (tripept.)	eukar. (Faux et al.)	prokar. (Faux et al.)
1. L3	**4552**	**1446**	**70(5)**
2. A3	**4046**	**5465(3)**	**251(3)**
3. G3	**2972**	**5002(5)**	**310(2)**
4. P3	**2258**	**4157(7)**	**217(4)**
5. S3	**1981**	**5424(4)**	**378(1)**
6. E3	**1630**	**4334(6)**	**67(6)**
7. R3	**1145**	**462**	**60(8)**
8. Q3	**802**	**8022(1)**	**52(9)**
9. K3	**535**	**1920(9)**	**25**
10. V3	414	94	9
11. H3	273	1049	32
12. D3	269	1554	34
13. T3	267	2492(8)	63(7)
14. I3	109	34	3
15. F3	**103**	**175**	**1**
16. C3	**92**	**38**	**0**
17. N3	79	6962(2)	31
18. M3	34	19	0
19. Y3	32	39	4
20. W3	14	3	0
	92%	**75%**	**89%**

EVOLUTION OF THE TRIPLET CODE

E. N. Trifonov, December 2007, Chart 101

Consensus temporal order of amino acids:

```
                     UCX         CUX     CGX AGY UGX AGR         UUY UAX
    Gly Ala Asp Val Ser Pro Glu Leu Thr Arg Ser TRM Arg Ile Gln Leu TRM Asn Lys His Phe Cys Met Tyr Trp Sec Pyl

 1  GGC-GCC  .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 2   |   |  GAC-GUC  .   .   .   .   .   .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 3  GGA--|---|---|--UCC  .   .   .   .   .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 4  GGG--|---|---|---|--CCC  .   .   .   .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 5   |   | (gag)-|---|---|--GAG-CUC  .   .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 6  GGU--|---|---|---|---|---|---|--ACC  .   .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 7   .  GCG--|---|---|---|---|---|---|--CGC  .   .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 8   .  GCU--|---|---|---|---|---|---|---|--AGC  .   .   .   .   .   .   .   . | .   .   .   .   .   .   .   .
 9   .  GCA--|---|---|---|---|---|---|---|---|--ugc  .   .   .   .   .   .   . | .   .  UGC  .   .   .   .   .
10   .   .   |   |   |  CCG--|---|---|--CGG  |   |   .   .   .   .   .   .   . | .   .   |   .   .   .   .   .
11   .   .   |   |   |  CCU--|---|---|---|---|---|--AGG  .   .   .   .   .   . | .   .   |   .   .   .   .   .
12   .   .   |   |   |  CCA--|---|---|---|---|--ugg  |   .   .   .   .   .   . | .   .   |   .   .  UGG  .   .
13   .   .   |   |  UCG------|---|---|--CGA  |   |   |   .   .   .   .   .   . | .   .   |   .   .   .   .   .
14   .   .   |   |  UCU------|---|---|---|---|---|--AGA  .   .   .   .   .   . | .   .   |   .   .   .   .   .
15   .   .   |   |  UCA------|---|---|---|---|--UGA  .   .   .   .   .   .   . | .   .   |   .   .   .  UGA  .
16   .   .   |   |   .   .   |   |  ACG-CGU  |   |   .   .   .   .   .   .   . | .   .   |   .   .   .   .   .
17   .   .   |   |   .   .   |   |  ACU-----AGU  |   .   .   .   .   .   .   . | .   .   |   .   .   .   .   .
18   .   .   |   |   .   .   |   |  ACA---------ugu  .   .   .   .   .   .   . | .   .  UGU  .   .   .   .   .
19   .   .  GAU--|-----------|---|----------------------AUC  .   .   .   .   . | .   .   .   .   .   .   .   .
20   .   .   .  GUG----------|---|-----------------------|--cac  .   .   .   . |CAC  .   .   .   .   .   .   .
21   .   .   .   |   .   .   |  CUG----------------------|--CAG  .   .   .   . | |   .   .   .   .   .   .   .
22   .   .   .   |   .   .   |   |   .   .   .   .   .  aug-cau  .   .   .   . |CAU  .   .  AUG  .   .   .   .
23   .   .   .   |   .   .  GAA--|-----------------------|---|--uuc  .   .   . | .  UUC  .   .   .   .   .   .
24   .   .   .  GUA--------------|-----------------------|---|---|--uac  .   . | .   |   .   .  UAC  .   .   .
25   .   .   .   |   .   .   .  CUA----------------------|---|---|--UAG  .   . | .   |   .   .   |   .   .  UAG
26   .   .   .  GUU--------------|-----------------------|---|---|---|--AAC  . | .   |   .   .   |   .   .   .
27   .   .   .   .   .   .   .  CUU----------------------|---|---|---|---|--AAG| .   |   .   .   |   .   .   .
28   .   .   .   .   .   .   .   .   .   .   .   .   .   |  CAA-UUG  |   |   | | .   |   .   .   |   .   .   .
29   .   .   .   .   .   .   .   .   .   .   .   .   .  AUA------|--uau  |   | | .   |   .   .  UAU  .   .   .
30   .   .   .   .   .   .   .   .   .   .   .   .   .  AUU------|---|--AAU  | | .   |   .   .   .   .   .   .
31   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .  UUA-UAA      | | .   |   .   .   .   .   .   .
32   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .  uuu---------AAA| .  UUU  .   .   .   .   .   .

                    CONSECUTIVE ASSIGNMENT OF 64 TRIPLETS                                  CODON CAPTURE
   aa "age":
    17  17  16  16  15  14  13  13  12  11              10   9           8   7   6   5   4   3   2   1
```

"... if **variations** useful to any organic being ever do occur, assuredly individuals thus characterized will have the best chance of being preserved in the struggle for life;
and from the strong principle of inheritance, these will tend to **produce offspring similarly characterized**“

Charles Darwin, Origin of Species (1859)

Rephrasing (ET):

Individuals with useful **variations** will **self-reproduce**

not Life yet

(self-reproduction only)

Life

(self-reproduction and variations)

```
       ↓                ↓
   Gly  Ala| Val  Asp  Ser  Pro ...
           |
1  GGC--GCC|
2   |    |   GUC--GAC
3  GGA---|----|----|---UCC
4  GGG---|----|----|----|---CCC
.
.
```

Life is self-reproduction with variations

Human Genome Composition

Protein-coding and RNA-coding	3%
Non-coding DNA	97%
of which	
Simple sequence repeats	3% (underestimate)
Transposable elements	45%

"repeat sequences account for at least 50% and, probably, much more"

From E. S. Lander *et al.* Initial sequencing and analysis of the human genome, Nature 409, 860-921, 2001

Could it be that protein sequences,
actually, are ALL originally made
from the aggressive repetitions?

And we don't see all the original repeats
just because they have
extensively mutated.

If this view is correct, then we should see in mRNA sequences

1. Ideal repeats of some codons - observed

2. The codons “sandwiched” between two identical codons should be their point mutation derivatives

3. Those codons which are more often in tandem repeats should be also of higher usage in non-repeats

We, thus, undertook analysis

of the largest non-reduntant database of mRNAs available,

of total ~5 000 000 000 codons,

eukaryotes, prokaryotes, viruses, organelles together

Z. Frenkel, E. Trifonov, JBSD, 30, 201-210 (2012)

Sorted occurrence of the triplet repeats for different groups ("aggressive" triplets)

	group of codons	Occurrence
1	**GCC, CCG, CGC, GGC, GCG, CGC**	**1 784302**
2	**GCA, CAG, AGC, UGC, GCU, CUG**	**1 436660**
3	**GAA, AAG, AGA, UUC, UCU, CUU**	**1 131214**
4	AAU, AUA, uaa, AUU, UUA, UAU	932105 (1 118526)
5	AUC, UCA, CAU, GAU, AUG, uga	735397 (882476)
6	ACC, CCA, CAC, GGU, GUG, UGG	726443
7	AGG, GGA, GAG, CCU, CUC, UCC	706484
8	AAC, ACA, CAA, GUU, UUG, UGU	694387
9	ACG, CGA, GAC, CGU, GUC, UCG	533888
10	ACU, CUA, UAC, AGU, GUA, uag	152747 (183296)

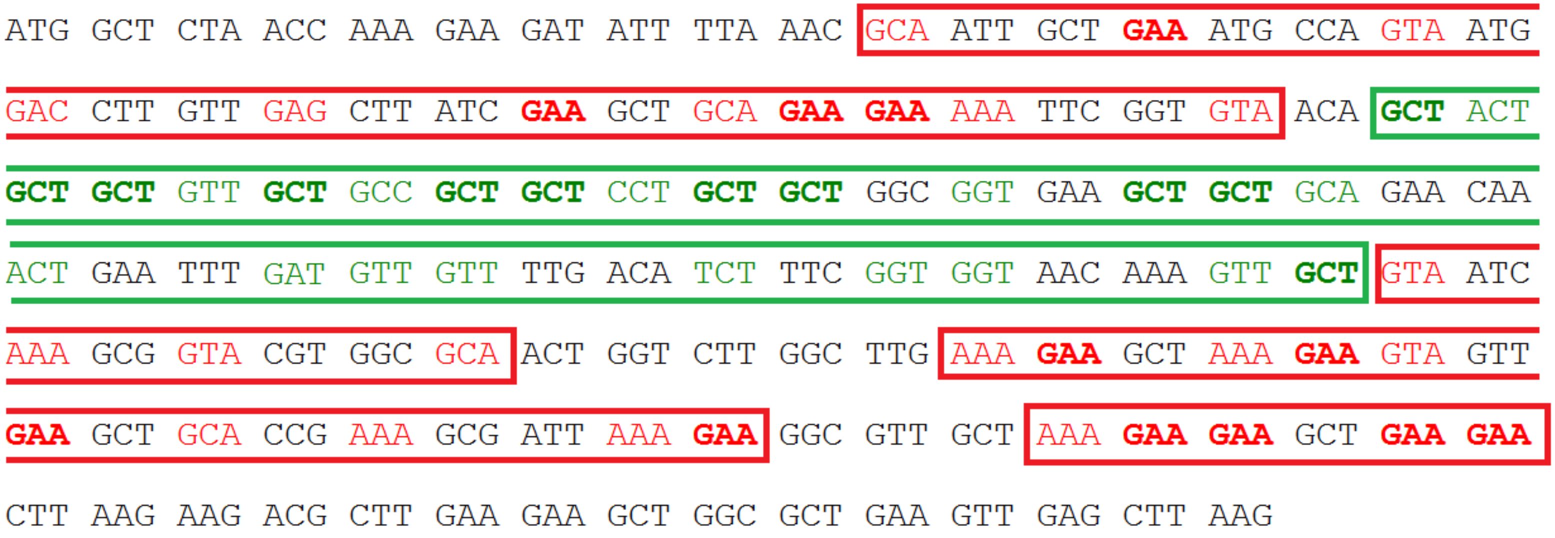
1. Tandem repeats of all 61 different codons are observed, strongest for aggressive groups, as expected

2. Middle codons abc in “sandwiches” GCUabcGCU (total 3 168 933) are most often first derivatives of GCU

GCU	243706	
GG**U**	125946	
GA**U**	115500	
GAA	114278	the topmost in codon usage
GU**U**	102550	
GCA	95493	
GCC	92153	
AUU	89648	
UUU	87861	
AAA	84194	next topmost in codon usage
UUA	80660	
GGA	74934	
GGC	71770	
	…	

This also holds for most of other codons

2. The first derivatives between the identical codons in mRNA keep memory of initial tandem repetition of the codons

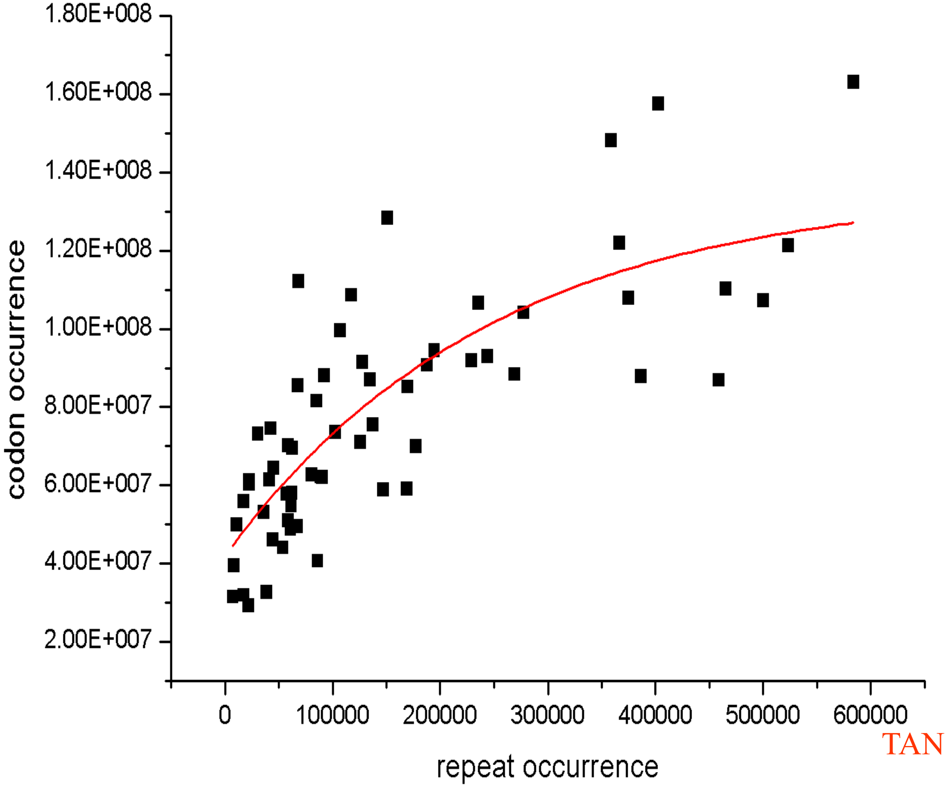


ATG GCT CTA ACC AAA GAA GAT ATT TTA AAC GCA ATT GCT **GAA** ATG CCA GTA ATG
GAC CTT GTT GAG CTT ATC **GAA** GCT GCA **GAA** **GAA** AAA TTC GGT GTA ACA **GCT** ACT
GCT **GCT** GTT **GCT** GCC **GCT** **GCT** CCT **GCT** **GCT** GGC GGT GAA **GCT** **GCT** GCA GAA CAA
ACT GAA TTT GAT GTT GTT TTG ACA TCT TTC GGT GGT AAC AAA GTT **GCT** GTA ATC
AAA GCG GTA CGT GGC GCA ACT GGT CTT GGC TTG AAA **GAA** GCT AAA **GAA** GTA GTT
GAA GCT GCA CCG AAA GCG ATT AAA **GAA** GGC GTT GCT AAA **GAA** **GAA** GCT **GAA** **GAA**
CTT AAG AAG ACG CTT GAA GAA GCT GGC GCT GAA GTT GAG CTT AAG

GAA and GCT “bricks” in mRNA of
ribosomal protein L12 of *Ps. atlantica*

3. The more frequently the codon appears in tandem the more frequent it is also in non-repeating regions of mRNA

NON-REPEATING REGIONS



TANDEMS

This result came as a surprize,
considering zelions of factors
known to influence the codon usage

More frequent codons keep memory of
tandem repetition of these codons
in the past

The triplet expansion of codons
is the major single factor
shaping the codon usage

Thus, life started with the replication (and expansion)
and subsequent mutations
of tandemly repeating triplets GGC and GCC.
(self-reproduction with variation)

Life continued then to spontaneously emerge
within the primitive early genomes and further on,
in form of replication and expansion
and subsequent mutations
of other tandem repeats as well
(self-reproduction with variation)

Life never stopped emerging

The tandem repeats have been considered as a class of
"selfish DNA" (Orgel and Crick, 1980; Doolittle and Sapienza, 1980).

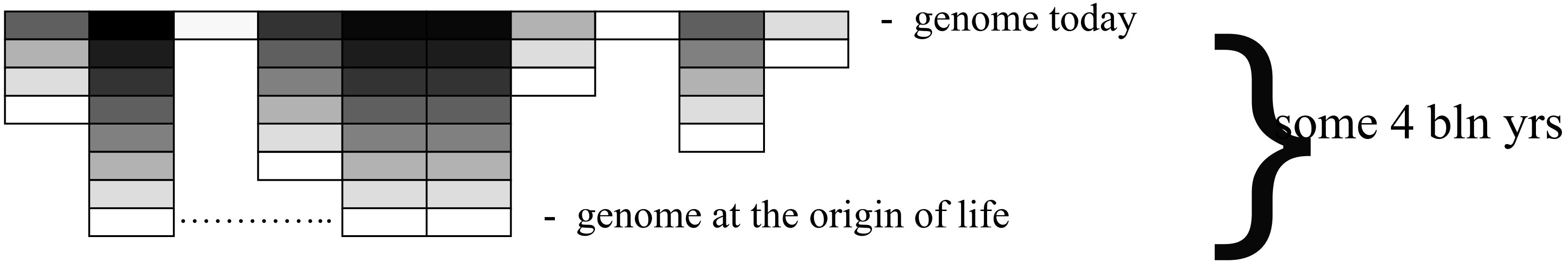
They are, actually, more than just parasites tolerated by genome.
They are even more than
building material for the genome (Ohno, Junk DNA, 1972).

The tandem repeats represent constantly emerging life,
and genomes are products of their everlasting domestication.

Genomes are built by the expansion and
mutational domestication of the tandem repeats

Genomes ARE the repeats
(some already unrecognizable)

Genes and protein sequences evolve as a mosaic of expanding
nucleotide and amino acid repeating sequences,
gradually mutating to their modern sequence appearance
not recognizable as repeats anymore

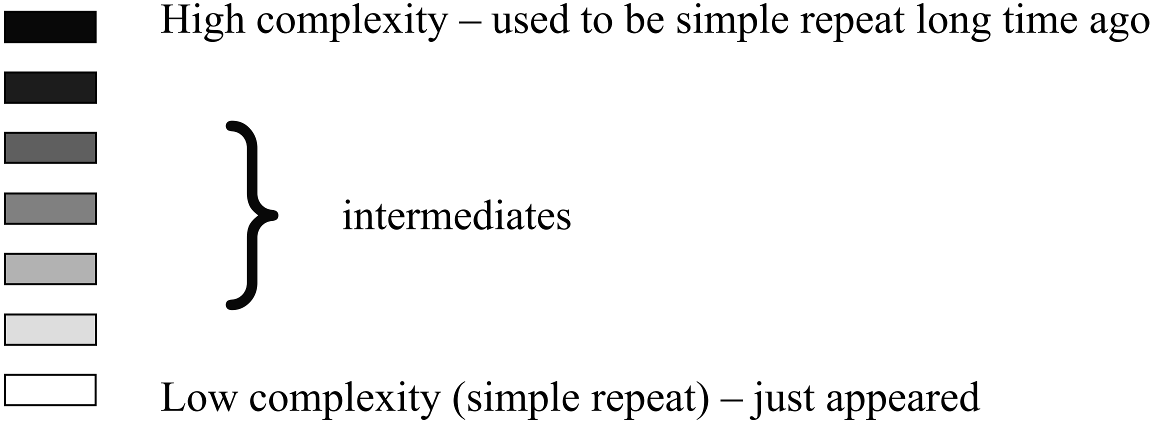


- genome today

- genome at the origin of life

some 4 bln yrs

Genomes are all built from simple repeats.
Just many of them already unrecognizable



High complexity – used to be simple repeat long time ago

intermediates

Low complexity (simple repeat) – just appeared

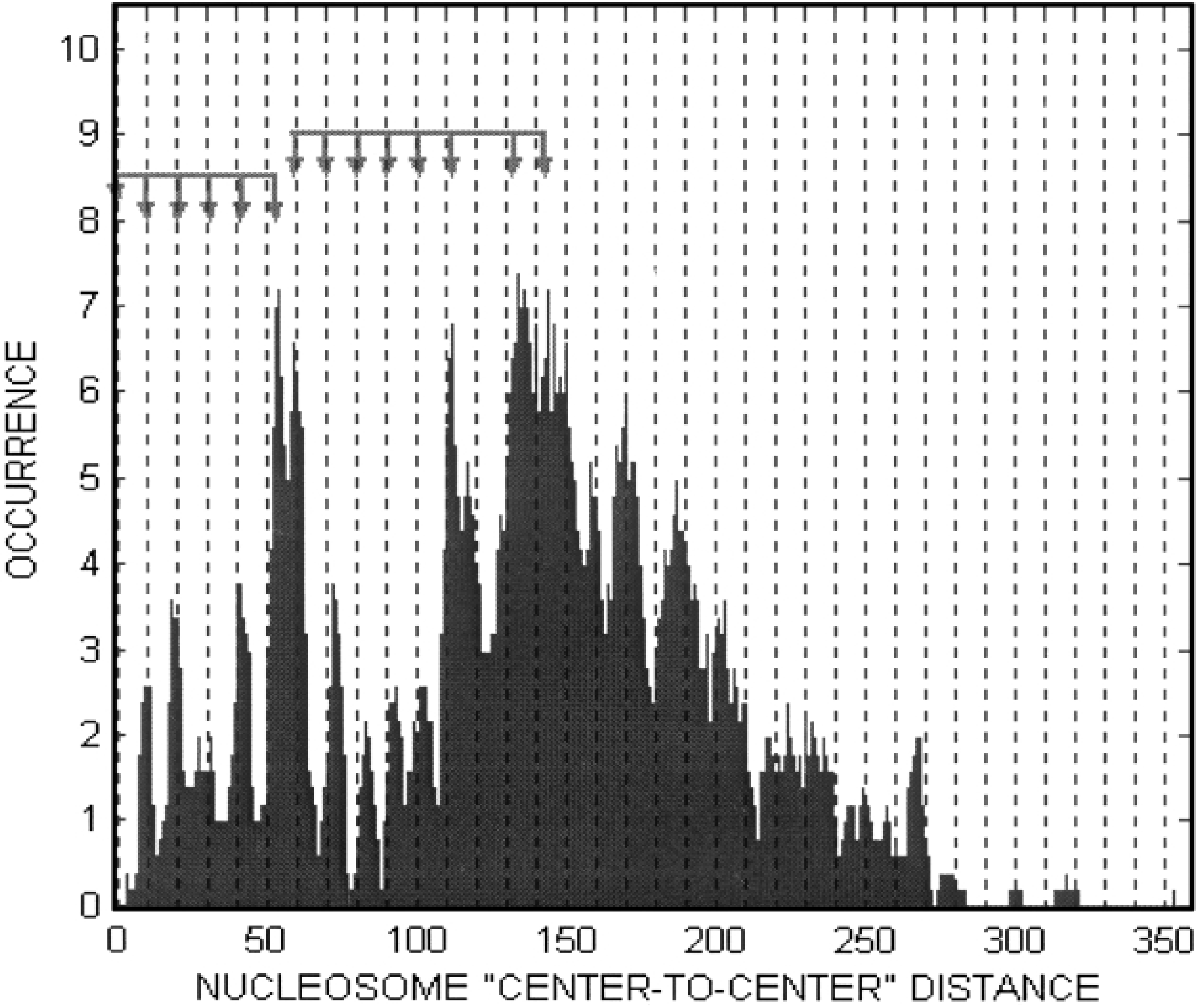
I wish you all success
in your studies, exams
and healthy interesting life

Total 406 slides (2014)

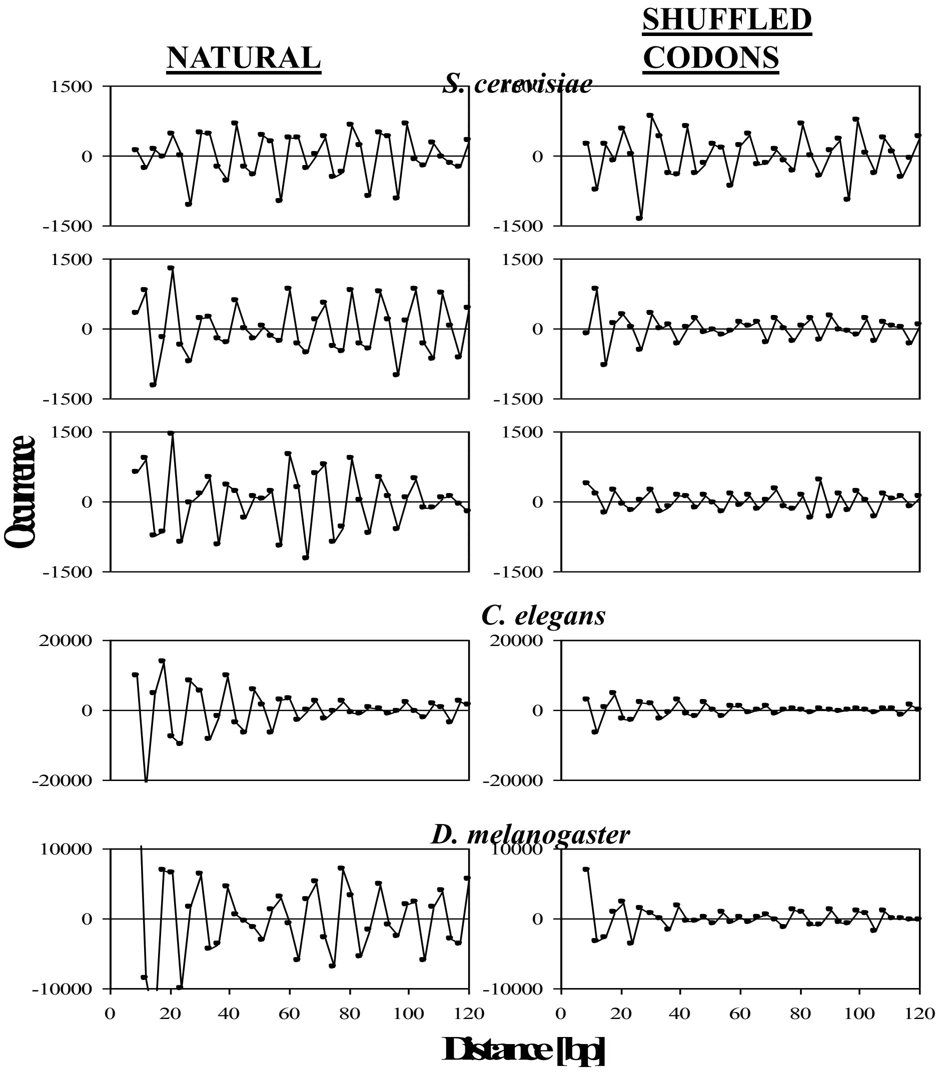
5-lectures course, 80 slides each

Edward N. Trifonov

(kakhol ve lavan)
(blue and white)


OCCURRENCE
0
1
2
3
4
5
6
7
8
9
10
0
50
100
150
200
250
300
350
NUCLEOSOME "CENTER-TO-CENTER" DISTANCE

AA-PERIODICITY DISAPPEARS WHEN THE THIRD POSITIONS ARE RANDOMIZED



CODON POSITIONS

1,2

2,3

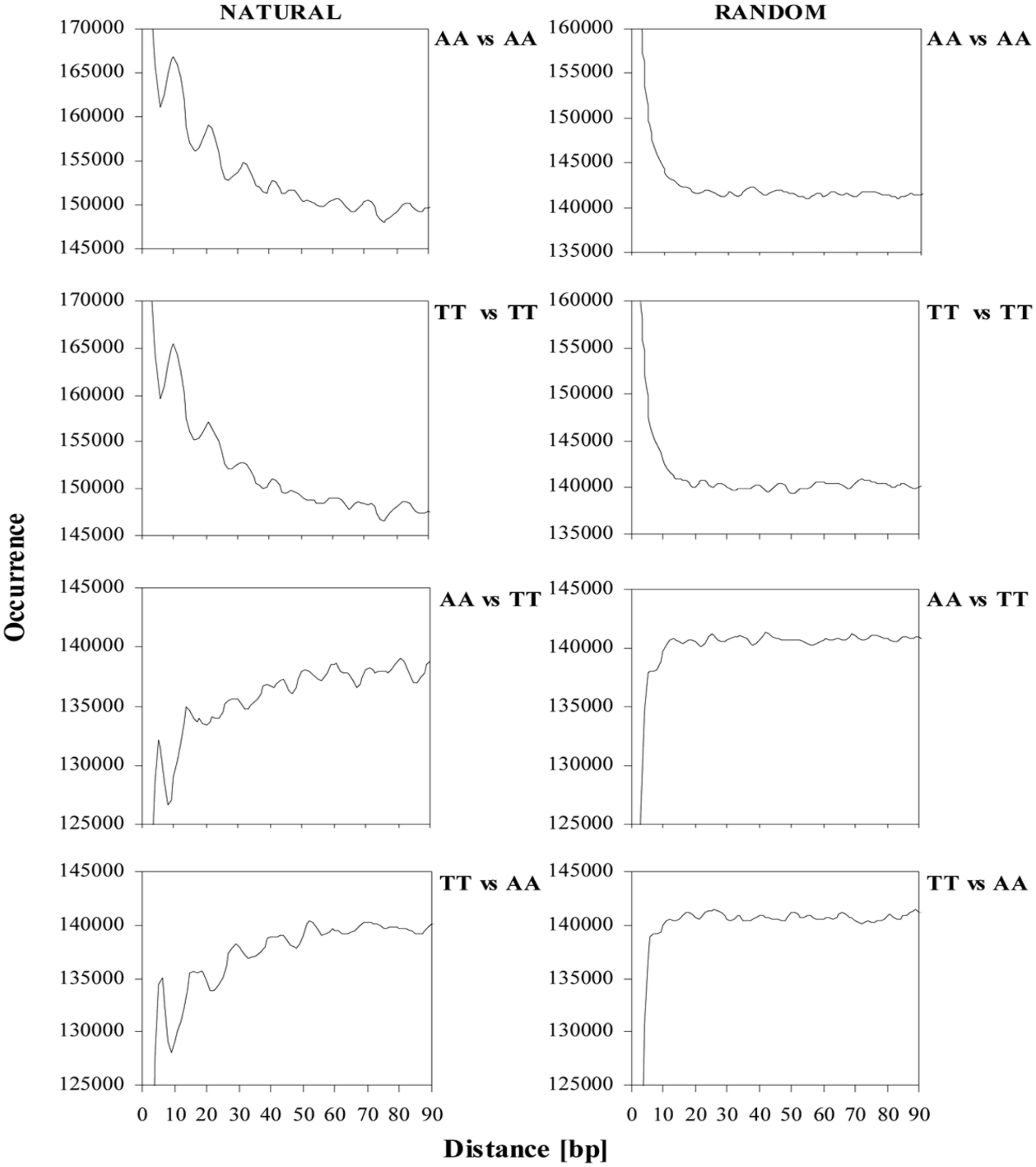
3,1

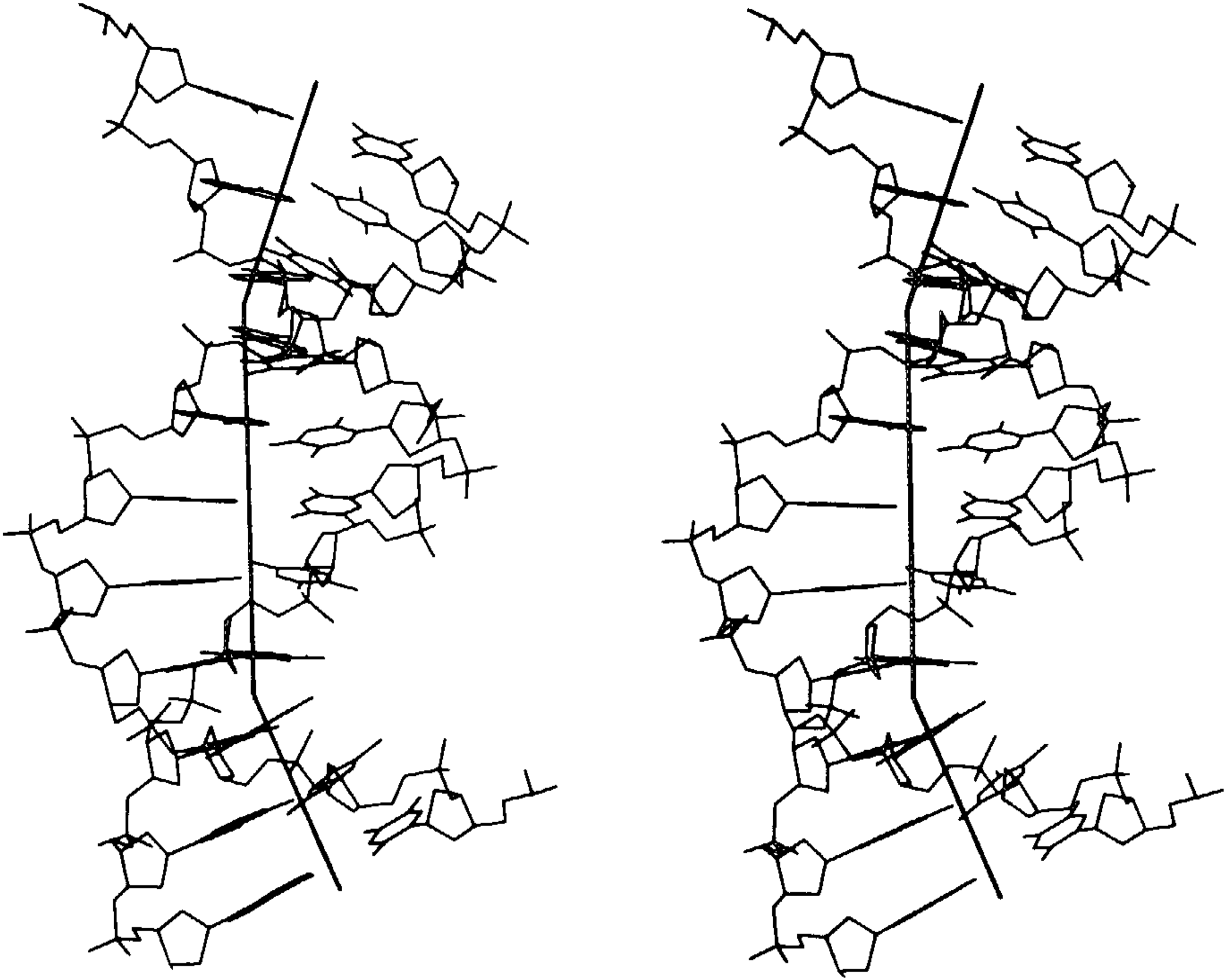
3,1

3,1

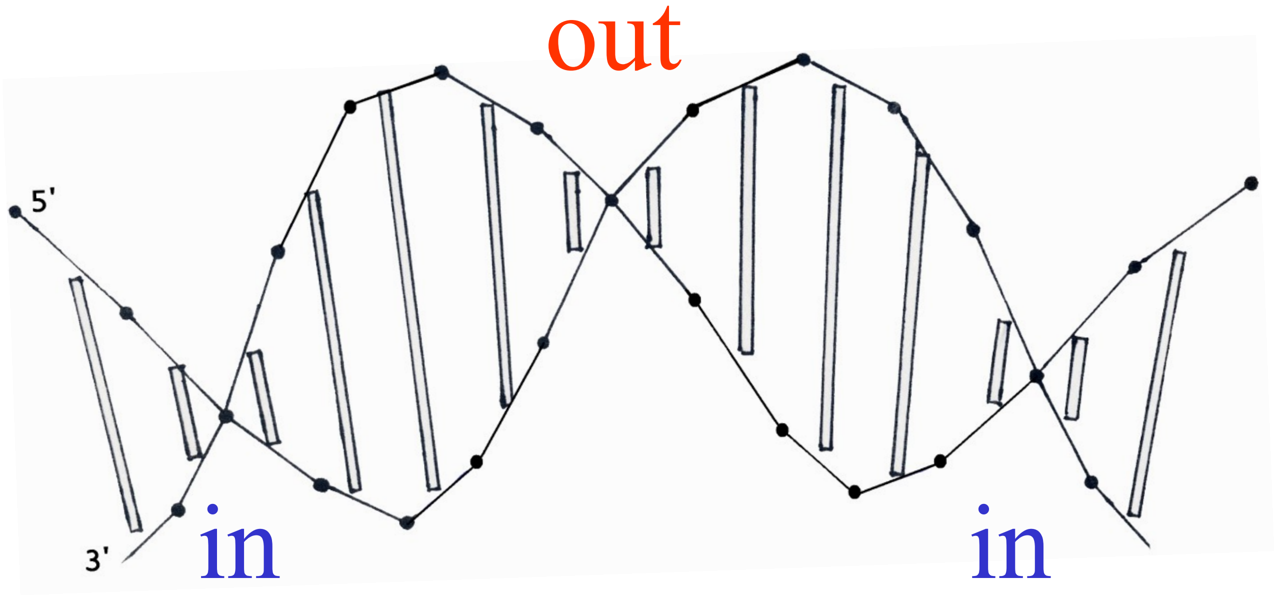
Cohanim 2006

Yeast
Cohanim, 2005





Ulyanov and Zhurkin, JBSD, 1984



Mere physics

SSSS WWWW SSSS ←

weak base pair stacks
should be OUT,
as they are easier
to deform (unstack).

YR RY YR ←

R stacks are on the surface,
i. e. IN (Zhurkin, 2010)

Y RRR YYY R ←

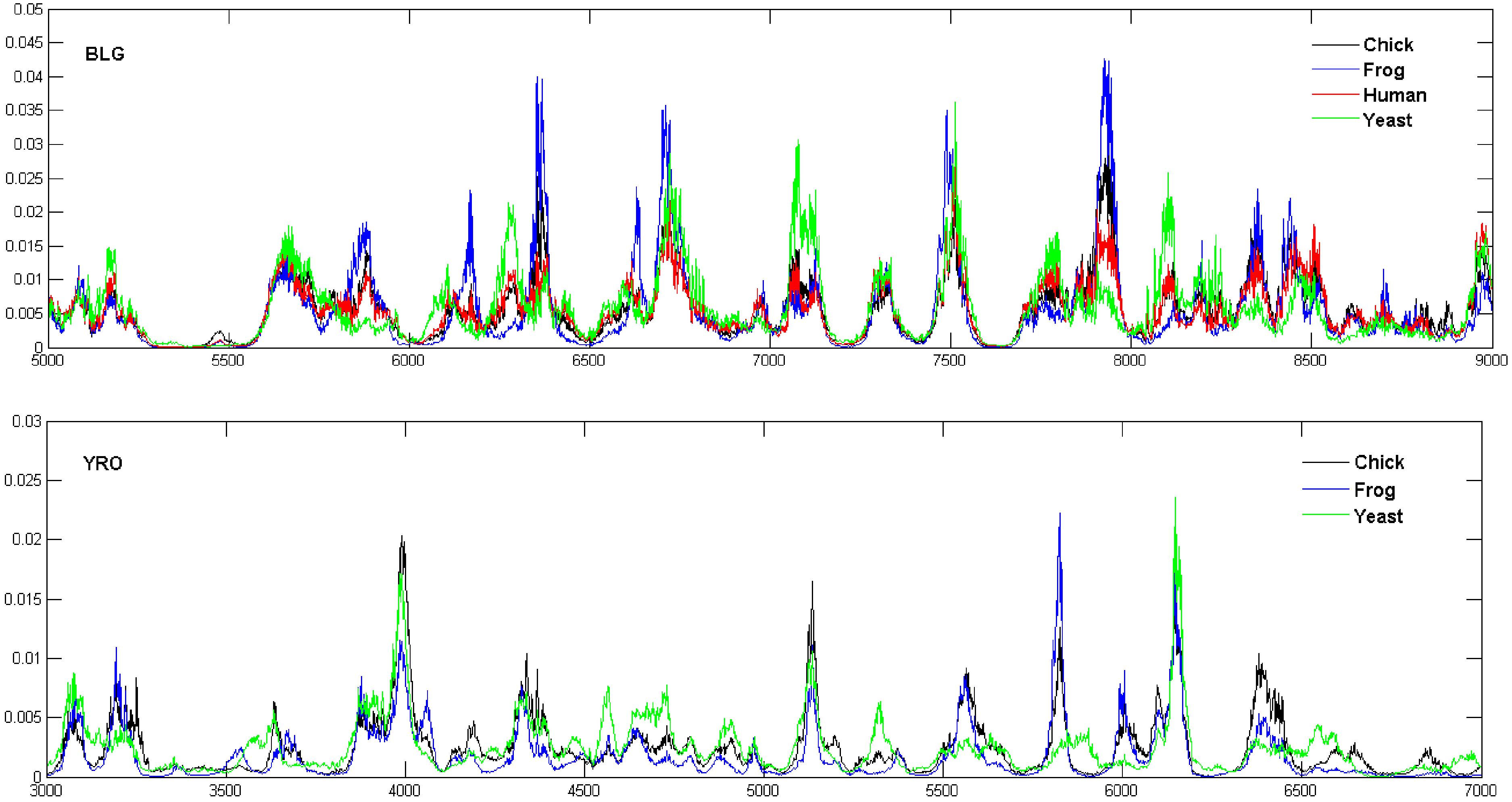
rines, with stronger stacking
between them,
should be on the surface

CCGGRAATTYCCGG ←

a unique merger
of the binary patterns

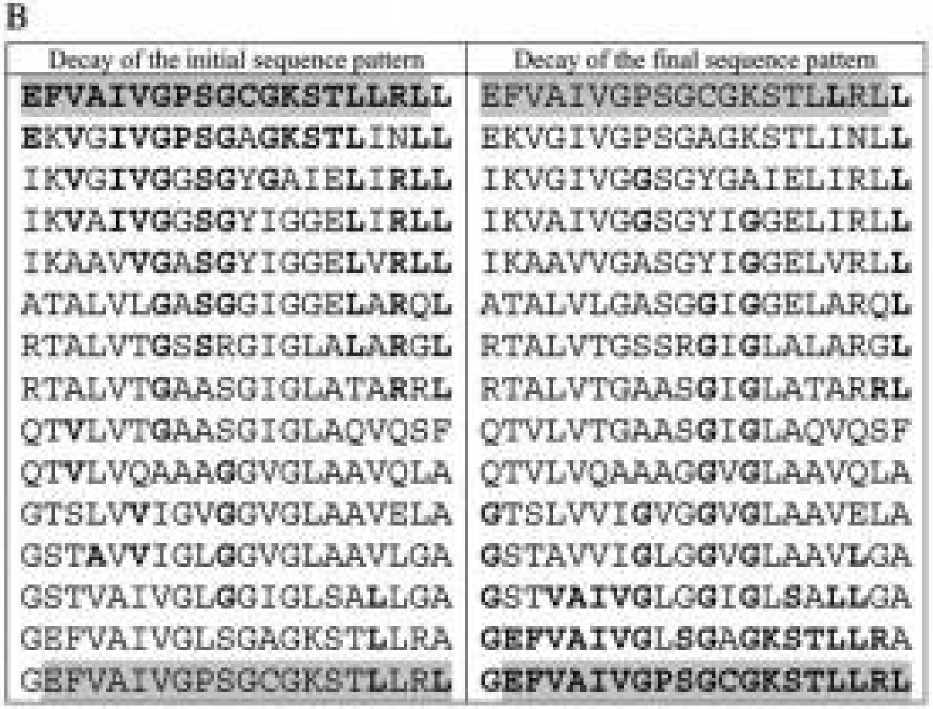
CCGGAAATTTCCGG ←

A+T rich genomes



Species-specificity of nucleosome positioning
Allan et al. JMB, 2010

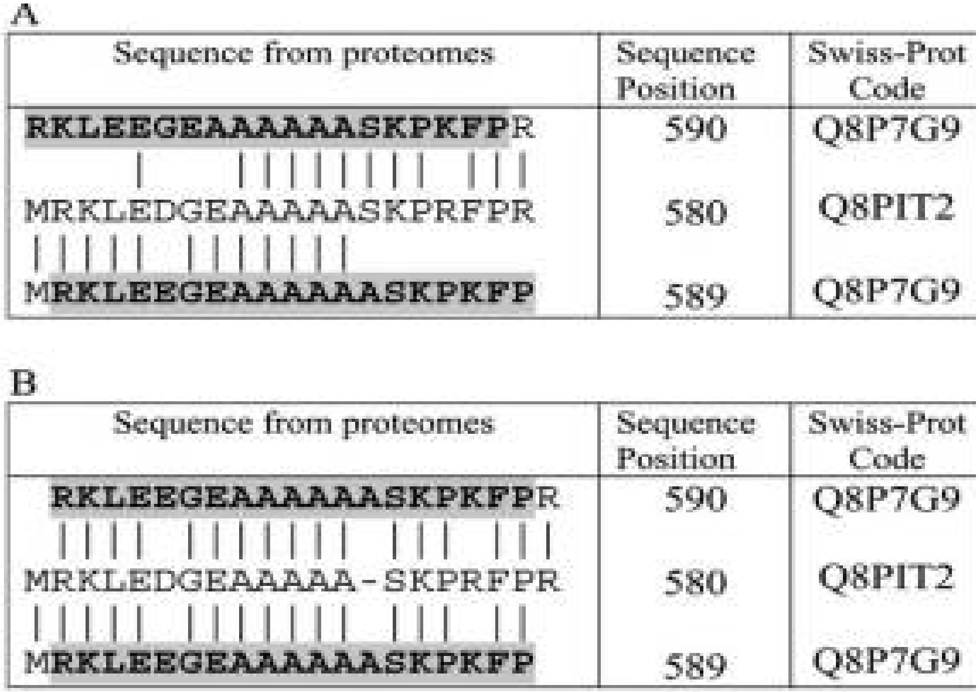
Sequences shifted by one residue may belong to the same network



B

Decay of the initial sequence pattern	Decay of the final sequence pattern
EFVAIVGPSGCGKSTLLRLL	EFVAIVGPSGCGKSTLLRLL
EKVGIVGPSGAGKSTLINLL	EKVGIVGPSGAGKSTLINLL
IKVGIVGGSGYGAIELIRLL	IKVGIVGGSGYGAIELIRLL
IKVAIVGGSGYIGGELIRLL	IKVAIVGGSGYIGGELIRLL
IKAAVVGASGYIGGELVRLL	IKAAVVGASGYIGGELVRLL
ATALVLGASGGIGGELARQL	ATALVLGASGGIGGELARQL
RTALVTGSSRGIGLALARGL	RTALVTGSSRGIGLALARGL
RTALVTGAASGIGLATARRL	RTALVTGAASGIGLATARRL
QTVLVTGAASGIGLAQVQSF	QTVLVTGAASGIGLAQVQSF
QTVLVQAAAGGVGLAAVQLA	QTVLVQAAAGGVGLAAVQLA
GTSLVVIGVGGVGLAAVELA	GTSLVVIGVGGVGLAAVELA
GSTAVVIGLGGVGLAAVLGA	GSTAVVIGLGGVGLAAVLGA
GSTVAIVGLGGIGLSALLGA	GSTVAIVGLGGIGLSALLGA
GEFVAIVGLSGAGKSTLLRA	GEFVAIVGLSGAGKSTLLRA
GEFVAIVGPSGCGKSTLLRL	GEFVAIVGPSGCGKSTLLRL

Formation of shifted self by deletion of repeating residue



A

<table>
<tr><th>Sequence from proteomes</th><th>Sequence Position</th><th>Swiss-Prot Code</th></tr>
<tr><td><pre>RKLEEGEAAAAAASKPKFPR</pre></td><td>590</td><td>Q8P7G9</td></tr>
<tr><td><pre> | |||||||| |||</pre></td><td></td><td></td></tr>
<tr><td><pre>MRKLEDGEAAAAASKPRFPR</pre></td><td>580</td><td>Q8PIT2</td></tr>
<tr><td><pre>||||| |||||||</pre></td><td></td><td></td></tr>
<tr><td><pre>MRKLEEGEAAAAAASKPKFP</pre></td><td>589</td><td>Q8P7G9</td></tr>
</table>

B

<table>
<tr><th>Sequence from proteomes</th><th>Sequence Position</th><th>Swiss-Prot Code</th></tr>
<tr><td><pre> RKLEEGEAAAAAASKPKFPR</pre></td><td>590</td><td>Q8P7G9</td></tr>
<tr><td><pre> |||| ||||||| ||| |||</pre></td><td></td><td></td></tr>
<tr><td><pre>MRKLEDGEAAAAA-SKPRFPR</pre></td><td>580</td><td>Q8PIT2</td></tr>
<tr><td><pre>||||| ||||||| ||| ||</pre></td><td></td><td></td></tr>
<tr><td><pre>MRKLEEGEAAAAAASKPKFP</pre></td><td>589</td><td>Q8P7G9</td></tr>
</table>