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")

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

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

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
since 2002	University of Haifa
2005, 2009	University of Rome "Sapienza", Italy
2007-2011	Masaryk University, Brno, Czech Republic

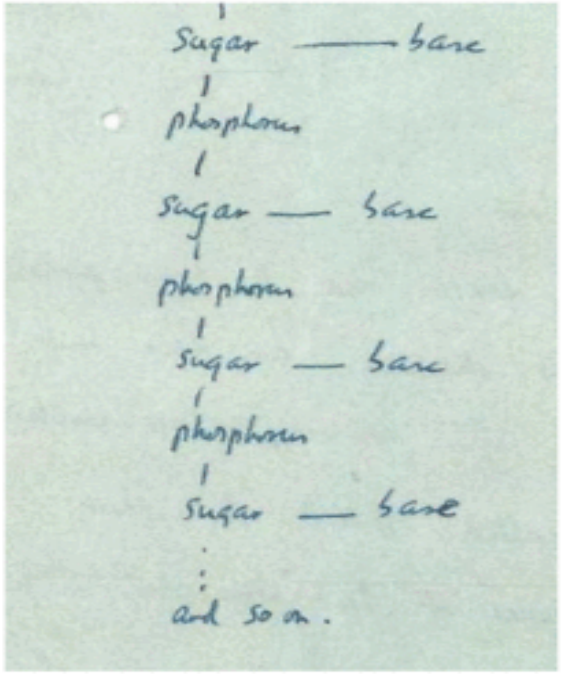
19 Portugal Place
Cambridge
19 March '53

My Dear Michael,

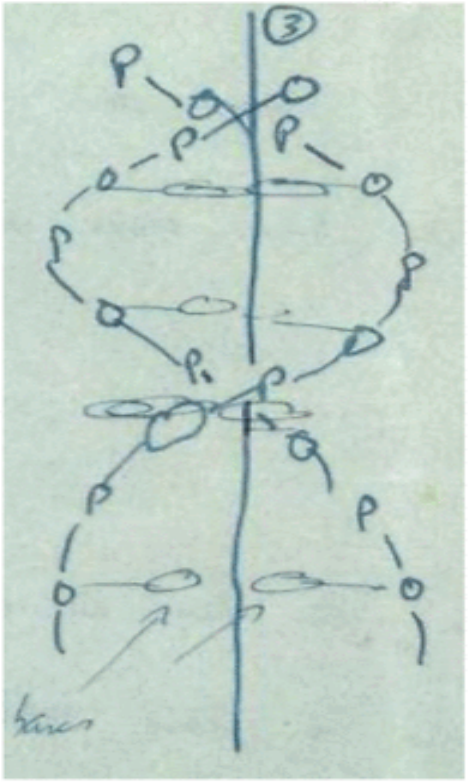
Jim Watson and I have probably made a most important discovery. We have built a model for the structure of des-oxy-ribose-nucleic-acid (read it carefully) called D.N.A. for short. You may remember that the genes of the chromosomes -- which carry the hereditary factors -- are made up of protein and D.N.A.

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]



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



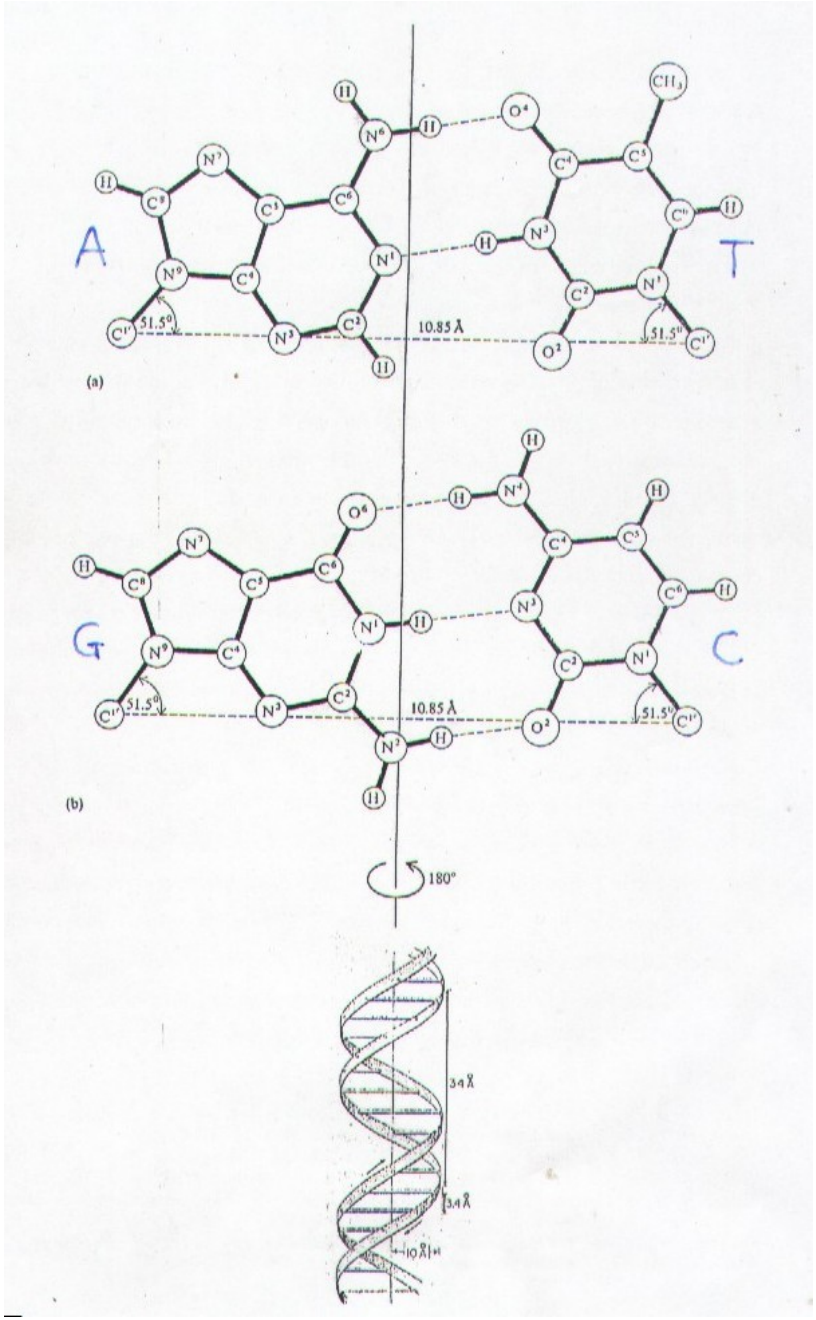
[drawing of double helix showing base pairings on inside]

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


A
T
10.85 Å
51.5°
(a)
G
C
10.85 Å
51.5°
(b)
180°
34 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

The idea on

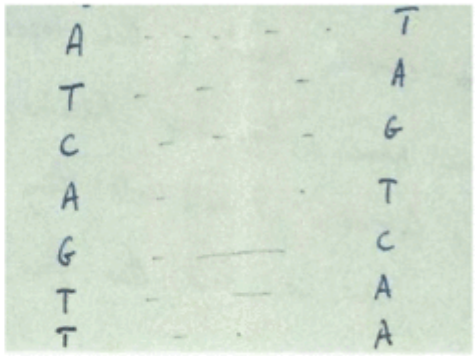
molecular complementarity
in macromolecular interactions

was outlined by
Linus Pauling and Max Delbruck
in 1940

Nature 371, 285, 1994

> then the second must go

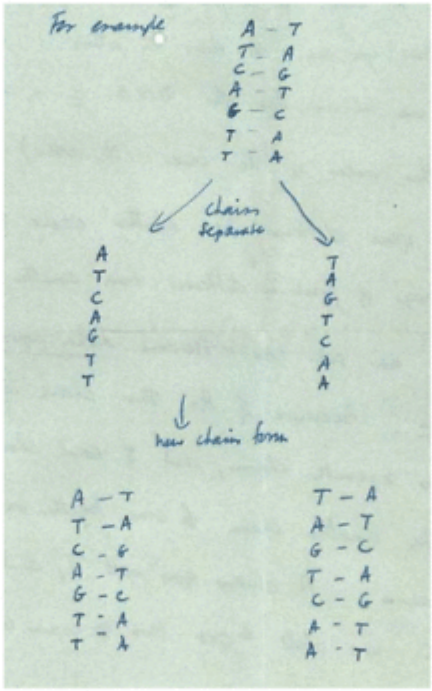
A - - - - - - - - - - - - - - - - - T
T - - - - - - - - - - - - - - - - - A
C - - - - - - - - - - - - - - - - - G
A - - - - - - - - - - - - - - - - - T
G - - - - - - - - - - - - - - - - - C
T - - - - - - - - - - - - - - - - - A
T - - - - - - - - - - - - - - - - - A



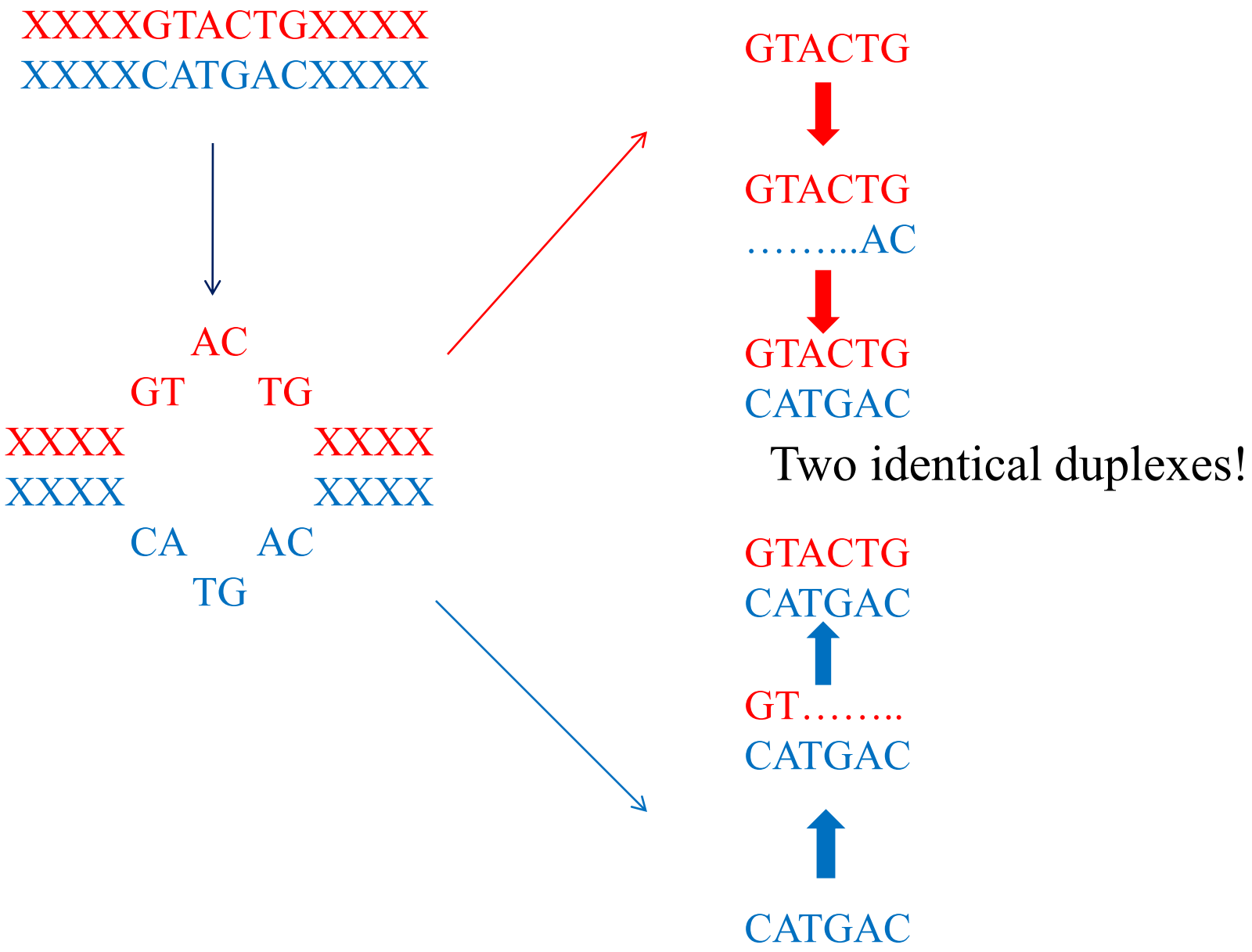
It is like a code. If you are given one set of letters you can write down the others.

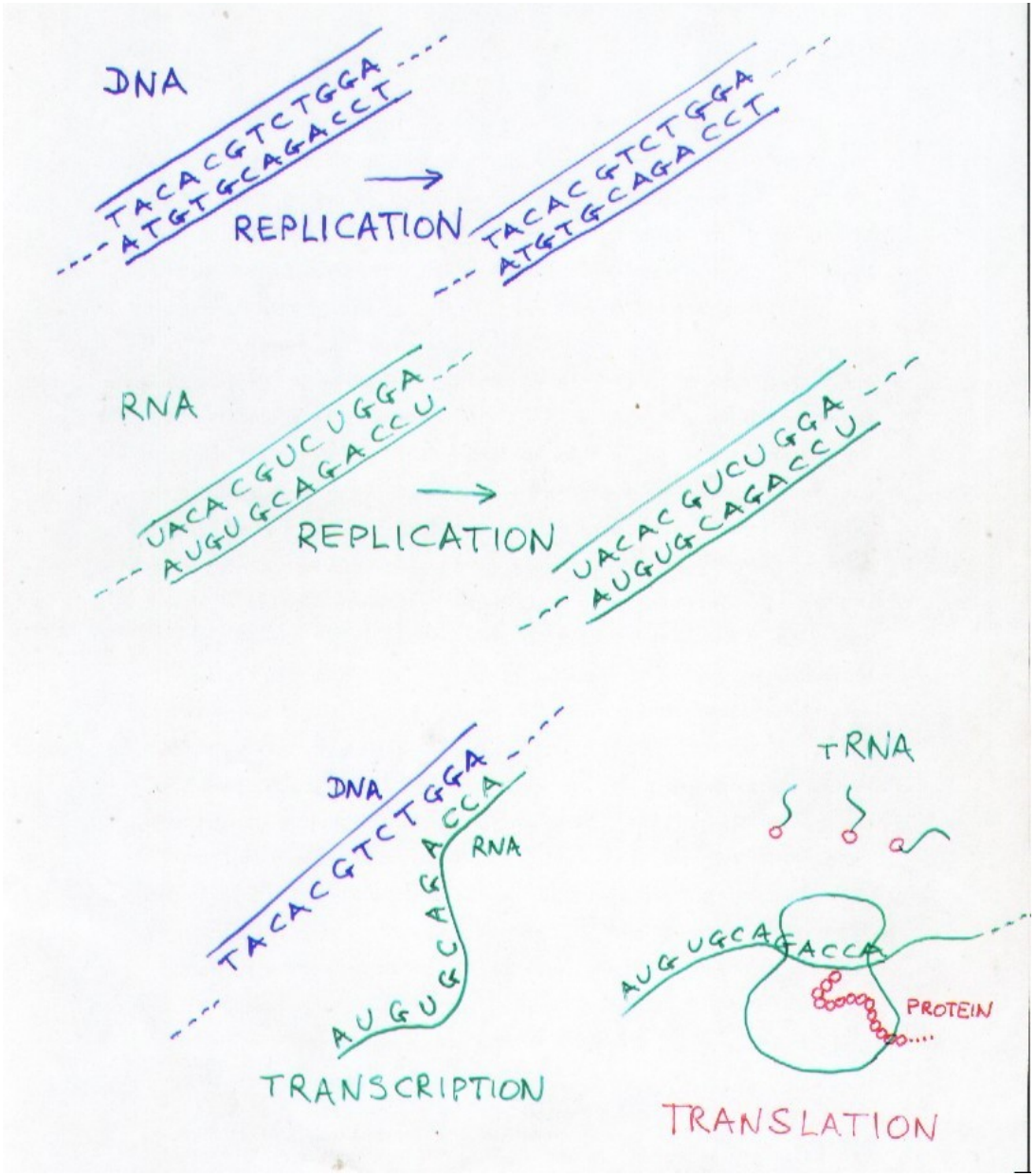
Now we believe that the D.N.A. is a code. That is, the order of the bases (the letters) makes one gene different from another gene (just as one page of print is different from another). You can now see how Nature makes copies of the genes. Because if the two chains unwind into two separate chains, and 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


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

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

Salvador Dali

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"

Astbury and Bell (1938) discovered
3.3 A 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".

Transforming activity of DNA

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

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

identical tetranucleotide units

(Steudel, 1906; Levene and Simms, 1925)

GALACIDALACIDESOXIRIBUNUCLEICACID

(HOMAGE TO CRICK AND WATSON)

Erwin Chargaff established the "Chargaff's rule" in 1948:

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

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.

```
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 ttttttttt ttttttagt 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
EPCQPKVPEPCQPKVPEPCQPKVPEPCQPKVPEPCQPKVPQPCQPKVPEPCQPKVPEPCQP
KVPEPCQSKVPQPCQPKVPEPCQTKQKMADNLSQSFDKSAMTEEERRHIKKEIRKQIVAFA
LMIFLTLMSFMAVATDVIPRSFAIPFIFILAVIQFALQLFFFMHMKDKDHGWANAFMISGI
FITVPIAALMLLLGVNKISKIVKFLKELATPSHSMEFFHKPASNSLLASELNFVRRNIKRE
DFGHEVLTGAFGTLKSPVIVSIFHSRIVACEGGDGEEHDILFHTVAEKKPTICLDGQVFKL
KHISSEGEVMYYMFRQCAKRYASSLPPNALKPAFGPPDKVAAQKFKESLMATEKHAKDTSN
MWVKISVWVALPAIALTAVNTYFVEKEHAEHREHLKHVPDSEWPRDYEFMNIRSKPFFWGD
GDKTLFWNPVVNRHIEHDDQSTVHIVGDNTGWSVPSSPNFYSQWAAGKTFRVGDSLQFNFP
ANAHNVHEMETKQSFDACNFVNSDNDVERTSPVIERLDELGMHYFVCTVGTHCSNGQKLSI
NVVAANATVSMPPPSSSPPSSVMPPPVMPPPSPS

PROKARYOTIC GENOME

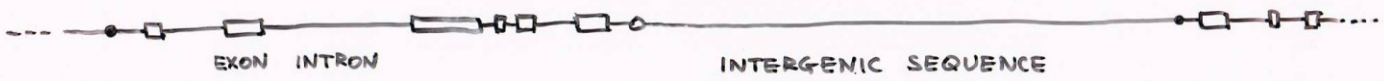
1 – 2 CIRCULAR CHROMOSOMES — 400 kBp – 4000 kBp

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

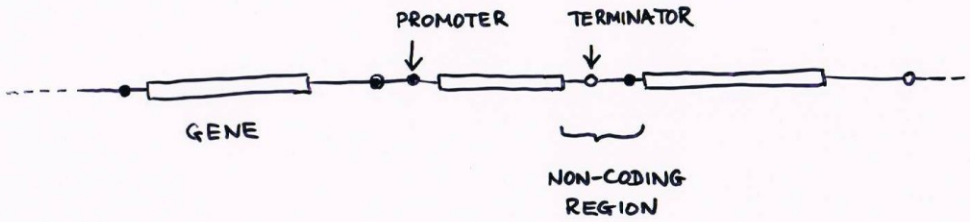


INTRONS & INTERGENIC SEQ-S:

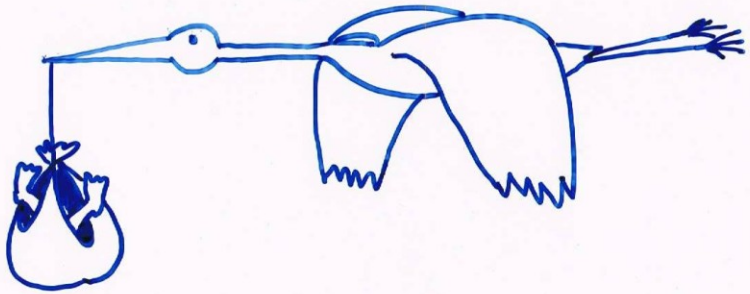
EXONS, rRNA GENES; tRNA	1 – 10 %
TRANSPOSONS & REPEATS:	20 – 40 %
INTRONS & UNASSIGNED SEQ-S:	50 – 70 %

VIRAL GENOME

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



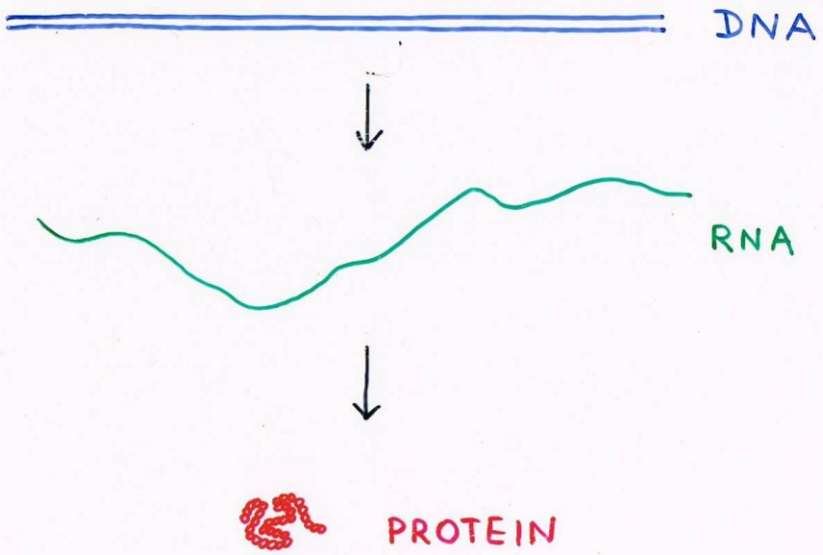
CODING REGIONS : ~ 80%



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

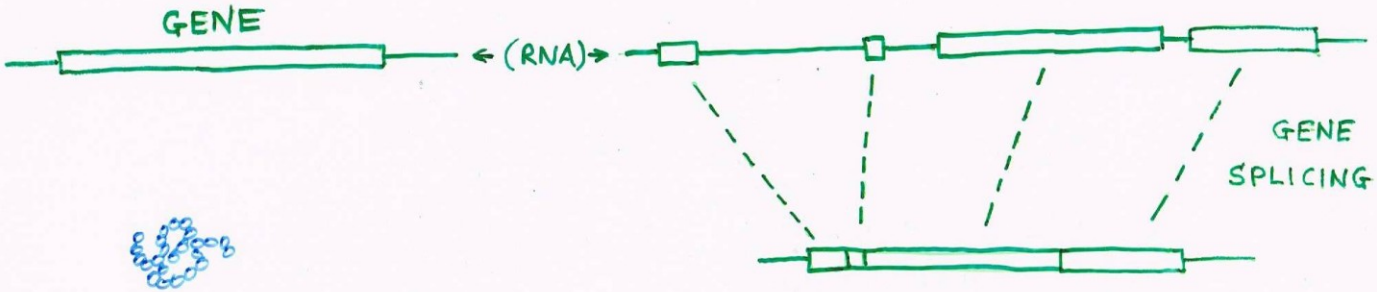
(Jacque Monod)

Jacque Monod died in 1976
Gene splicing was discovered in 1977


DNA
RNA
PROTEIN

BACTERIA

ANIMALS, PLANTS



PROTEIN

PROTEIN

Linguistics of genetic sequences

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)

NOW NO SWIMS ON MON

NOW NO SWIMS ON MON

dyad symmetry

G G A T C C

C C T A G G

Bam H1 restriction site

When placed in one sequence

….GGATCCxxxxxxxxxxxGGATTC….

the Bam H1 sites will make a hairpin with xxxxxxxxxxx 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

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 Pompeii

A B R A C A D A B R A

A B R A C A D A B R

A B R A C A D A B

A B R A C A D A

A B R A C A D

A B R A C A

A B R A C

A B R A

A B R

A B

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

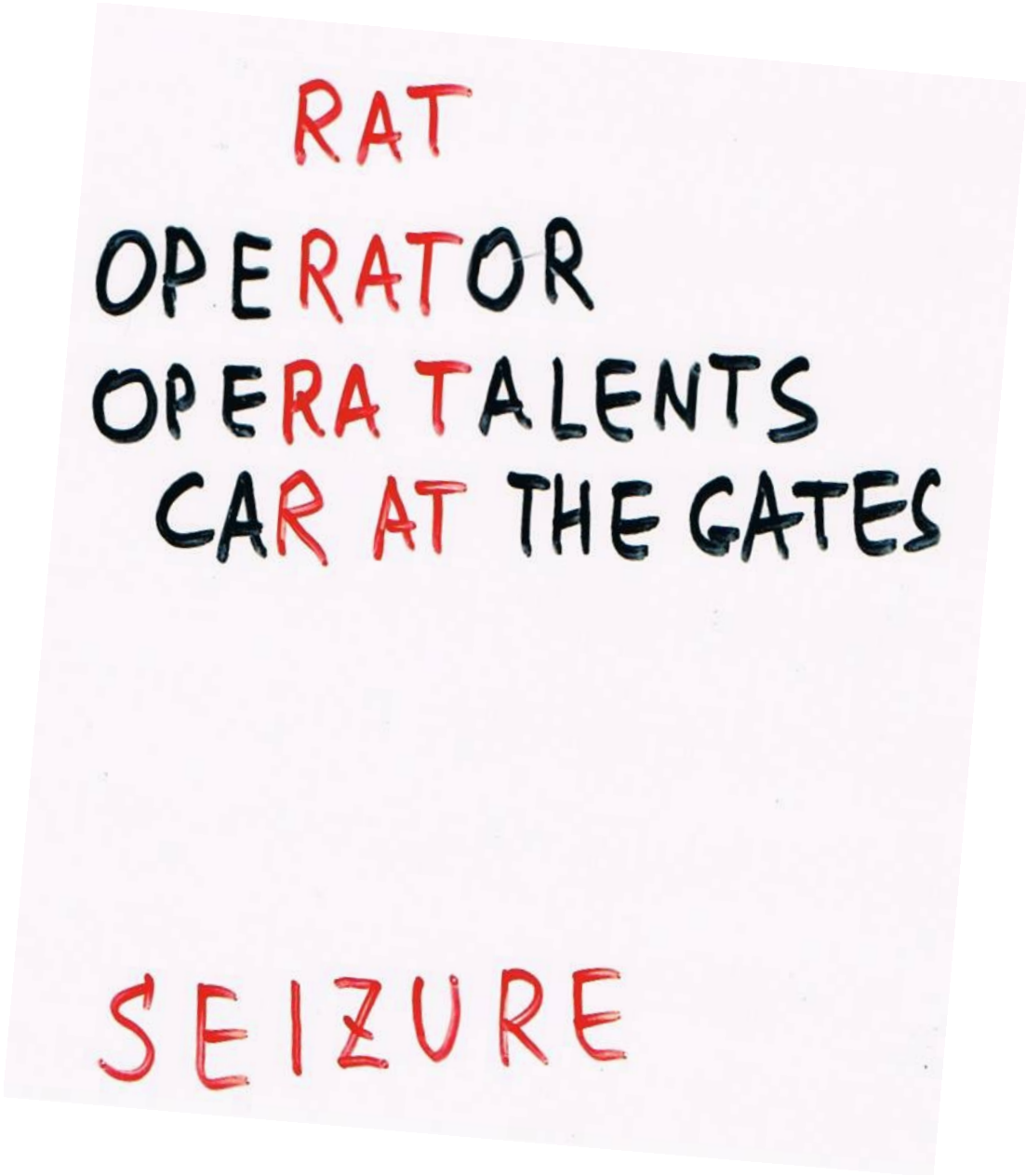
Various sequence types may be characterized

by so-called contrast words –

the words that expand uniquely

from inside of the word,

but continue randomly outside

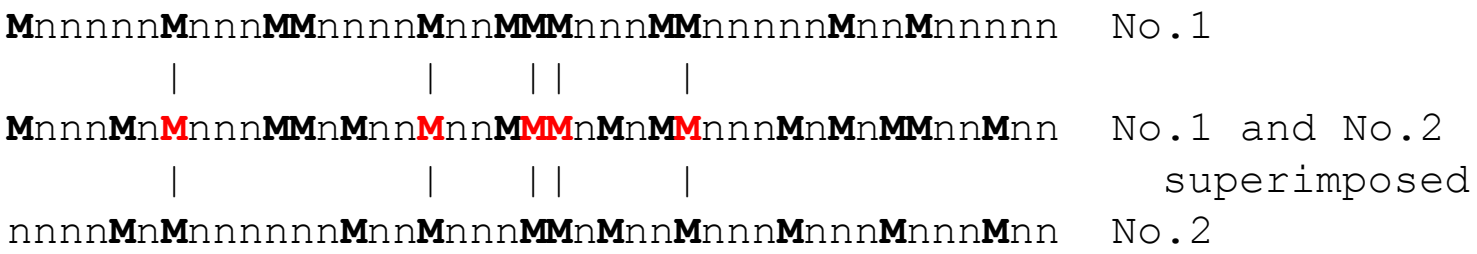

RAT
OPERATOR
OPERA TALENTS
CAR AT THE GATES
SEIZURE

TRIPLET CODE

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

Multiple overlapping codes

in the biological sequences


Mnnnnn Mnnn MM nnnn Mnn MMM nnn MM nnnnnn Mnn Mnnnnn No.1
Mnnn Mn Mnnn MM n Mnn Mnn MMM n Mn MM nnn Mn Mn MM nn Mnn No.1 and No.2 superimposed
nnnn Mn Mnnnnnn Mnn Mnnn MM n Mnn Mnnn Mnnn Mnnn Mnn No.2


Just because of you don't understand, you can't call us "Junk"!
http://www.biocomicals.com

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.

Definition of the sequence code:

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

(ENT, 1989)

There are, thus, many codes

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

TRIPLET CODE

G x x G x x G x x G x x G x x G x x ...

FRAMING CODE

AG x x x x x x x x AG x x x x x x x x AG x x ...
AAA x x x x x x x AAA x x x x x x x AAA x ...
GC x x x AG x CG x x CT x x x x TT x x x x ...

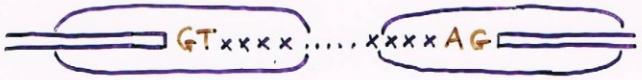
DNA SHAPE CODE

RR x x x YY x x x R R x x x YY x x x RR x x ...

CHROMATIN CODE

TGTG x x x x x x x x x x x x ...
TGTGTG x x x x x x x x x x ...
TGTGTGTG x x x x x x x x ...
TGTGTGTGTG x x x x x x ...
TGTGTGTGTGTG x x x x ...

MODULATION CODE

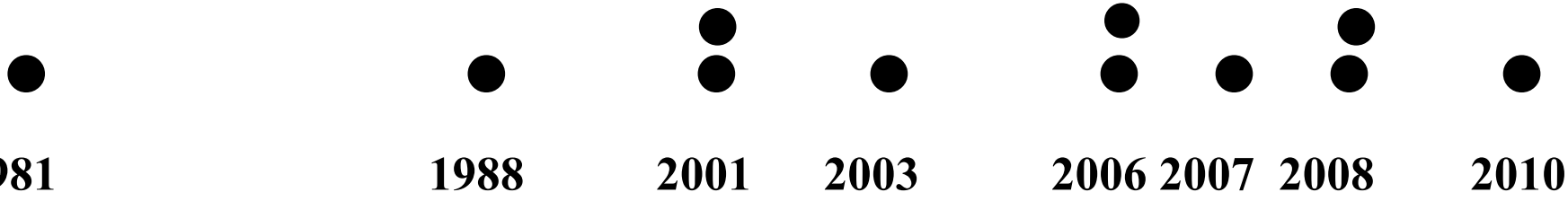


GENE SPLICING CODE

MET x x x x x x ... x x MET x x x x x x ... x x MET x x ...

GENOME SEGMENTATION CODE

The tale of 11 Second Genetic Codes



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

work is important, but hardly most of the answer to the puzzle

that some call "the second genetic code“
and others call "the protein recognition problem."

C. Vaughan, Science News, May 28, 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,"
S*cientific 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

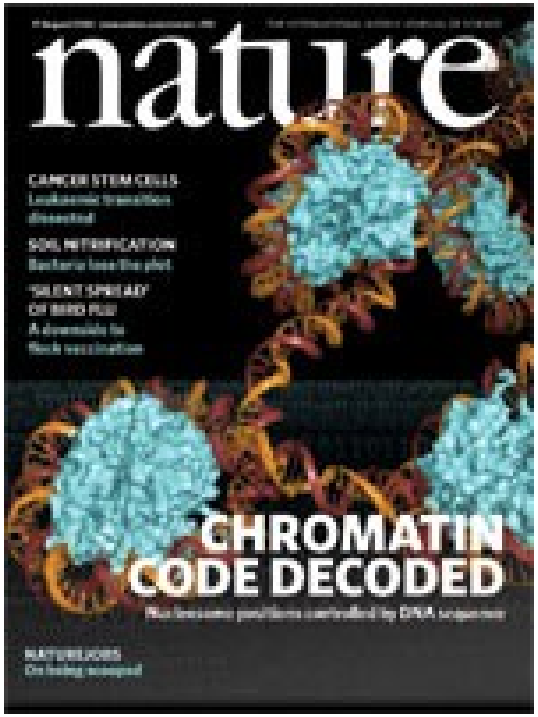
Eran Segal, Yvonne Fondufe-Mittendorf, Lingyi Chen, AnnChristine Thastrom,
Yair Field, Irene K. Moore, Ji-Ping Z. Wang & Jonathan Widom

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



2006

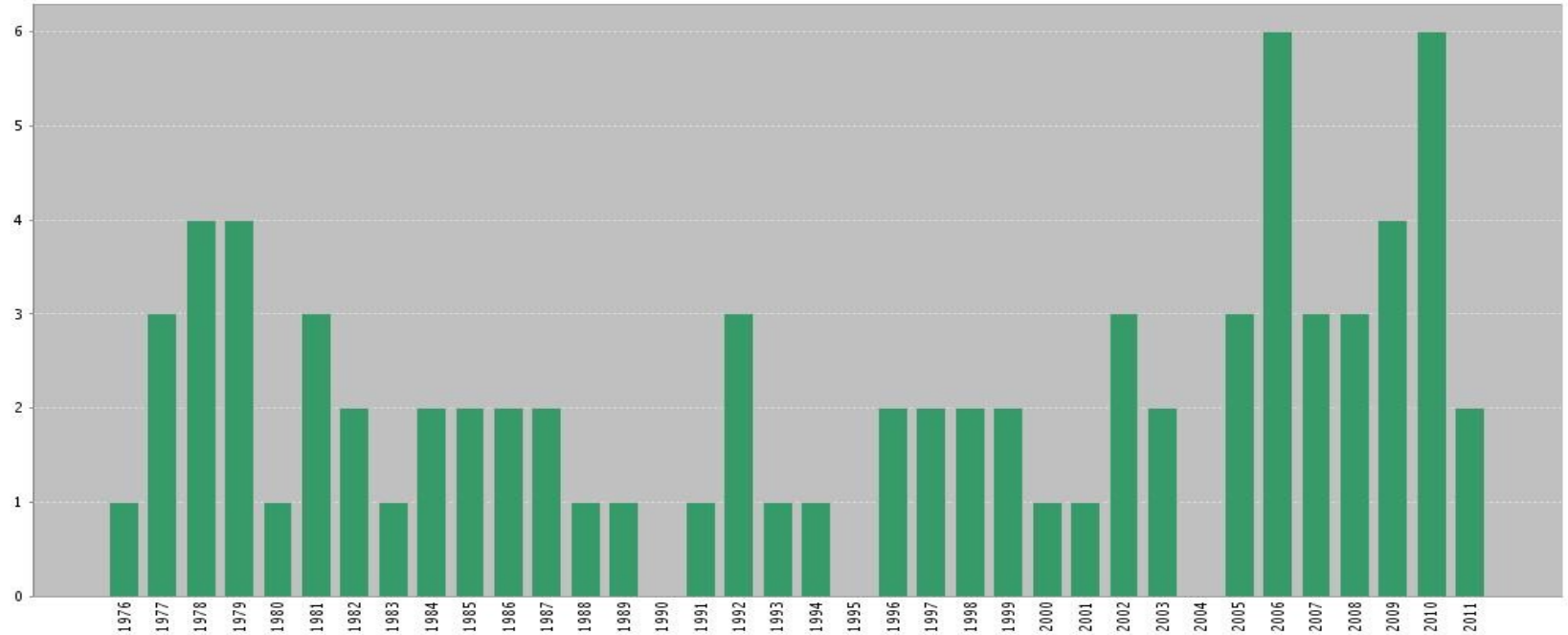
The tendency of the dinucleotides to fit to … 10.5 or so base frame … can be considered as another message… two codes …

Trifonov, Nucl. Acids Res. 1980

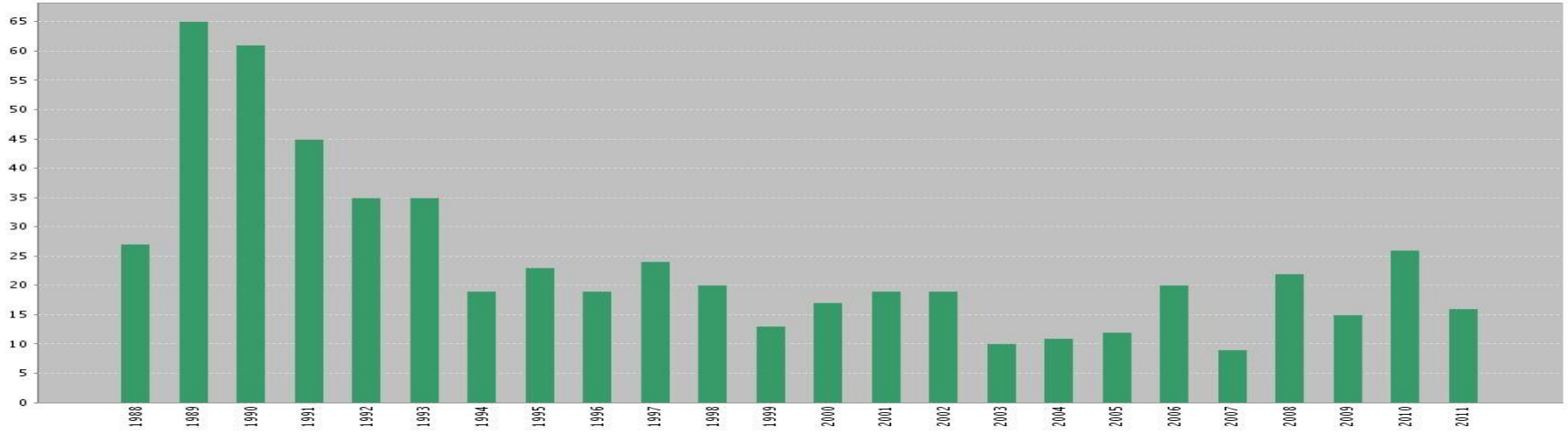
"Second code of chromatin DNA" –

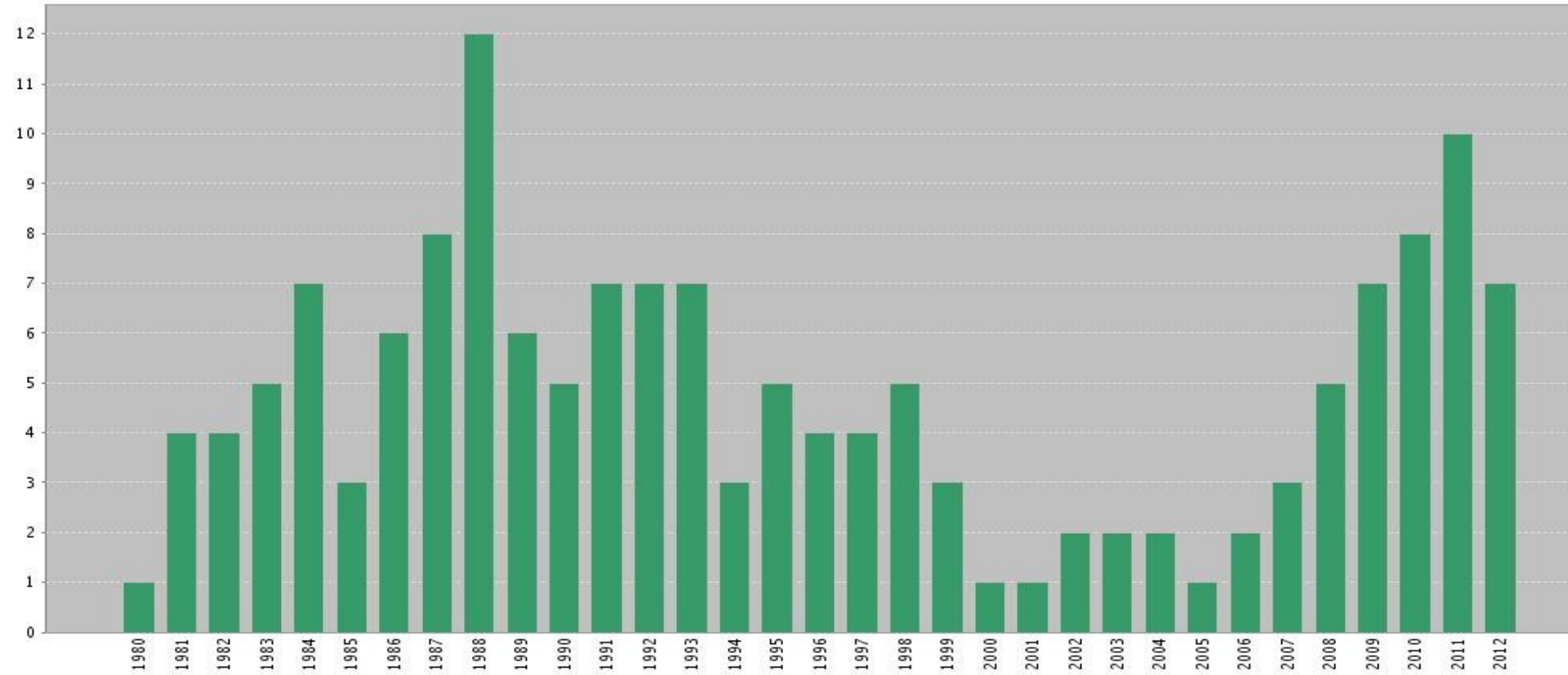
chapter by Trifonov in
"International Cell Biology 1980-1981"

Zuckerkandl, J Mol Evol 1977



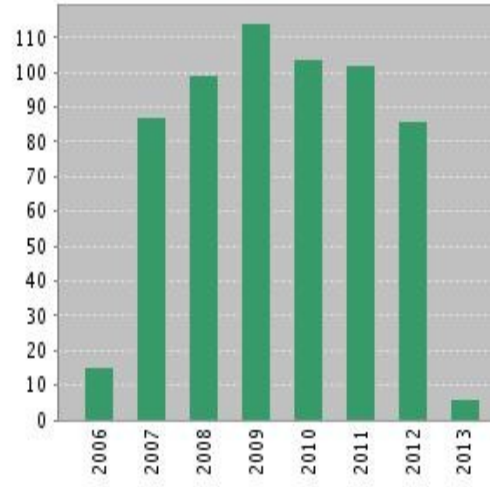
Holliday R, Science 1987





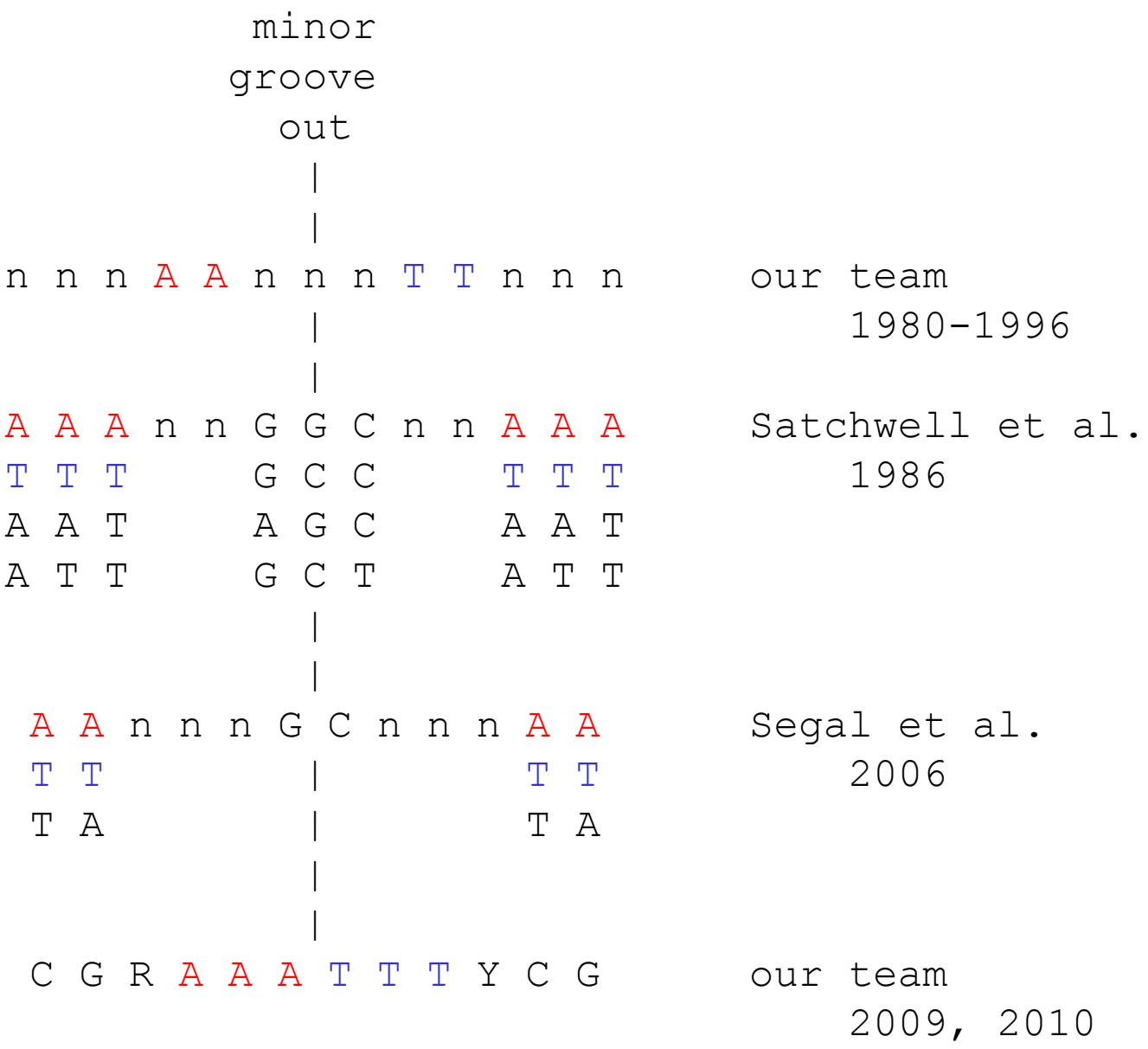
E. N. Trifonov,
Nucl Acids Res 1980
First “second genetic code”-
Chromatin code

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)


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
T T T G C C T T T
A A T A G C A A T
A T T G C T A T T
Satchwell et al.
1986
A A n n n G C n n n A A
T T T T
T A T A
Segal et al.
2006
C G R A A A T T T Y C G
our team
2009, 2010

“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

eleven SECOND CODES:

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

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)

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

	discovered	cracked
1. RNA-protein translation (triplet) code	(1961)	(1961)
2. Genomic code (isochores)	(1973)	(1973-1990)
3. Chromatin (nucleosome positioning) code	(1980,1981)	(1980-2009)
4. DNA shape code (curved DNA)	(1980,1981)	(1980-1996)
5. Gene splicing code (Chambon rules)	(1981)	not yet
6. N-end rule (protein lifetime)	(1986)	(1986-1996)
7. Translation framing code	(1987)	(1987)
8. Fast adaptation (modulation) code	(1989)	(1989)
9. Genome segmentation code	(1994)	not yet
10. Codes of small RNAs	(1998)	(1998)
11. Translation pausing code	(2002)	(2002)
12. Proteomic code (proteins)	(2003)	(2003-2008)
13. 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	UAU TYR Y	UGU CYS C
UUC PHE	UCC SER S	UAC TYR	UGC CYS
UUA LEU L	UCA SER	UAA STOP	UGA STOP W
UUG LEU	UCG SER	UAG STOP	UGG TRP
CUU LEU	CCU PRO	CAU HIS H	CGU ARG
CUC LEU L	CCC PRO P	CAC HIS	CGC ARG R
CUA LEU	CCA PRO	CAA GLN Q	CGA ARG
CUG LEU	CCG PRO	CAG GLN	CGG ARG
AUU ILE	ACU THR	AAU ASN N	AGU SER S
AUC ILE I	ACC THR T	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	GCU ALA	GAU ASP D	GGU GLY
GUC VAL V	GCC ALA A	GAC ASP	GGC GLY G
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 C 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 C 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 . . .

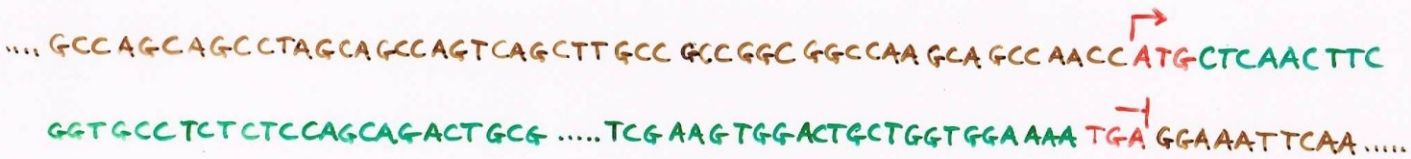
A C	G T	G T	A C	C T	A T	G G	G T	G T	A C	A T	T A A		1) end of frdD gene
thr	val	val	thr	leu	ile	gly	val	val	thr	ile	term		
	G	G				G	G	G					2) framing of frdD
	T T G	C A						T A	A A T				3) promoter P1 of ampC gene

(c)

. . . T C G A A C T G G A C T G C T G G T G G A A A A T C A G G 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 C	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			
		S			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 ATG CTCAACTTC
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	

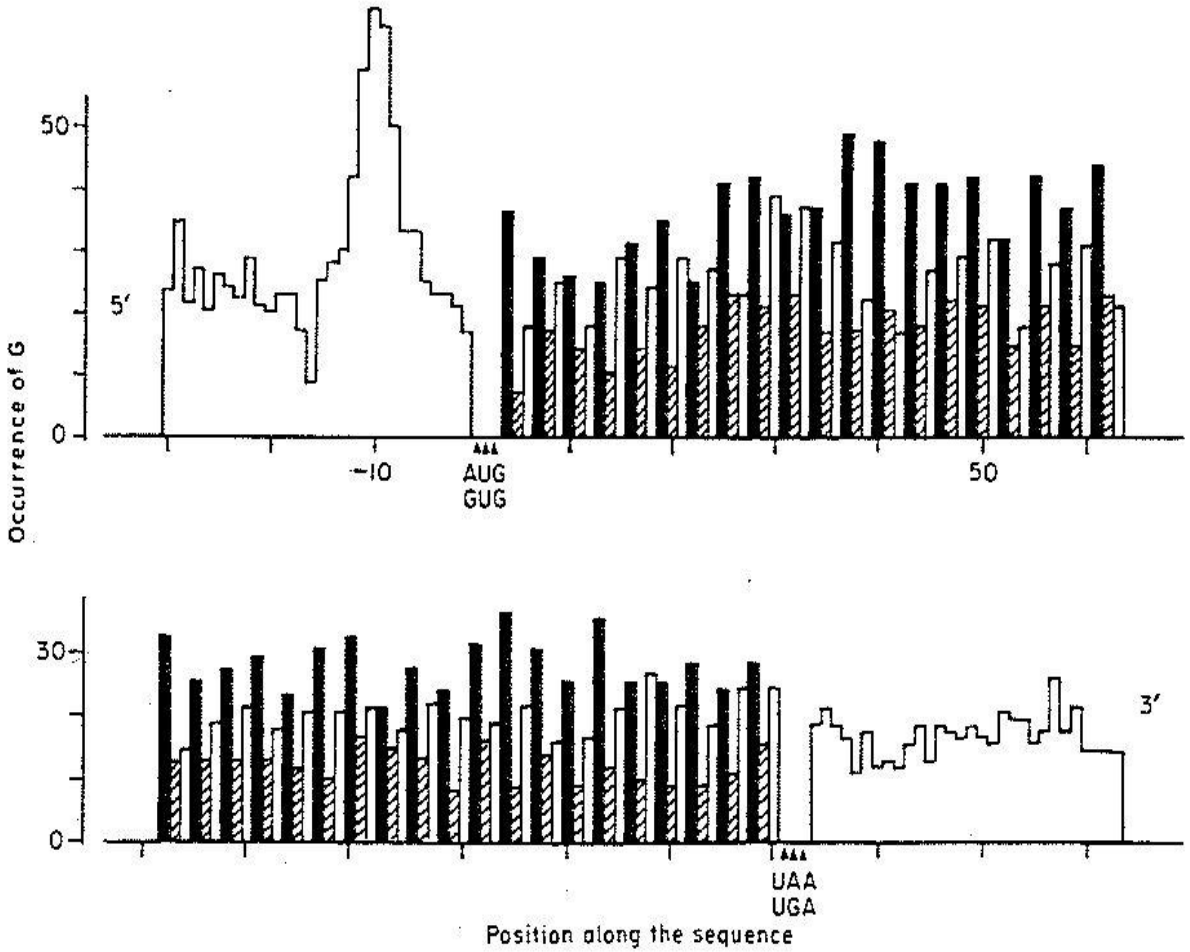


Figure 1. Distribution of guanines along *E. coli* mRNA. Filled bars, first positions of the codons; hatched bars, second positions. Only the first and last 60 bases of the coding regions are presented.

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)

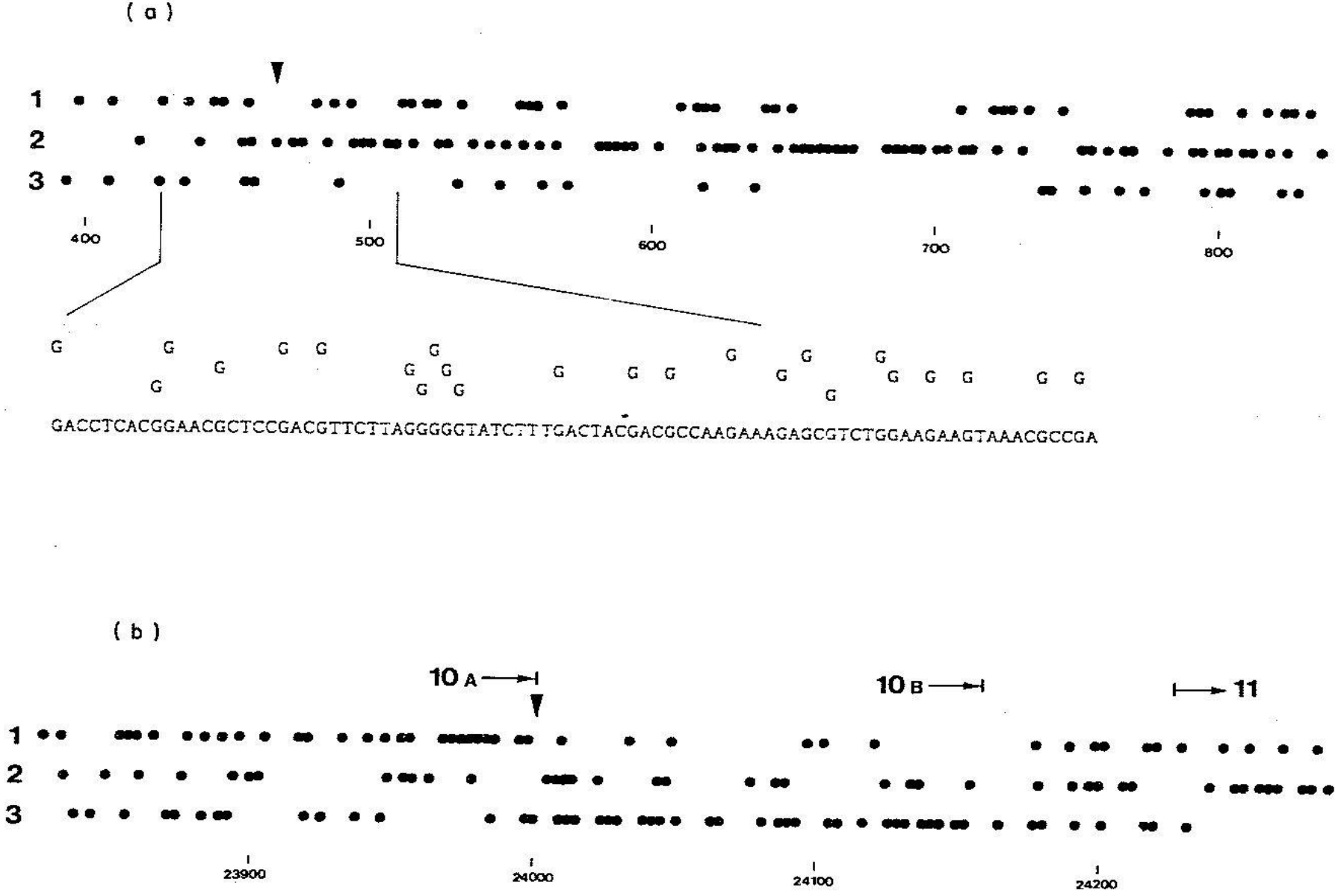
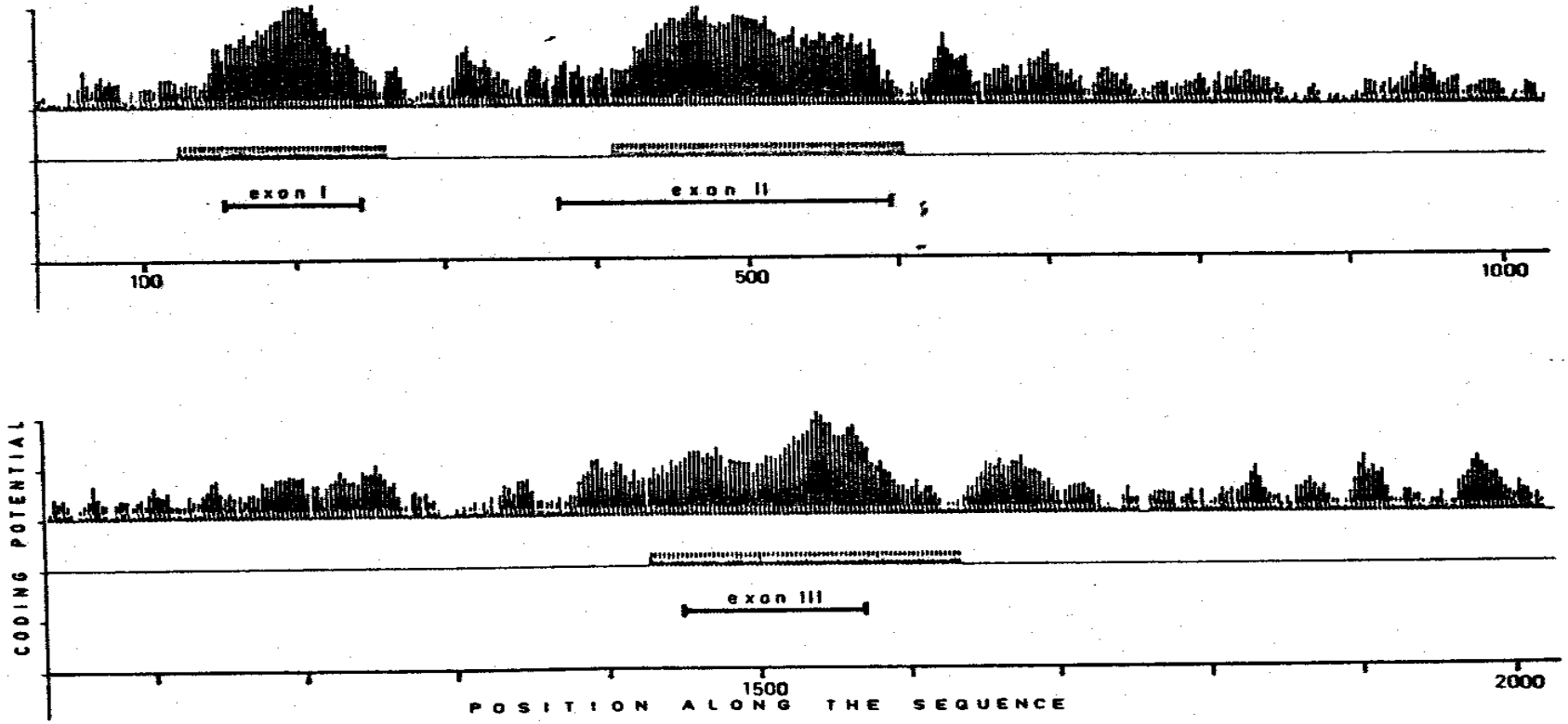
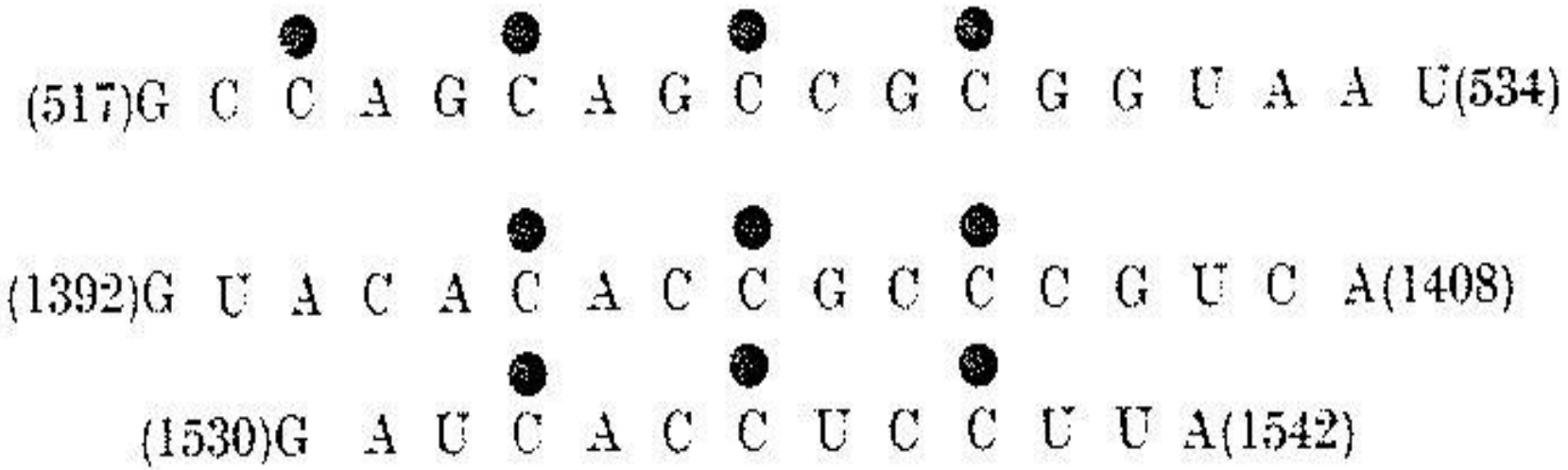


Figure 3. Actual distribution of guanines in 3 frames of the *RF-2* gene of *E. coli* (a) and the *10A,B* gene of bacteriophage T7 (b). The sequence around the ribosome slippage site is also shown (a). Every occurrence of G is indicated by a dot. Arrowheads indicate positions of ribosome frameshifting.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


exon I
exon II
exon III
100
500
1000
1500
2000
CODING POTENTIAL
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)geCagCagC'egC	1·17	+
(629)aaCugCauC	1·15	
(499)agCacCggC'	1·13	
(1061)guC'guCagC'	1·13	
(803)guC'caC'geC'	1·11	
(306)acC'agC'caC'	1·11	
(1312)guC'ugC'aaC'	1·10	
(874)guC'gaC'egC'	0·97	
(1531)auC'acC'ucC'	0·96	+
(891)uaC'ggC'egC'	0·92	
(993)gaC'auC'caC'	0·89	
(1095)ucC'egC'aaC'	0·88	
(1257)agC'gaC'cuC'	0·80	
(730)ggC'ggC'ccC'	0·73	
(1320)cuC'gaC'ucC'	0·52	
(337)gaC'ucC'uaC'	0·44	


(517)G C C A G C A G C C G C G G U A A U(534)
(1392)G U A C A C A C C G C C C G U C A(1408)
(1530)G A U C A C C U C 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)
```

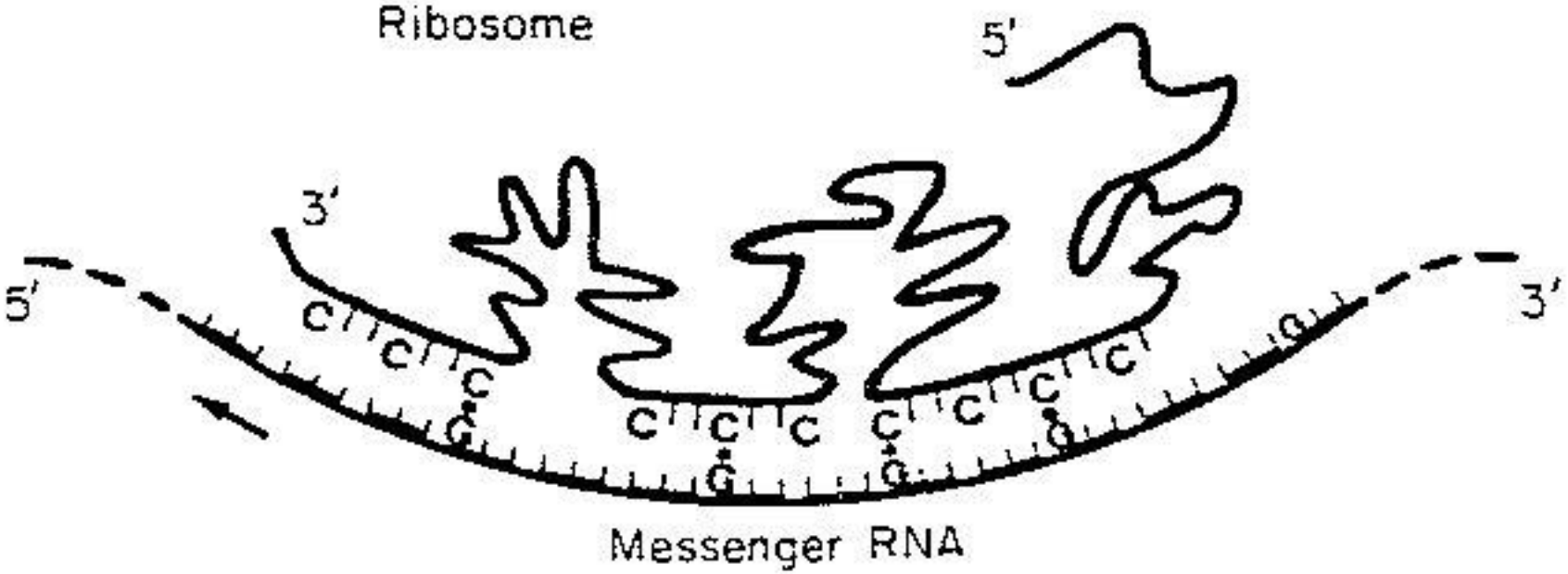
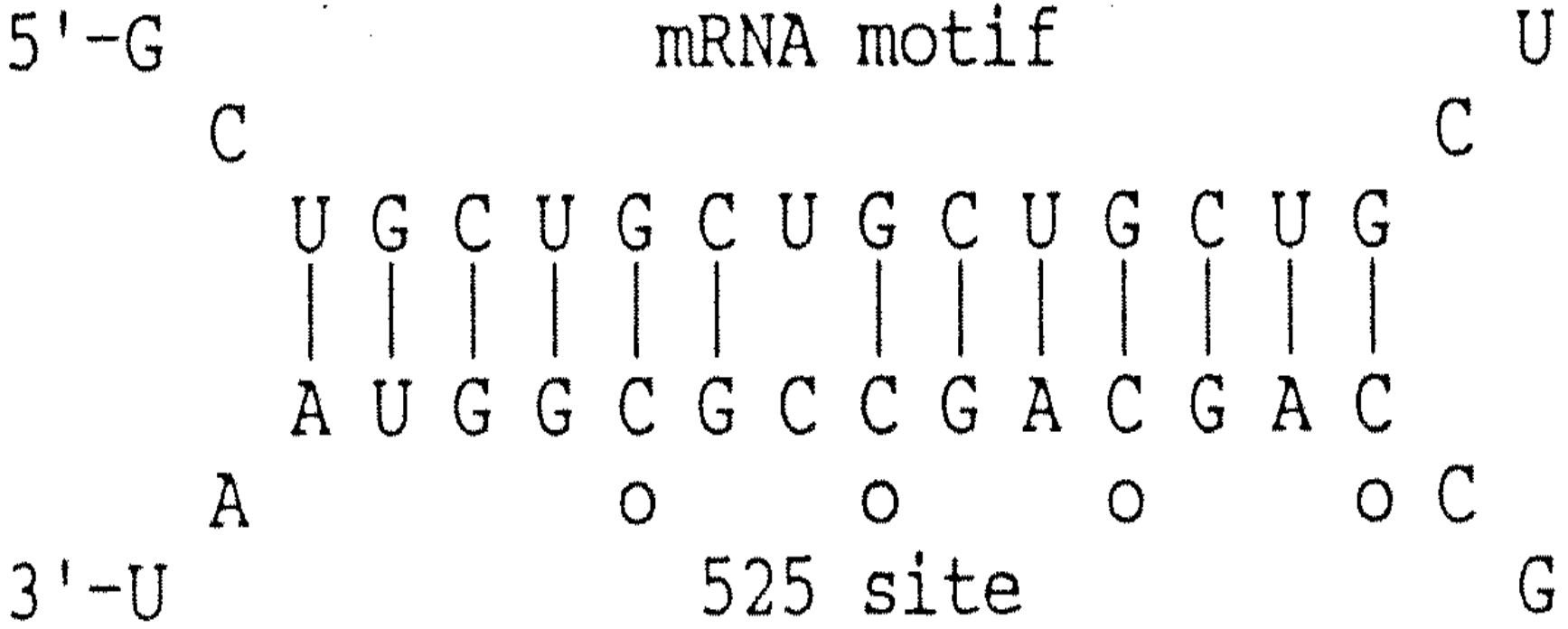


Figure 4. Scheme of the translation frame-monitoring mechanism.

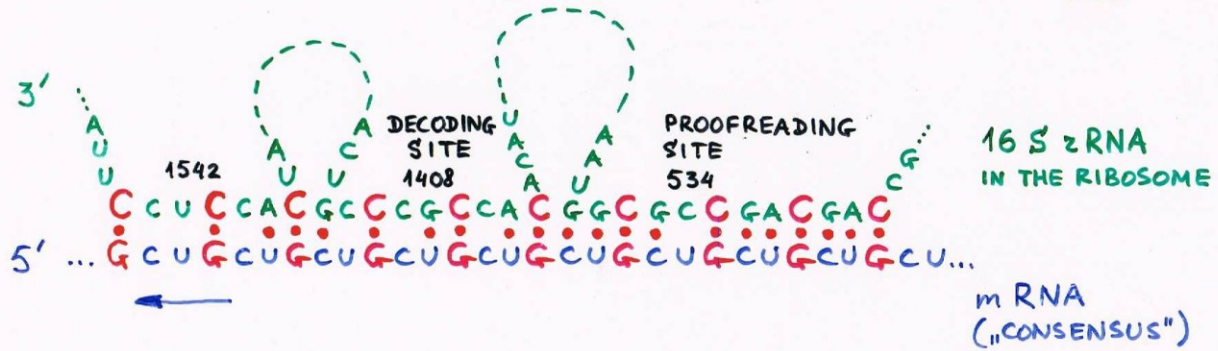
ENT, 1987



Which one is more ancient?

TRANSLATION FRAMING CODE

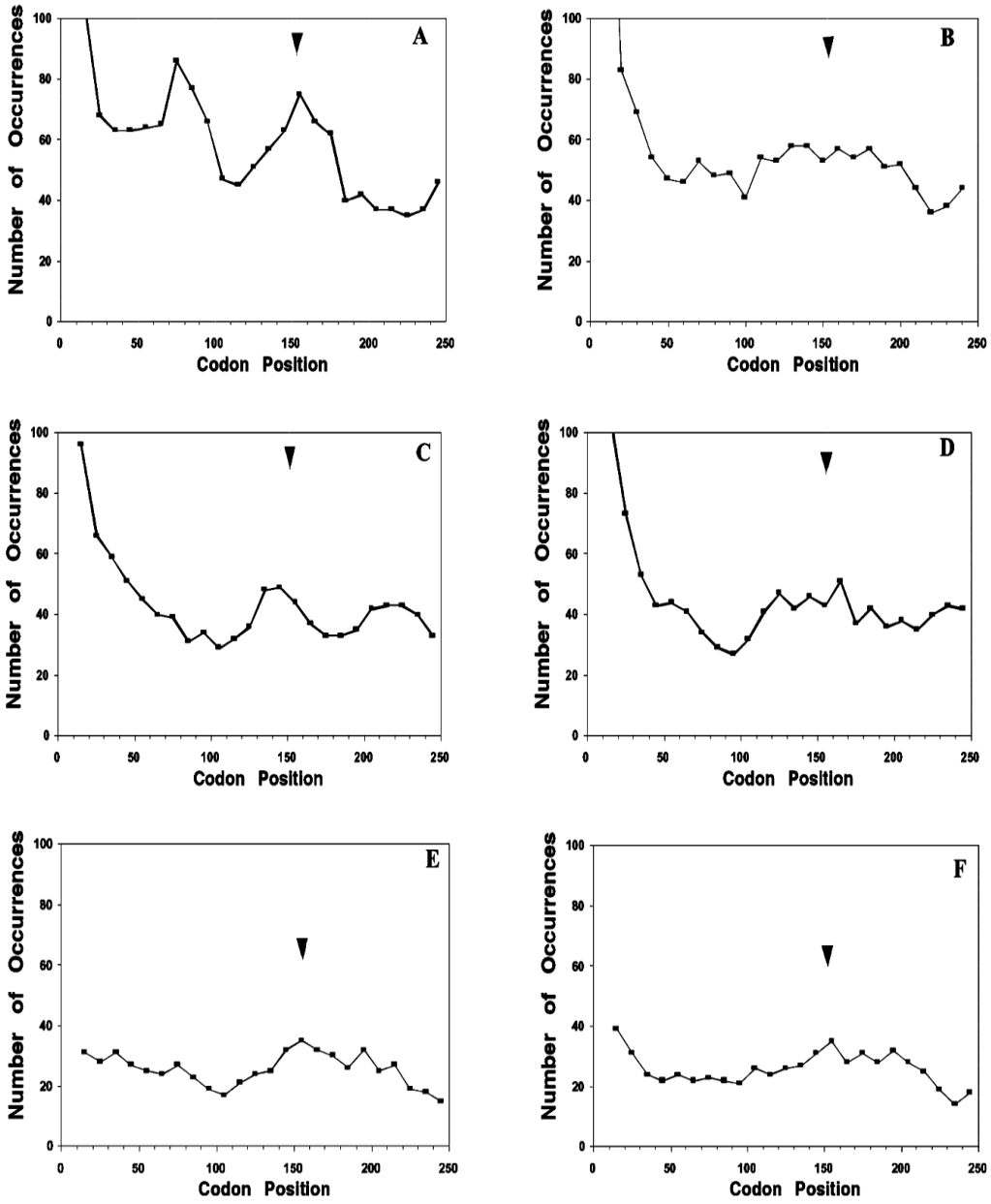
$(GCU)_n$ - mRNA „CONSENSUS"
(J. Lagunez-Otero, E. Trifonov)
1992

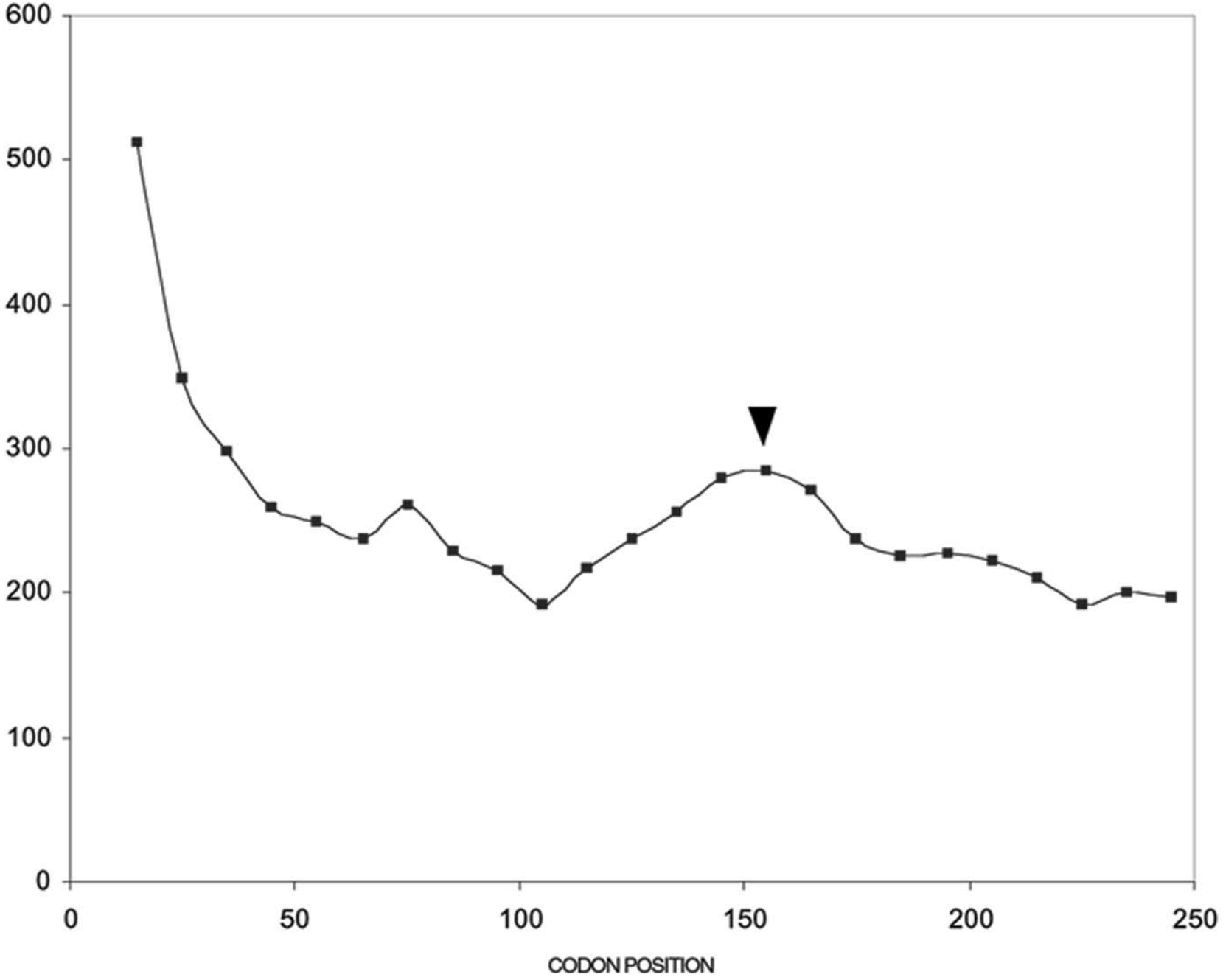


THE IN-FRAME COMPLEMENTARITY
PREVENTS RIBOSOME SHIFTING TO WRONG FRAME

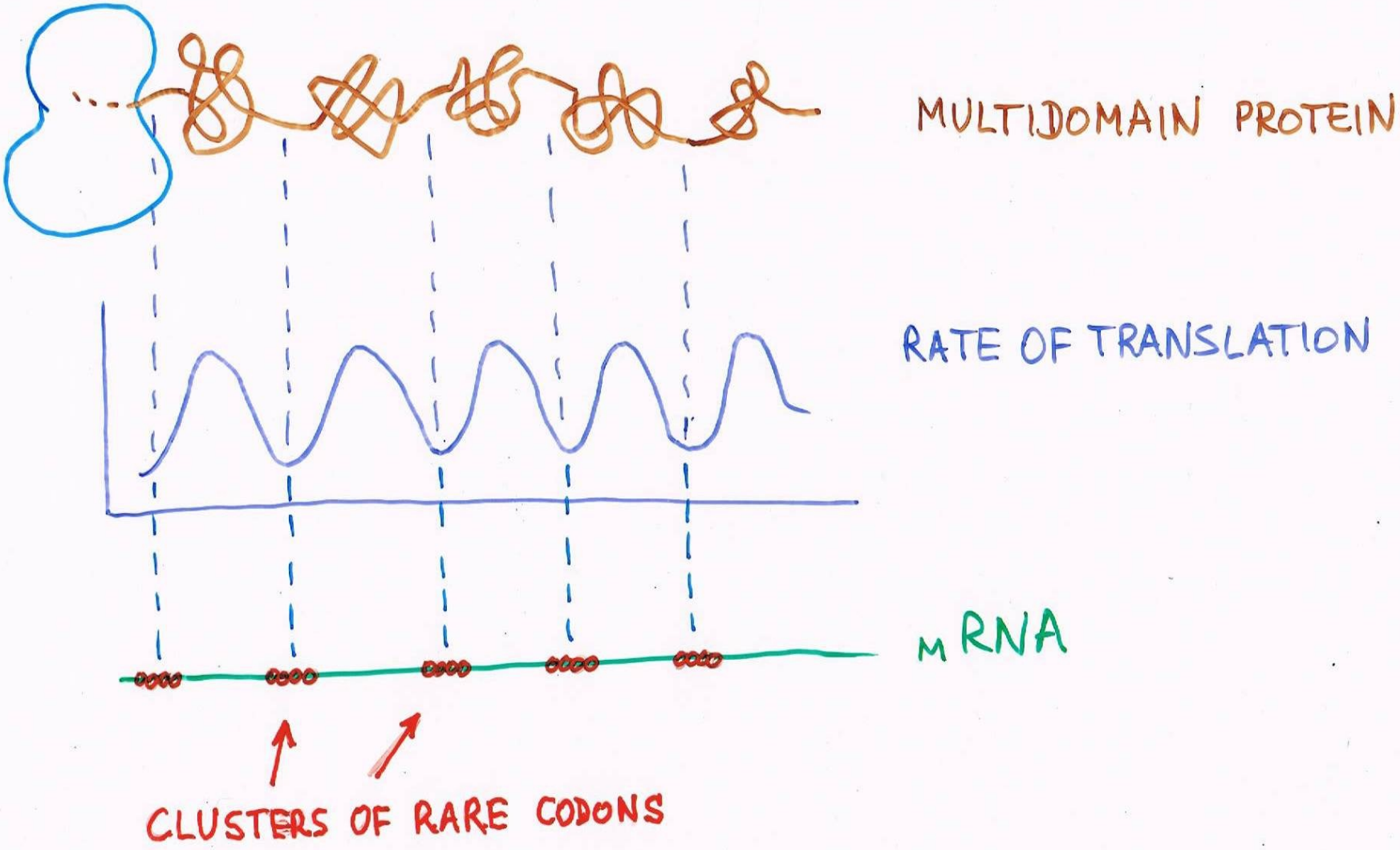
THIS IS IMPORTANT FOR LARGE PROTEINS

Translation pausing code

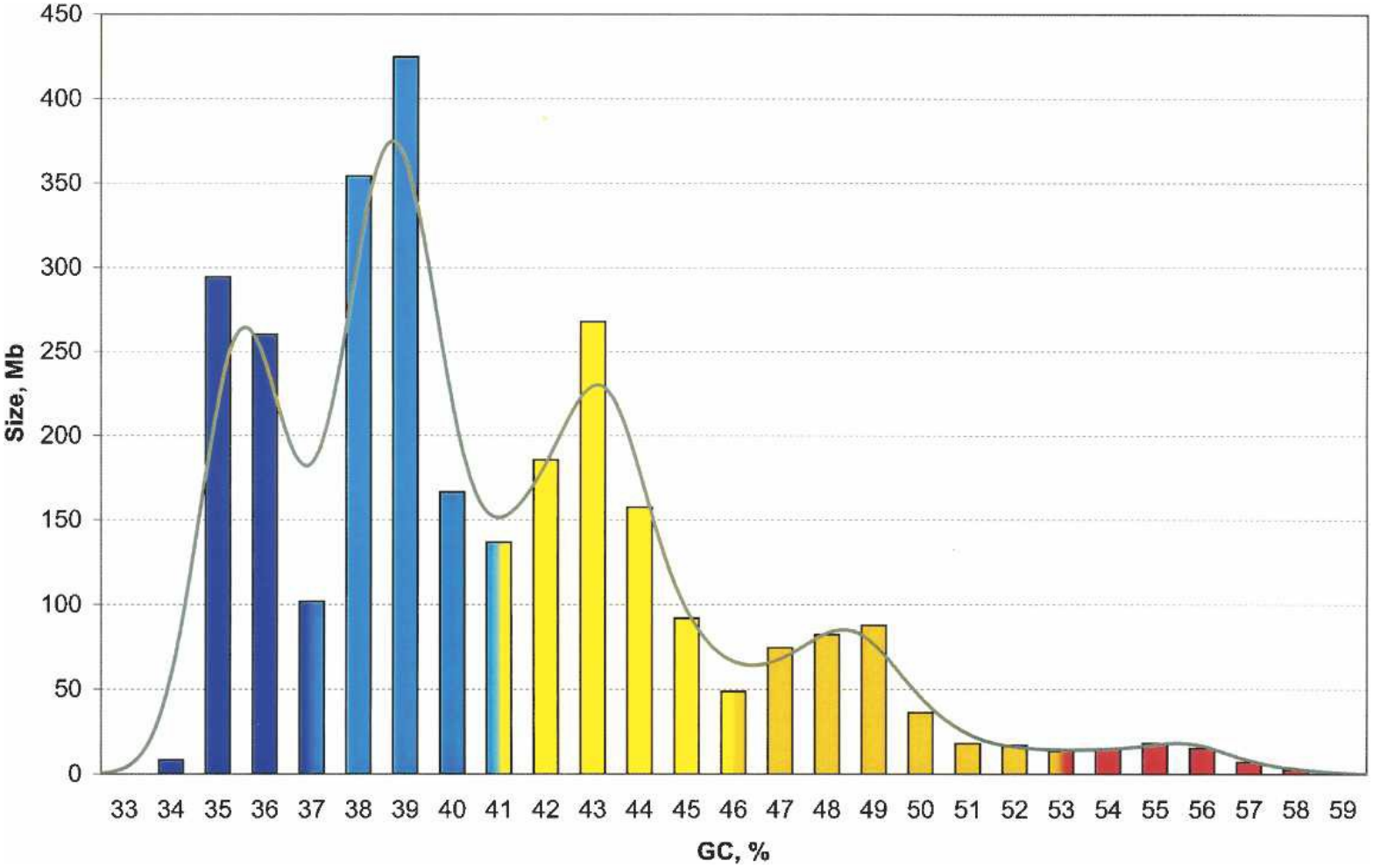

A
B
C
D
E
F
Number of Occurrences
Codon Position
0
20
40
60
80
100
0
50
100
150
200
250

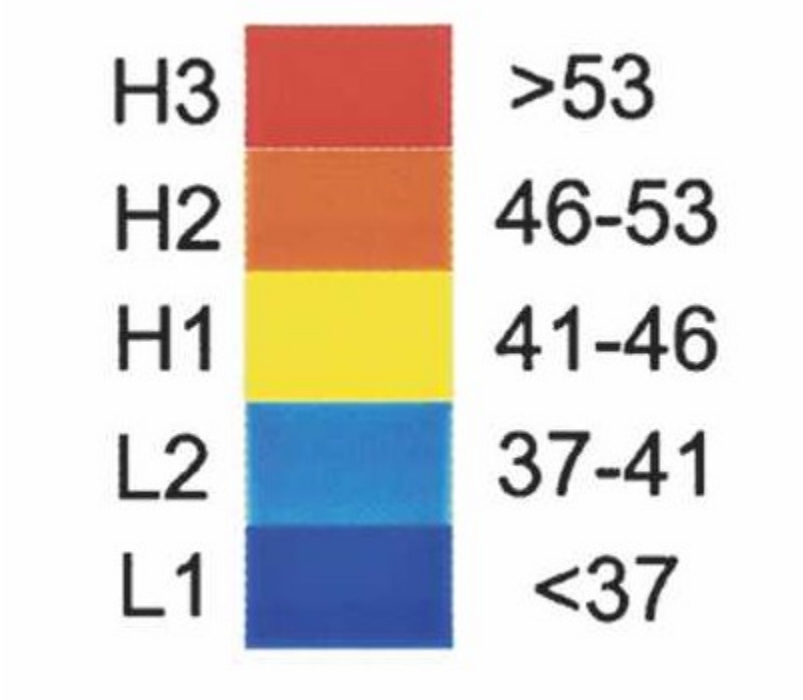

600
500
400
300
200
100
0
0
50
100
150
200
250
CODON POSITION

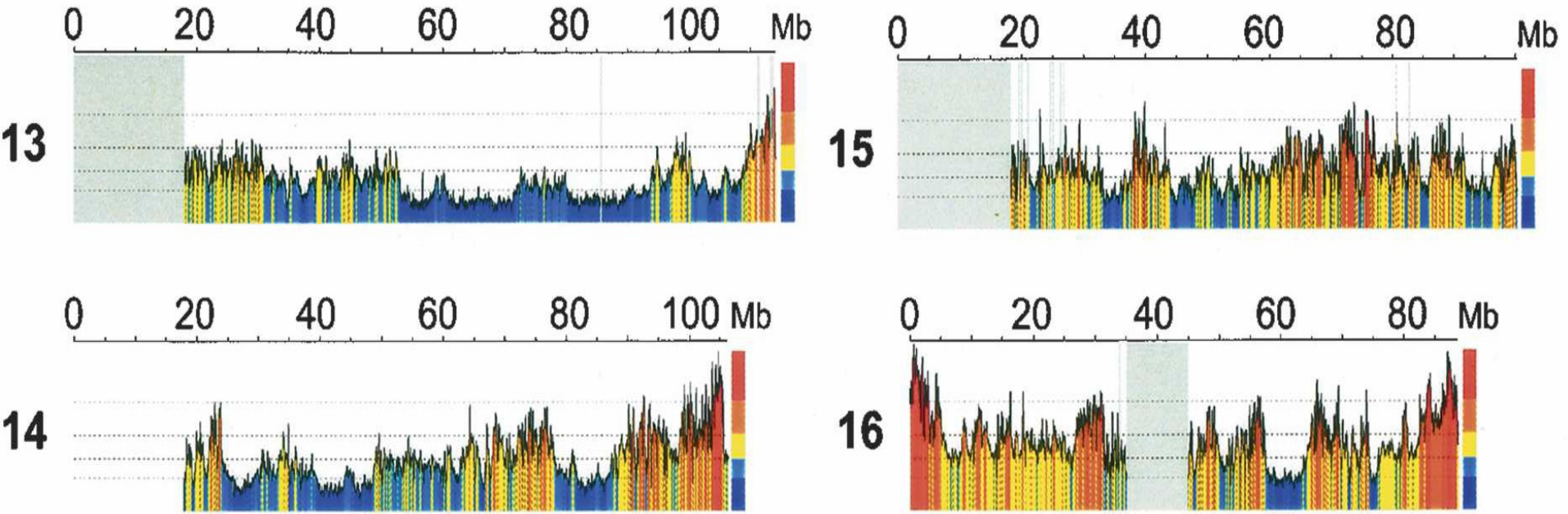
TRANSLATION PAUSING CODE



Genomic code (isochores)


Size, Mb
450
400
350
300
250
200
150
100
50
0
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

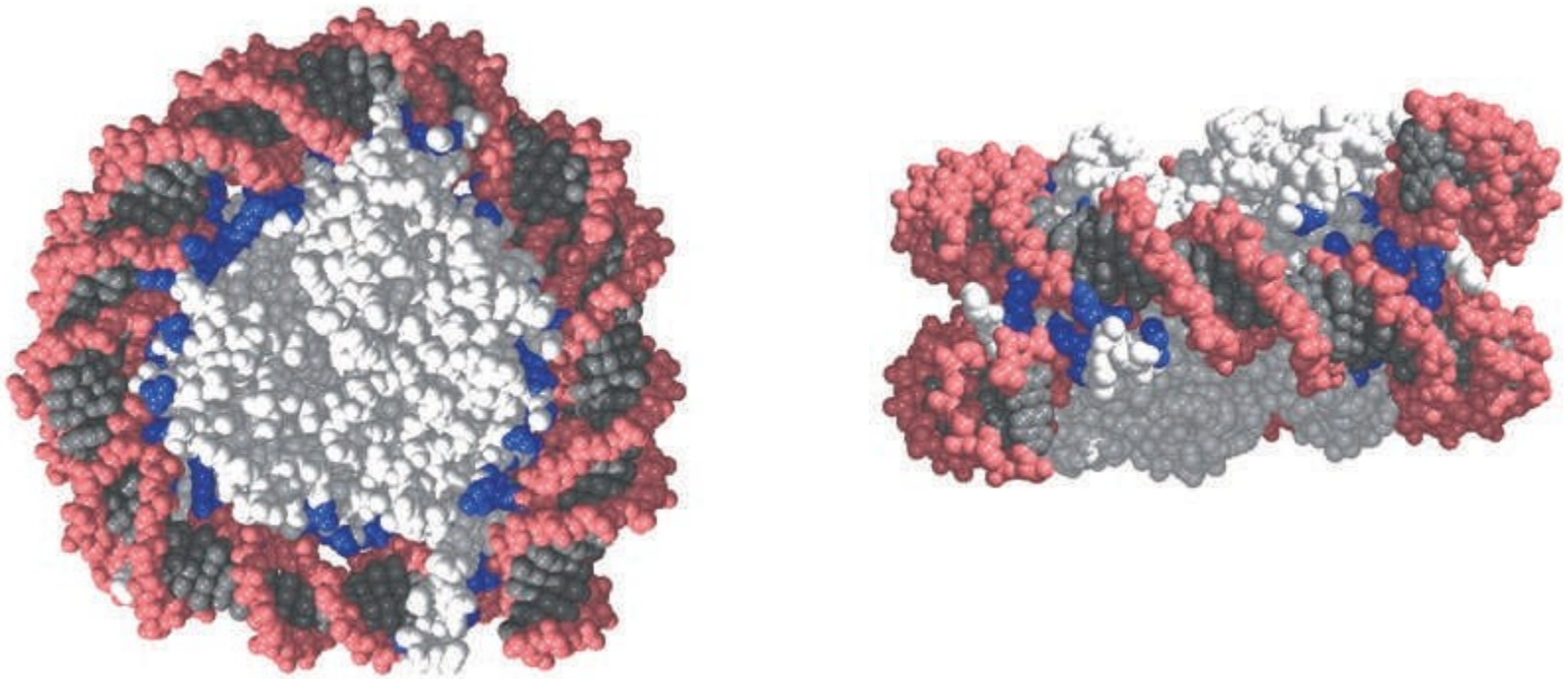
This results in different usage of transcription factors
in different isochores

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

Increments of 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

In Google “bent” is found 287 000 000 times, while
“curved” – only 76 800 000 times, 3.7 times less often (2011)

Object of arc-like shape is called

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

Krzywy domek (Curved house), Sopot, Poland

From Google :

	2007	2008	2011
"Curved DNA" was used of total "Curved DNA" and "Bent DNA"	44%	47%	48%

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 (with no strain)

Bent DNA (force applied)

} DIFFERENT THINGS

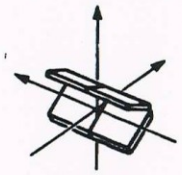
Strongest nucleosome motif: GAAAATTTTC

Strongest curvature motifs: AAAAATGACT

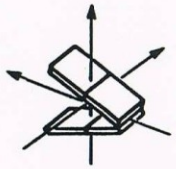
and AAAAACGCGA

BP to BP

Twist (Ω)



Roll (ρ)



Tilt (τ)

Rise (Dz)

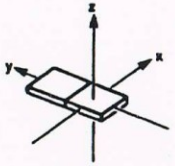
Slide (Dy)

Shift (Dx)

BP to AXIS

Tip (θ)

Inclination (η)



y displacement (dy)

x displacement (dx)

BASE to BASE

Opening (σ)

Propeller twist (ω)

Buckle (κ)

Stagger (Sz)

Stretch (Sy)

Shear (Sx)

III 1

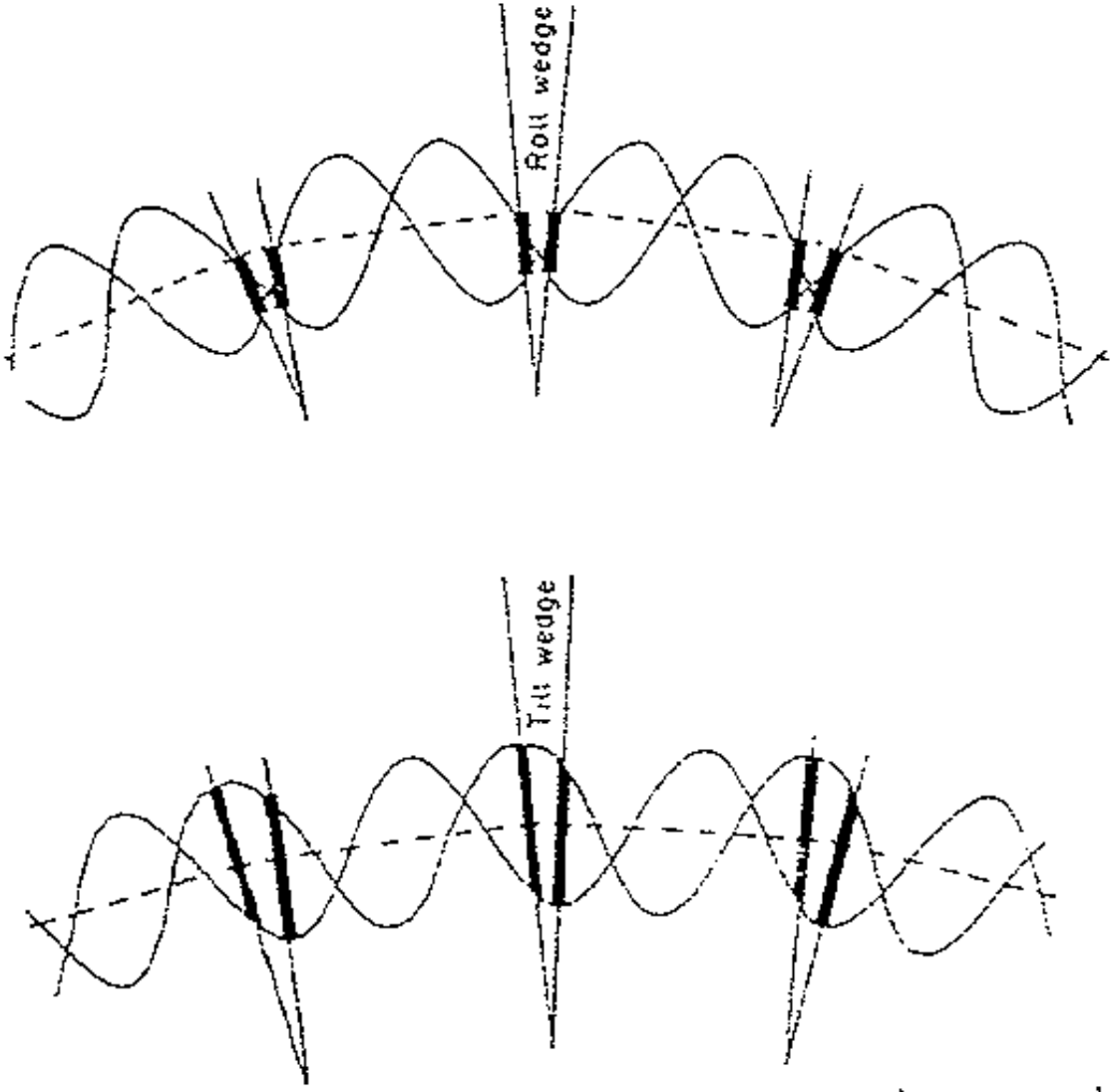
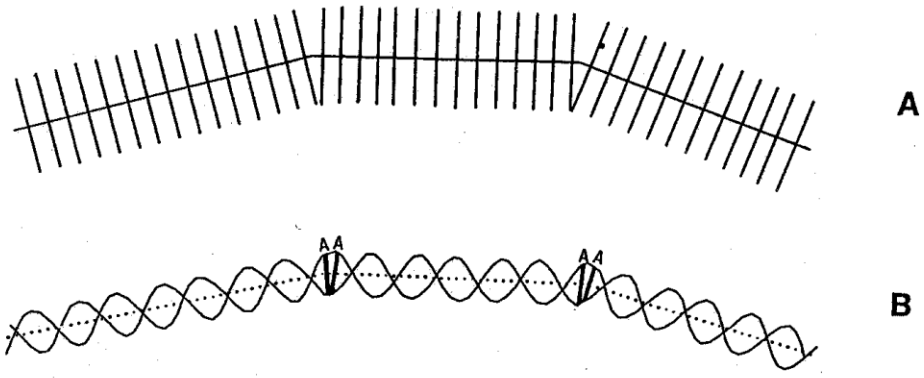


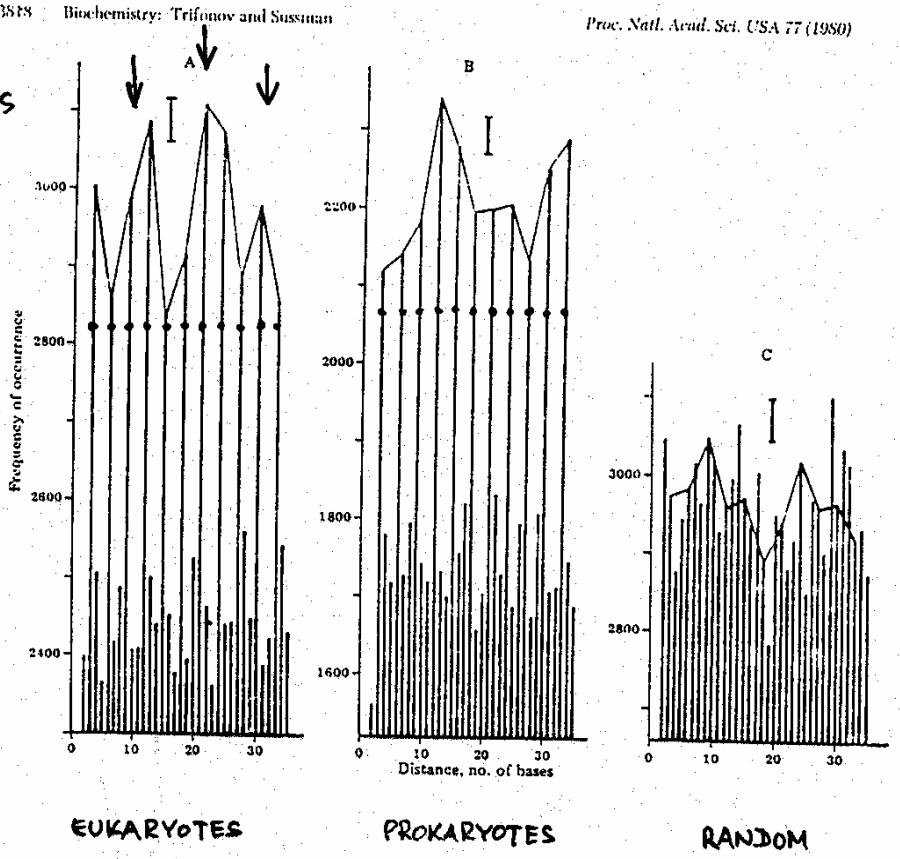
Figure 2. Wedge components of curved DNA (scheme). two interwound strands of double helical DNA molecule are presented by their sinusoidal projections. Only those base-pairs are shown which are non-parallel making the coresponding angles in their in-plane projections (From Ulanovsky and Trifonov, 1987,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


A
B

TRIFONOV, SUSSMAN, 1980

~ 10.5 BASES

3 BASES



EUKARYOTES PROKARYOTES RANDOM

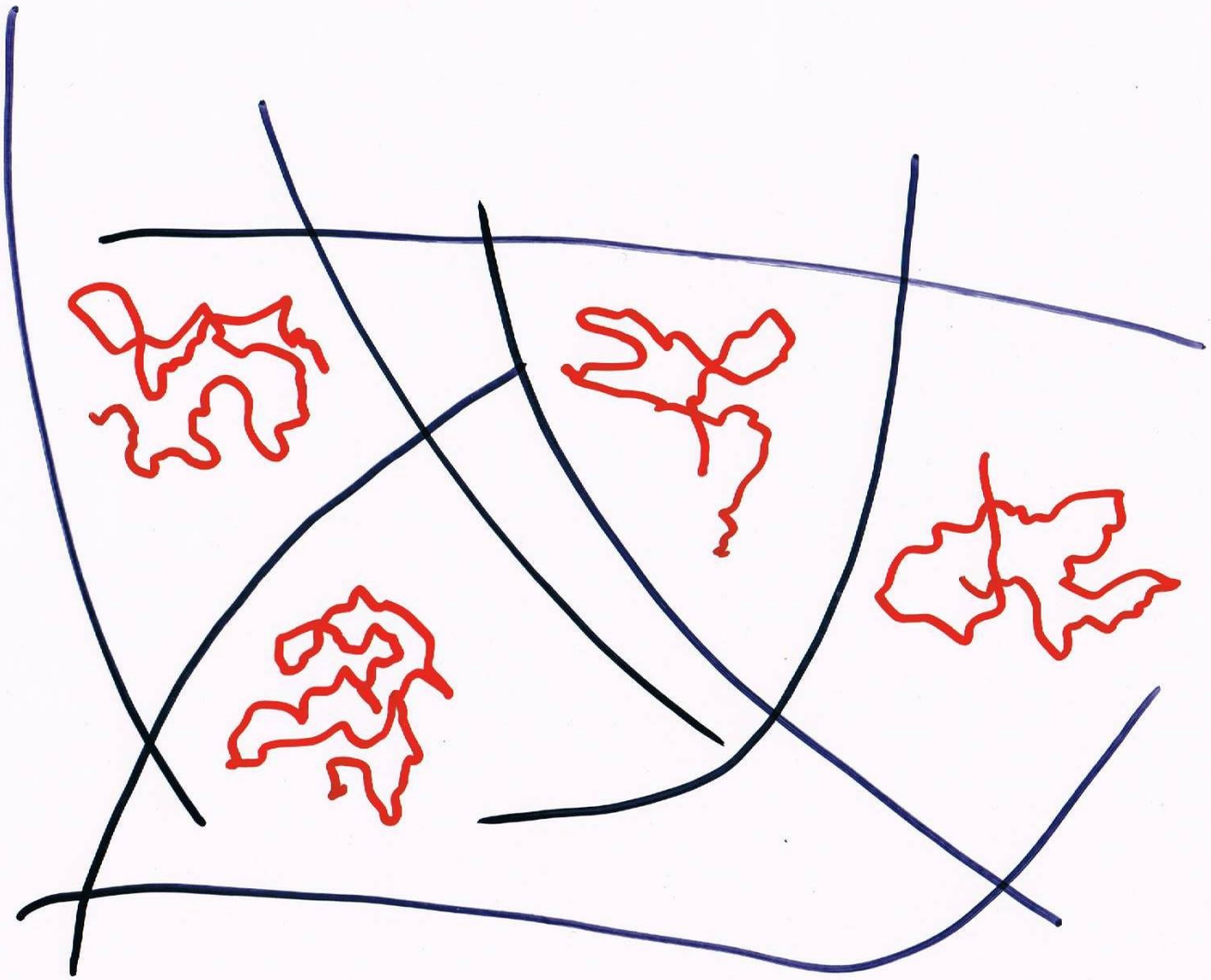
~ 30 000 BASES

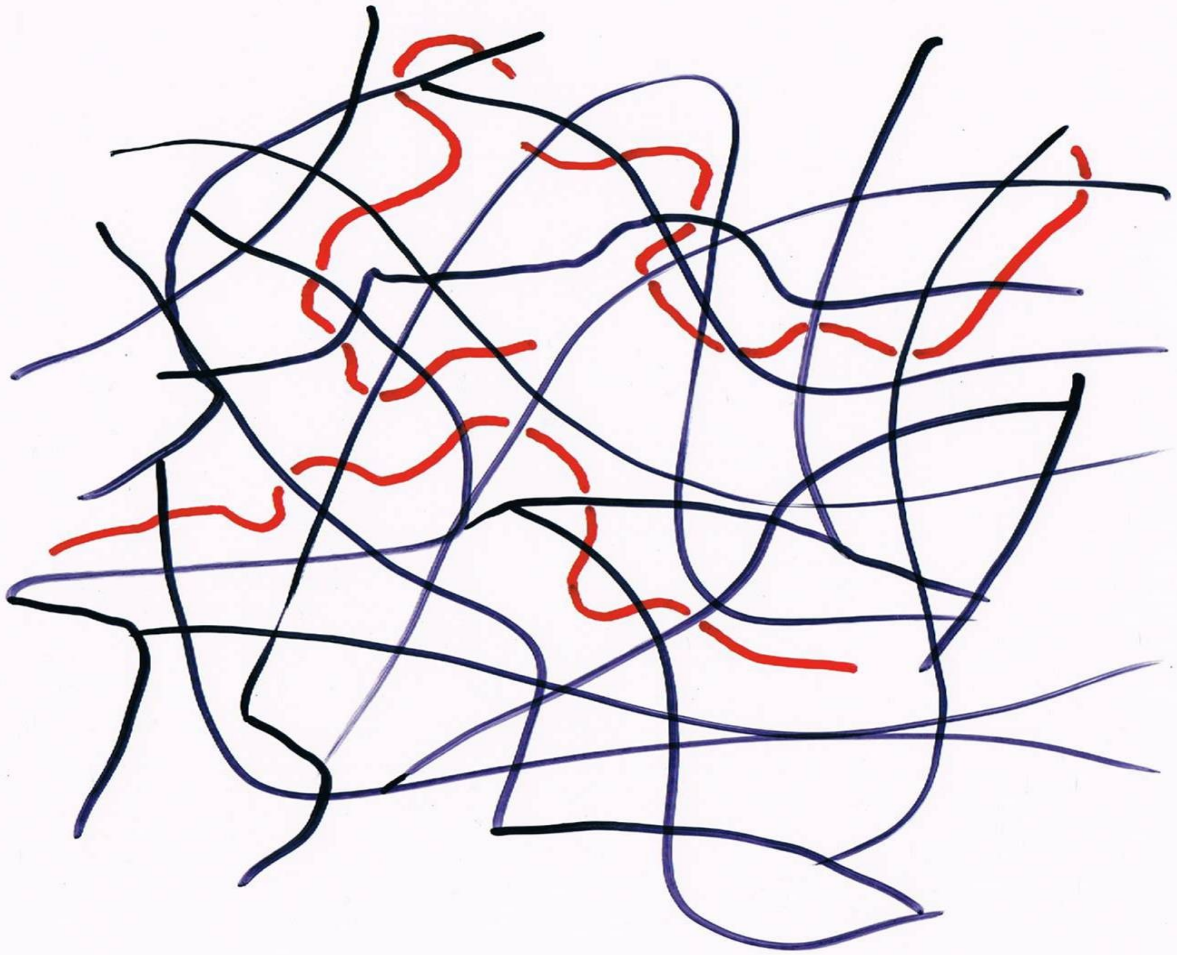
```
aacaagctaagtaccgtactgaagcgcattttaattacgataaggcttatcttaatttcgccgatggcaatgaatgacgtaagcttac
.  .    .              .            .          .              .              .   .       .
0  3    8             21           32         41             53             68  72      80
   0    5             18           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
```

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

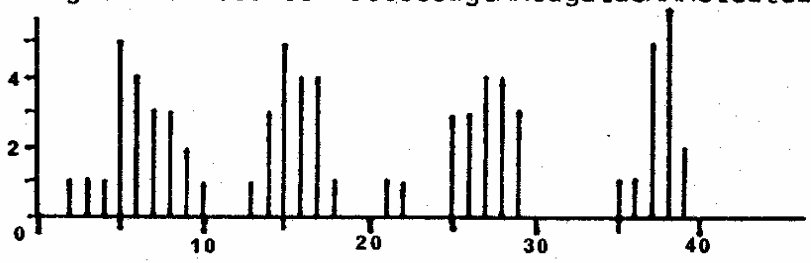
DNA moves head-on through the narrow pores of the polyacrylamide gel – reptation

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

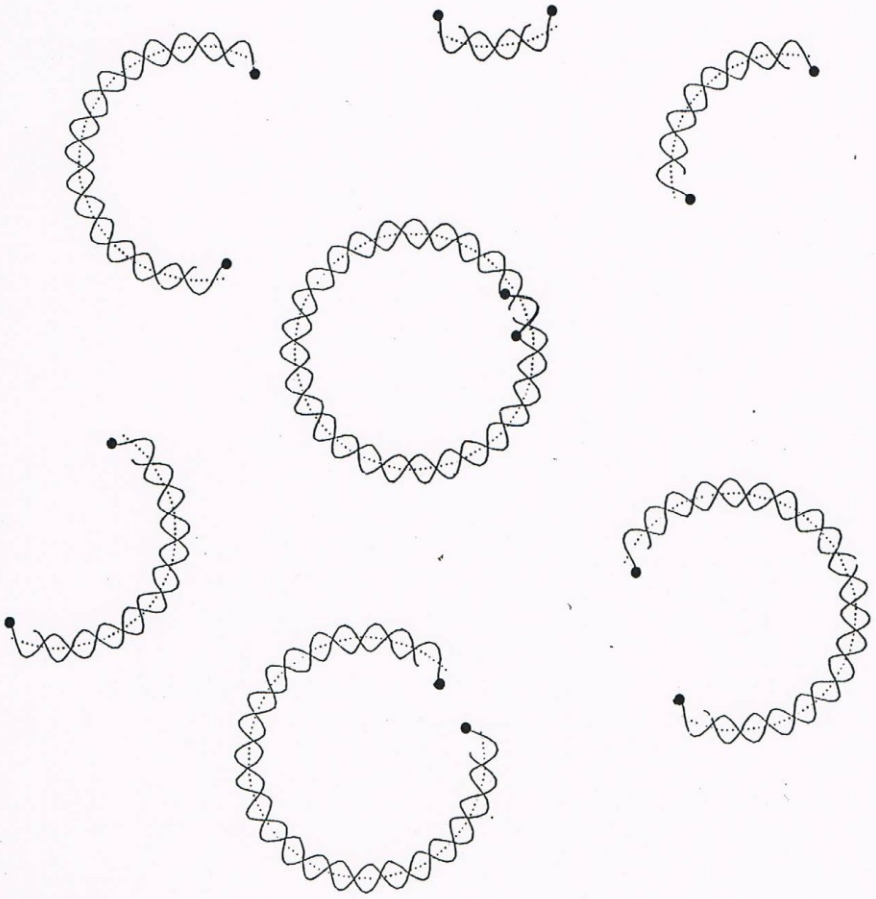




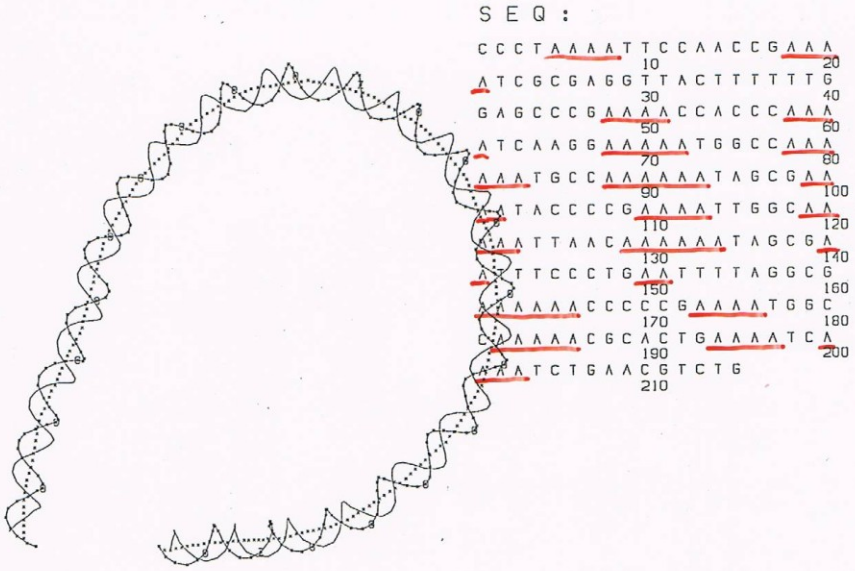
A	tcccAAAAAtgtcAAAAAAtaggcAAAAAAtgccAAAAAtccc	kDNA
B	gtatAAAAAAgctgAAcgagAAAcgtAAAAtgatatAAAtatc	attP
C	gatcgAAAAcAAAAAAtgctttAAAtagcattttAAAAcata	Ch. thummi th.
D	acacAAAAAActcatgAAAAtggtgctggAAAAcccattcAAggt	SV40 Hind F
E	cctcAAAAcgagggAAAAtcccctAAAAcgagggatAAAAcatccctcAAAttgg	ORI lambda
F	tgccAAttcatccattAActtctcagtAAcagatacAAActcatcacgAAcgtc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

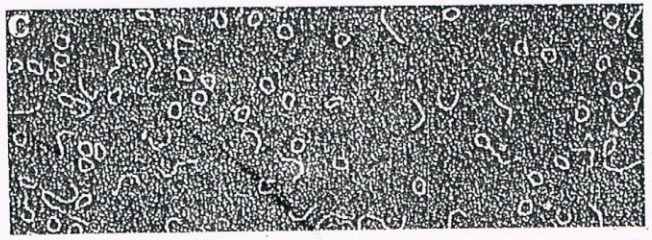


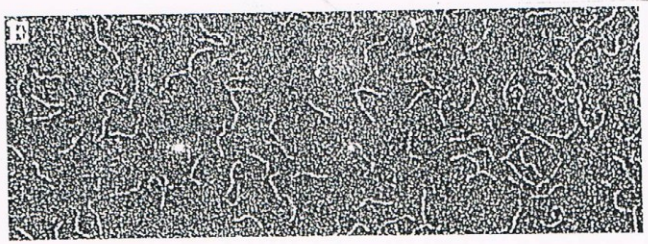
TCTCTAAAAAATATATAAAAA




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

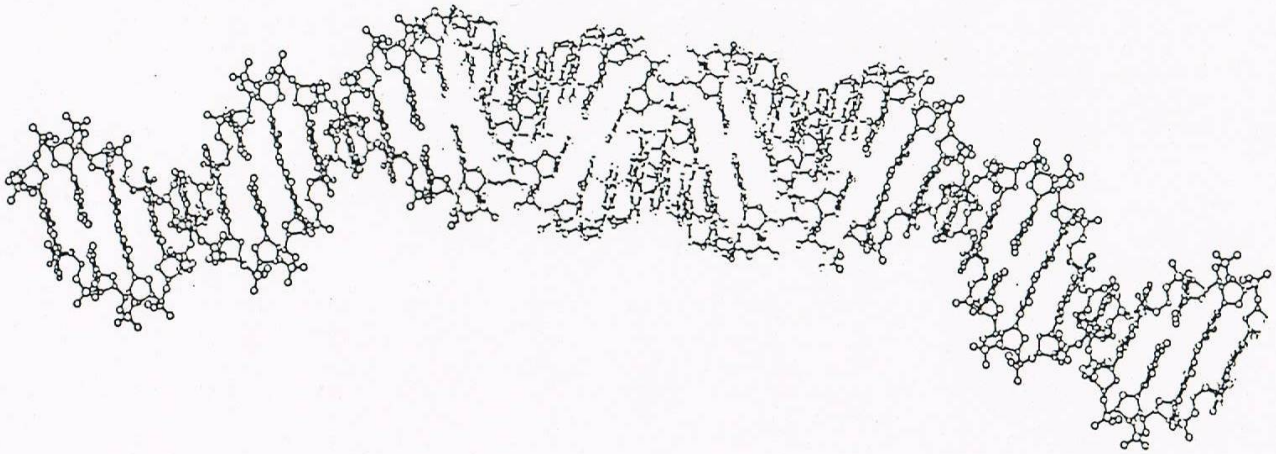






Griffith et al. Cell 46, 717-724 (1986)

JUNCTION MODEL OF DON CROTHERS



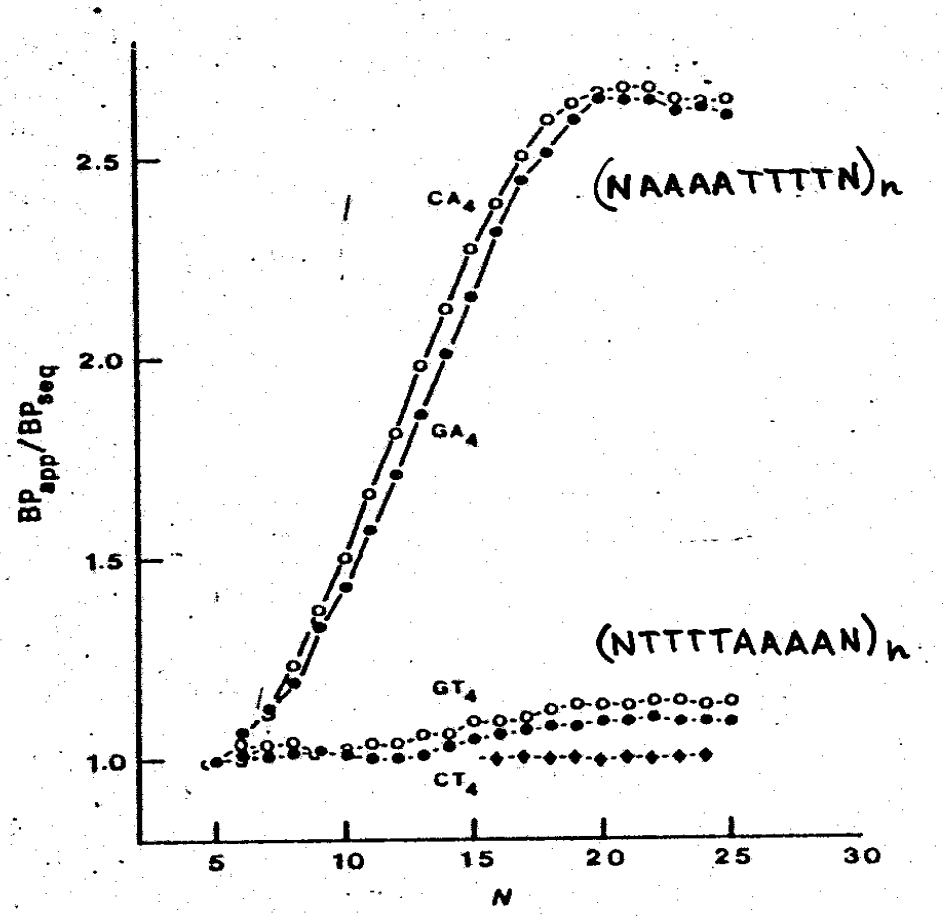
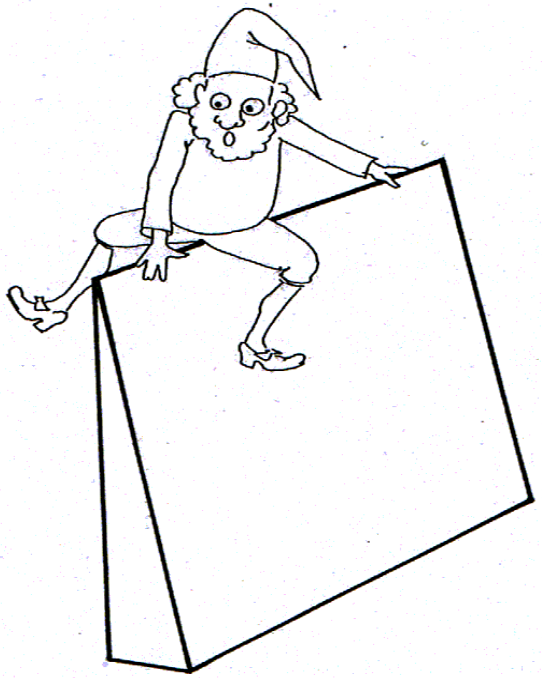


Fig. 2 Gel electrophoretic behaviours of duplex polymers having a repeating decamer motif. CA_4, $[CA_4T_4G]_N$; GA_4, $[GA_4T_4C]_N$; GT_4, $[GT_4A_4C]_N$; CT_4, $[CT_4A_4G]_N$. Mobilities of the various polymers, represented as the ratio of the apparent number of base pairs (BP_{app}) to the true number 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).

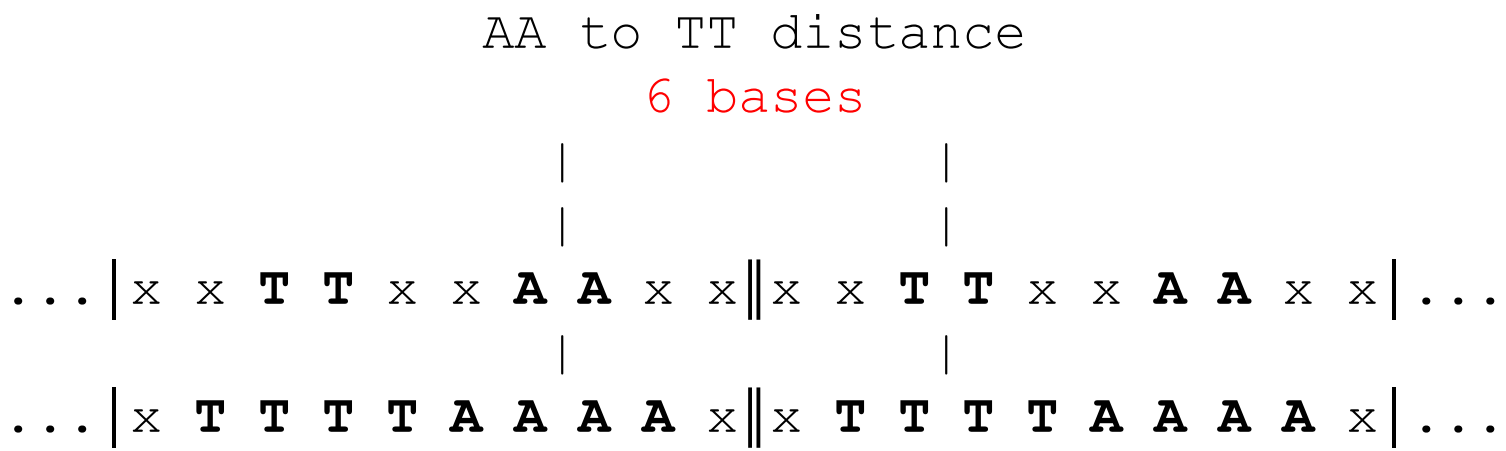
Nature 321, 449, 1986



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


AA to TT distance
4 bases
...|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
...|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|...

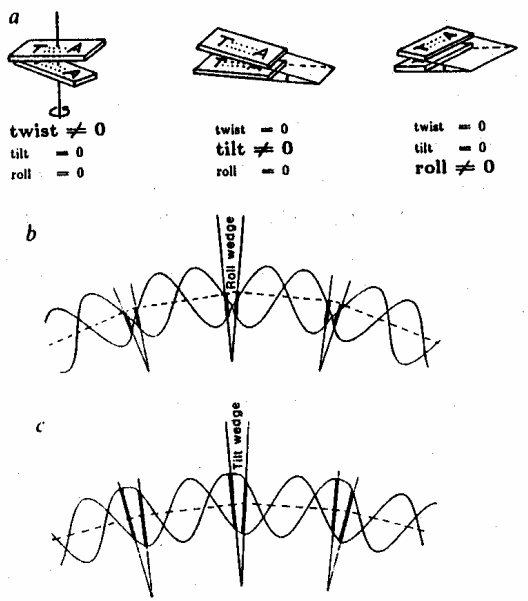


Fig. 1 Tilt and roll angles. *a*, Twist, tilt and roll angles formed by two adjacent base pairs. *b*, Curvature by roll components of the wedges, opening towards the major groove. *c*, Curvature by tilt components of the wedges, opening towards the backbone. Note that *b* and *c* show mutually perpendicular 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

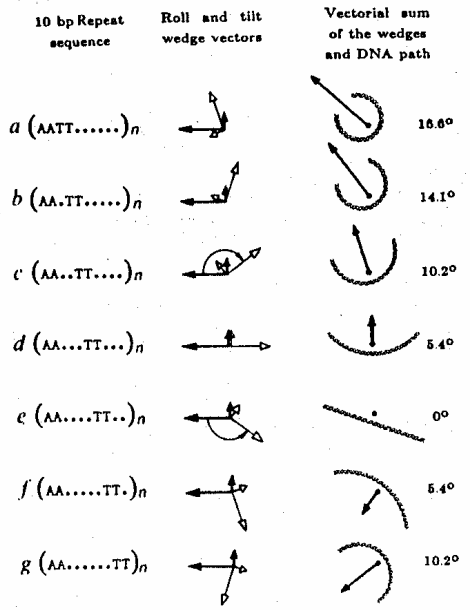
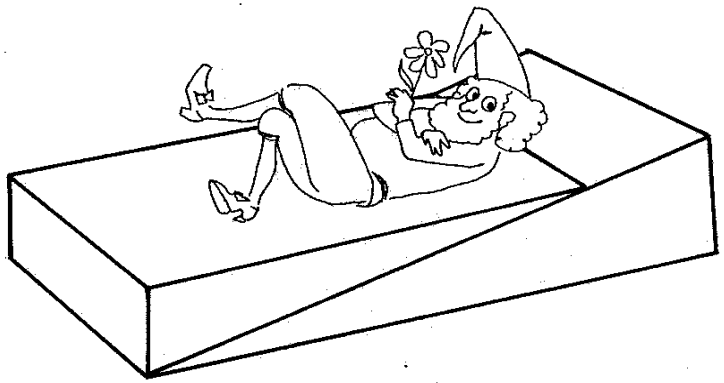


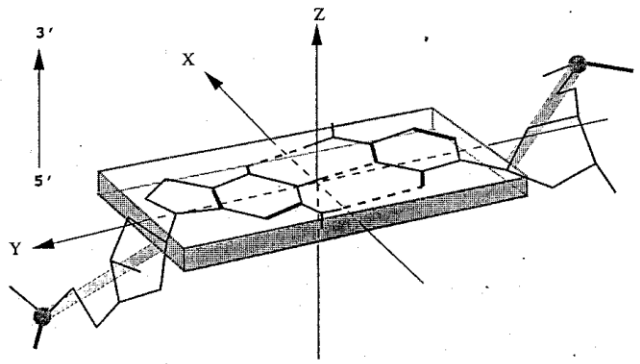
Fig. 2 Curvature caused by interplay of AA and TT wedges in a 10-bp repeat. Separating TT from AA by one more base results in a 36° rotation of TT versus AA wedge components denoted by unfilled (TT) and filled (AA) arrowheads in the central column, as viewed along the axis of the DNA. Each wedge component 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

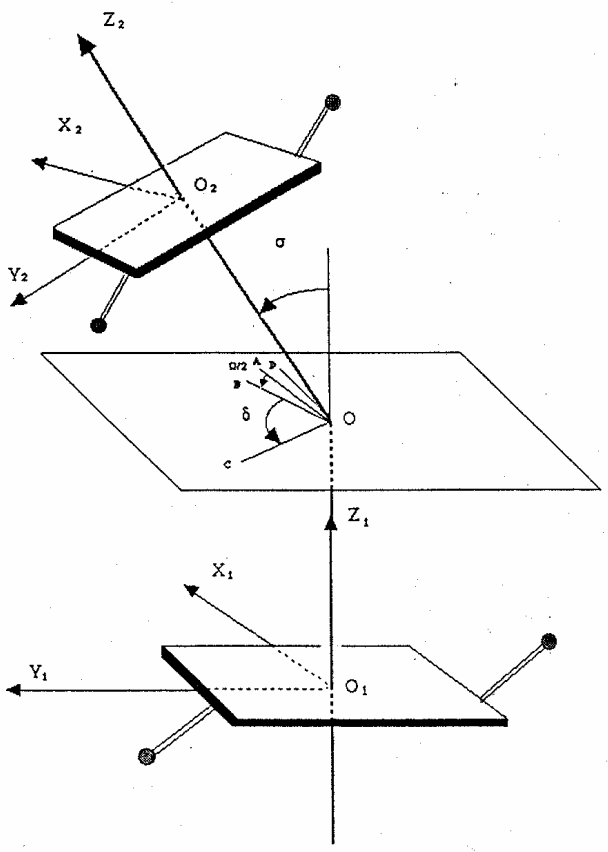
III 1

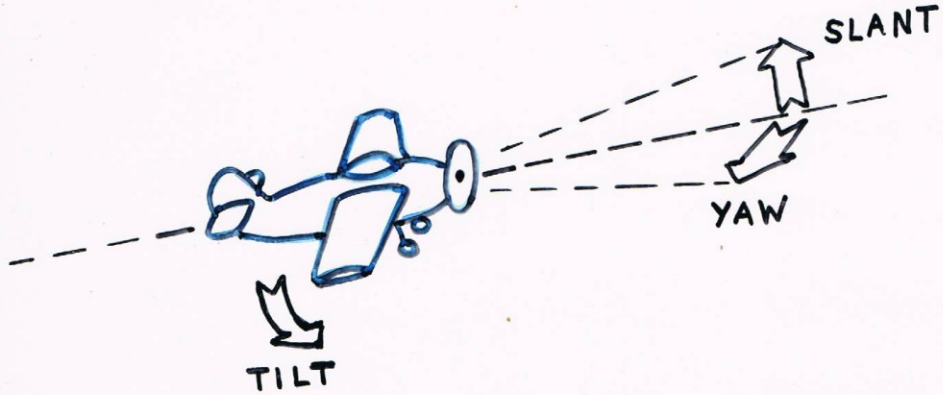
$(5'\text{-CAAAATTTTG-}3')_6$

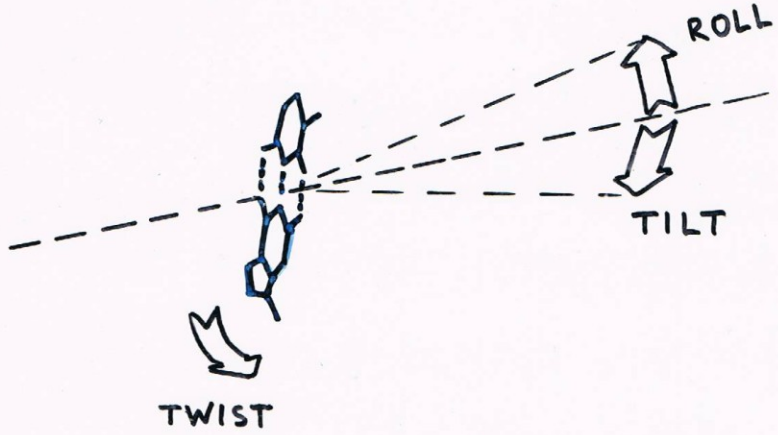
$(5'\text{-CTTTTAAAAG-}3')_6$




3′
5′
Z
X
Y


Z₂
X₂
O₂
σ
Y₂
α/2
δ
O
c
Z₁
X₁
Y₁
O₁


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.

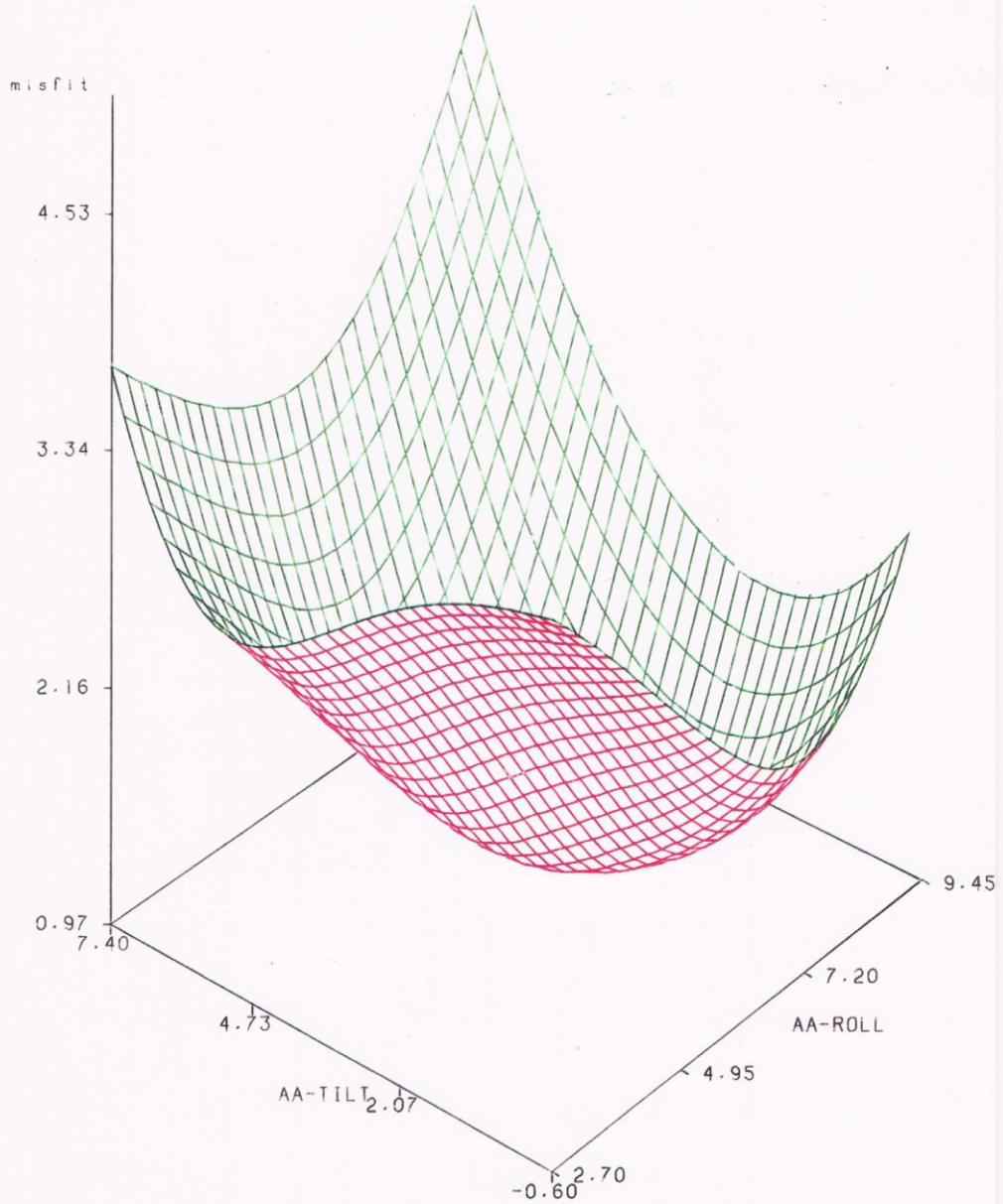
Today both Alex and myself are Professors
in the Institute of Evolution, Haifa.

To ne kazhdyi svladne

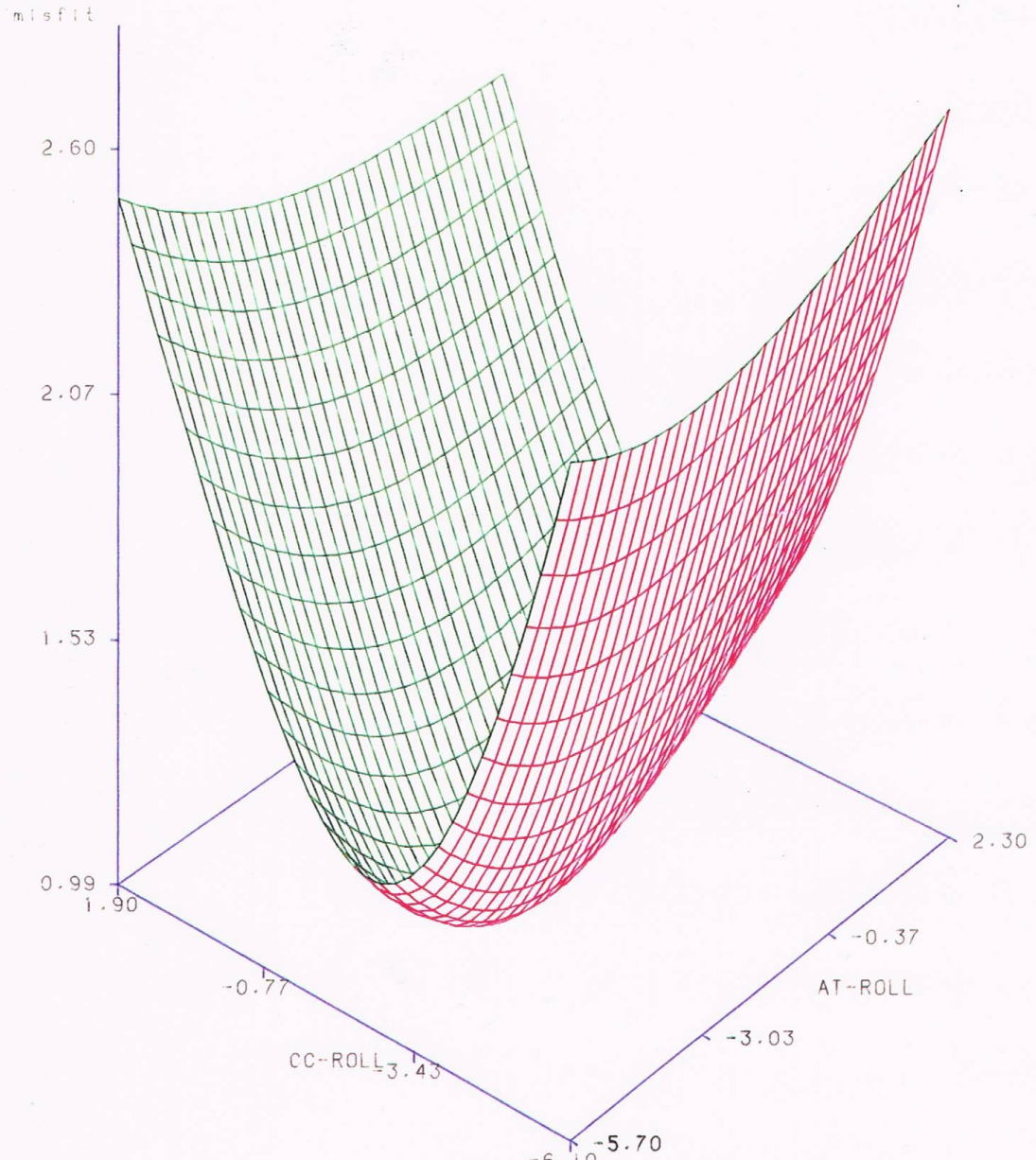
Table 1. Curved and straight synthetic DNA fragments.

	Repeat unit	Curvature (k-factor)		Misfit(std)
	Circles	Experimental curvature	Calculated curvature	
1	TCTGTAAAAAATATATAAAAA	0.59cu (0.06)	0.586	0.0
2	TCAAATTCGGGGCAAAGATCCC	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	GTTTTAAAAG	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	GGGTGGGCAAAAAACGGGCAA	1.26 (0.03)	1.21	1.5
	AAAACGGCAAAAAACGGGCTCC			
13	GGGTGGGCAAAAAACGGCAAA	1.19 (0.03)	1.21	0.7
	AAACGGCTGC			
14	GGGTGGGCAAAAAACGGGCTCC	1.14 (0.03)	1.13	0.3
15	GGGCAGGGTCGGGCAAAAAACG	1.07 (0.03)	1.02	1.6
	GCTGGATCGC			
16	GGCAGGCCGGTCGACGGGCAA	1.06 (0.03)	1.05	0.3
	AAAACGGCGGTCGGGCGGATCC			
17	GGGCAAAAACGGCAAAATTTT	1.11 (0.03)	1.16	1.5
	GCCGCCGGGCC			
18	GGGCAAAAACGGGCGGCCAAA	1.01 (0.02)	1.01	0.0
	ATTTTGCCGC			
19	AAAAAAATTTTTTTTTTAAA	1.00 (0.02)	1.03	1.5
20	AAAAAAAAAAAAAAAAAAAA	0.98 (0.03)	1.01	1.0
21	TGTCGTTCTTGGTTCTCTTCTC	1.00 (0.02)	1.02	0.8
22	GCCCCGCGGG	1.05 (0.06)	1.01	0.7
23	GACAGGACTG	1.01 (0.03)	1.03	0.8
24	GCATCGATGG	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	GGGTGGGCAAAAAACGGCTCC	1.14 (0.02)	1.13	0.4
29	ACGTGGGCAAAAAACGGCTCC	1.14 (0.02)	1.15	0.4
30	GGGTCACCAAAAAACGGCTCC	1.12 (0.02)	1.08	2.0
31	TCAGTTATATAAAAAATATAT	1.13 (0.02)	1.14	0.5
32	TCGCTTATATAAAAAATATAT	1.13 (0.02)	1.12	0.3
33	GCCCGTAAAAAGCCCCTTTTA	1.12 (0.02)	1.13	0.4
34	GTGGGAGAAAGTGCCCACAAA	1.06 (0.02)	1.06	0.1
35	GTGTGAAAAAACACACTTTTT	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	GGGGTTTTTAAAAACCGGGCC	1.03 (0.03)	1.02	0.2
39	GGGGTTTTTAAAAAAACCGCC	1.07 (0.03)	1.09	0.6
40	GGGGTTTTTAAAAAAAAACCC	1.15 (0.03)	1.12	0.9
41	GGGGTTTTTTAAAAAAACCC	1.21 (0.03)	1.22	0.2
42	CGGAGCCGTTTTTTGCCCAGC	1.15 (0.03)	1.13	0.6
43	CCGGCCAAAAAAAACGCGCGC	1.09 (0.03)	1.04	1.6
44	CCGGCCAAAAAAAAAACGCGC	1.04 (0.03)	1.01	1.0
45	CCGGCCAAAAAAAAAAAAACGC	1.01 (0.03)	1.02	0.3
46	CCCCCAAAAAAAAAAAAACG	1.05 (0.03)	1.06	0.4
47	CCCGAACAAAAAAAAAAAAG	1.07 (0.03)	1.08	0.4
	non-AA fragments			
48	CATGTCACGCACGCATCACGG	1.07 (0.02)	1.02	2.3
49	TCCGCAGACGTCCCCACCAGG	1.02 (0.02)	1.01	0.3
50	GCGAGACGGTACCGAGATGTC	1.10 (0.02)	1.06	2.0
51	TGTGAGAGCGCCATGAGATCA	1.11 (0.02)	1.11	0.2
52	TACGGATCTCGCCATGACTCTC	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

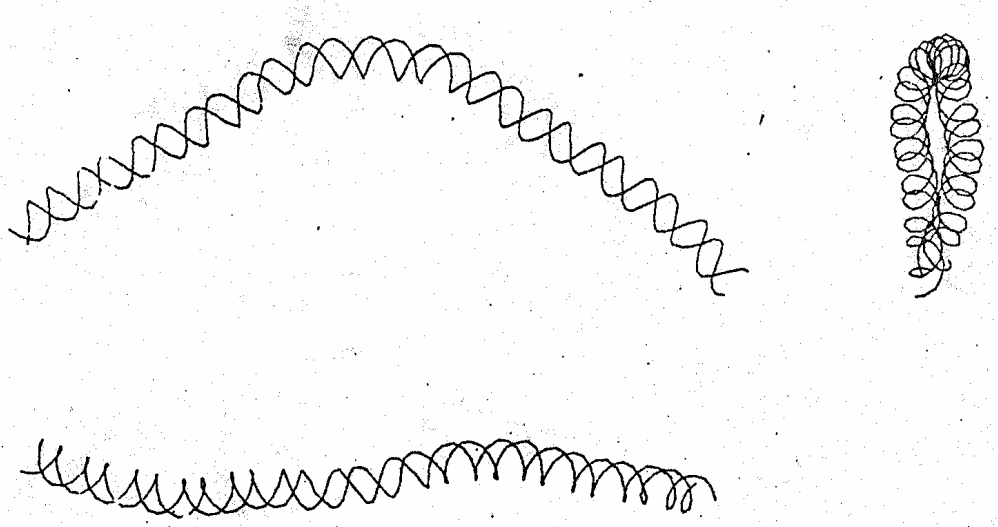


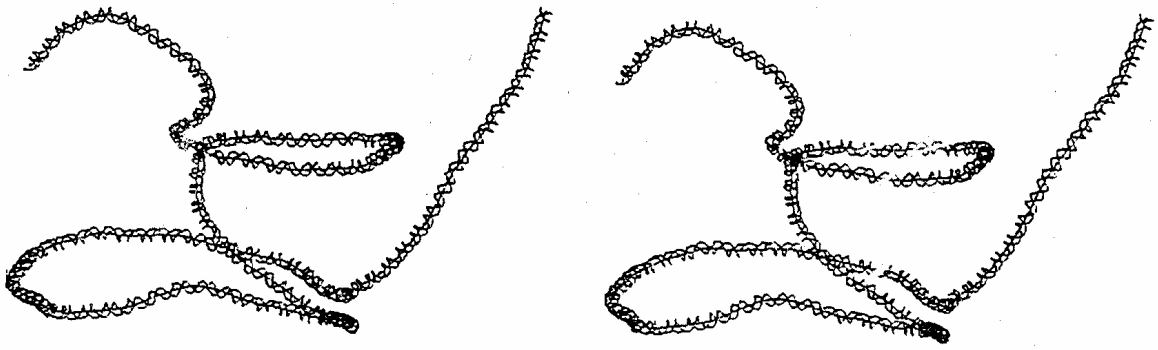
ANGLES DESCRIBING SHAPE OF DNA (DNA SHAPE CODE)

	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

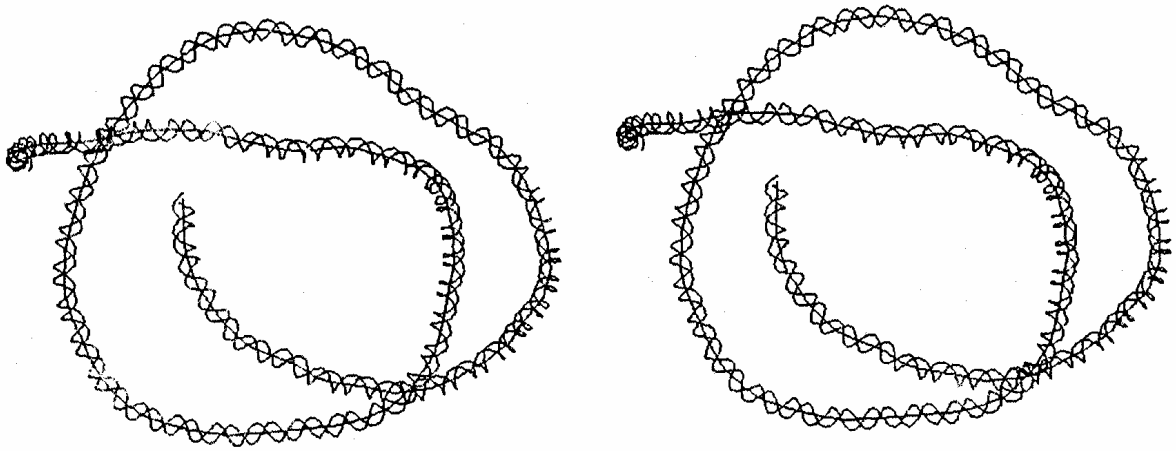
Bolshoy et al., 1991
Kabsch et al., 1982





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

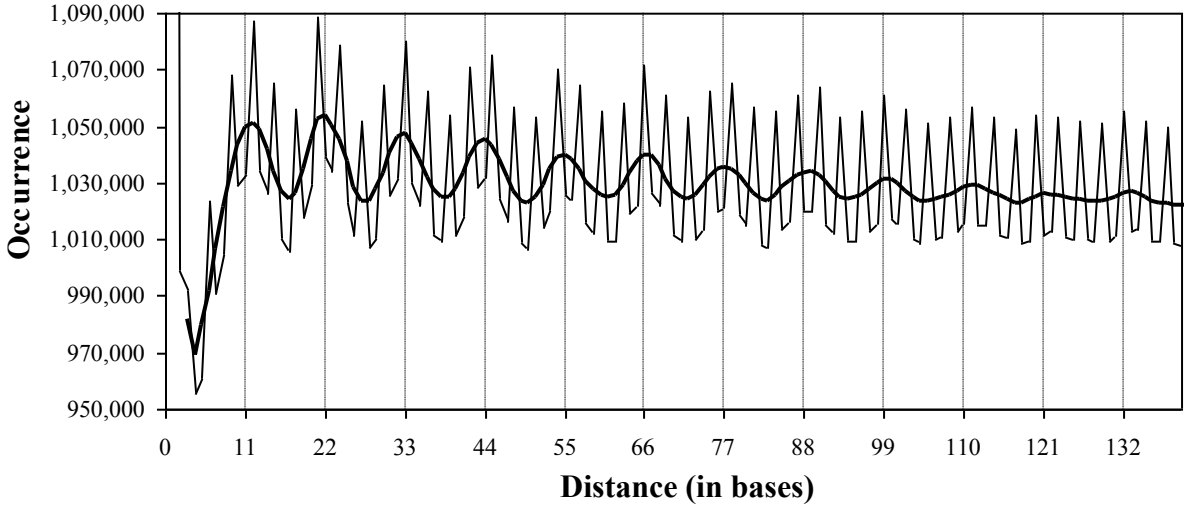
III 1



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

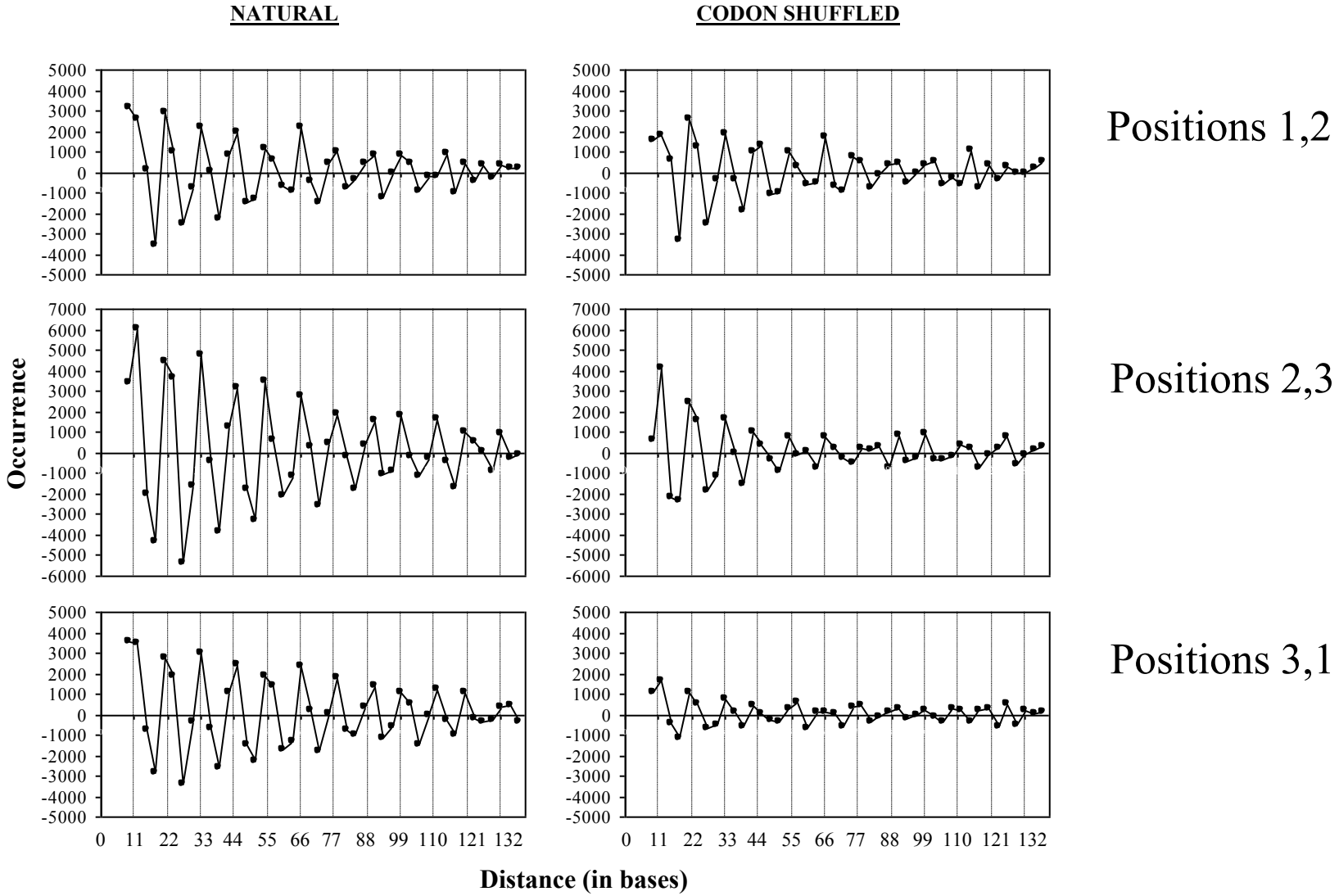
Later on it turned out that prokaryotic genomes are periodical as well, apparently to maintain DNA superhelicity

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)



Cohanim, 2006
Eubacteria

Randomizing third positions brings the oscillations down



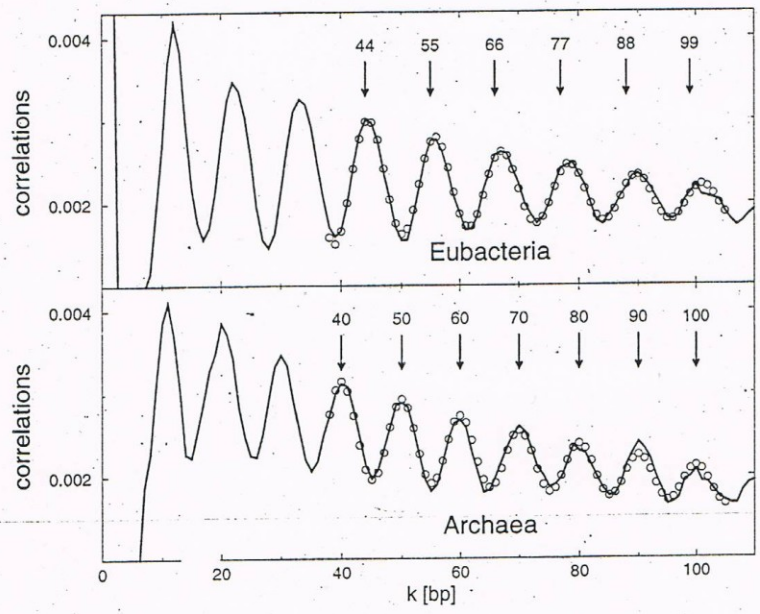


Fig. 2 Comparison of correlation functions from Eubacteria and Archaea. The functions represent the arithmetic means of WW-correlation functions from 8 eubacterial genomes and 3 archaeal genomes (listed in Table 1). The circles are obtained by nonlinear curve fitting. In order to highlight the difference 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

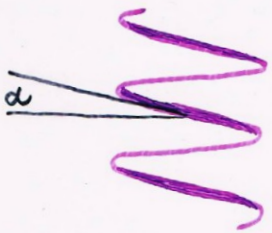
Table 1: Periodicities of genomic DNA

	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



CRICK (1976):

$$\text{TWIST} = N \cdot \sin\alpha$$

N: NUMBER OF TURNS OF THE SUPERHELIX

α: ASCENDING ANGLE

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

* FOR LEFT-HANDED SUPERHELIX $P < P_o$

* FOR RIGHT-HANDED SUPERHELIX $P > P_o$

P_o: HELICAL REPEAT OF NON-CONSTRAINED DNA

TAKING KNOWN GEOMETRY OF THE NUCLEOSOME SUPERHELIX ONE GETS:

$$P_{\text{NUCL.}} = P_{o\,\text{FREE}} - 0.15\ \text{BP}$$

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

10 BP REPEAT

11 BP REPEAT

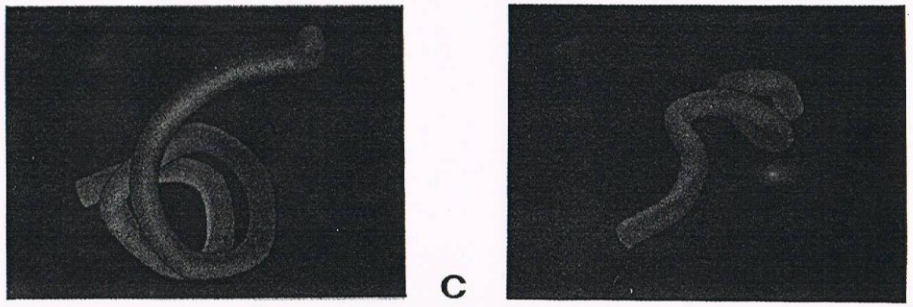


Fig. 2 Stereo micrographs of $[(A)_5TGCCC]_{54}$ DNA molecules and a 3D reconstructions of one molecule. For cryo-EM the DNA molecules are suspended in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH. 8.0) (refs 6,9). The molecules, in a thin vitrified layer of buffer are confined to a thickness of about 50 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 $[(A)_6TGCCC]_n$ 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.

structural biology volume 1 number 6 june 1994

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

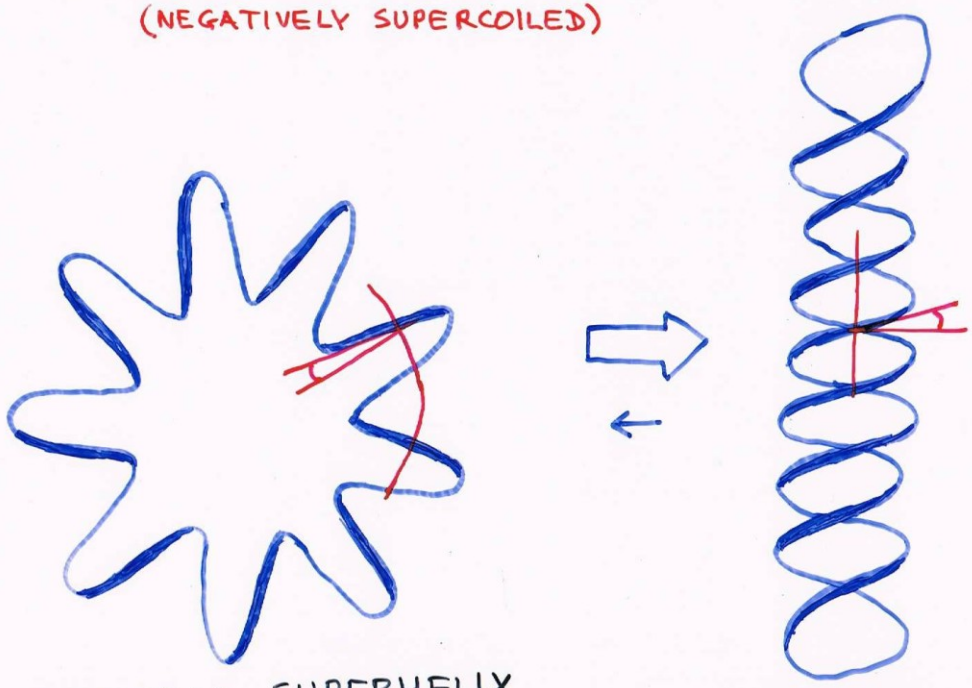
FREE DNA ($\alpha = 0$): 10.54 BP/TURN

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

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

TOPOLOGICALLY EQUIVALENT SUPERHELICAL STRUCTURES

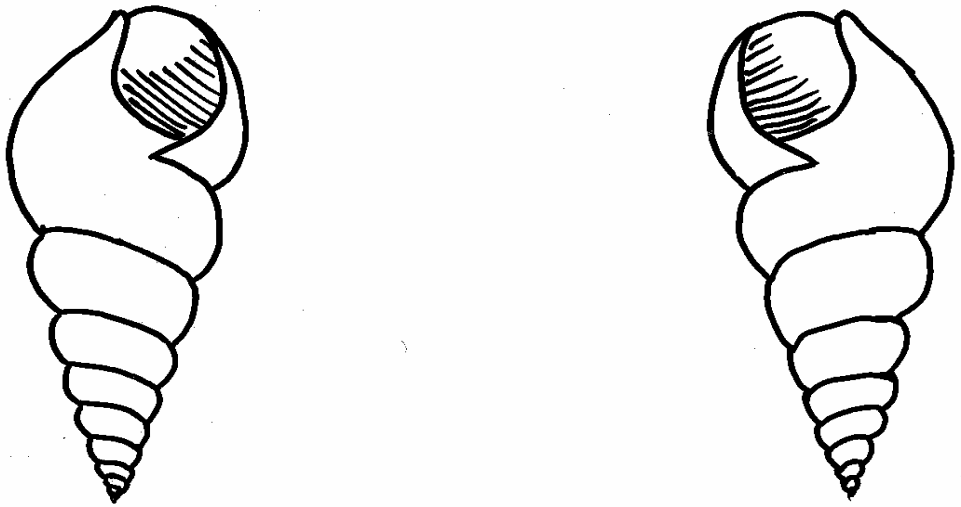
(NEGATIVELY SUPERCOILED)

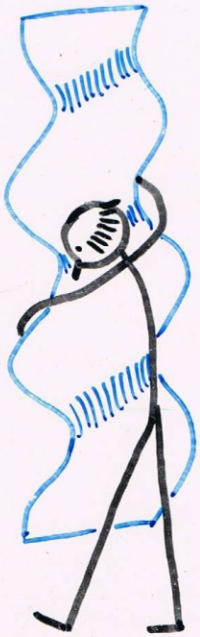


TOROIDAL SUPERHELIX

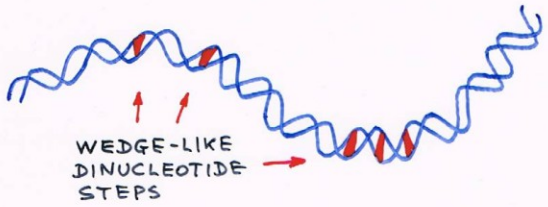
INTERWOUND SUPERHELIX

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





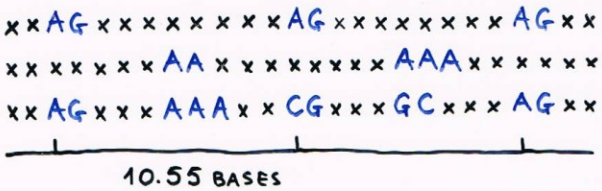
DNA SHAPE CODE



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:

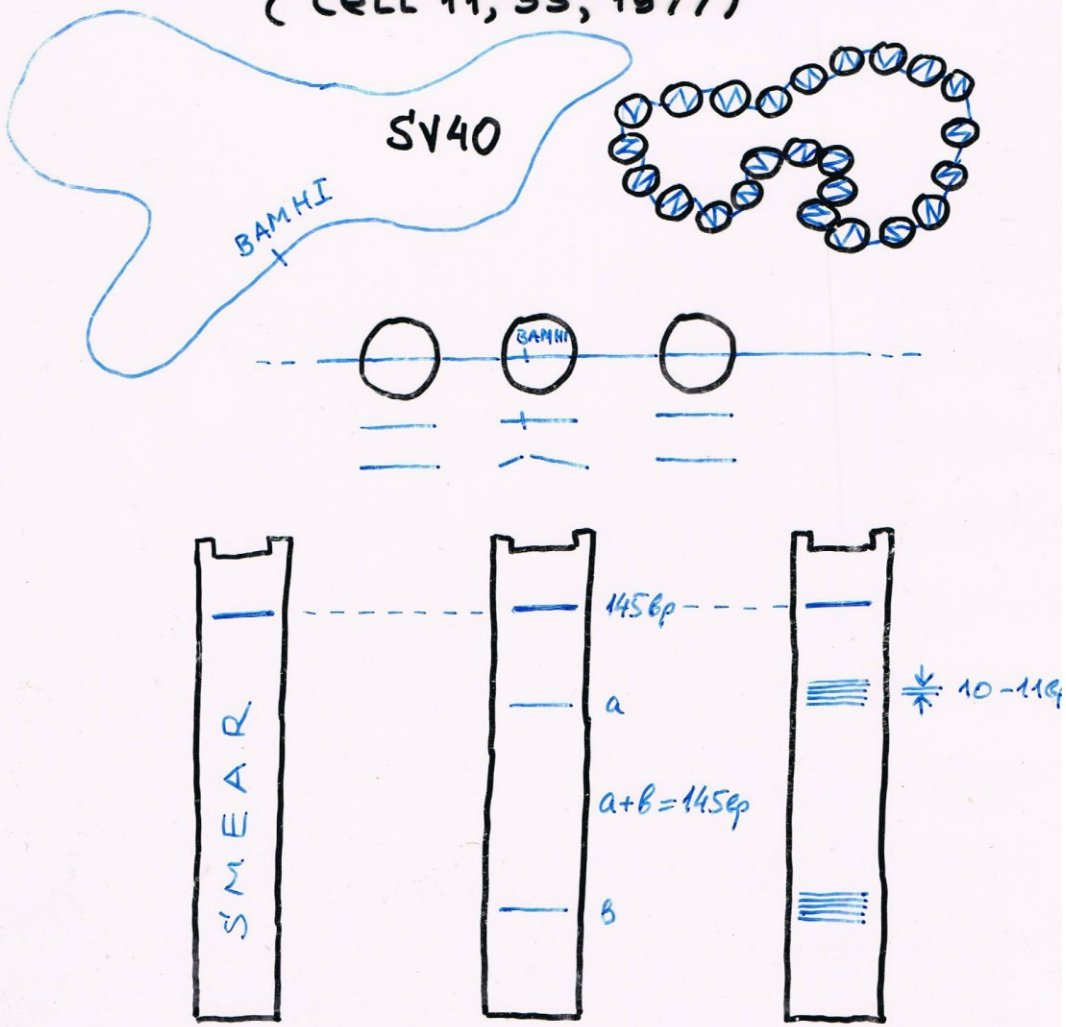


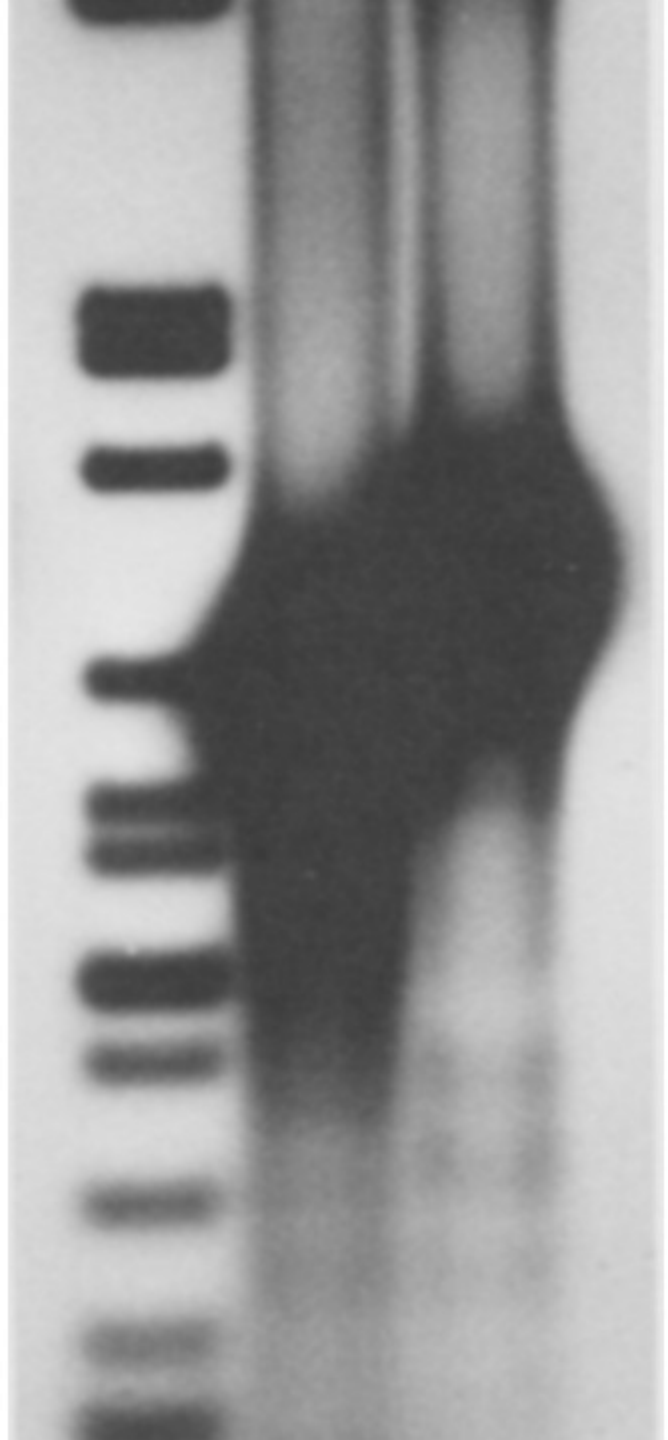
WRITHE:

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

~145bp

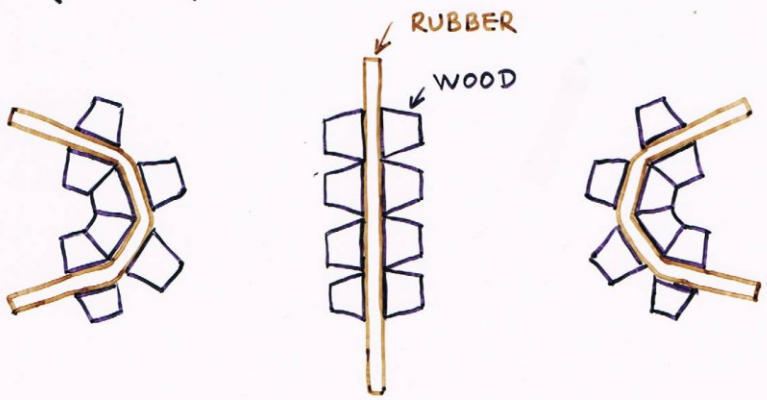
~93bp

~83bp

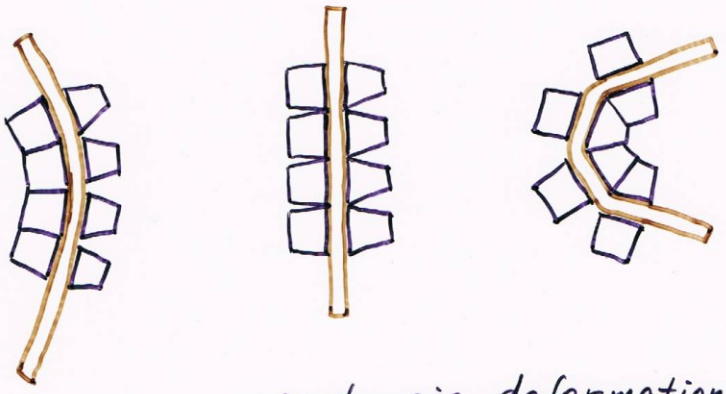
~73bp

~63bp

DEFORMATIONAL ANISOTROPY (IN 2D)

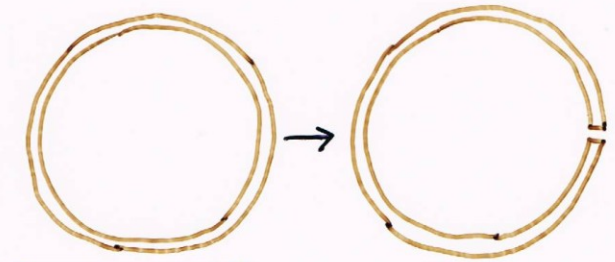


isotropic deformation



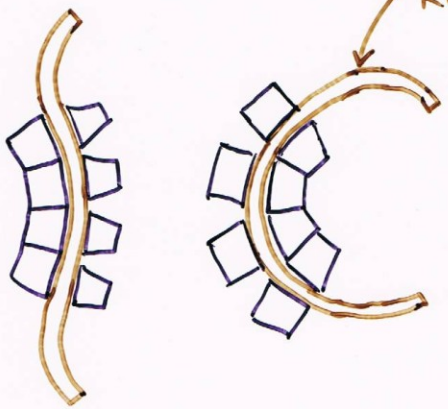
anisotropic deformation

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



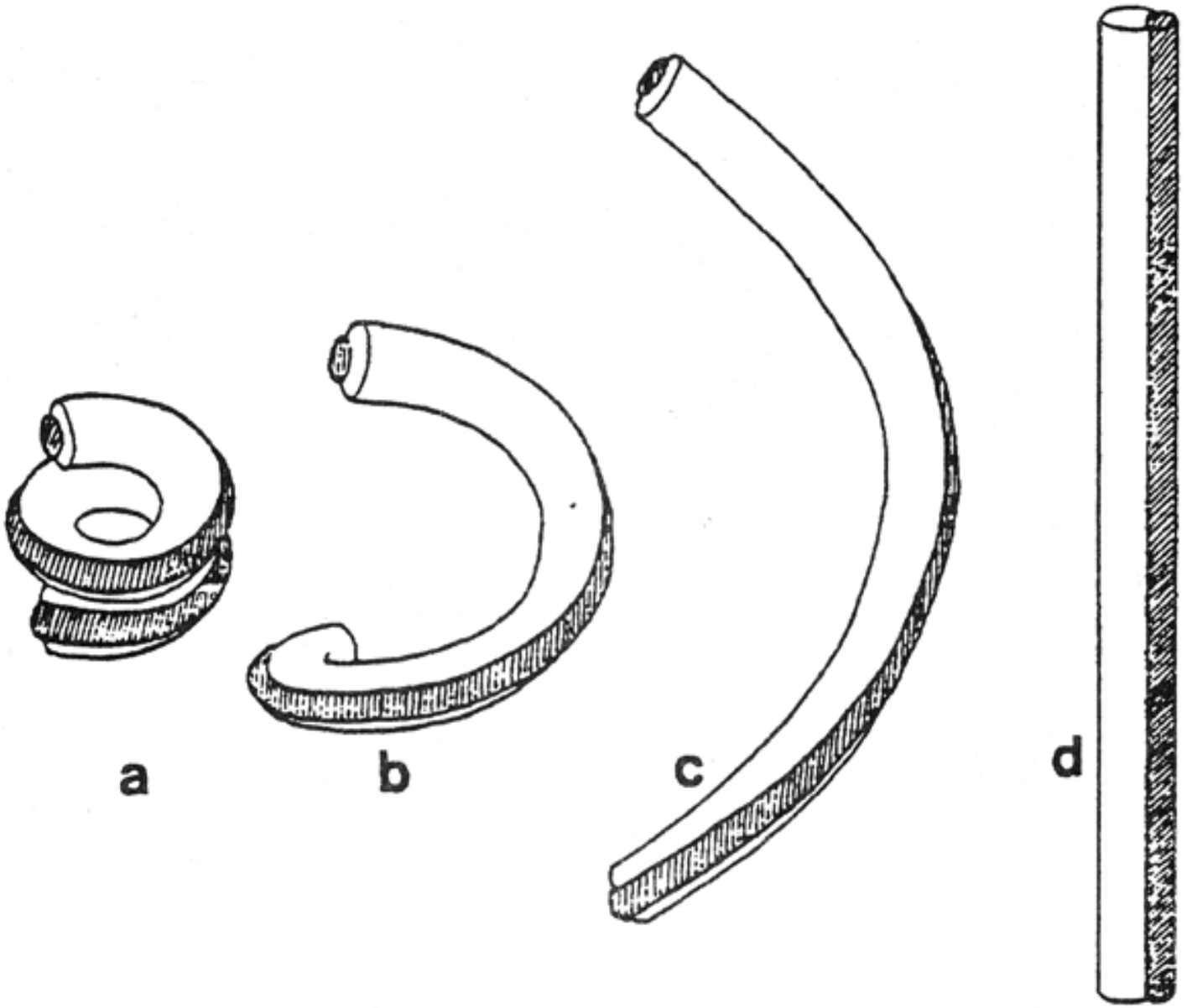
RUBBER RING
(TUBE CUT)

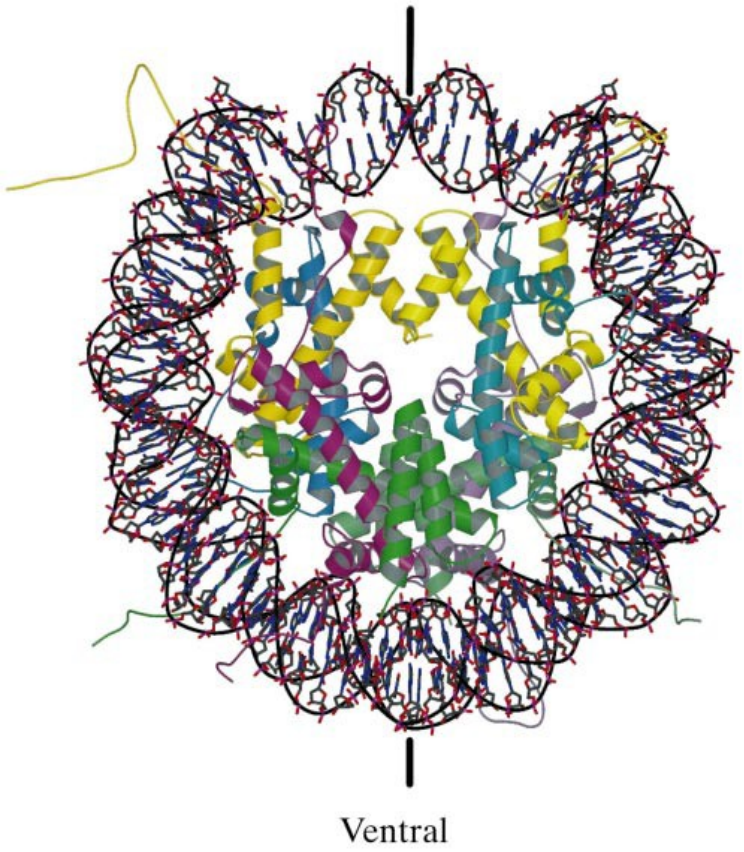
CURVED
RUBBER



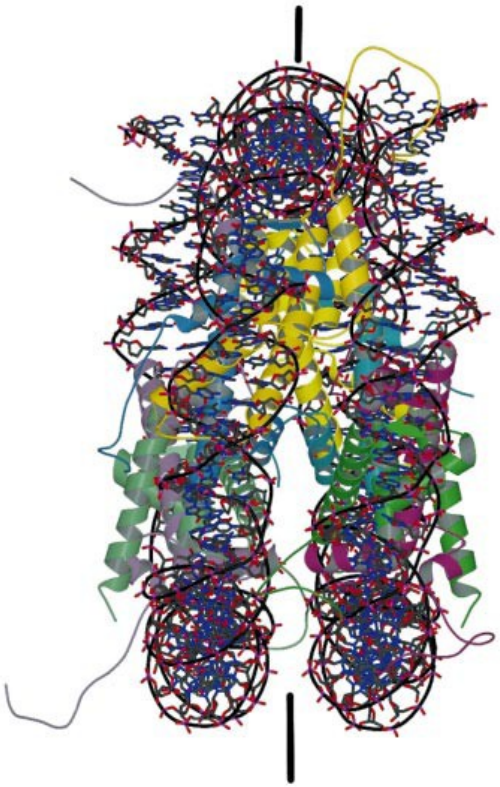
NO BENDING
IN DIRECTION
OF CURVATURE

BETTER BENDING
AGAINST CURVATURE

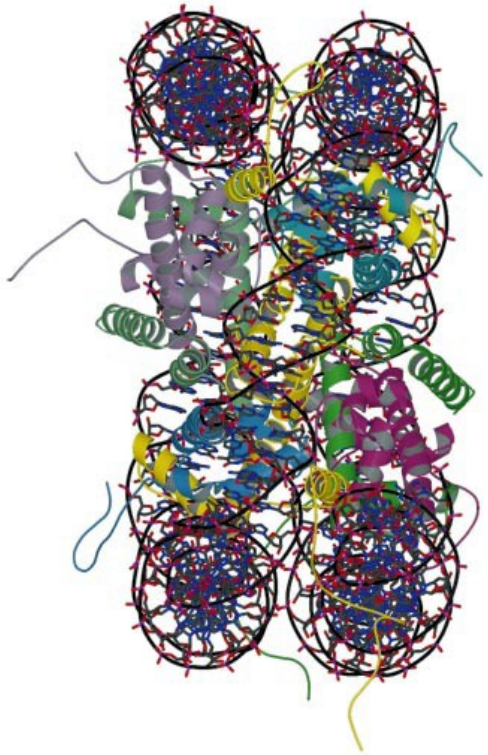

a
b
c
d



Ventral



Side



Dyad

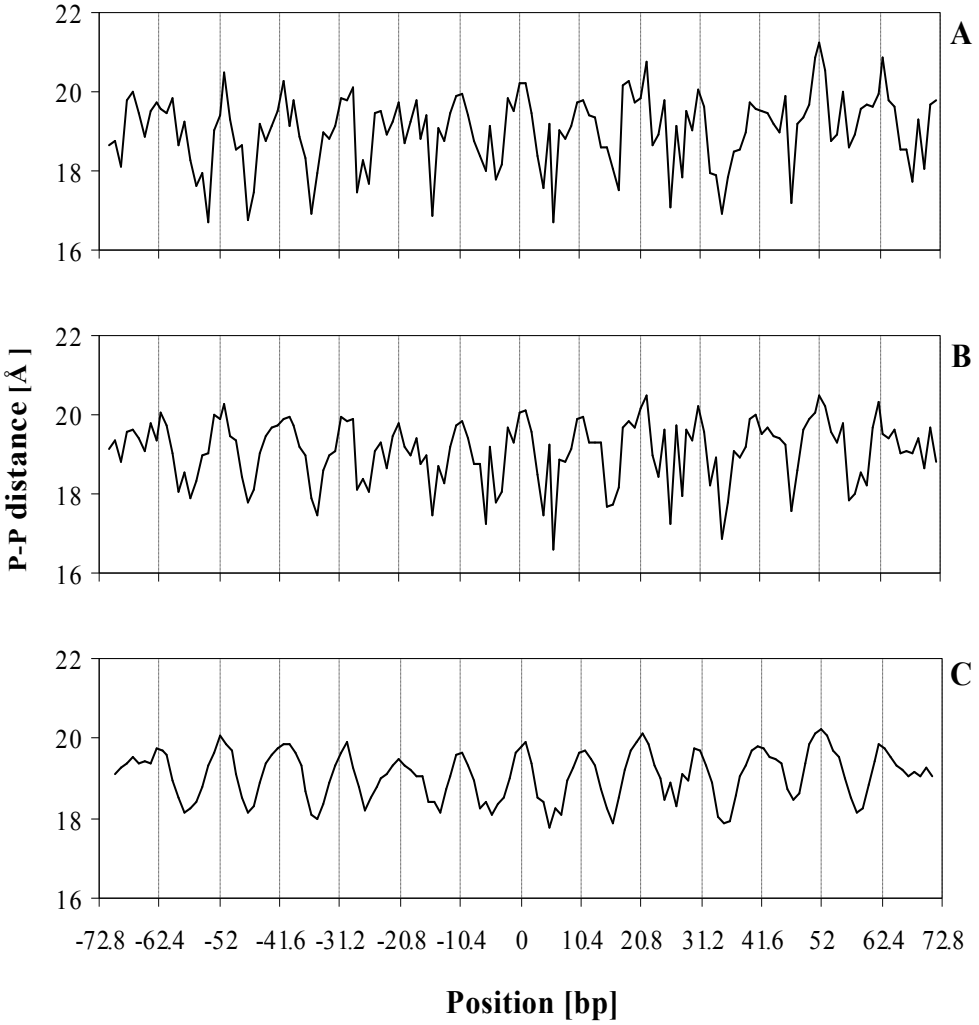
Lab of G. Bunick, 2000

The nucleosome DNA structural period is between 10.333 and 10.400

pitch of DNA (base pairs)	local dyads 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

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

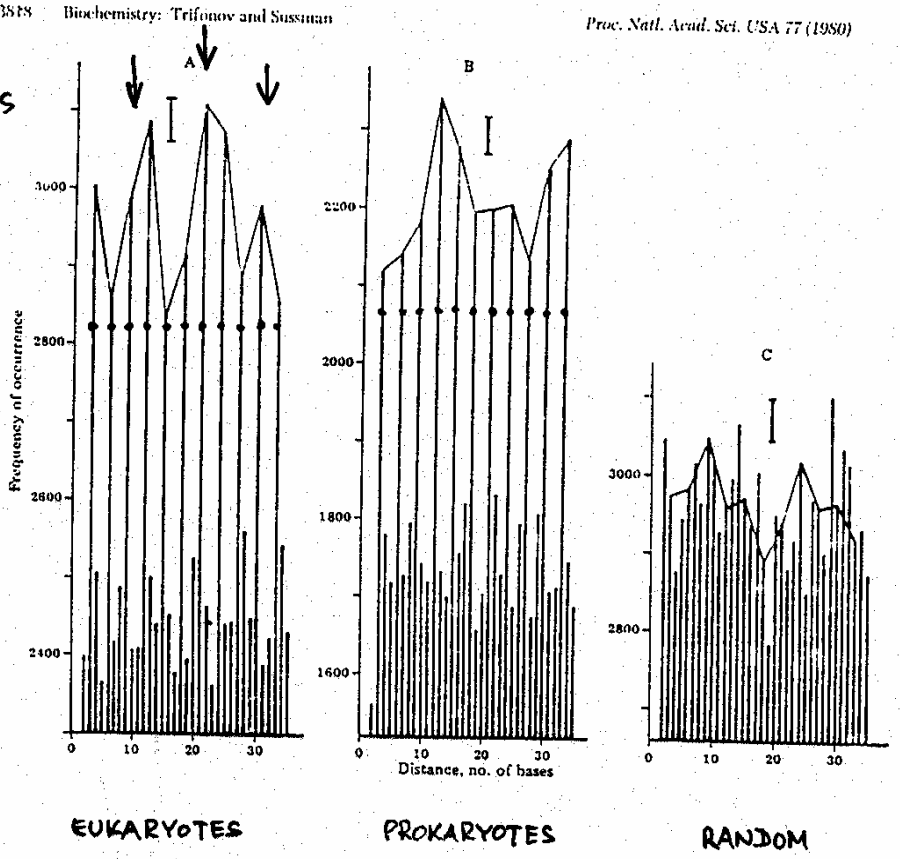
It contains ~125 bp of DNA
with structural period 10.4 bp

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

TRIFONOV, SUSSMAN, 1980

~ 10.5 BASES

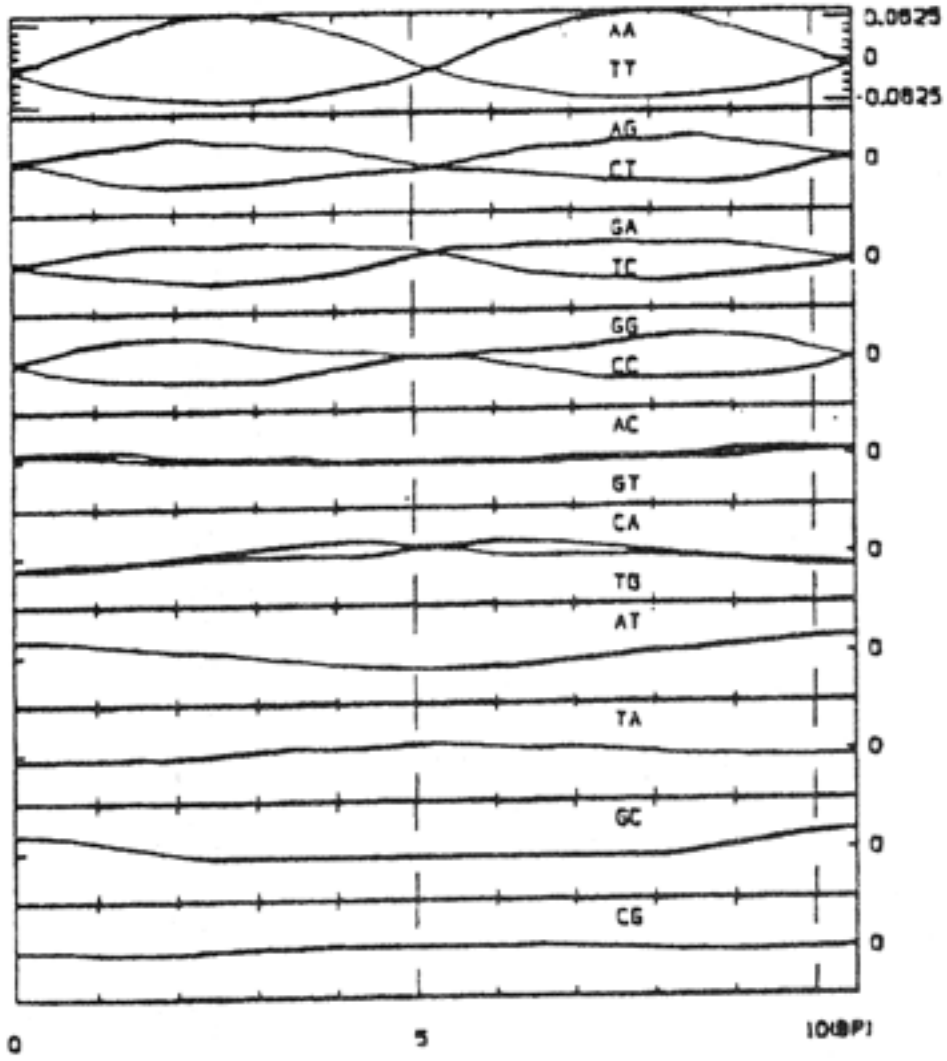
3 BASES



EUKARYOTES PROKARYOTES RANDOM

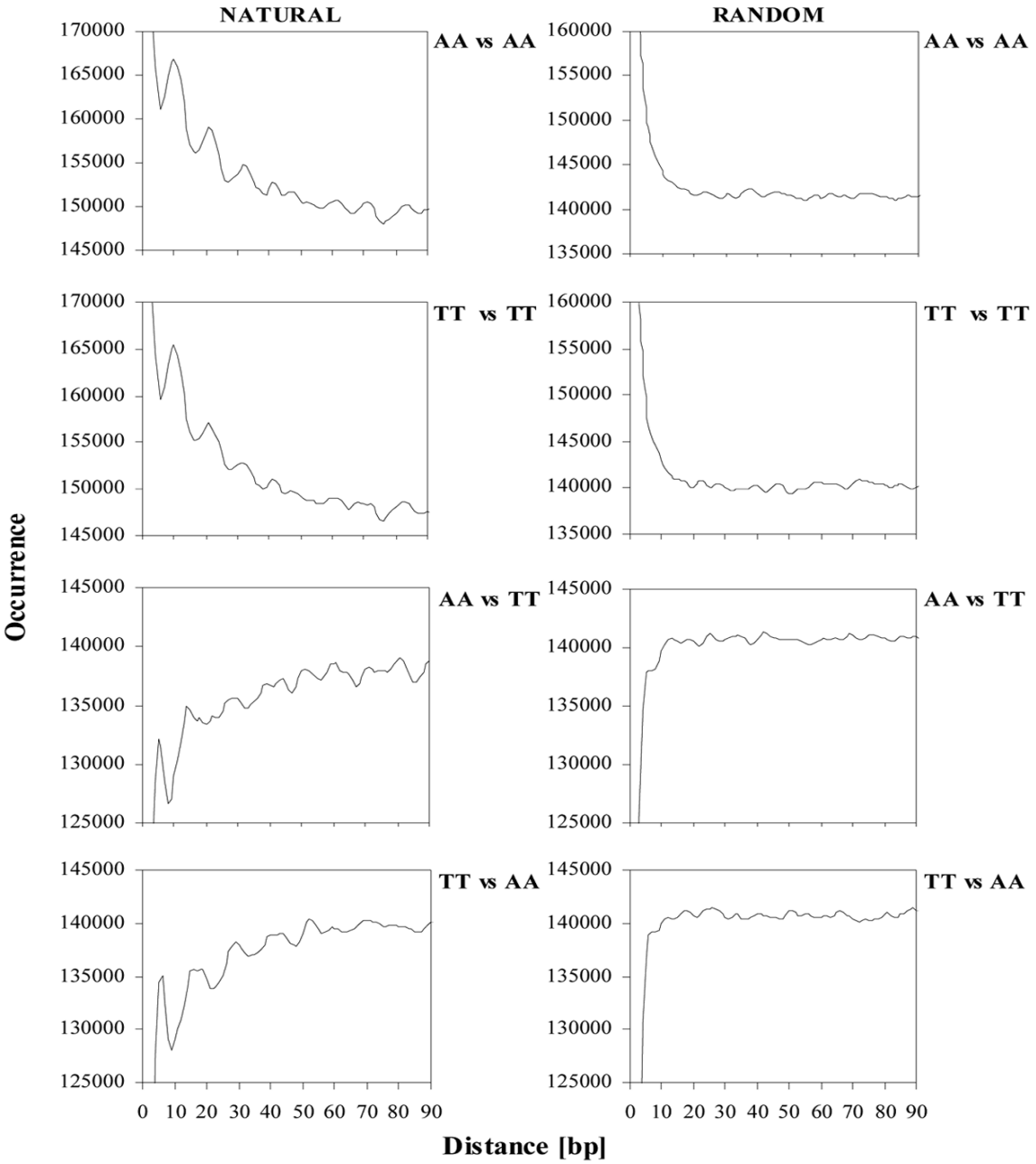
~ 30 000 BASES

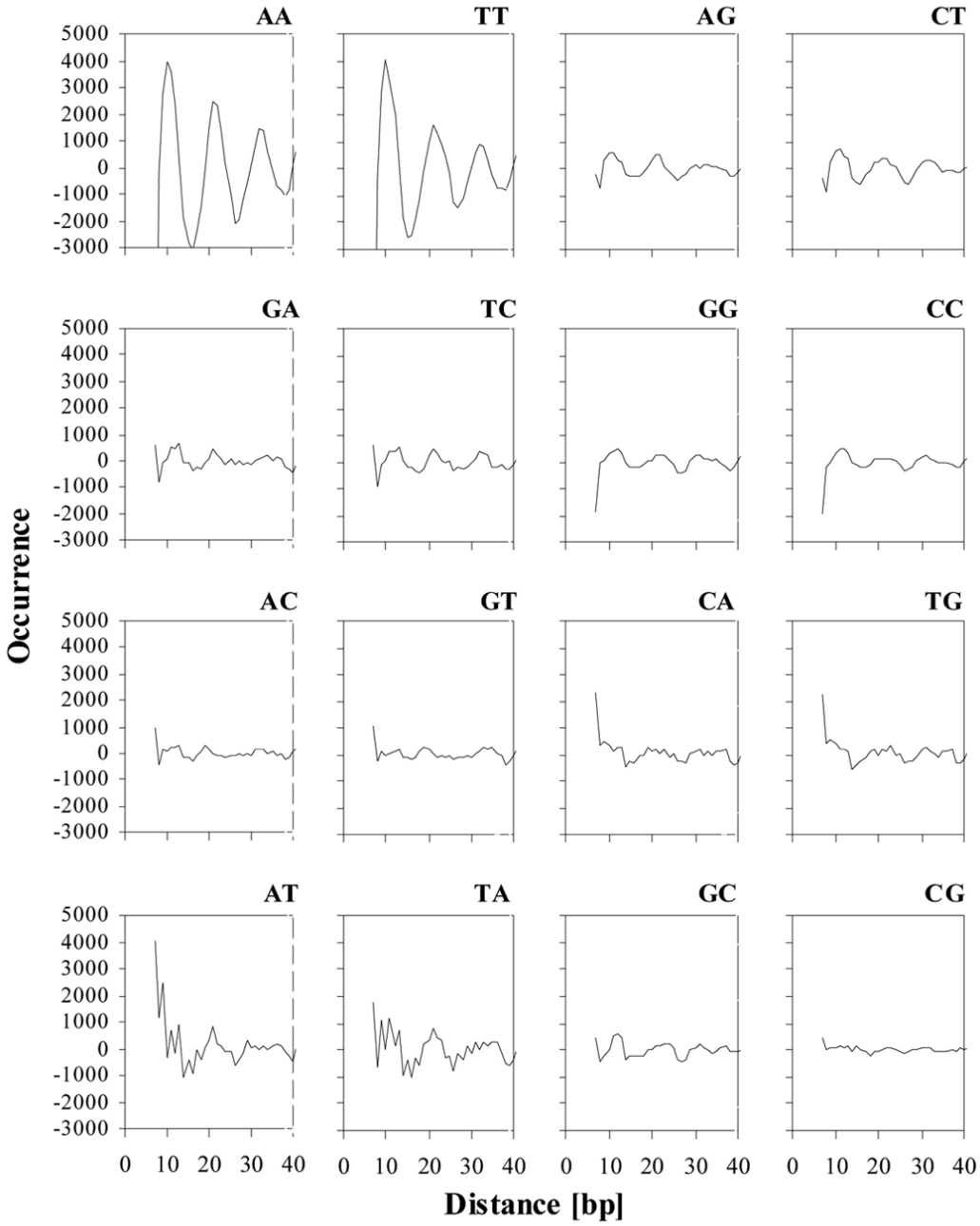
First matrix of nucleosome DNA bendability

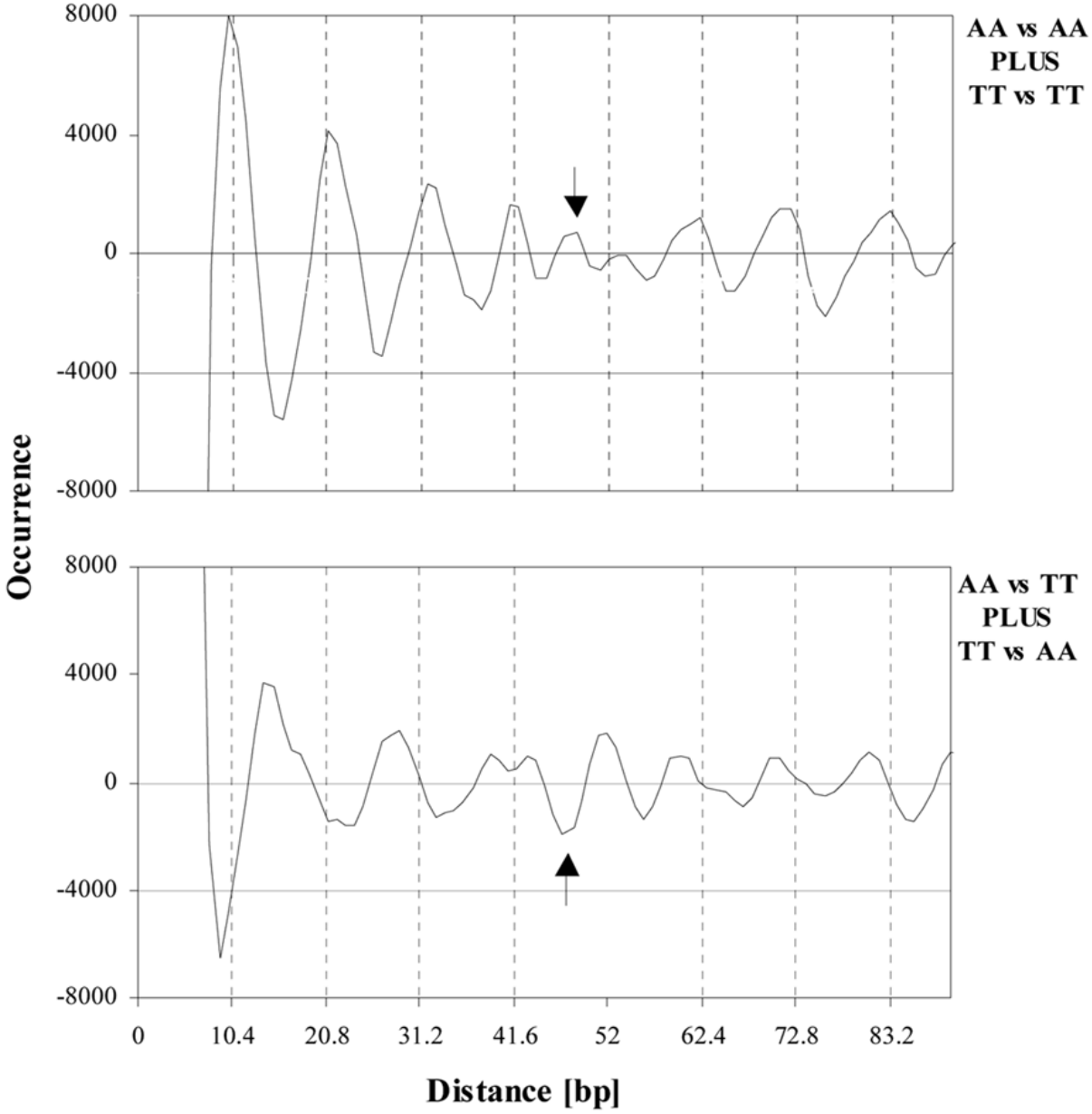


Mengeritsky and ENT, 1983

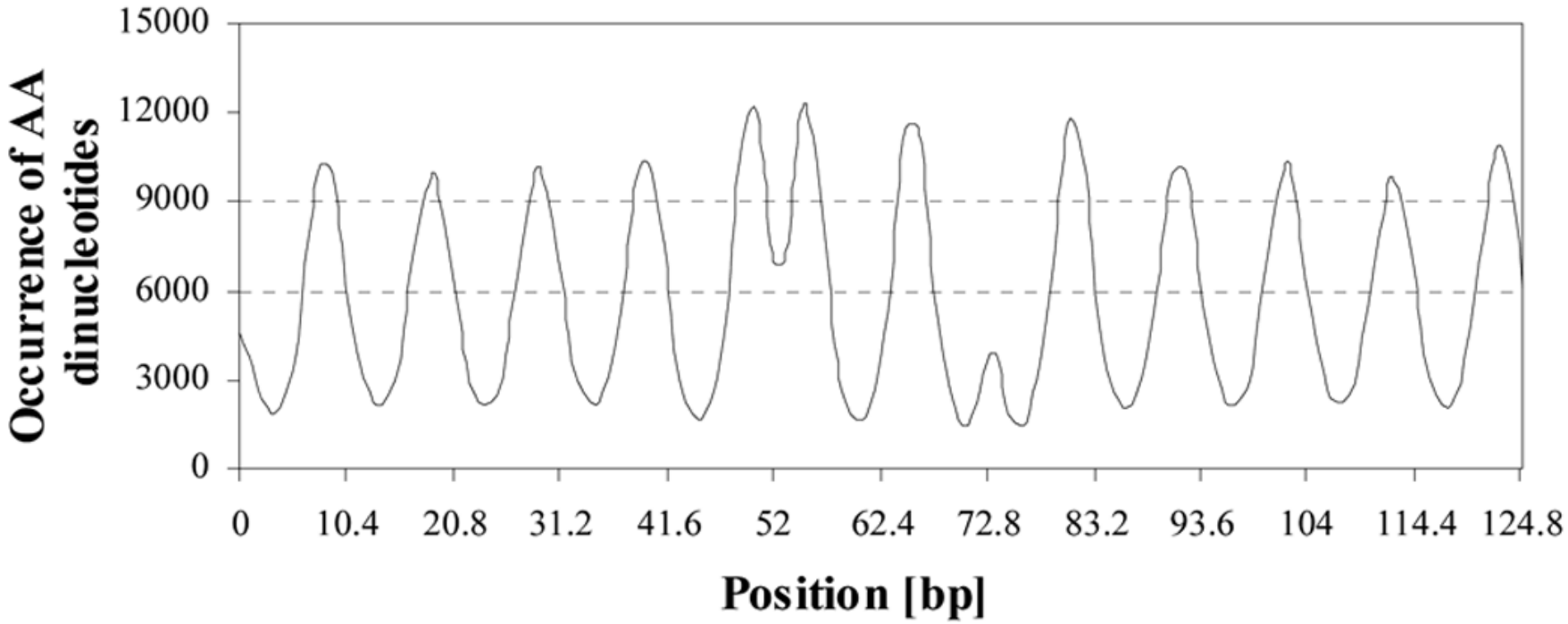
Yeast
Cohanim, 2005




AA
TT
AG
CT
GA
TC
GG
CC
AC
GT
CA
TG
AT
TA
GC
CG
5000
4000
3000
2000
1000
0
-1000
-2000
-3000
0 10 20 30 40
Occurrence
Distance [bp]


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

Calculated nucleosome positioning pattern for yeast genome (Cohanim, 2005)



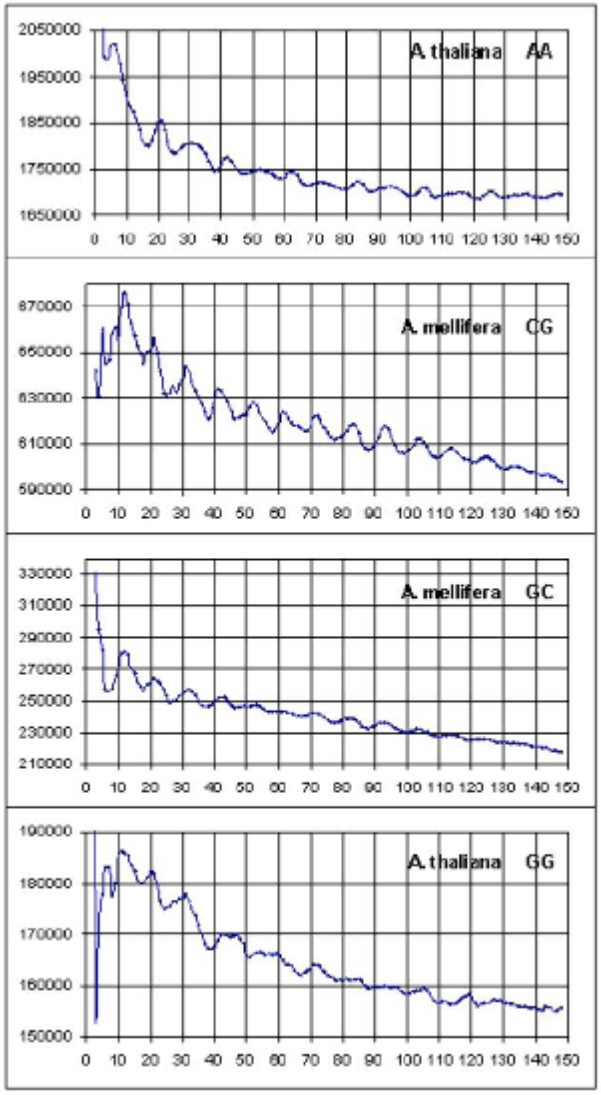

A. thaliana AA
2050000
1950000
1850000
1750000
1650000
A. mellifera CG
670000
650000
630000
610000
590000
A. mellifera GC
330000
310000
290000
270000
250000
230000
210000
A. thaliana GG
190000
180000
170000
160000
150000
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150

Figure 1

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 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

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

ccggaaatttccggaaatttc

The ideal sequence
to which they both match

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

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)

Regeneration of signal from its incomplete versions:

AA

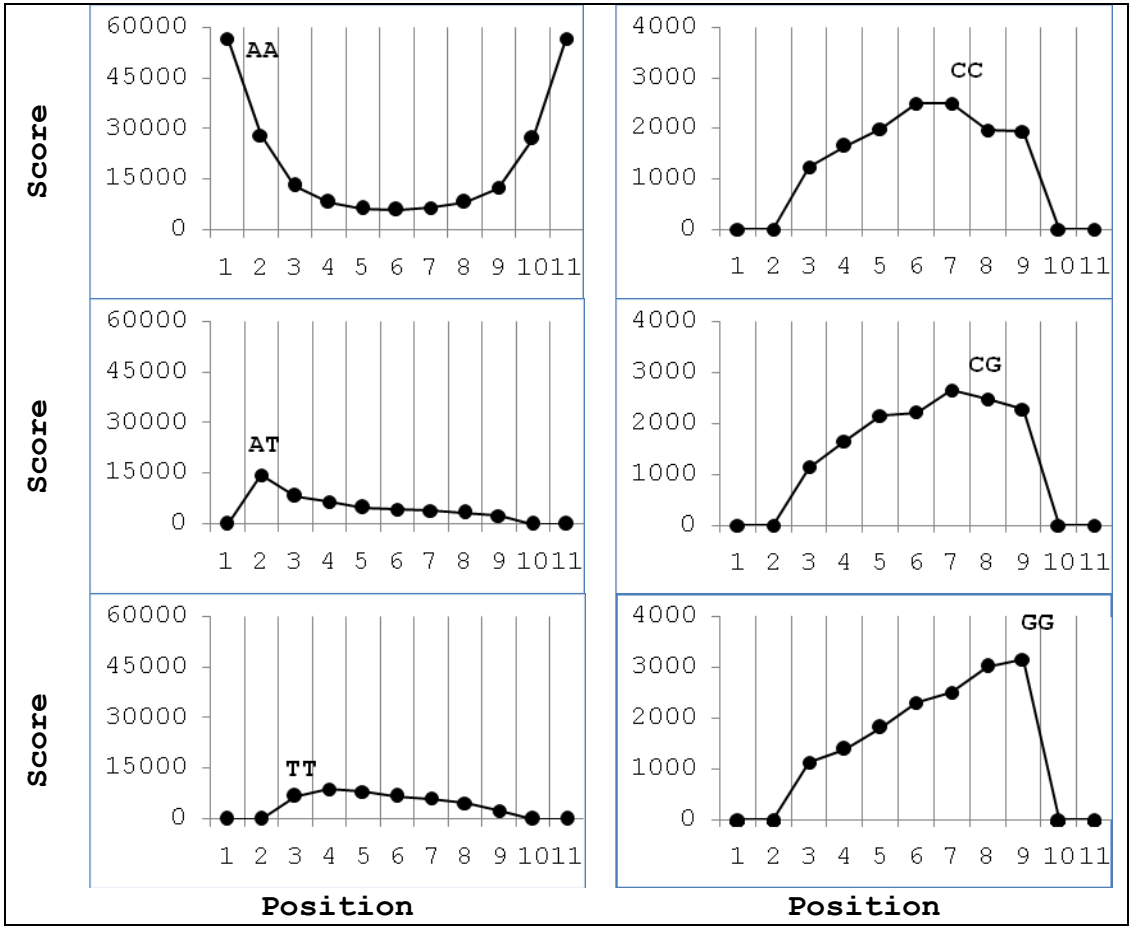
positional autocorrelation

AAnnnnnnnnAA

regeneration

AAnnnCCnnnAA

AAnnnnnnnnAA repeat structure (*C. elegans*)



Regenerated pattern (AAATTTCCGG)(AAAT…

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

1. Physical (deformational) preference.

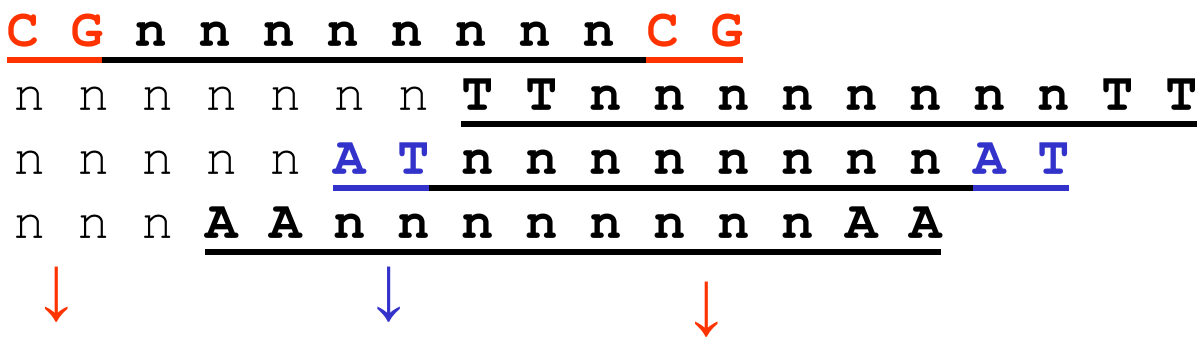
2. Sequence linkage (inclusion effect). Dinucleotide AB has to have neighbors NA and BN.

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)

Combination of four matrices:



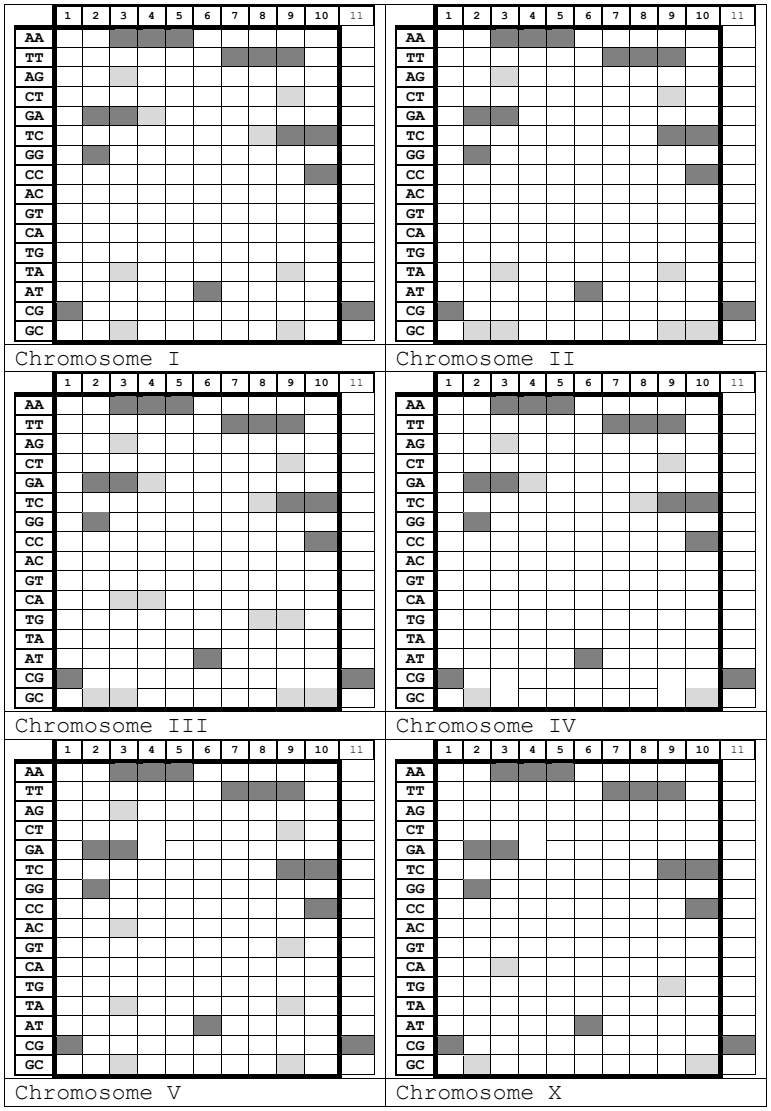
	1	2	3	4	5	6	7	8	9	10	11
AA	2	2	**4**	**4**	**4**	2	2	0	0	0	2
TT	2	0	0	0	2	2	**4**	**4**	**4**	2	2
AG	3	3	**4**	3	2	2	1	1	0	1	3
CT	3	1	0	1	1	2	2	3	**4**	3	3
GA	3	**4**	**4**	**4**	2	0	0	0	1	2	3
TC	3	2	0	0	1	0	2	**4**	**4**	**4**	3
GG	3	**4**	3	1	1	1	0	1	**4**	2	3
CC	3	2	3	1	0	1	1	2	**4**	**4**	3
AC	2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

The matrix turns out to be complementarily symmetrical.

Indeed, symmetrically positioned complementary base-pair stacks should have the same deformations.

Matrices of positional preferences
for six chromosomes
of *C. elegans*

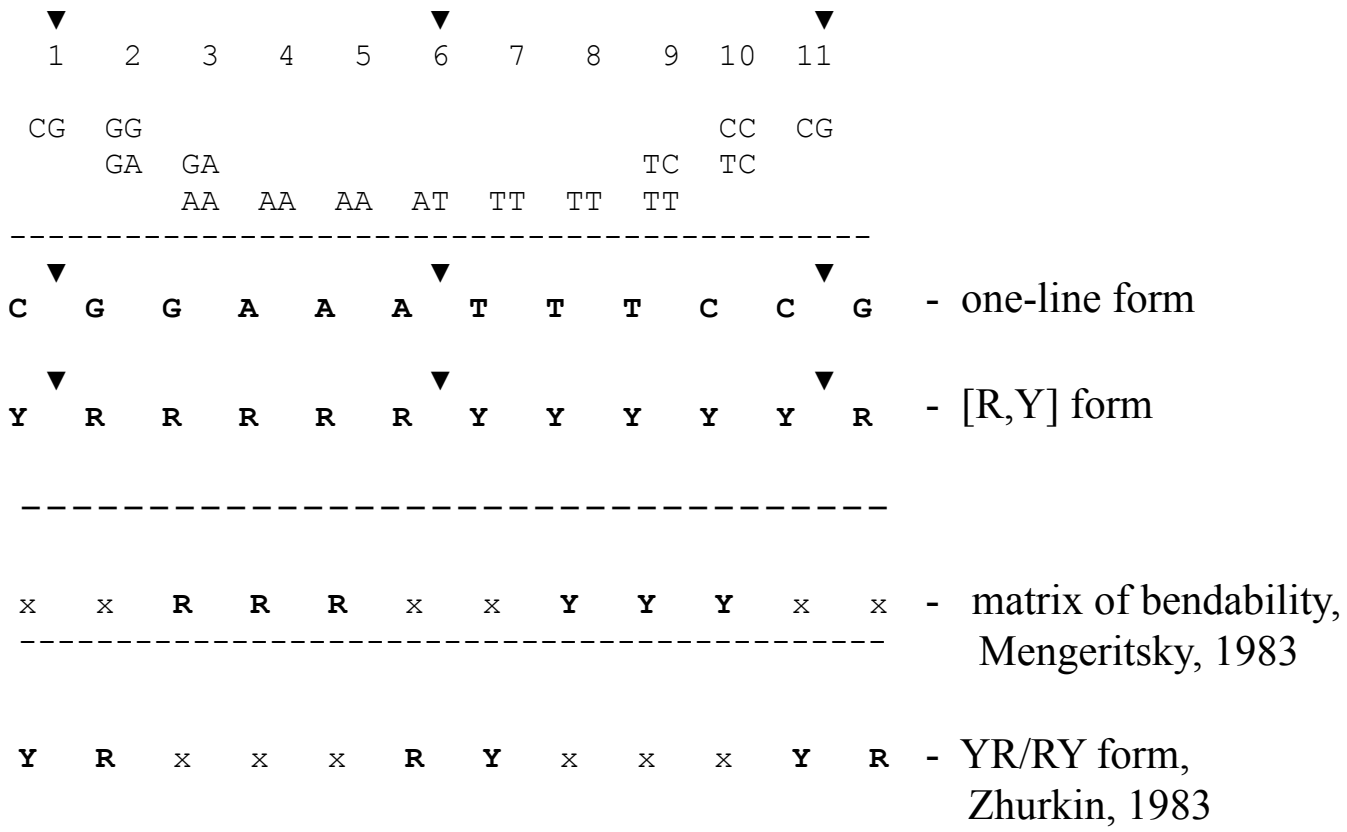
Common symmetrical elements:
AA/TT, GA/TC, GG/CC, AT and CG



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

Same in simplified forms:



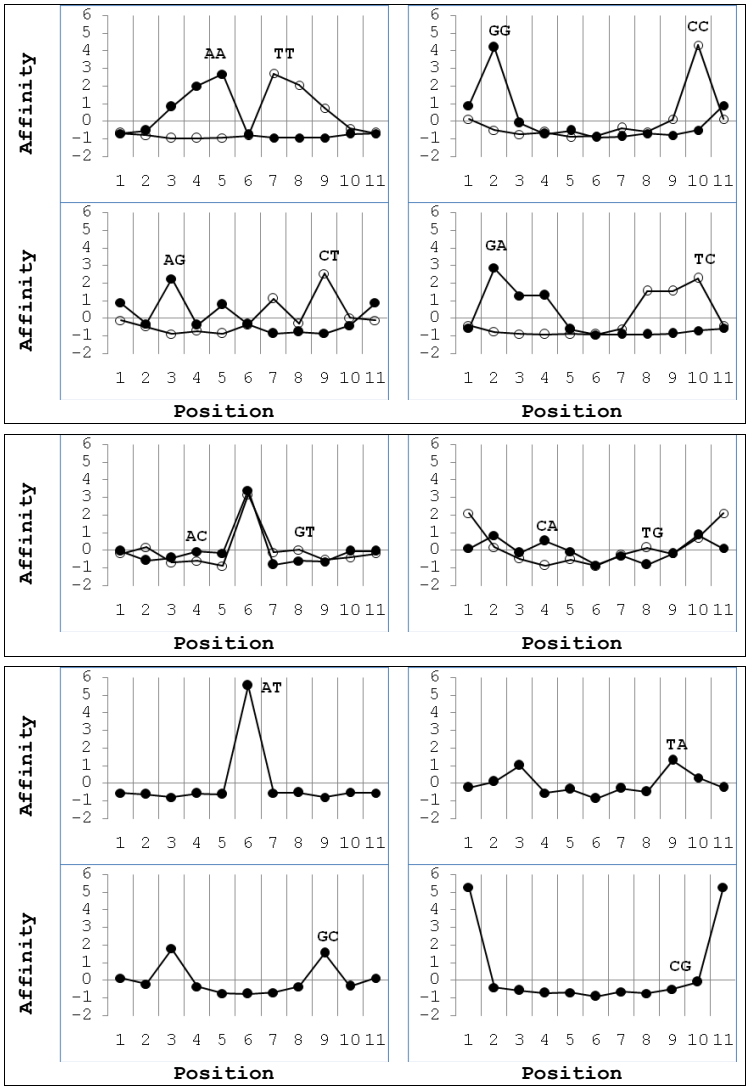
LINEAR FORM OF
THE POSITIONAL MATRIX OF BENDABILITY:

CGRAAATTTYCG

Matrix of bendability

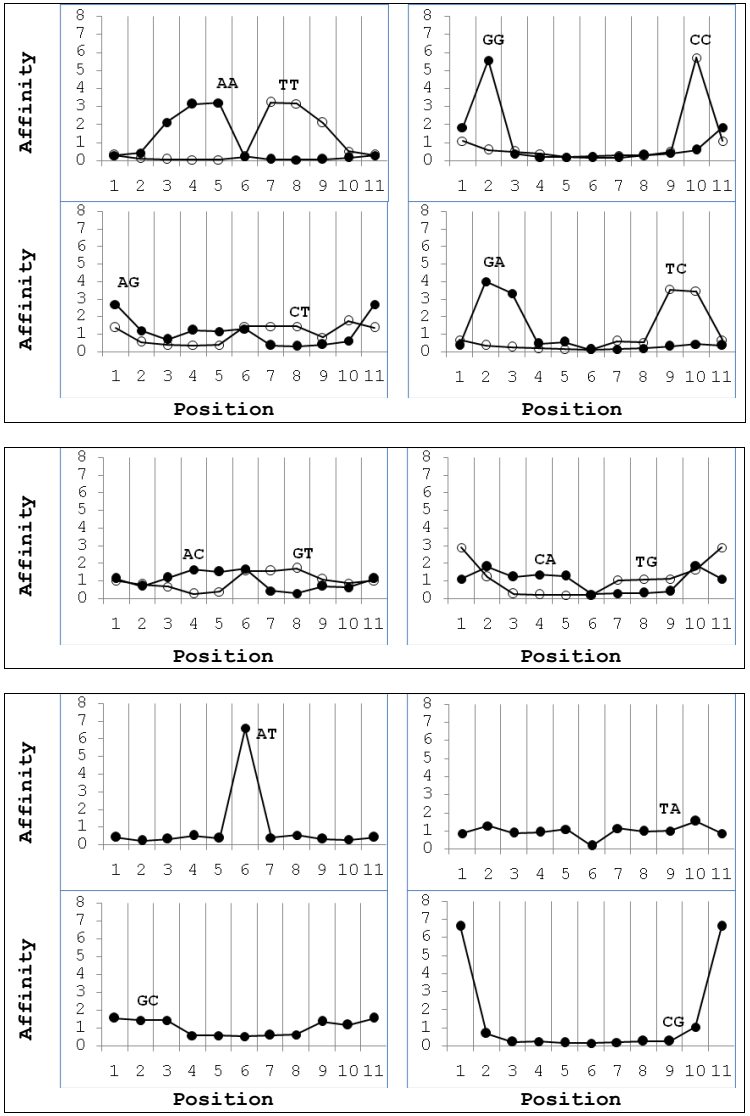
for Chromosome I

(no symmetrization applied)



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

NUCLEOSOME DNA PATTERNS IN 2-LETTER ALPHABETS

R = A, G Y = C, T

```
          |         |         |
. . . Y Y Y R R R R R Y Y Y Y Y R R R . . .
```

G. Mengeritsky, E. Trifonov, 1983

V. Zhurkin, 1983

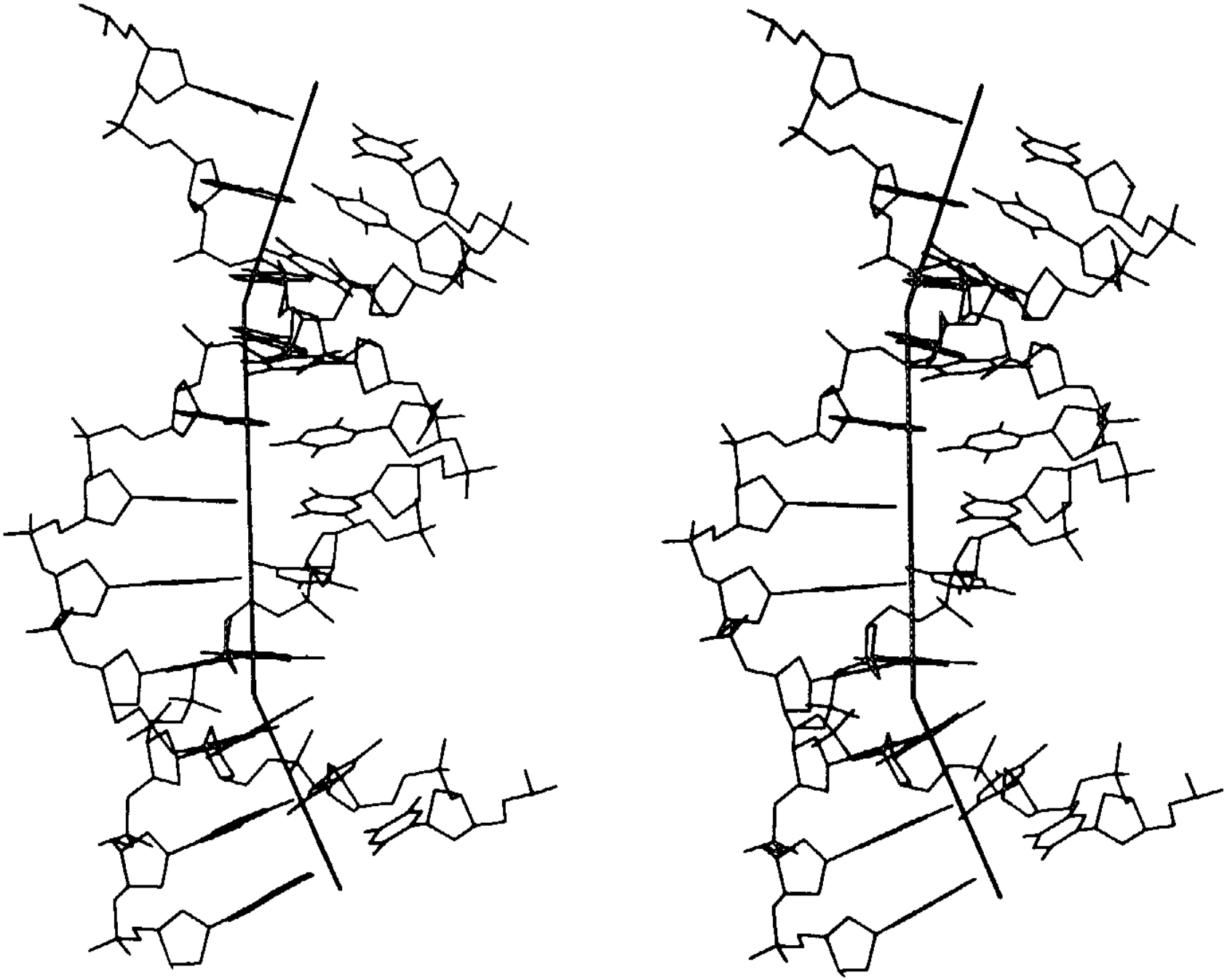
F. Salih et al, 2007, 2008

E. N. Trifonov, 2010

S = G, C W = A, T

```
          |         |         |
. . . S S S W W W W W S S S S S W W W . . .
```

S. Satchwell et al, 1986
H. Chung, M. Vingron, 2009



Ulyanov and Zhurkin, JBSD, 1984

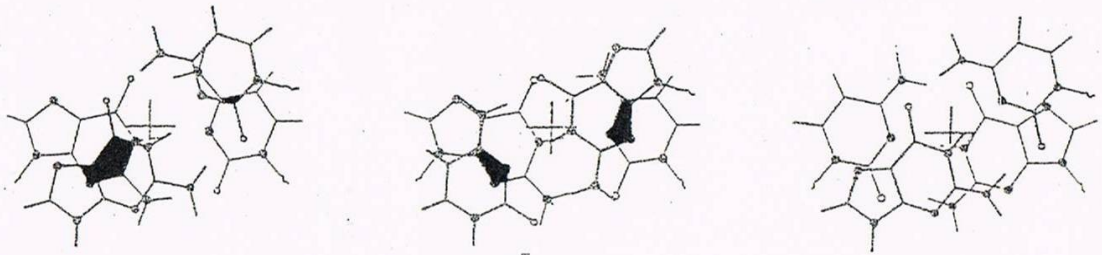
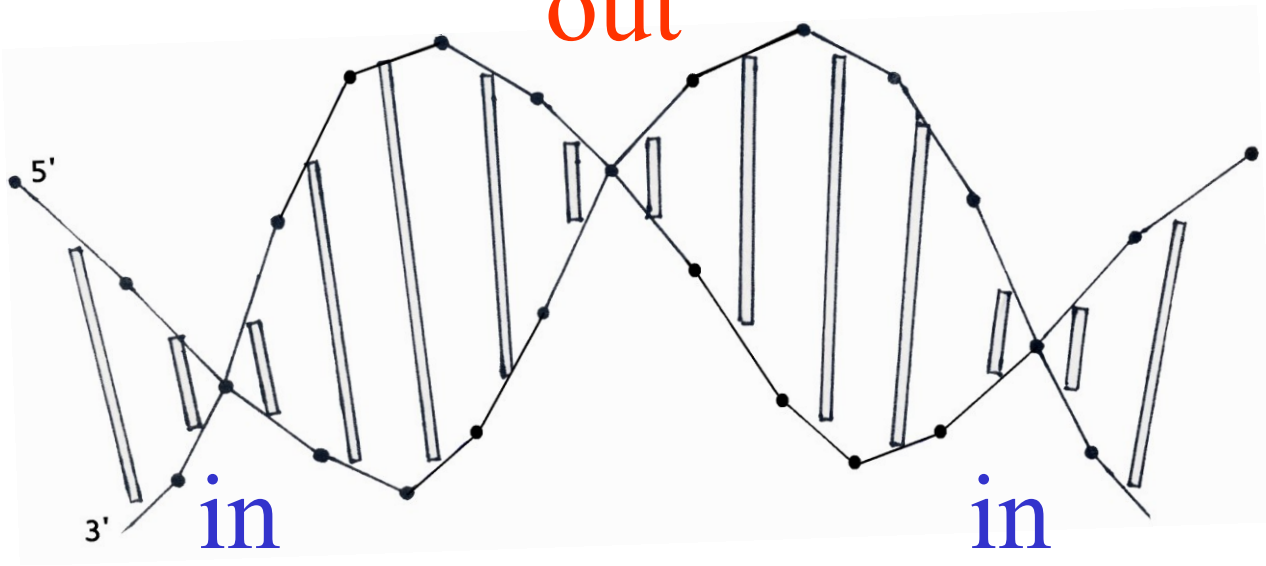


FIGURE 1. Projected views of two successive base-pairs of B DNA. Three possible cases of purine and pyrimidine base overlap are shown. Helix axes (perpendicular to the base-pairs) are indicated by crosses. Overlapping of the heterocyclic rings is shown in black. (From Arnott, S., Dover, S. D., and Wonacott,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

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



Mere physics

SSSS WWWW SSSS ←

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

YR RY YR ←

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

Y RRR YYY R ←

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

CCGGRAATTYCCGG ←

a unique merger
of the binary patterns

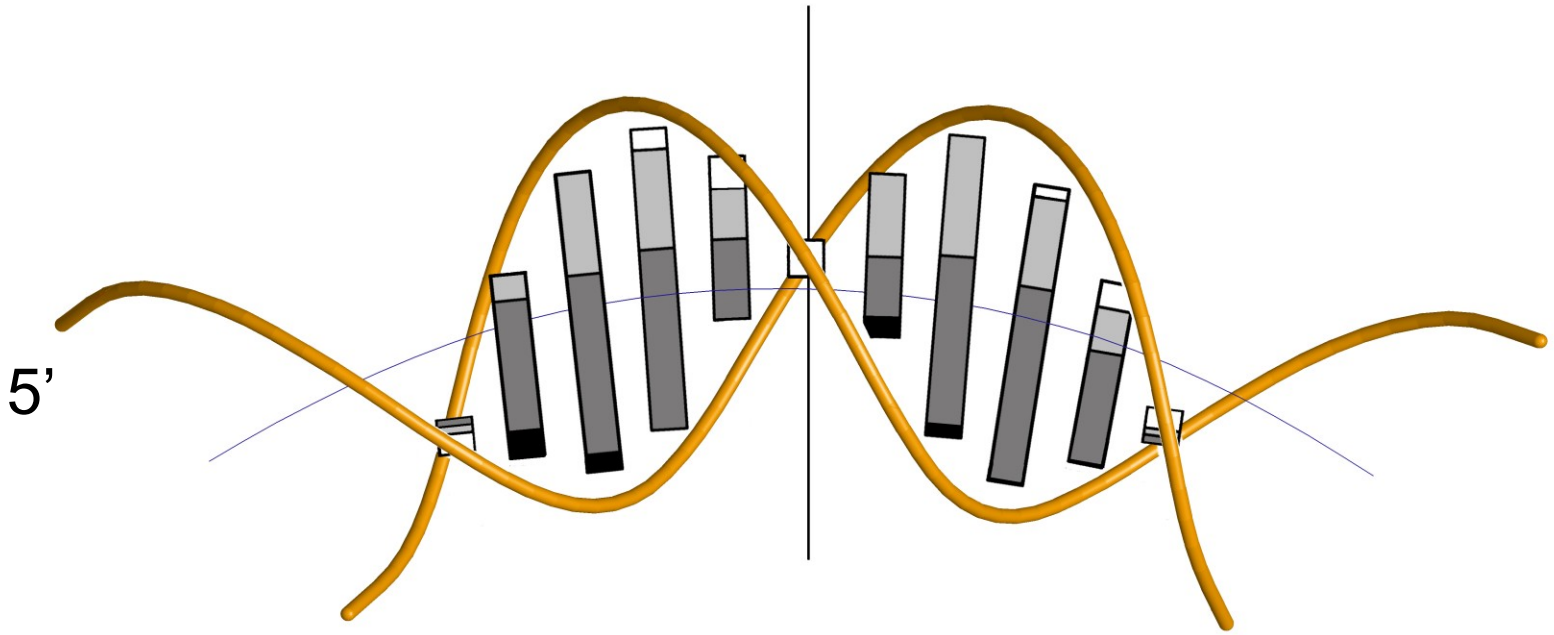
CCGGAAATTTCCGG ←

A+T rich genomes

10.4 base periodical contributions of SS and WW dinucleotides in various species

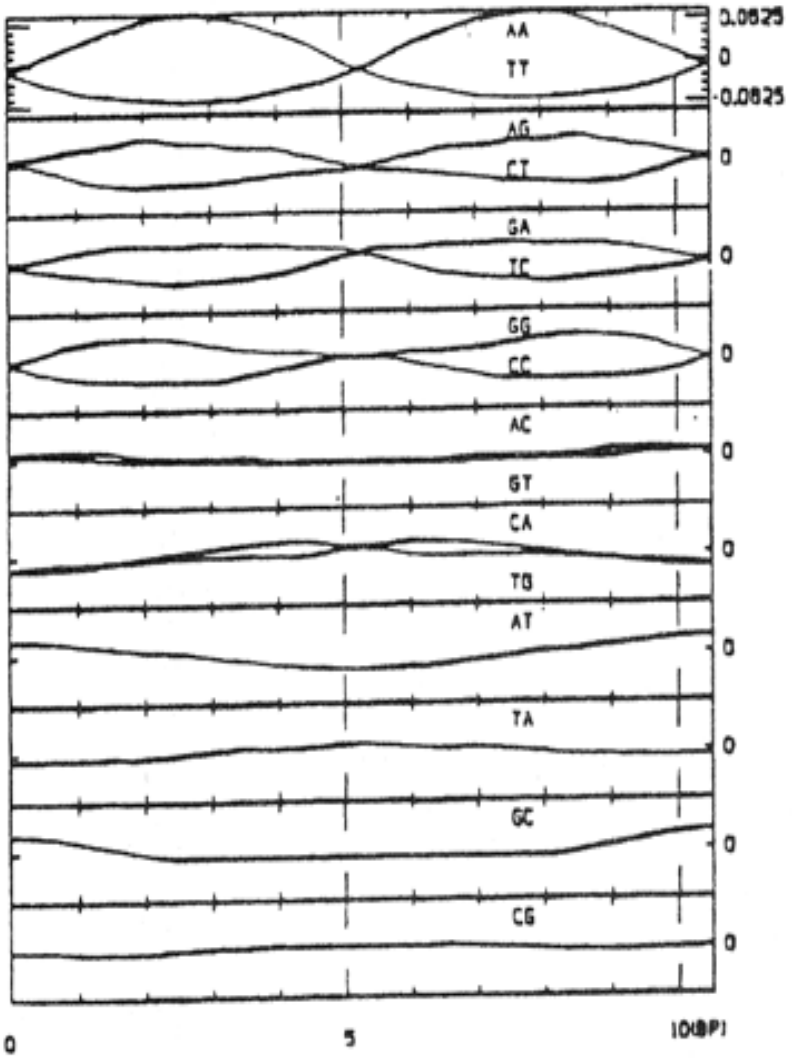
	Human	Mouse	Arabidopsis	C. elegans
SS	0.312	0.286	0.099	~0
WW	~0	0.050	0.092	0.185

S. Kogan, 2005



5’…YYYRRRRRYYYYYYRRR…

5’...RRRYYYYYYRRRRRRYYY...



First matrix of nucleosome DNA bendability

Mengeritsky and ENT, 1983

Sequence analysis: CGRAAATTTYCG

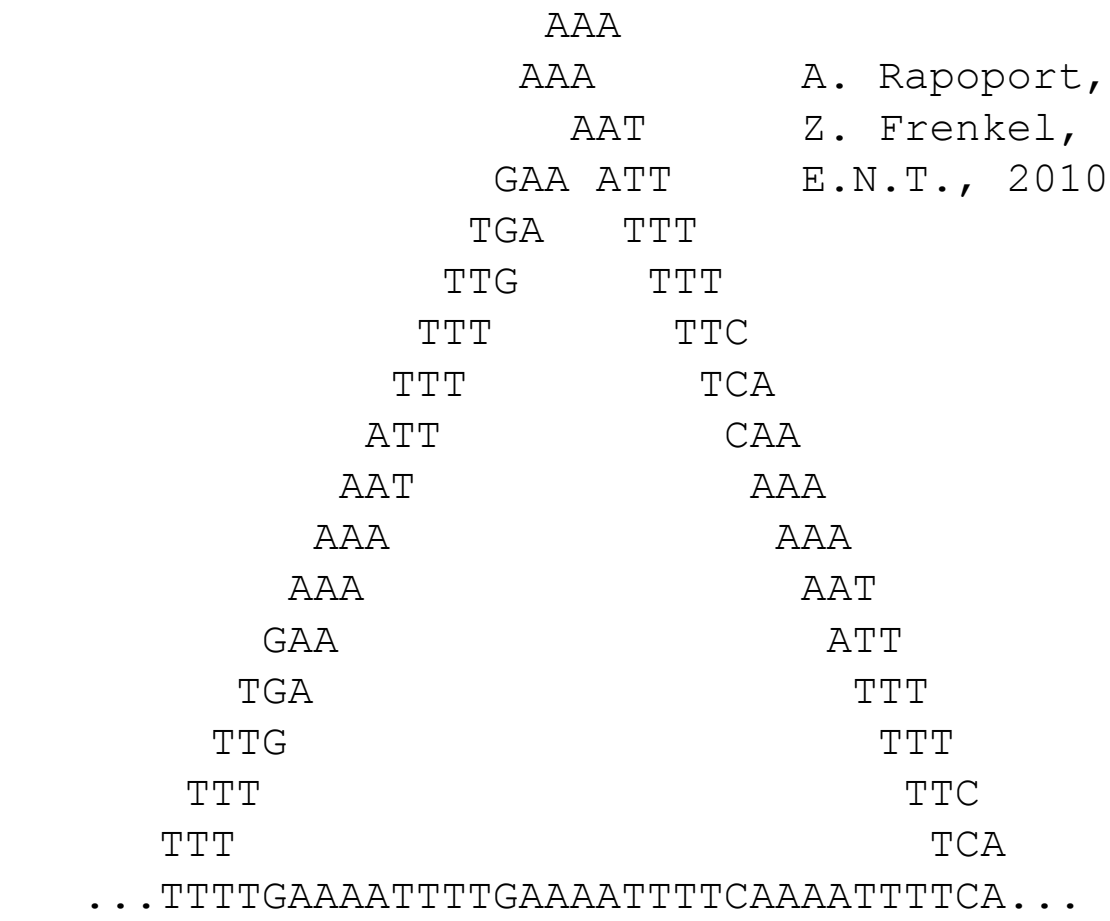
Physics: CGGAAATTTCCG

YRRRRRYYYYYR

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... : TTTtgAAAATTTTcaAAA

...CGA... : TTTcgAAAATTTTcgAAA

regeneration : TTYCGRAAATTTYCGRAA

TOPMOST TRINUCLEOTIDES MAKE TOGETHER THE DOMINANT PATTERN

GAAAATTTTC:

GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC
GAAAATTTTC

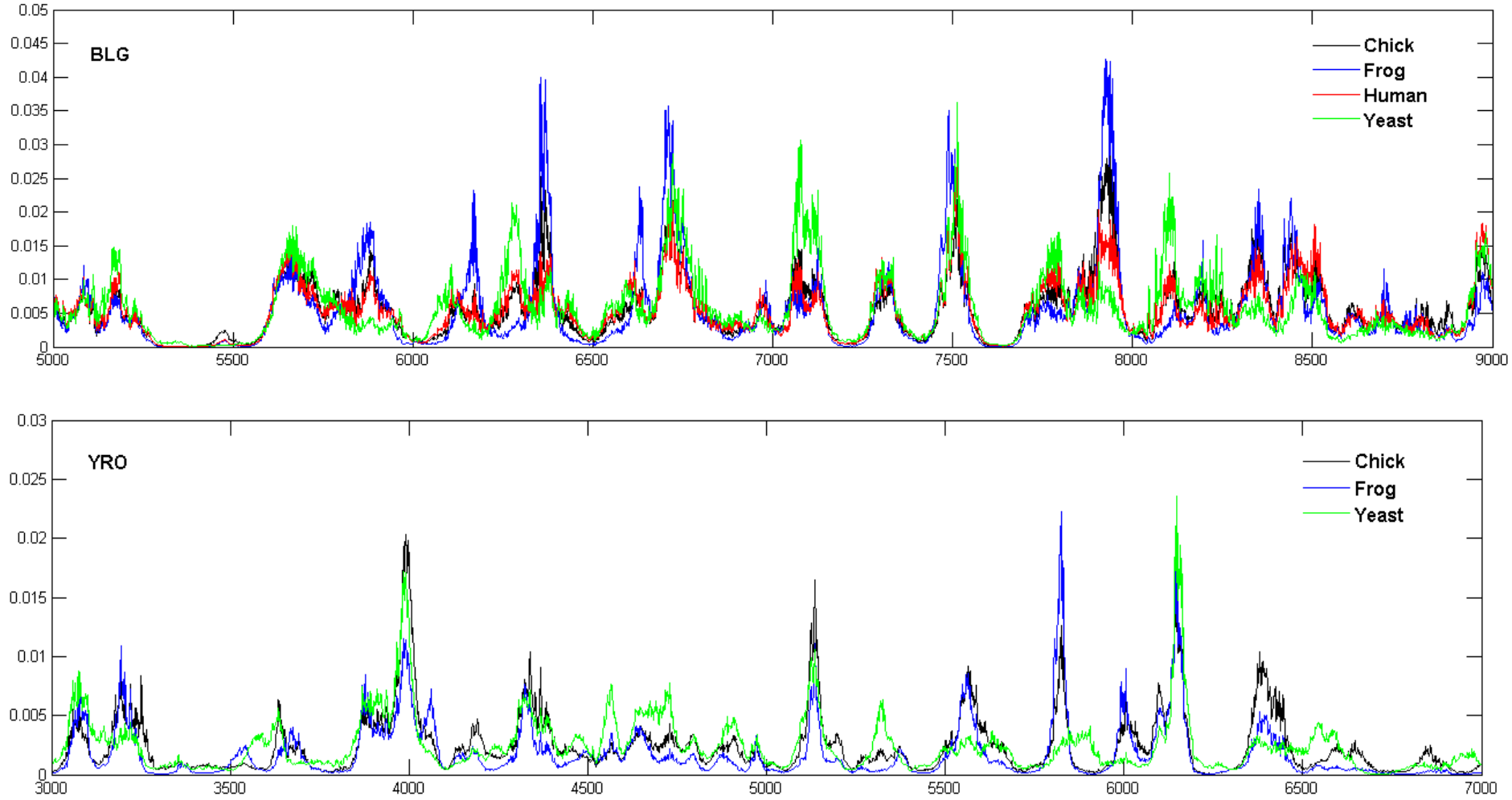
extention motifs	species	starting triplets
C AAAAA TTTTT G	A.gamb	TTT
T AAAAA TTTTT A	A.mell	TTT
AAAAA TTTTT	A.thali	AAA
TTTTC AAAAA TTTTT GAAAA	C.albic	AAA
GAAAA TTTTC	C.eleg	AAA
GG CC	C.reinh	GGC
AAAAA TTTTT	D.disc	AAA
C AAAAA TTTTT G	D.melan	AAA
AAAAA TTTTT	D.rerio	AAA
C AGAAA TTTCT G	G.gall	TTT
AAAAA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



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

It is derived by 3 independent methods:

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

TA/GC pattern (Segal/Widom, 2006)

at 5 bases distance

The pattern **TA/GC** is derived from SELEX experiments (*artificial sequences*)

CG/AT pattern is derived from *natural ones* (nematode, confirmed in other eukaryotes)

TA*TA stack is of the lowest stacking energy.
In symmetrical groove positions it would readily kink.
That would create mutational hot spot.

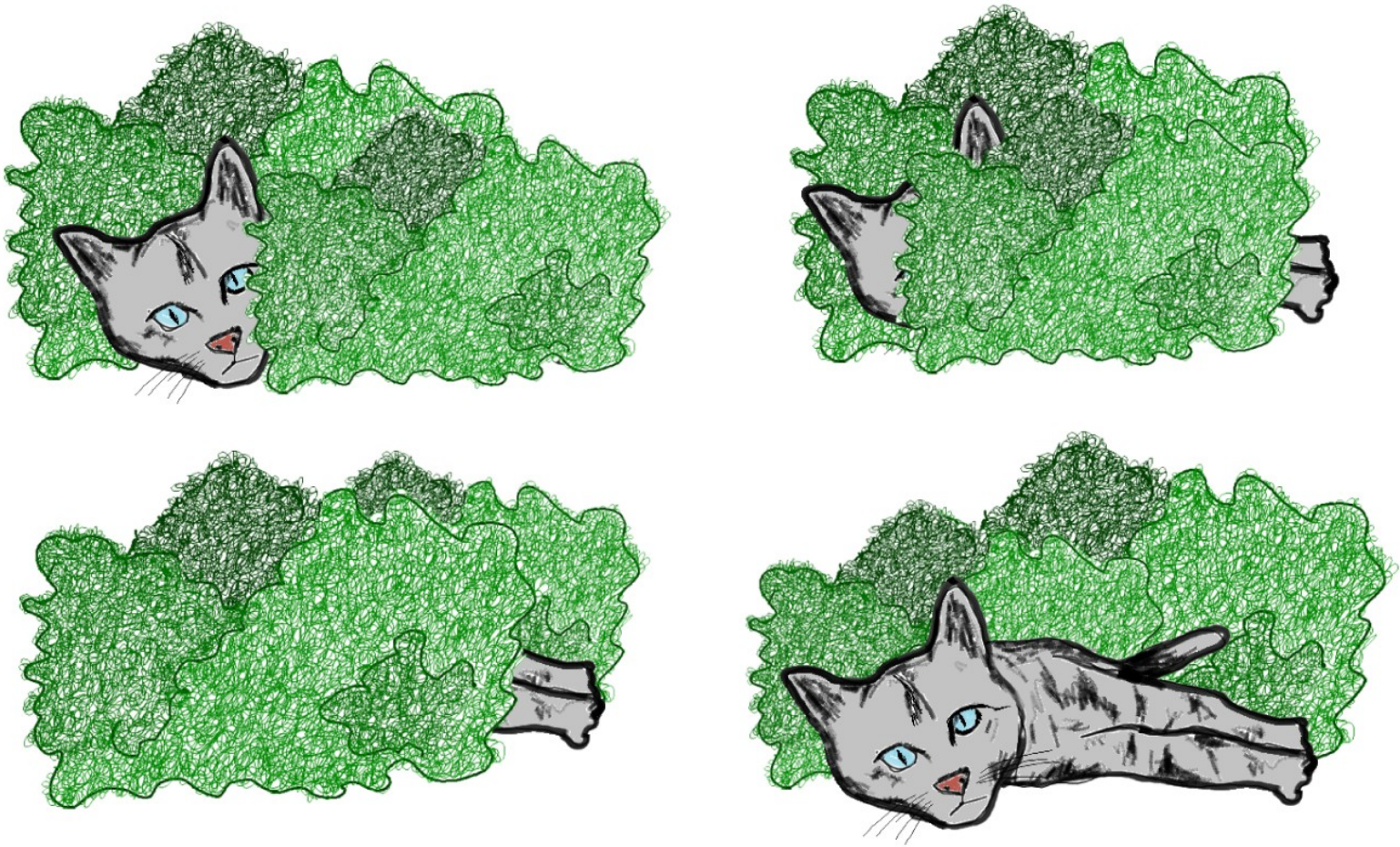
The hidden chromatin code is described by the motif:

CGRAAATTTYCG

o o o

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

CGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCGRAAATTTYCG



Cat in bushes. Courtesy of I. Gabdank

...TTTCCGGAAATTTCCGGAAA...

...ATTCGTTCCATTGAAGGCCG...

...CGAACGCTTGGTTAGCGATT...

...CCAGAATAAATACAGTCCAA...

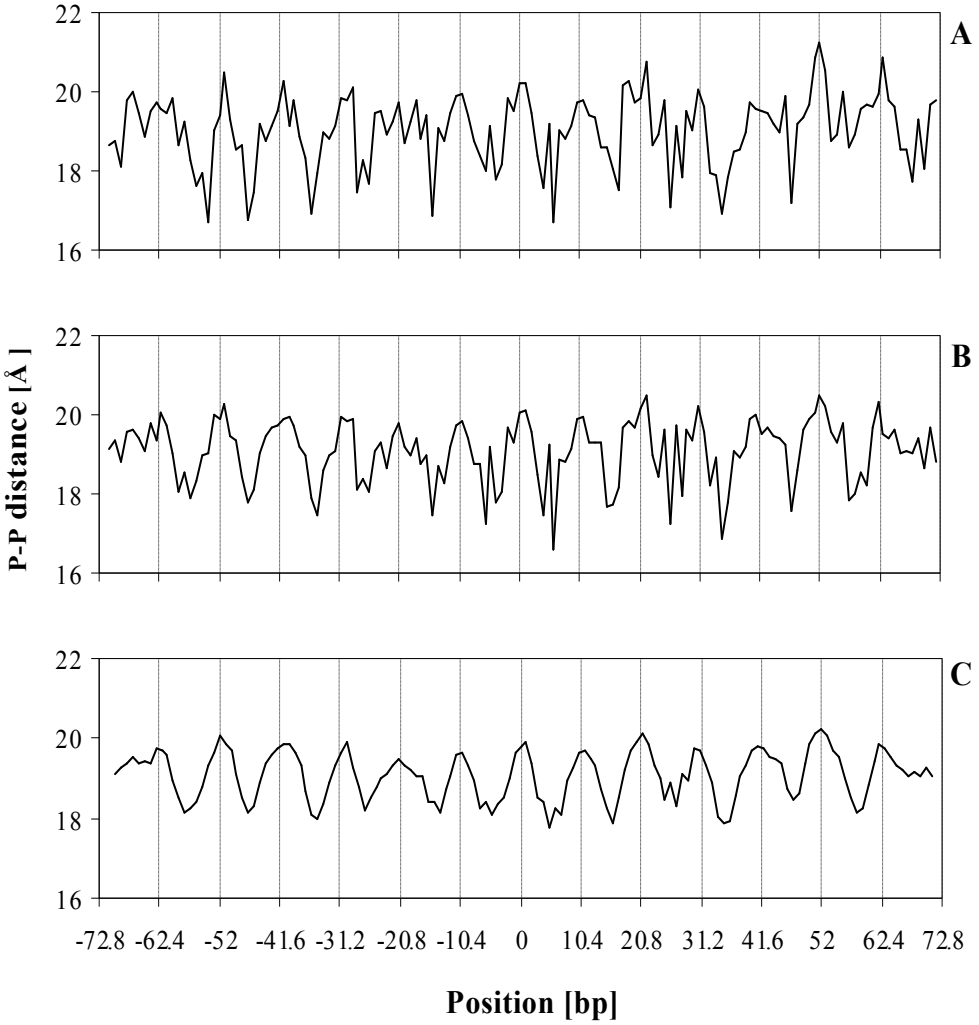
...AATCGCCTTTAAAGGGGTTT...

...GAGTTCGACTCCAATCAGGG...

...CGGTACCCTCAGACCCATTC...

...CATCTATTCCAAATTTTCGC...

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

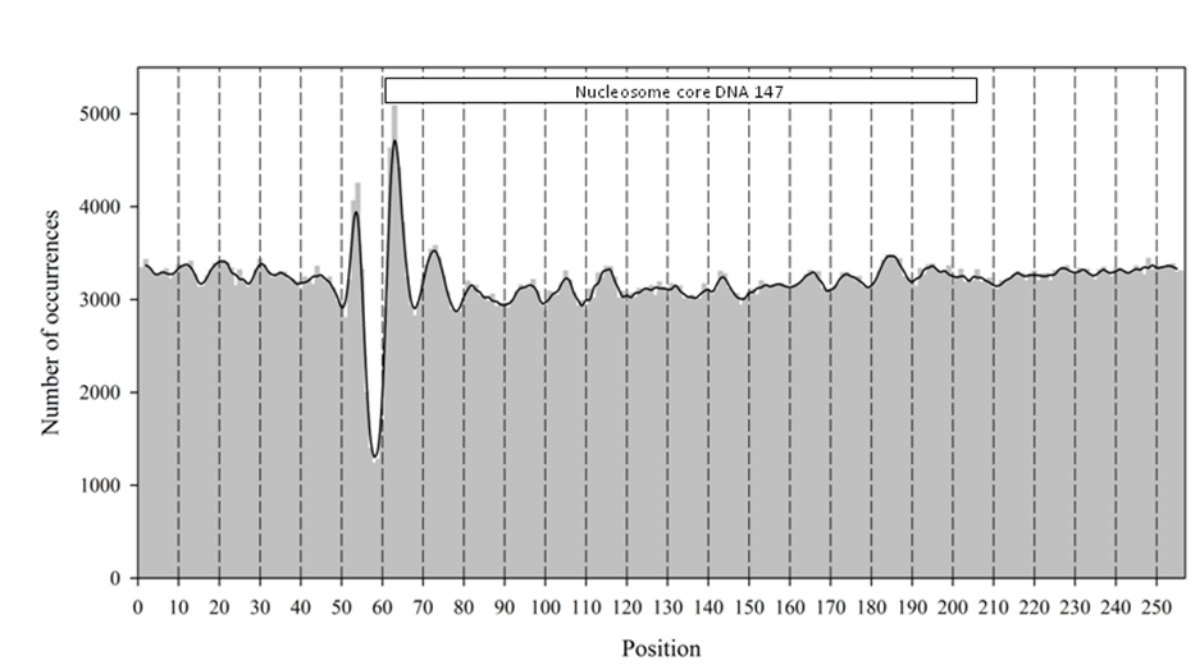
Same,
smoothed

There are 12 contact sites of the minor grooves with the histones – 12 positions for CG.

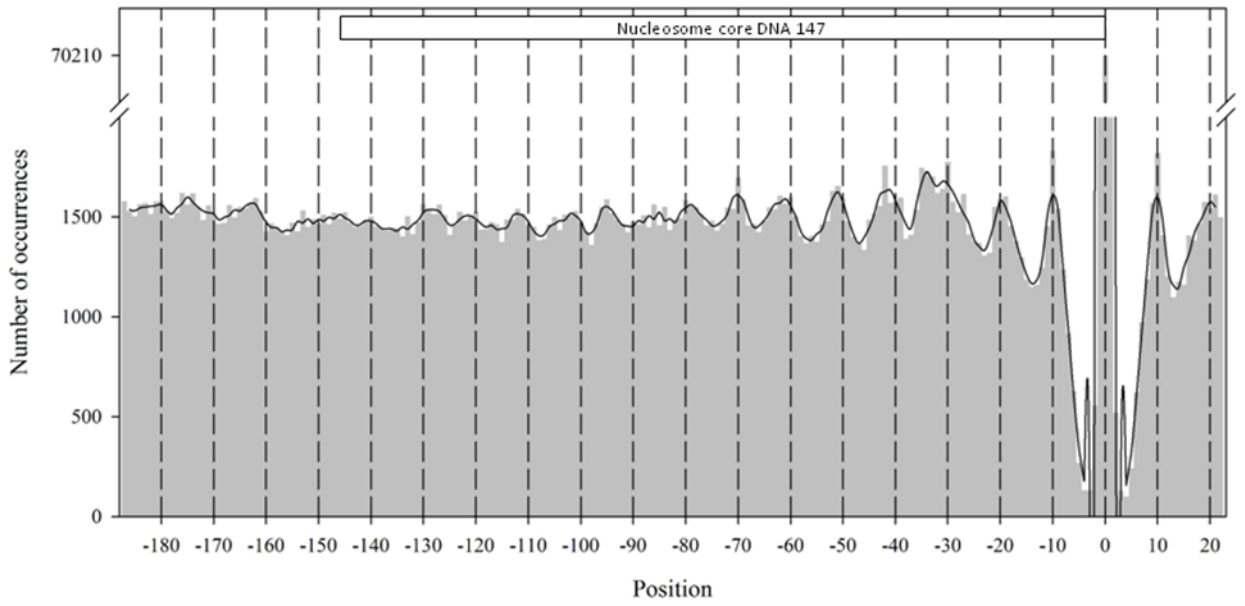
Total length of the DNA in contact with histone octamers is 10.4x11+1 = 115 bp

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

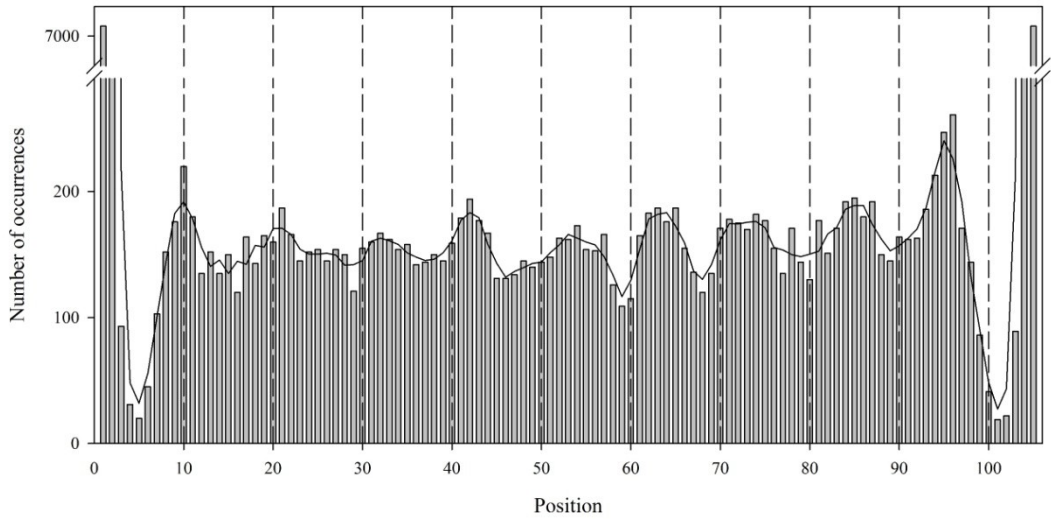
Alignment of nucleosome DNA sequences (C.elegans) by left ends

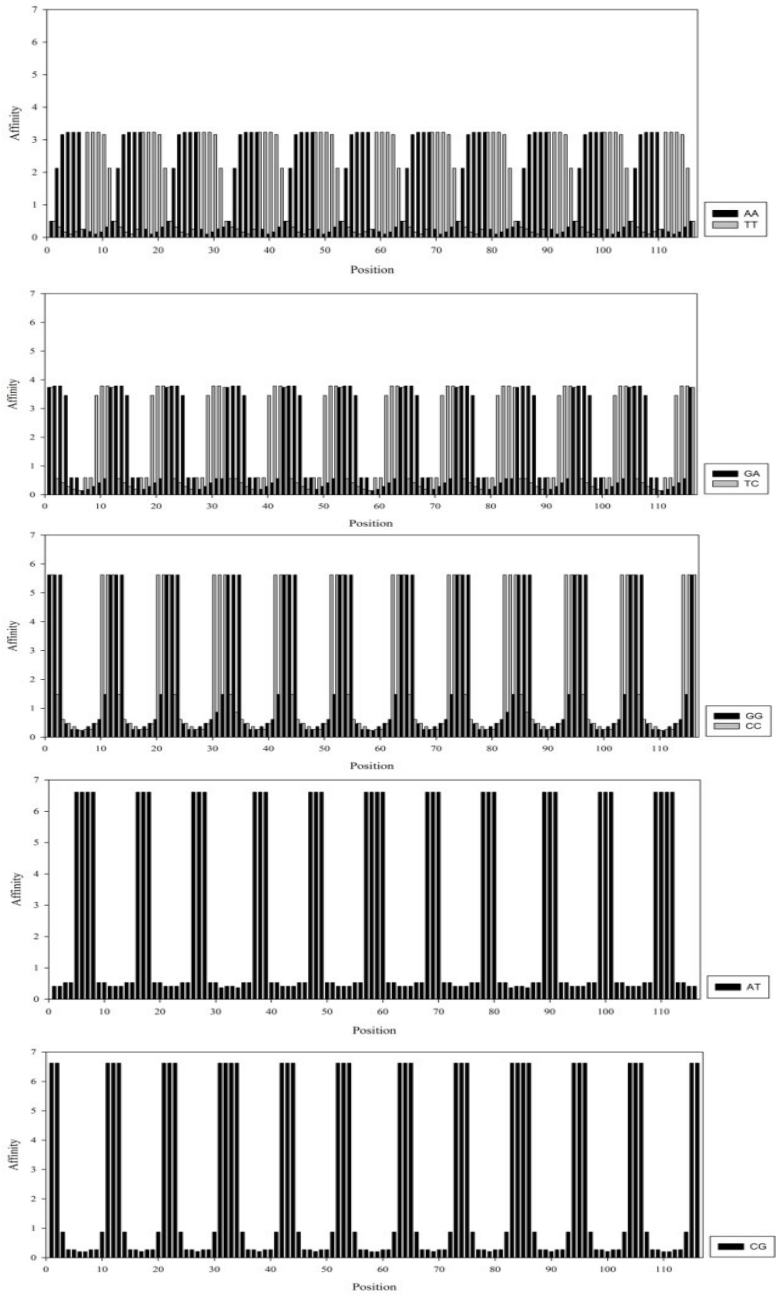


Alignment by right ends

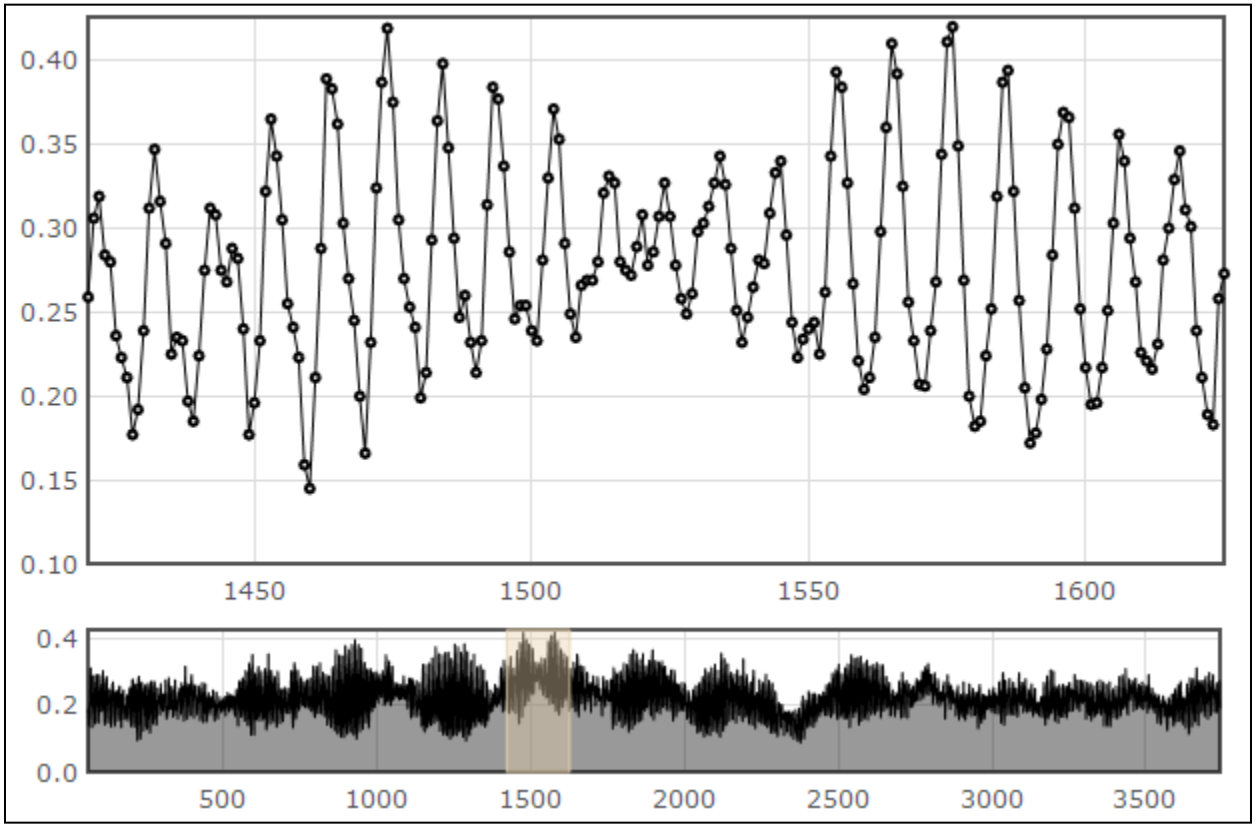


Periodicity all along



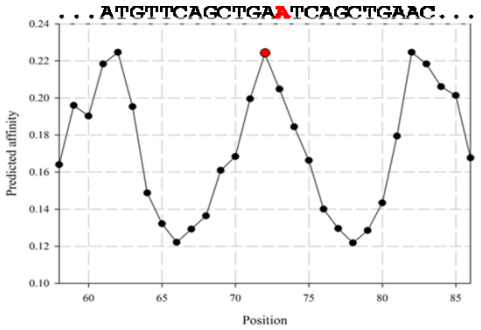


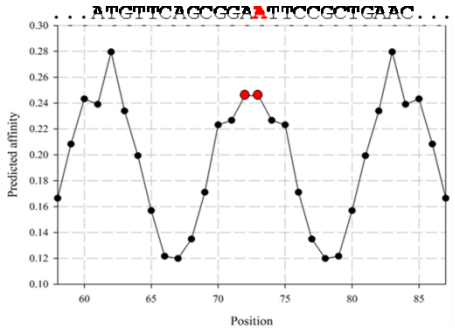
Full length (11 periods) matrix of bendability – nucleosome probe

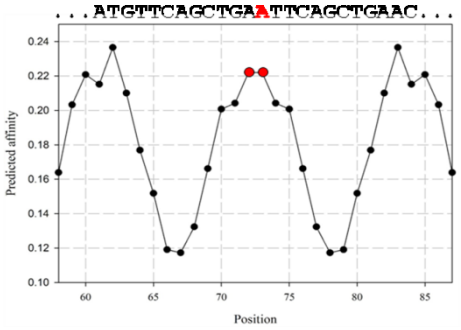


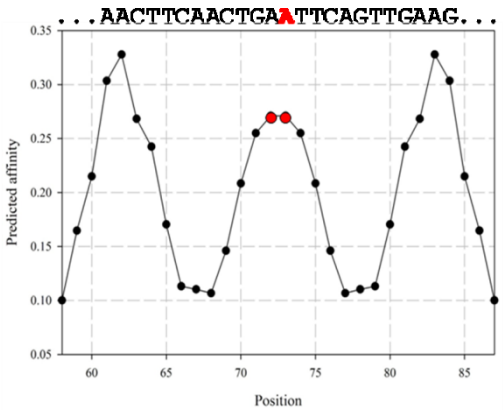
Example of the output from the nucleosome mapping server http://www.cs.bgu.ac.il/~nucleom

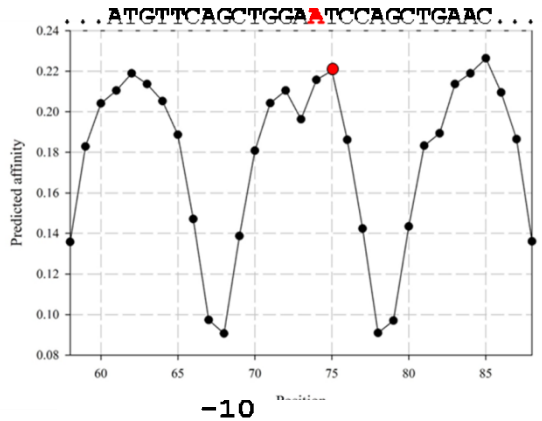
Examples of mapping of sharply positioned nucleosomes

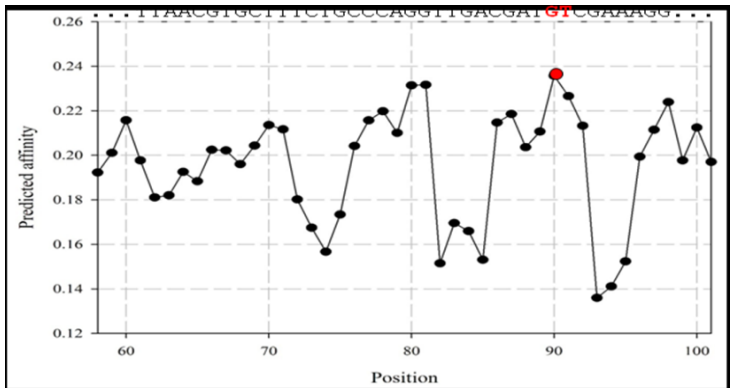


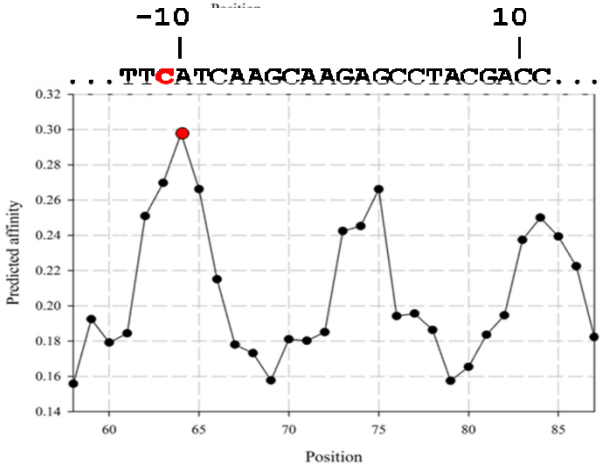


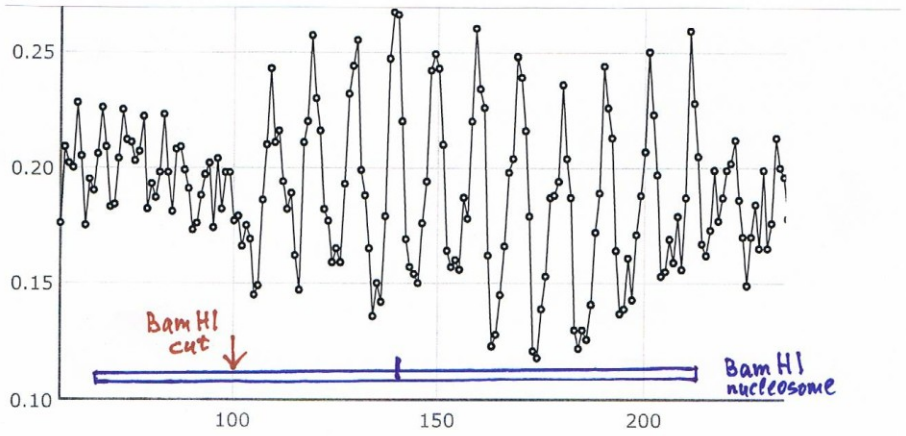












BamHI nucleosome of Ponder and Crawford, 1977

BamHI fragments of BamHI nucleosome DNA

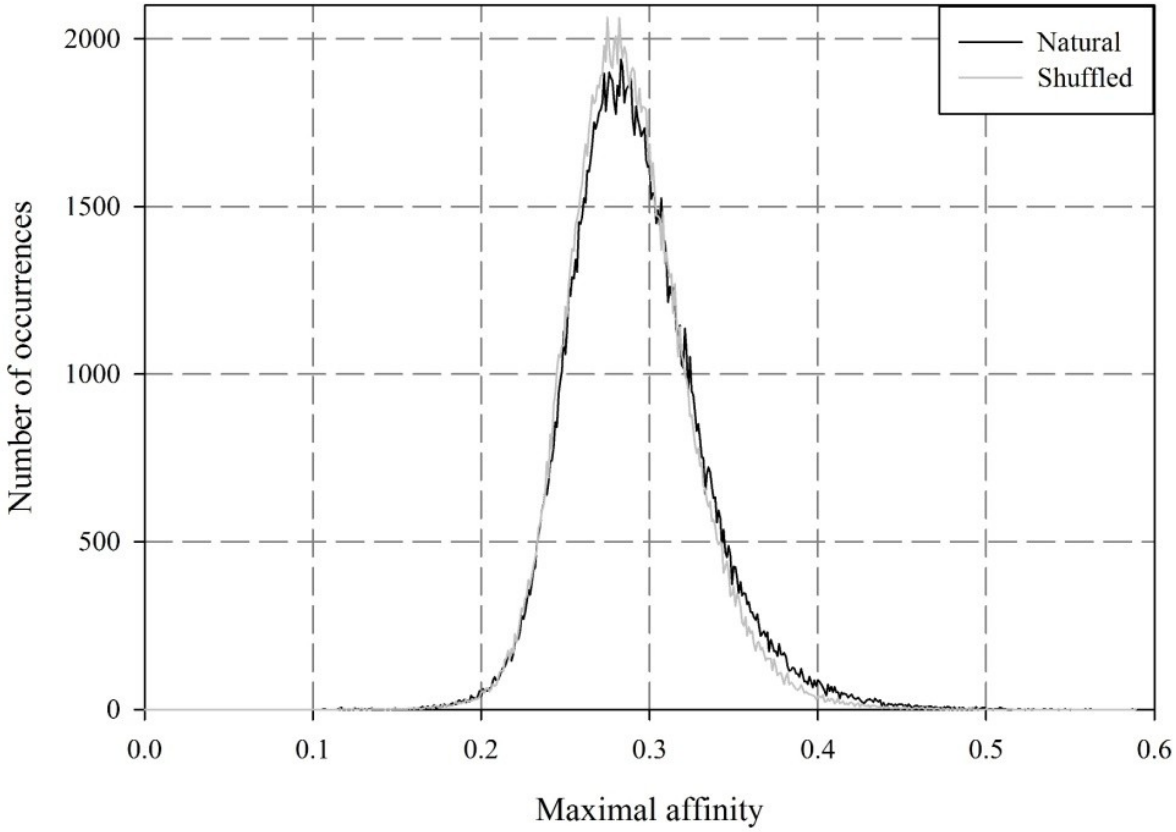
Calculated	Observable in the gel	
24		
34		
43		
54	~53 |	
64	~63 |	misfit
	(~73) |	1 base
82	~83 |	
92	~93 |	
103		
112		
122		

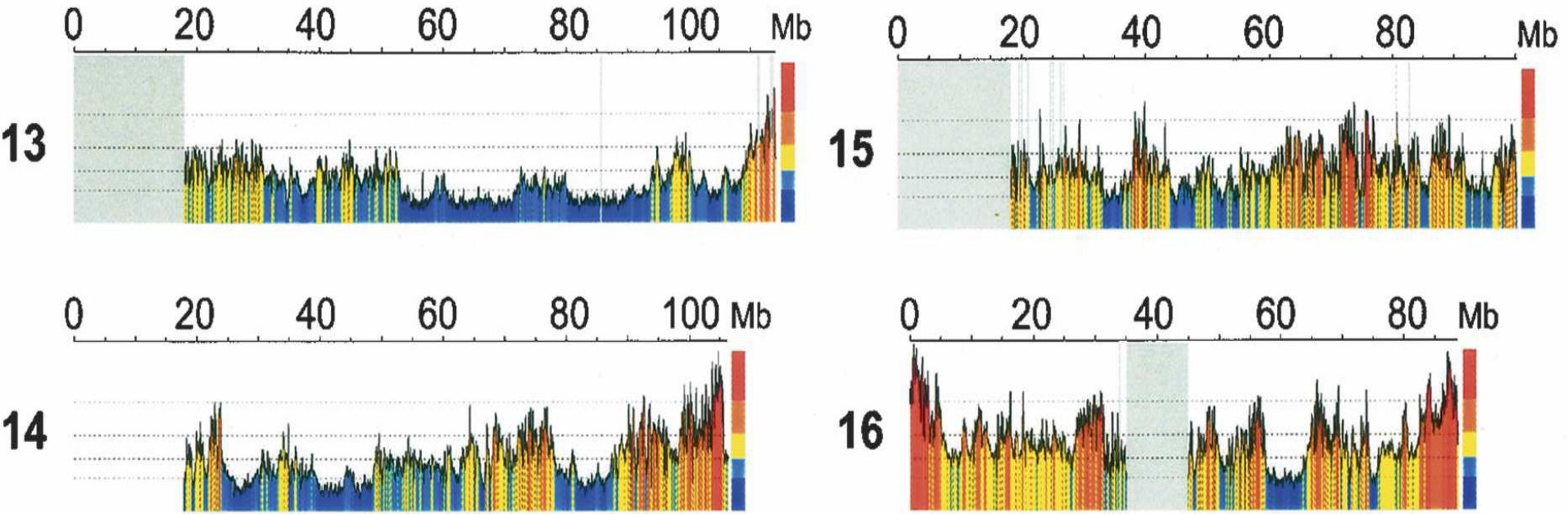
Match of the BamHI nucleosome to the standard nucleosome probe $(GAAAATTTTC)_n$

CGGAAATTTTCCGGAAATTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTCCGGAAATTTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTCCGGAAATTTTCC
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

Natural nucleosome sequence periodicity is only slightly higher than in random sequences.

Match to simple periodical probe:





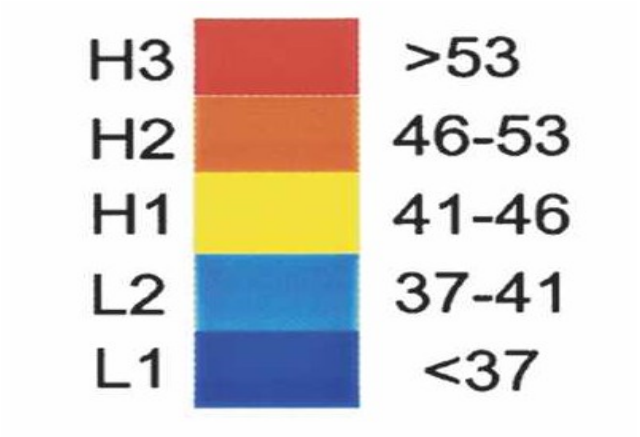
Human isochores

Lab of G. Bernardi, 2006

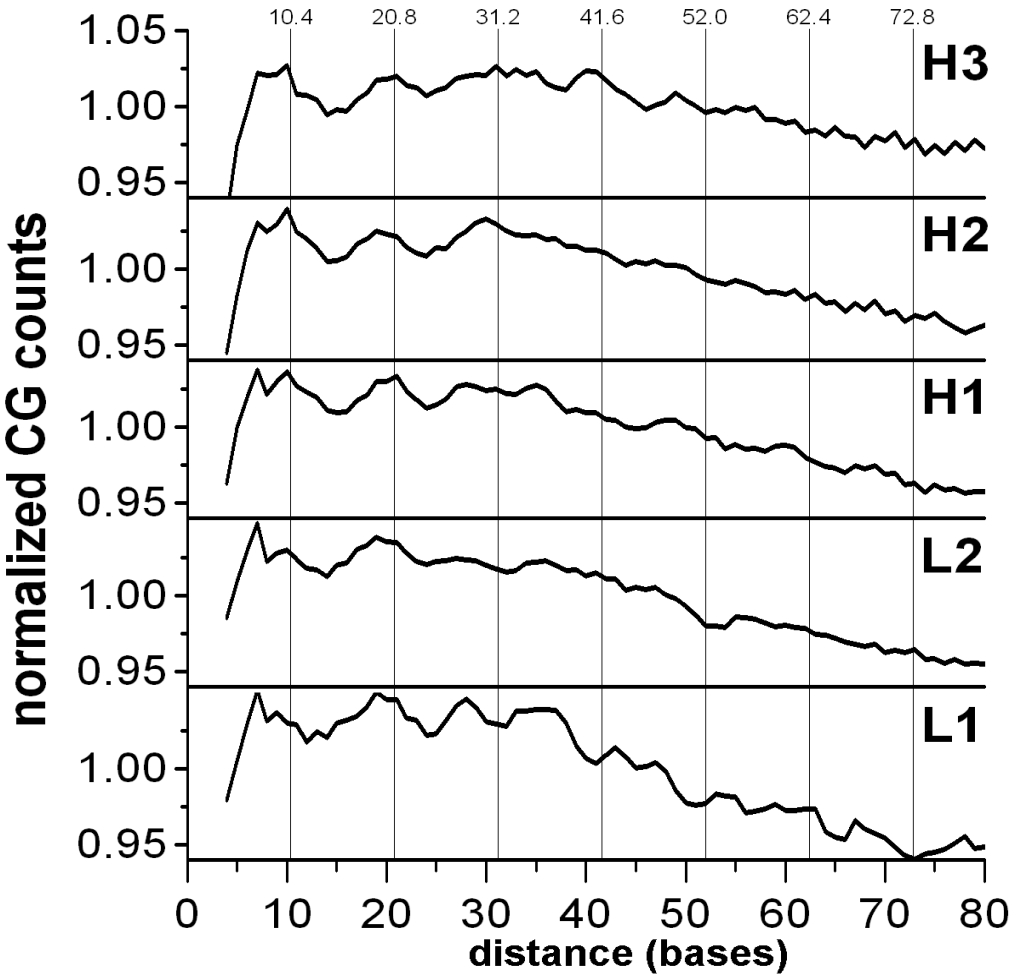
Nucleosome positioning patterns
of various isochores (Frenkel et al., 2011)
by N-gram extension

isochores G+C %

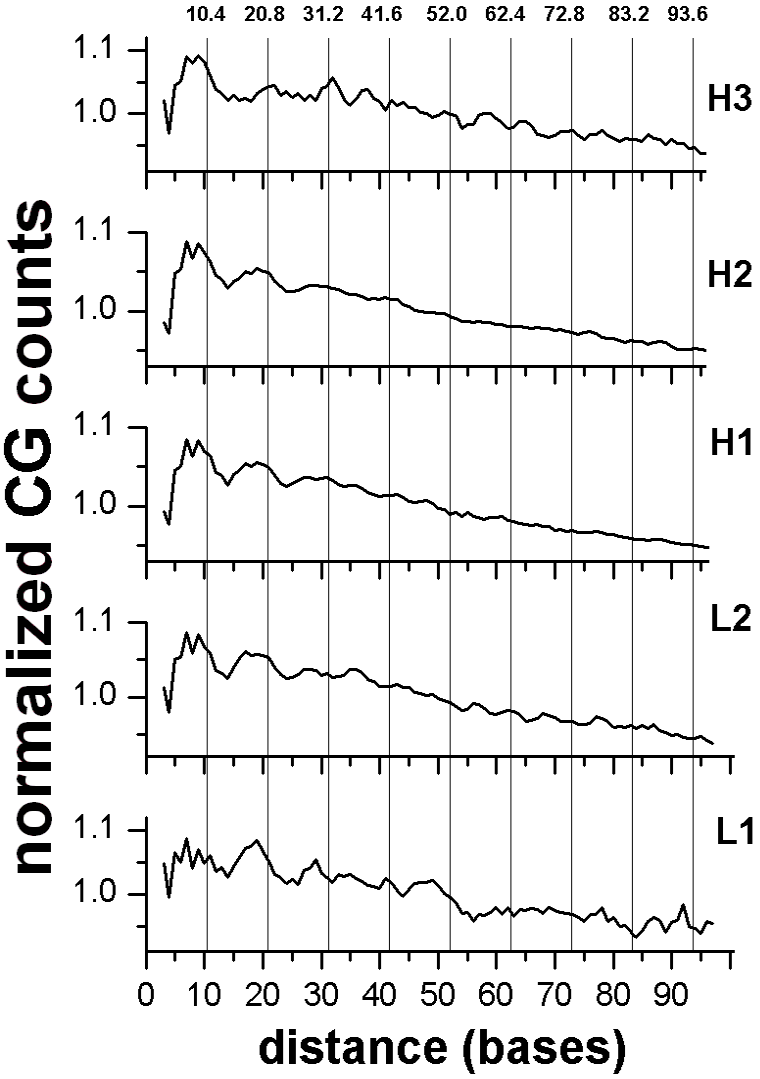
```
C AGGGG CCCCT G
C GGGGA TCCCC G
C AGAAA TTTCT G
T AAAAA TTTTT A
T AAAAA TTTTT A

Y RRRRR YYYYY R
```



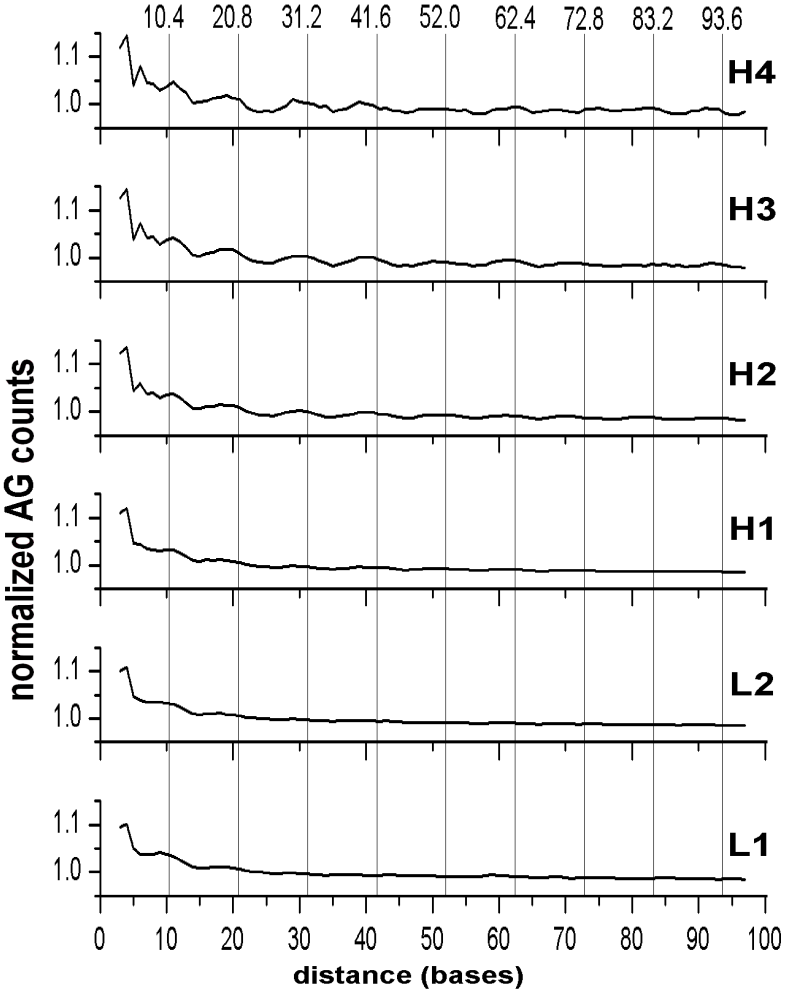

R Y Y Y Y Y R R R R R Y Y Y Y Y R R R R R Y
A|T T T T T|A A A A A|T T T T T|A A A A A|T
T|G T|G
A|T T T T | A A A A|T T T T | A A A A|T
C|A C|A
A|T T T T C|G A A A A|T T T T C|G A A A A|T
A|T T T T C C|G G A A A|T T T T C C|G G A A A|T
A|T T T C C C|G G G A A|T T T C C C|G G G A A|T
A|T C C C C C|G G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


10.4
20.8
31.2
41.6
52.0
62.4
72.8
H3
H2
H1
L2
L1
normalized CG counts
1.05
1.00
0.95
0
10
20
30
40
50
60
70
80
distance (bases)

human


10.4
20.8
31.2
41.6
52.0
62.4
72.8
83.2
93.6
H3
H2
H1
L2
L1
1.1
1.0
normalized CG counts
0
10
20
30
40
50
60
70
80
90
distance (bases)

mouse


10.4
20.8
31.2
41.6
52.0
62.4
72.8
83.2
93.6
H4
H3
H2
H1
L2
L1
1.1
1.0
normalized AG counts
0
10
20
30
40
50
60
70
80
90
100
distance (bases)

chicken

extention motifs	isochores	starting triplets
AAAAA TTTTT	L1	TTT (top)
AAAAA TTTTT	L2	TTT (top)
C AGAAA TTTCT G	H1	TTT (top)
C AGAAA TTTCC GGAAA TTTCT G	H1	CGG
TCCCC AGGGG	H2	CAG (top)
CCCCT GGGGA	H2	CTG (top)
TCCCC GGGGA	H2	CCG
AGGGG CCCCT	H3	GGG (top)
AGGGG CCCCC GGGGG CCCCT	H3	CGG

Y RRRRR YYYYY RRRRR YYYYY R human

extention motifs				isochores	starting triplets (top)
AAAAA	TTTTT			L1	TTT
AAAAA	TTTTT			L2	AAA
	TTTCT	G		H1	TTT
		C	AGAAA	H1	AAA
	TCCCC	AGGGG		H2	CAG
	CCCCT	GGGGA		H2	CTG
AGGGG	CCCCT	GGGGG	CCCCC	H3	CTG
GGGGG	CCCCC	AGGGG	CCCCT	H3	CAG

RRRRR YYYYY RRRRR YYYYY

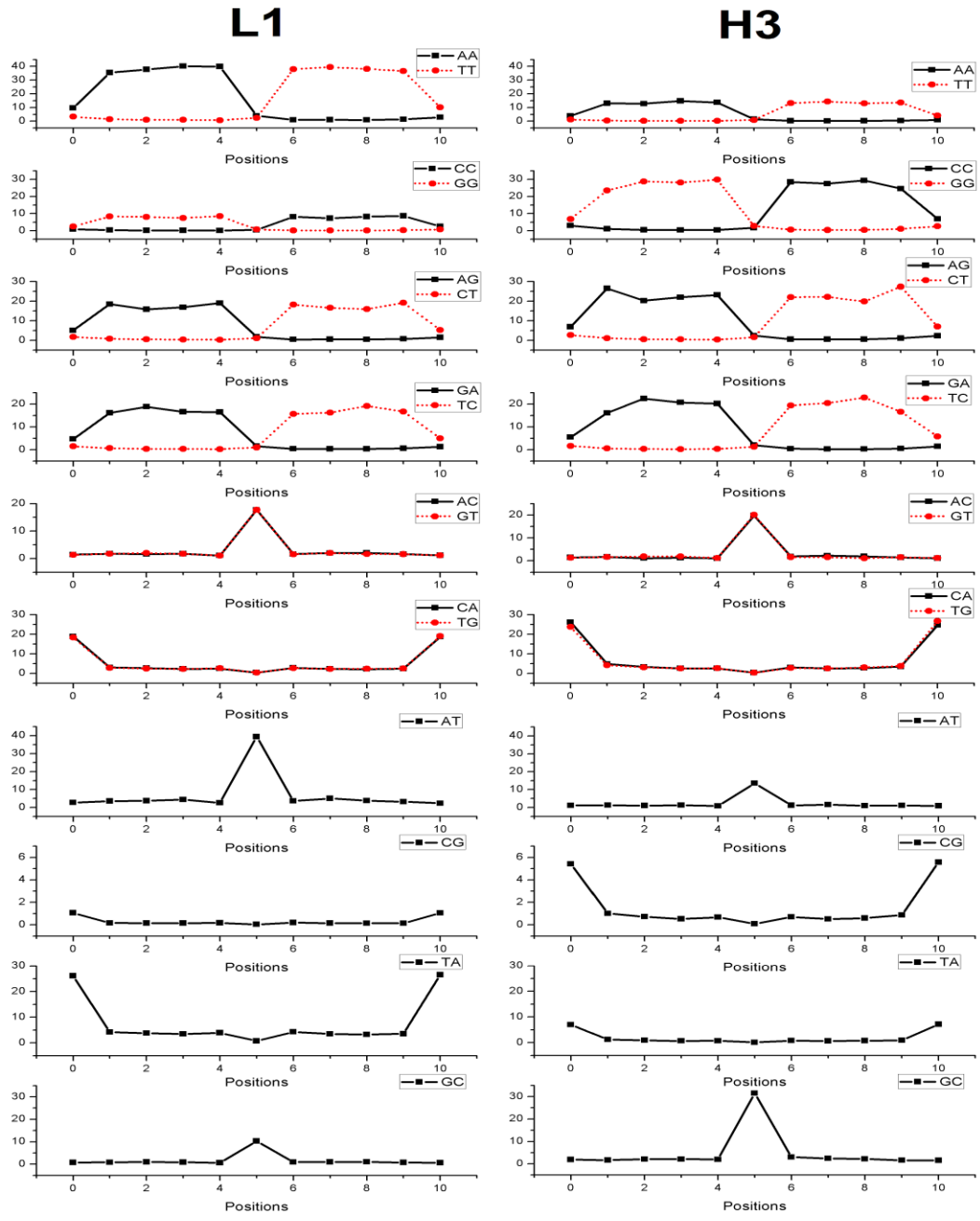
mouse

extention motifs	isochores	starting triplets
AAAAA TTTTT	L1	AAA (top)
GAAAA TTTTC	L2	TTT (top)
TTTCT G	H1	TTT (top)
C AGAAA	H1	AAA (top)
G CTCCC GGGAG C	H2	CCG
G CTCCC GGGAG C	H3	CCG
TG CCCCC GGGGG CA	H4	CCG

Y RRRRR YYYYY RRRRR Y chicken

```
human      AAAAA TTTTT
mouse      AAAAA TTTTT                L1
chicken    AAAAA TTTTT

human      AAAAA TTTTT
mouse      AAAAA TTTTT                L2
chicken    GAAAA TTTTC

human    C AGAAA TTTCT G              H1
mouse             TTTCT G
         C AGAAA
chicken           TTTCT G
         C AGAAA

human             TCCCC AGGGG
                  CCCCT GGGGA
mouse             TCCCC AGGGG
                  CCCCT GGGGA
chicken         G CTCCC GGGAG C
Consensus         YCCCY RGGGR          H2

human      AGGGG CCCCT
mouse      AGGGG CCCCT GGGGG CCCCC
           GGGGG CCCCC AGGGG CCCCT
chicken        G CTCCC GGGAG C
Consensus  RGGGG CCCCY RGGGG CCCCY    H3

chicken       TG CCCCC GGGGG CA       H4


         Y RRRRR YYYYY RRRRR YYYYY
```


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
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 T C C|G G A A A|T T T T C C|G G A A A|T
A|T T T C C C|G G G A A|T T T C C C|G G G A A|T
A|T C C C C C|G G G G A|T C C C C C|G G G G A|T
A|C A|C A|C
C C C C C|G G G G C C C C C|G G G G
G|T G|T G|T
G|C C C C C C|G G G G G|C C C C C C|G G G G G|C
isochores L1
most frequent patterns
isochores H3



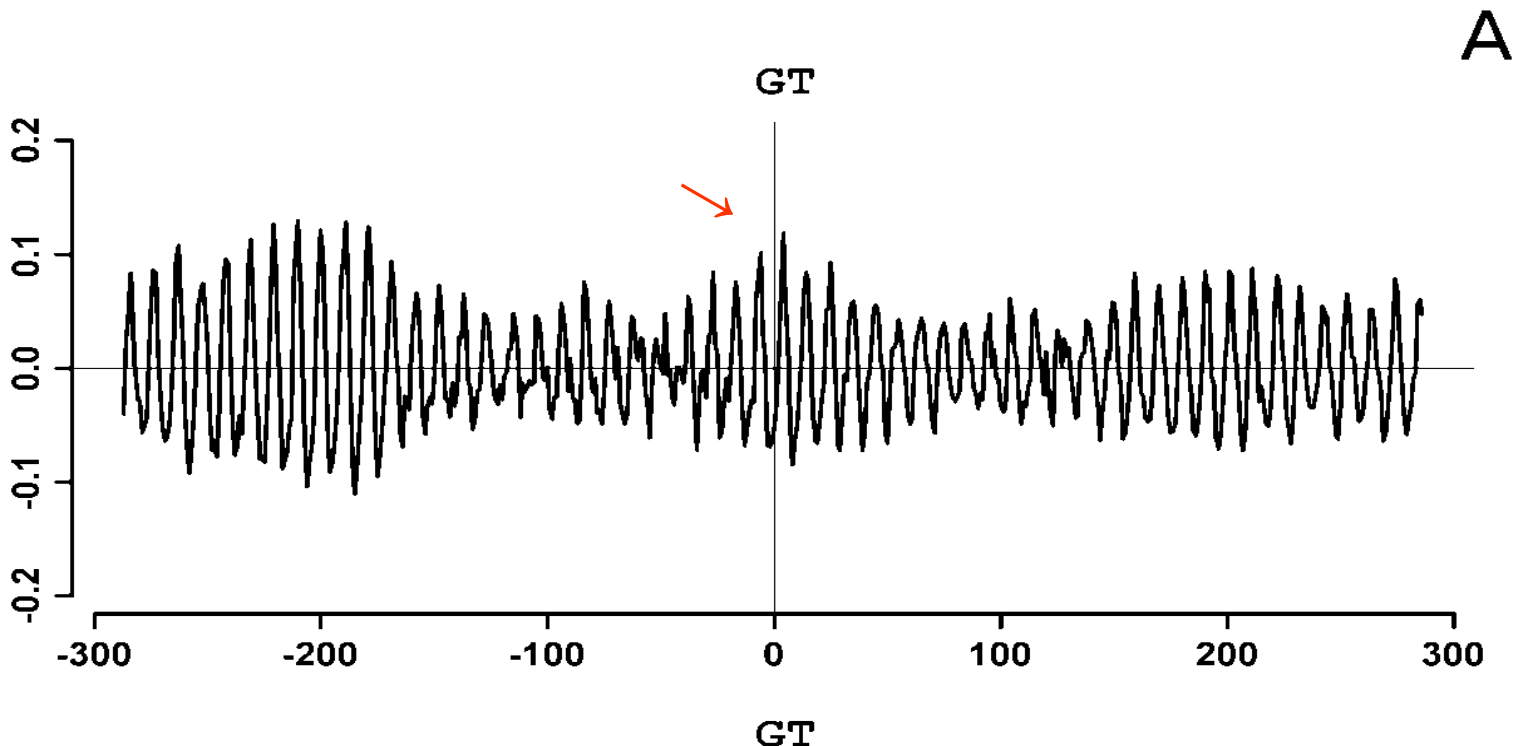
Nucleosome positioning patterns for human isochores L1 and H3 derived by signal regeneration from apoptotic nucleosomes:

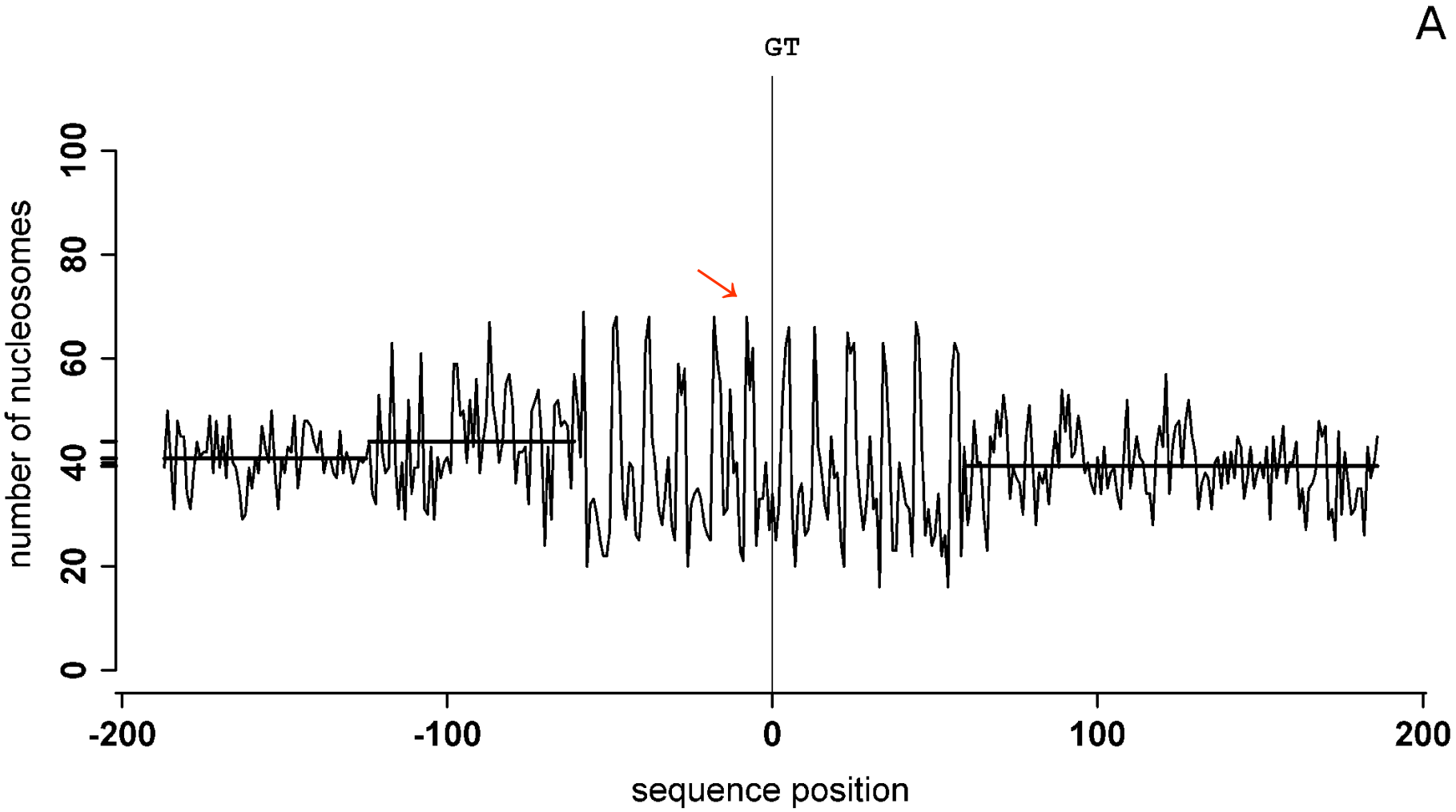
```
L1:   T AAAAA TTTTT A
H3:   C AGGGG CCCCT G
```

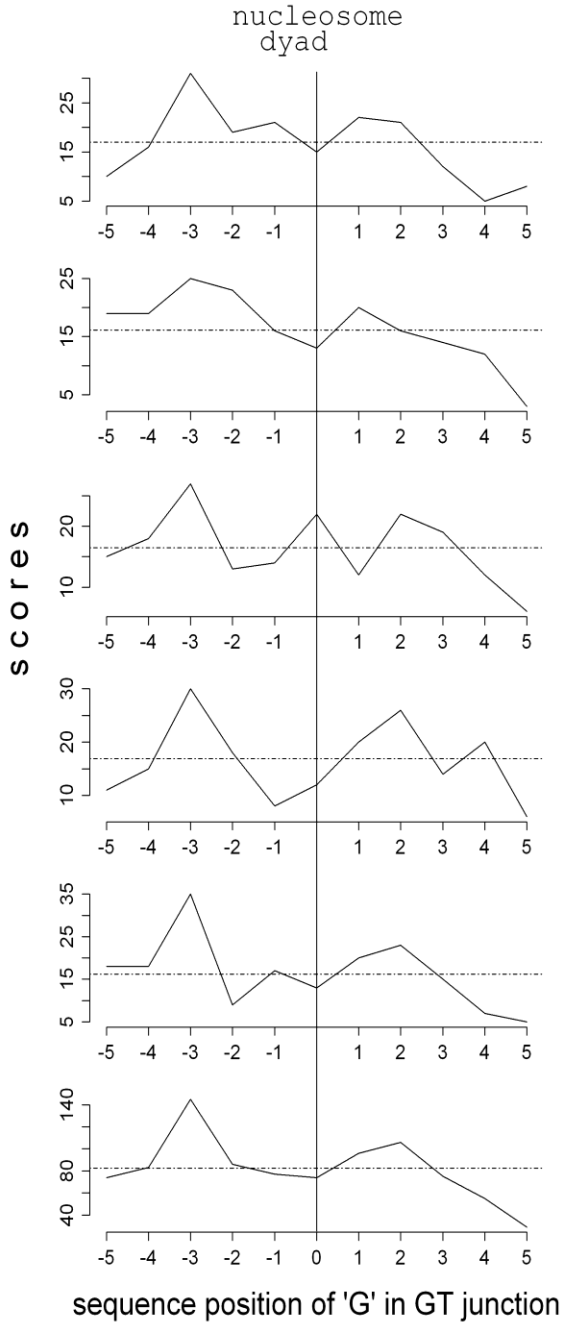
Frenkel et al., 2011

Example of the nucleosomes at and around GT splice junction

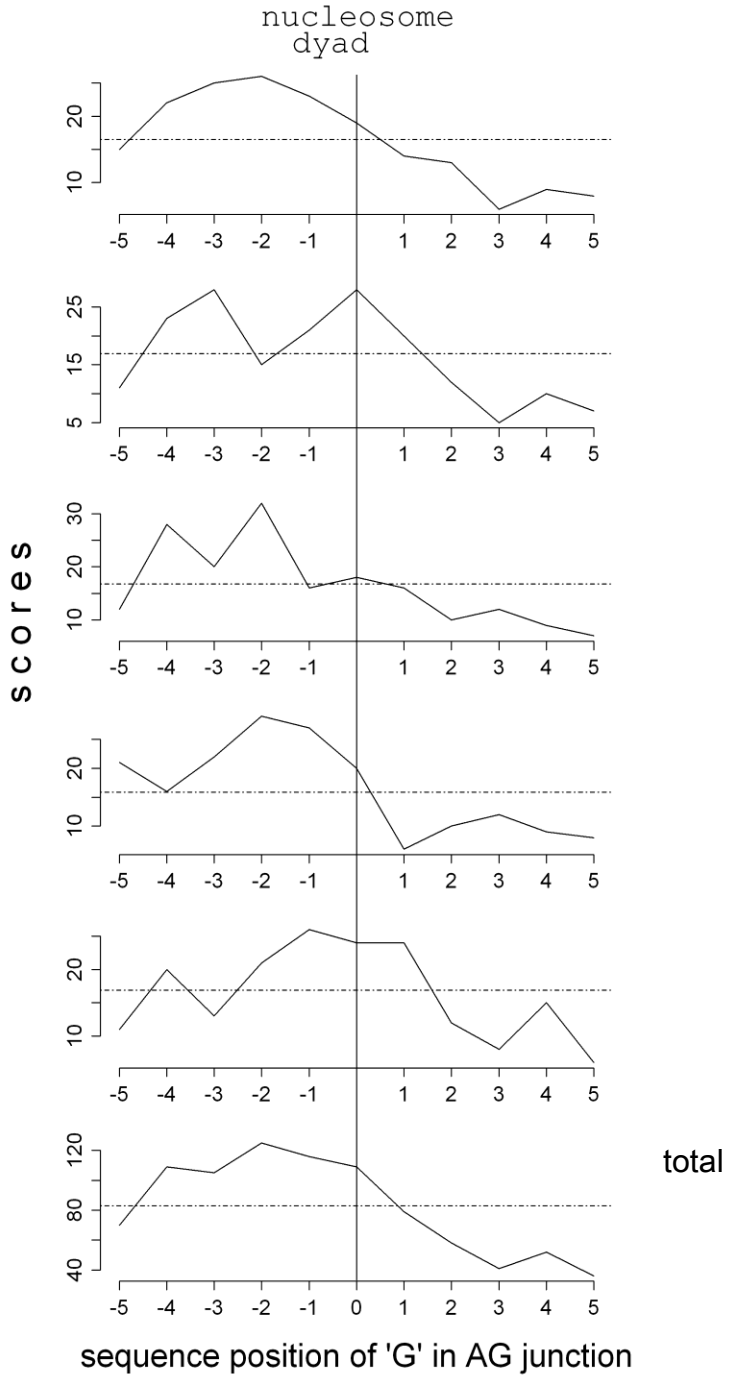
Hapala, 2011



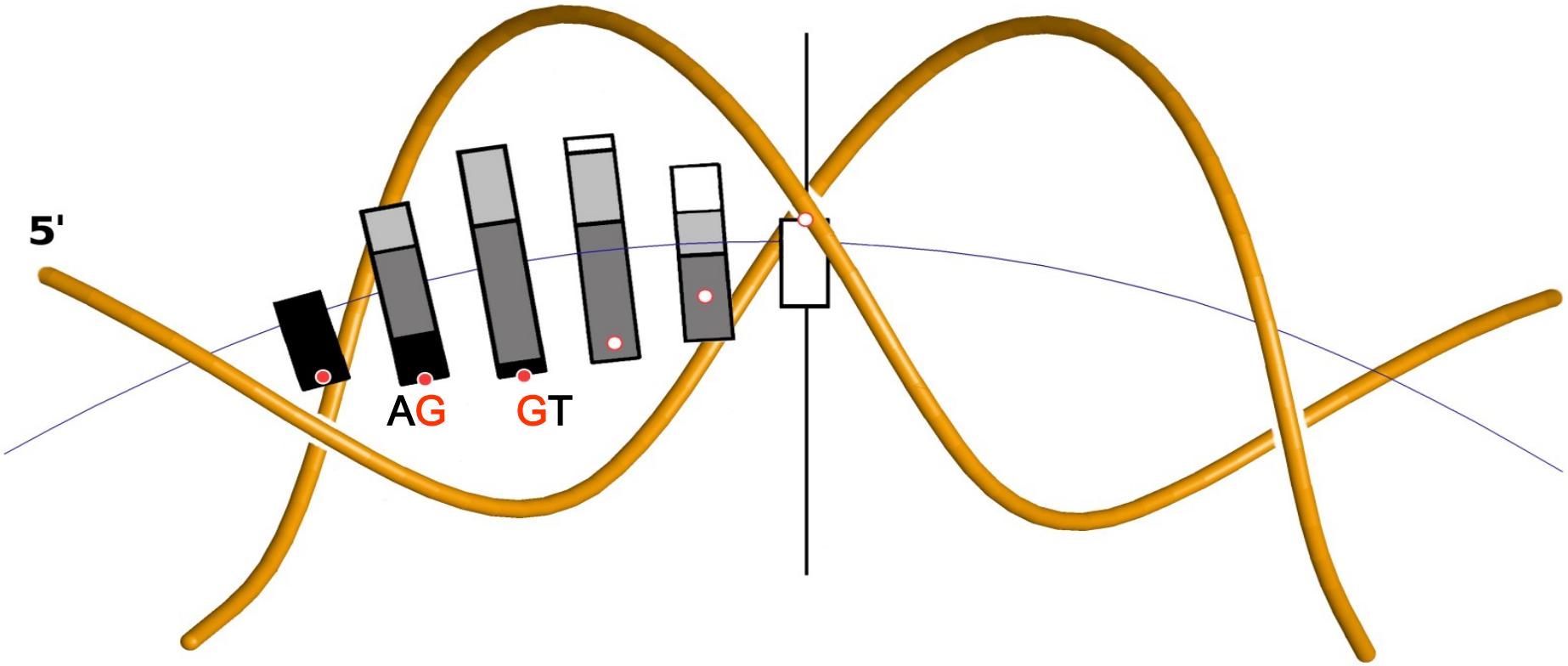

A
GT
number of nucleosomes
0
20
40
60
80
100
-200
-100
0
100
200
sequence position



Position -3 preferred



Position -2
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

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.

Origin of the chromatin code is to be looked for in

prokaryotes

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	polar amino acids
ala	**arg**
gly	**asn**
ile	asp
leu	cys
met	**glu**
phe	gln
pro	his
val	**lys**
	ser
	thr
	trp
	tyr

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

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)
Konstantin 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)
Idan Gabdank (2009)
Danny Barash (2009)
Zakharia Frenkel (2009)
Alexandra Rapoport (2010)
Jan Hapala (2010)

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 cgcggtgg  15
ctcacgcc tgtaatcc cagcactt tgggaggc  47
CGaggcgg gcggatca cctgaggt caggagtt  79
CGagacca gcctggc- caacatgg tgaaaccc 110
CGtctcta ctaaaaat acaaaaat tagccggg 142
CGtggtgg cgcgcgcc tgtaatcc cagctact 174
CGggaggc tgaggcag gagaatcg cttgaacc 206
CGggaggc ggaggttg cagtgagc cgagatcg 238
CGccactg cact-cca -gcctggg cgacagag 268
CGagactc cgtctcaa aaaaaa
Yrrrrxxx Yrrrrxxx Yrrrrxxx Yrrrrxxx
```

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 agcactttgggaggc**CG**aggcgggaggatcacttgagcccaggagtt**CG**agaccagcctgggcaacatagtgaaaccc**CG**tctctacaaaaaatacaaaaattagccggg**CG**tggtggcgcgcgcct

AluSx agcactttgggaggc**CG**aggcgggcggatcacctgaggtcaggagtt**CG**agaccagcctggccaacatggtgaaaccc**CG**tctctactaaaaatacaaaaattagccggg**CG**tggtggcgcgcgcct

AluSq agcactttgggaggc**CG**aggcgggtggatcacctgaggtcaggagtt**CG**agaccagcctggccaacatggtgaaaccc**CG**tctctactaaaaatacaaaaattagccggg**CG**tggtggcgggcgcct

AluSp agcactttgggaggc**CG**aggcgggcggatcacctgaggtcgggagtt**CG**agaccagcctgaccaacatggagaaaccc**CG**tctctactaaaaatacaaaaattagccggg**CG**tggtggcgcatgcct

AluSc ccagcactttgggaggc**CG**aggcgggcggatcacgaggtcaagagat**CG**agaccatcctggccaacatggtgaaaccc**CG**tctctactaaaaatacaaaaattagctggg**CG**tggtggcgcgcgcct

AluY cagcactttgggaggc**CG**aggcgggcggatcacgaggtcaggagat**CG**agaccatcctggctaacacggtgaaaccc**CG**tctctactaaaaatacaaaaaattagccggg**CG**tggtggcgggcgcct

AluYa5 cagcactttgggaggc**CG**aggcgggcggatcacgaggtcaggagat**CG**agaccatcccggctaaaacggtgaaaccc**CG**tctctactaaaaatacaaaaaattagccggg**CG**tagtggcgggcgcct

AluYa8 ccagcactttgggaggc**CG**aggcgggcggatcacgaggtcaggagat**CG**agaccatcccggctaaaacggtgaaaccc**CG**tctctactaaaactacaaaaaatagccggg**CG**tagtggcgggcgcct

AluYb8 cagcactttgggaggc**CG**aggcgggtggatcatgaggtcaggagat**CG**agaccatcctggctaacaaggtgaaaccc**CG**tctctactaaaaatacaaaaaattagccggg**CG**cggtggcgggcgcct

223 ↓

nucleosome 2 bends:

AluJ gtagtcccagctact**CG**ggaggctgaggcaggagaatcgcttgaacc**CG**ggaggcggaggttgcagtgagccgtgat**CGCG**ccactgcactccagcctgggcgacagag**CG**agaccctgtctcaaa

AluSx gtaatcccagctact**CG**ggaggctgaggcaggagaatcgcttgaacc**CG**ggaggcggaggttgcagtgagccgagat**CGCG**ccactgcactccagcctgggcgacagag**CG**agactccgtctcaaa

AluSq gtaatcccagctact**CG**ggaggctgaggcaggagaatcgcttgaacc**CG**ggaggcggaggttgcagtgagccgagat**CGCG**ccactgcactccagcctgggcaacaagag**CG**aaactccgtctcaa

AluSp gtaatcccagctact**CG**ggaggctgaggcaggagaatcgcttgaacc**CG**ggaggcggaggttgcggtgagccgagat**CGCG**ccattgcactccagcctgggcaacaagag**CG**aaactccgtctcaa

AluSc tgtagtcccagctact**CG**ggaggctgaggcaggagaatcgcttgaacc**CG**ggaggcggaggttgcagtgagccgagat**CG**cgccactgcactccagcctggcgacagag**CG**agactccgtctcaaa

AluY tgtagtcccagctact**CG**ggaggctgaggcaggagaatggcgtgaacc**CG**ggaggcgcaggttgcagtgagccgagat**CG**cgccactgcactccagcctgggcgacagag**CG**agactccgtctcaa

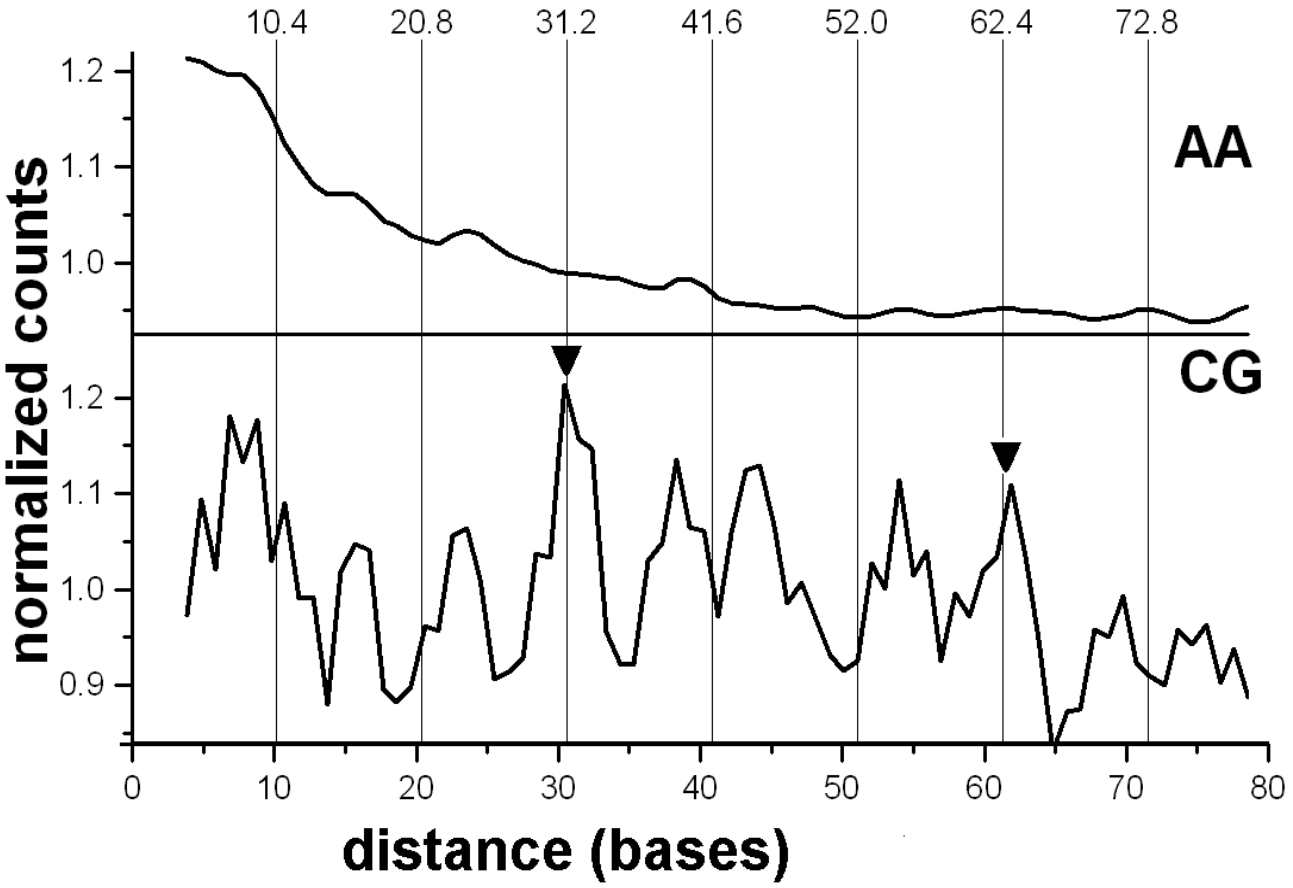
AluYa5 gtagtcccagctacttgggaggctgaggcaggagaatggcgtgaacc**CG**ggaggcgcaggttgcagtgagccgagatcc**CG**ccactgcactccagcctgggcgacagag**CG**agactccgtctcaaa

AluYa8 gtagtcctagctacttgggaggctgaggcaggagaatggcgtgaacc**CG**ggaggcgcaggttgcagtgagccgagatcc**CG**ccactgcactccagcctgggcgacagag**CG**agactccgtctcaaa

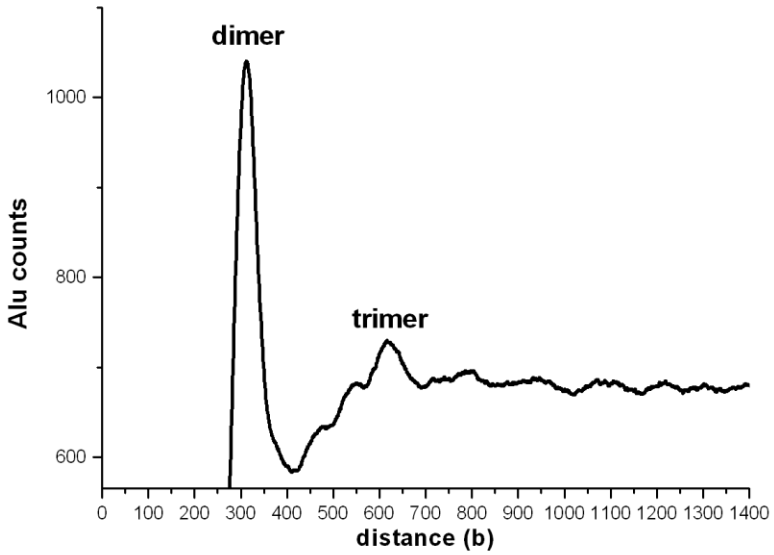
AluYb8 gtagtcccagctact**CG**ggaggctgaggcaggagaatggcgtgaacc**CG**ggaagcgcaggttgcagtgagccgagattg**CG**ccactgcagtccagcagtccggcctggg**CG**acagagcgagactcc

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

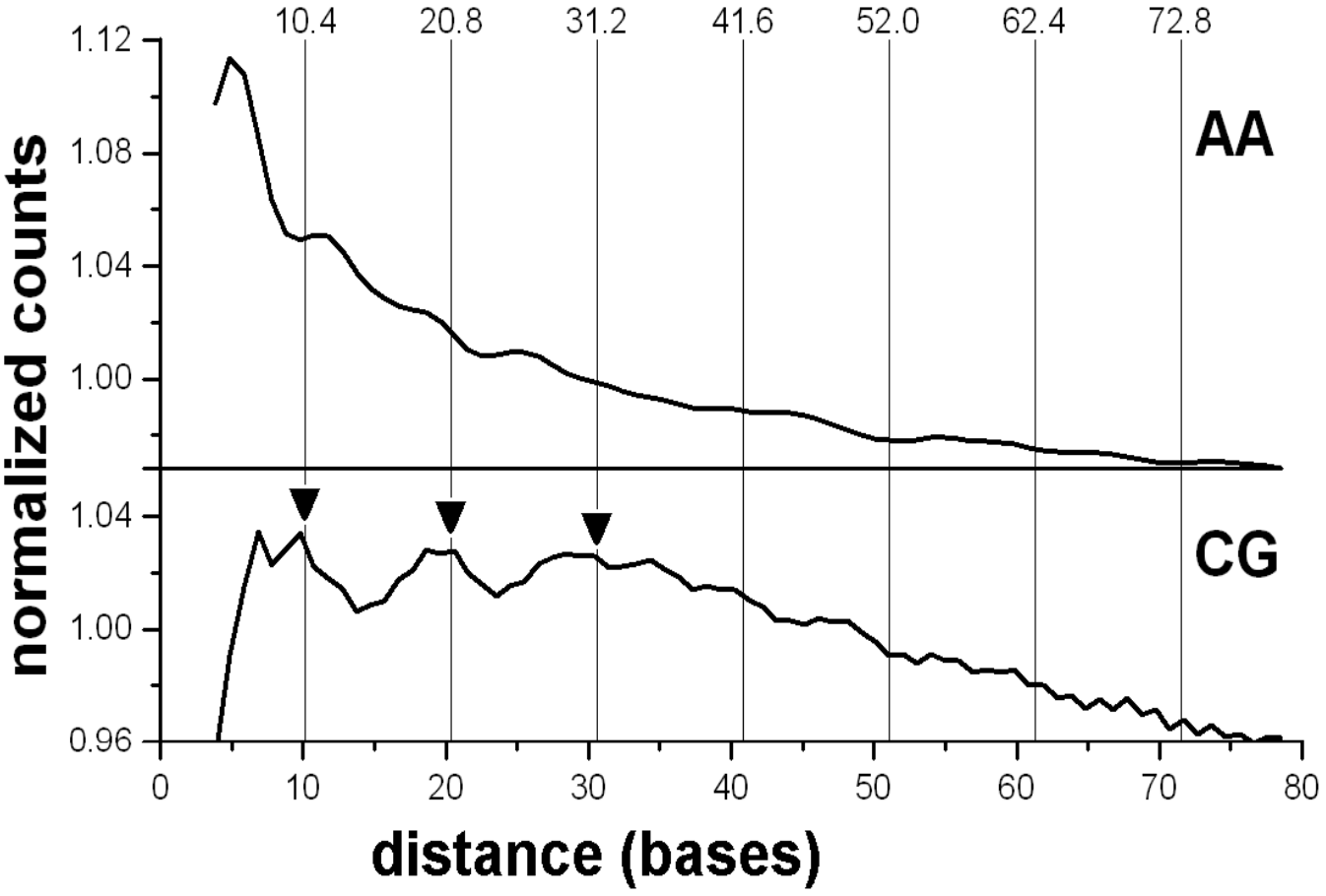
Whole genome (human) shows only 31n periodicity



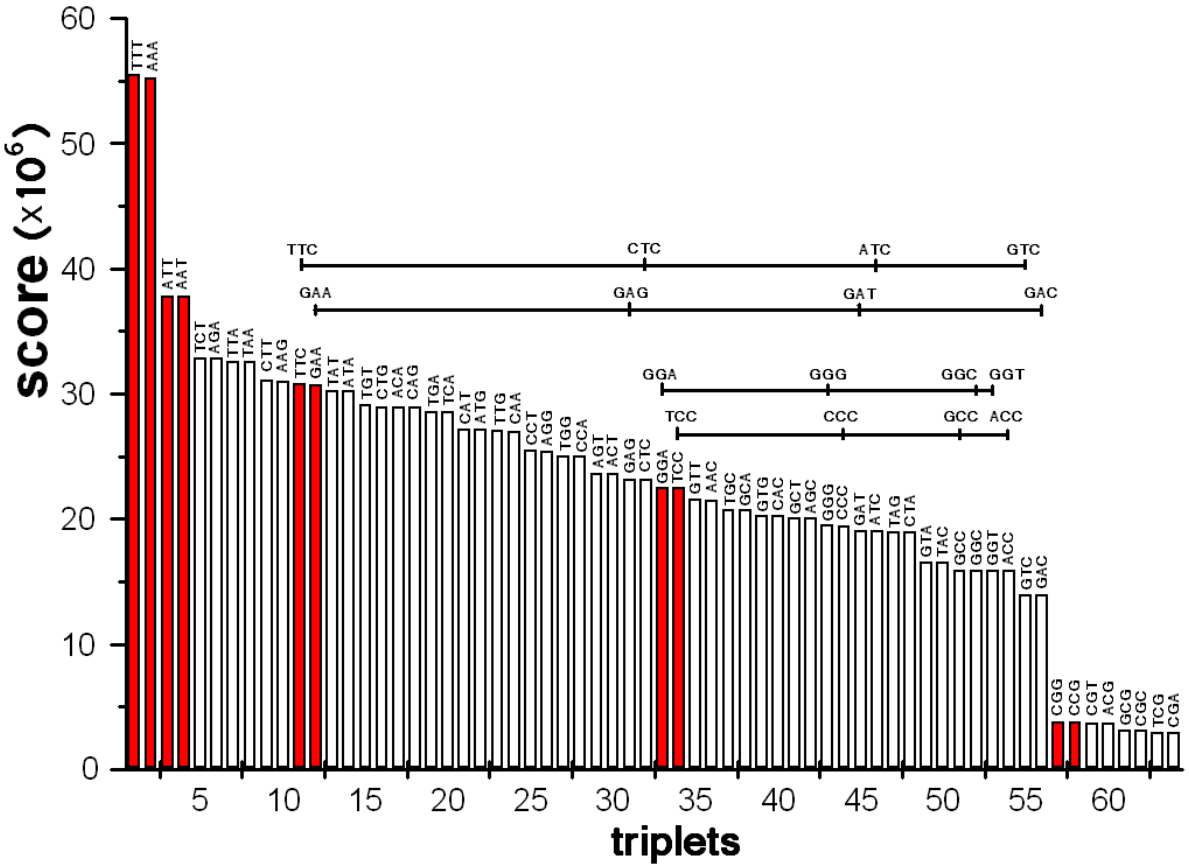
Alu sequences often make tandem clusters



After removal of Alu sequences CG periodicity is seen



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



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

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
99, 893, 1981

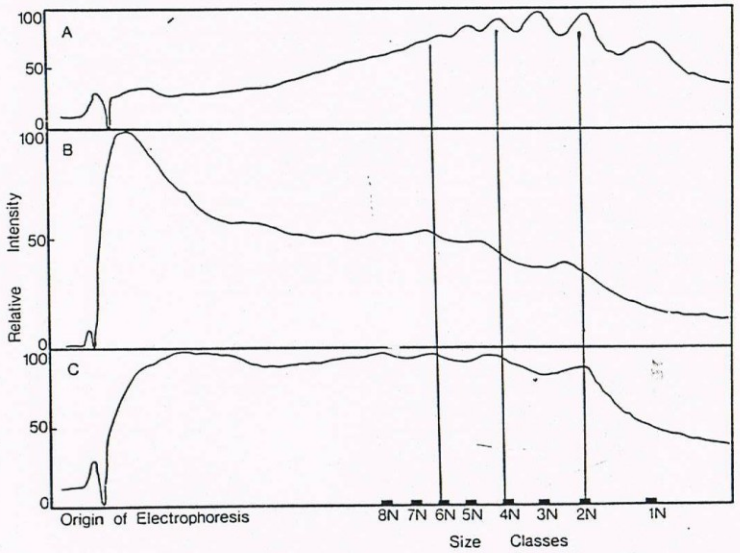
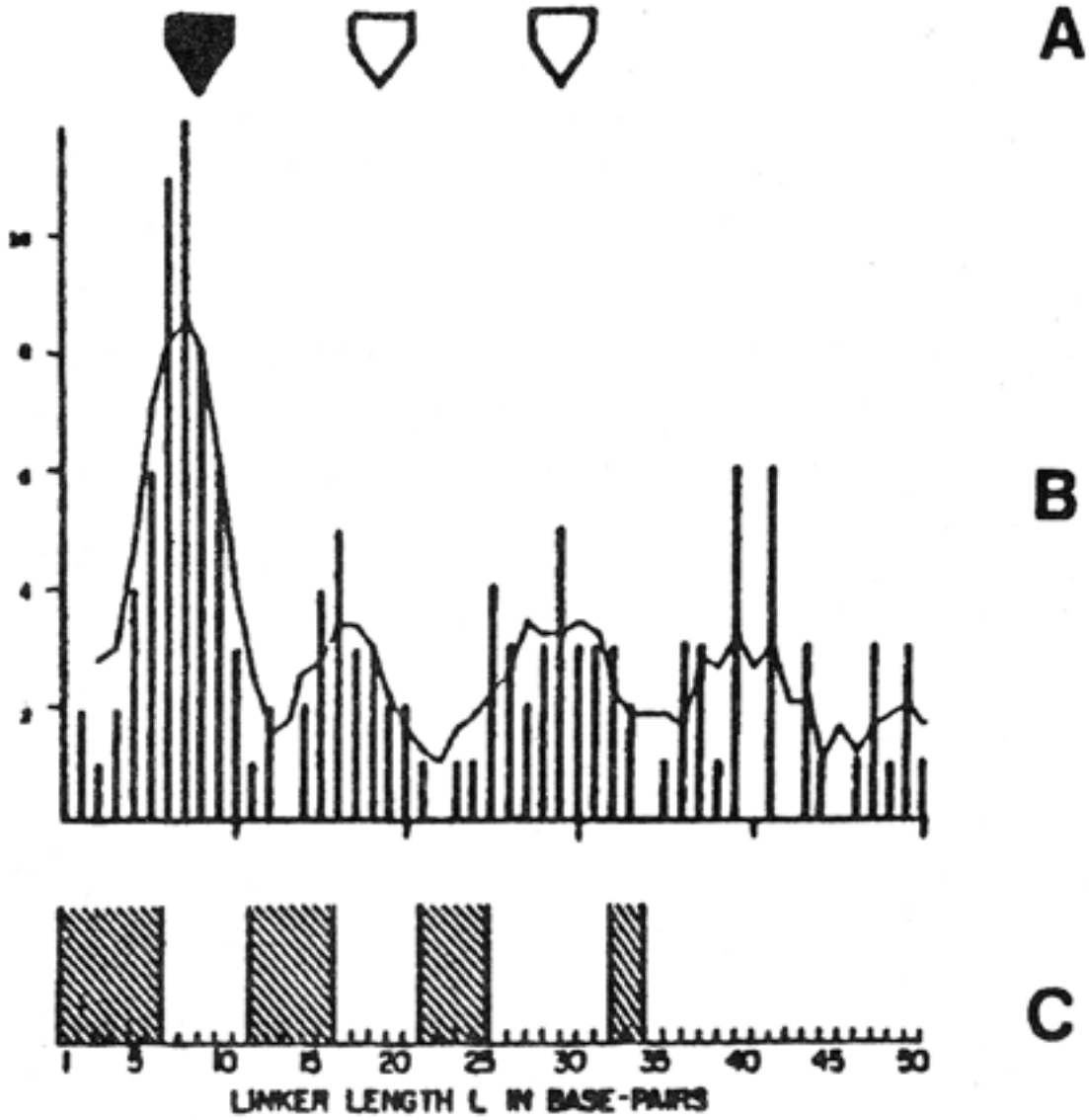
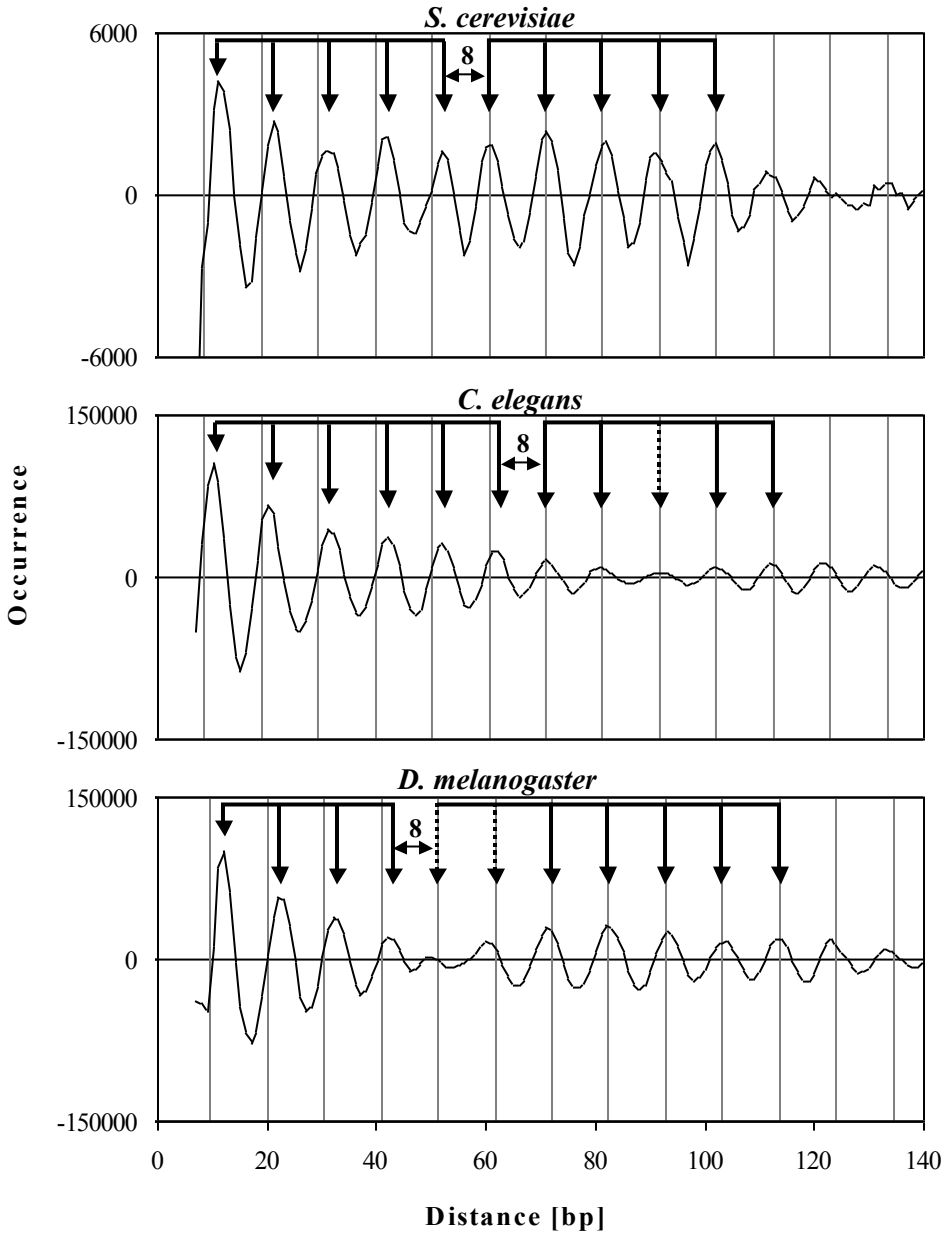


FIGURE 3 Ferritin based, DNAase-I armed probe attack on rat liver nuclei.
All conditions as for the experiment shown in Figure 2. 25 mins digestion time. Curve A - Standard 1N, 2N, etc. series producted by autolysis of rat liver nuclei by their intrinsic Ca-Mg nuclease. Curve B - Rat liver nuclei digested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

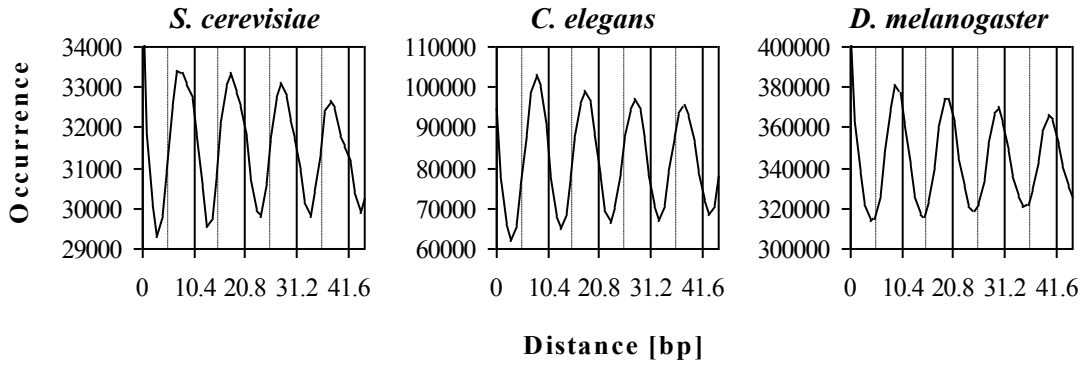
The deformational properties of DNA
is not the only sequence-dependent
factor of nucleosome positioning.

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

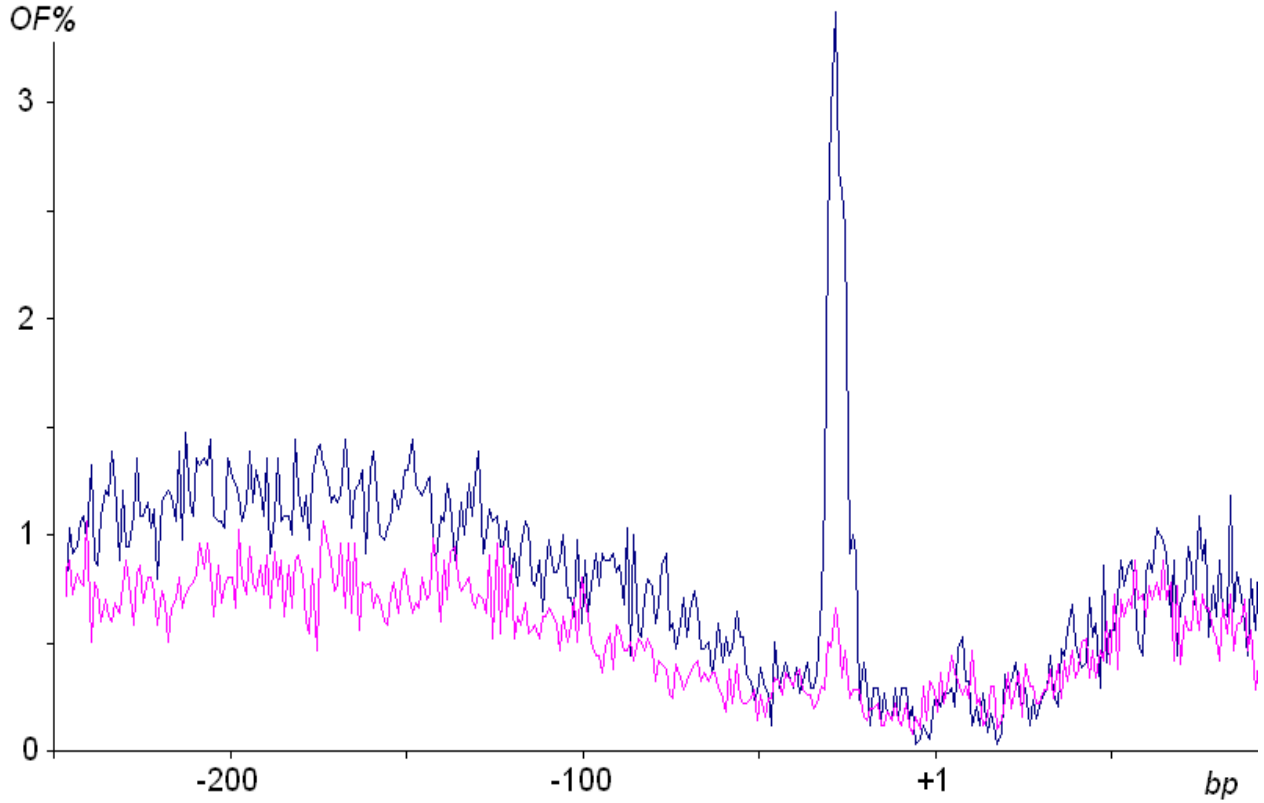

A
B
C
1
5
10
15
20
25
30
35
40
45
50
LINKER LENGTH L IN BASE-PAIRS


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

Linker lengths are 7-8 10.4•n bp



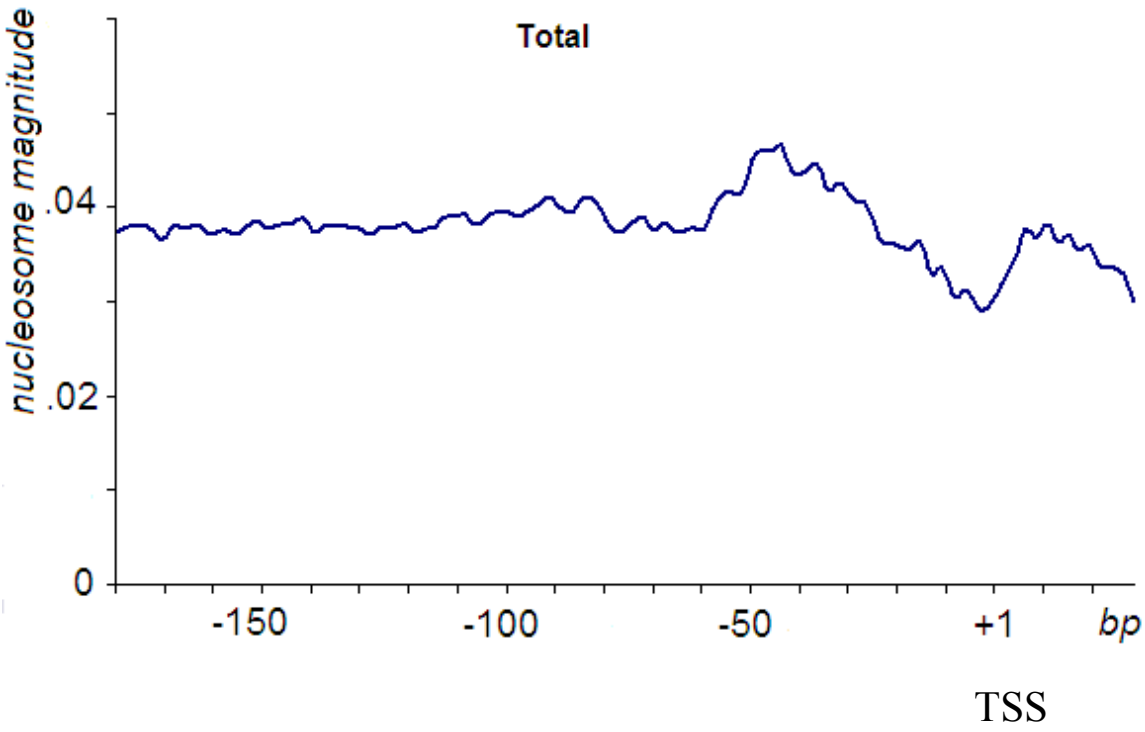
TATA-box



TSS

Gershenzon, Drosophila, 2006

Nucleosomes around transcription start sites (Drosophila)



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**
CG periodicity, honey bee	10.36-10.44 bp	Bettecken, **2009**
DNase I digestion of chromatin	10.36-10.44 bp	Duke University, **2013**

Common range 10.36-10.40 bp

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.

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

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

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

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

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. last year) nobody ever had seen

that the nucleosome sequences

look, indeed, periodical

From the bendability matrices

for the strong nucleosomes:

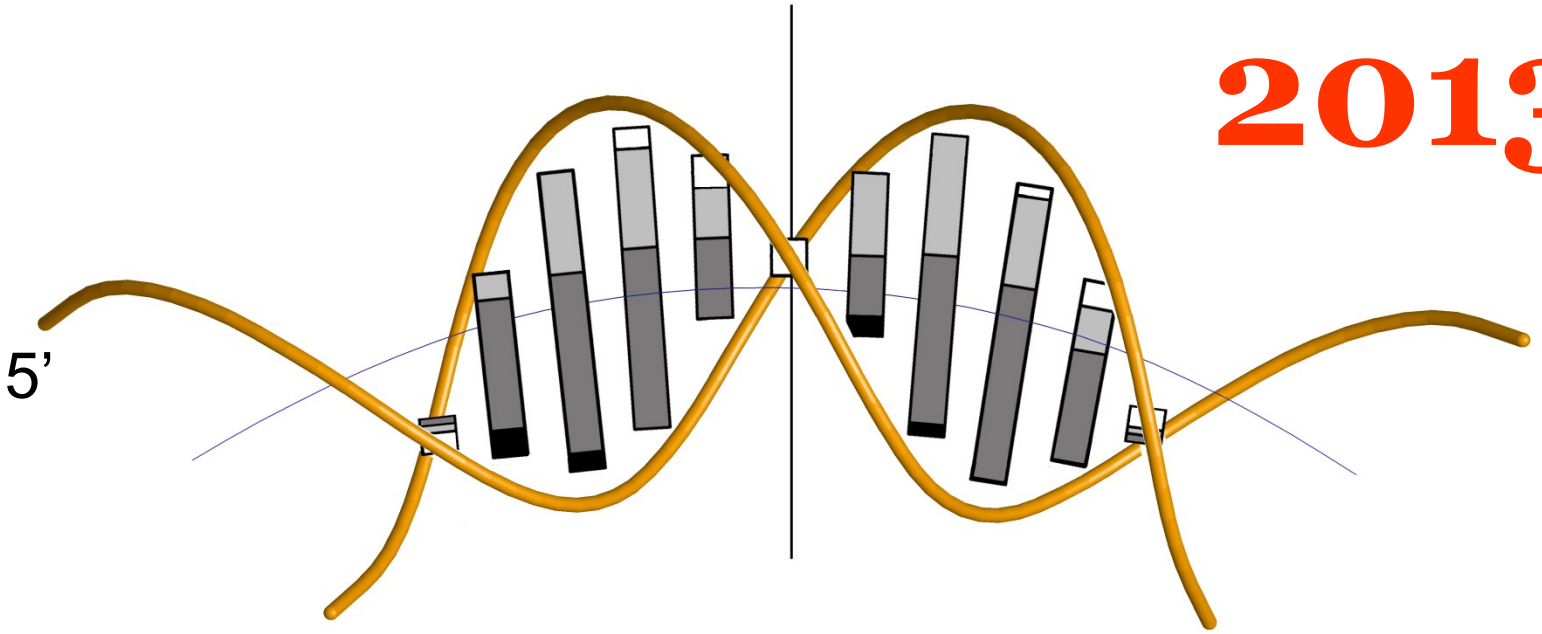
T AGAGG CCTCT A	Lowary and Widom
T AAAAA TTTTT A	A.thaliana
T AAAAA TTTTT A	C.elegans
T AAAAA TTTTT A	H.sapiens
T AAAAA TTTTT A	isochores L1, L2, H1 and H2
C GGGGG CCCCC G	isochores H3
Y RRRRR YYYYY R	common for all

A. thaliana	T AAAAA TTTTT A	strong nucleosomes
	T AAAAA TTTTT A	Shannon extension
C. elegans	T AAAAA TTTTT A	strong nucleosomes
	c grAAA 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 gAAA TTT**c**T **g**	Shannon extension
isochores H2	T AAAAA TTTTT A	strong nucleosomes
	c ggggA 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

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

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.

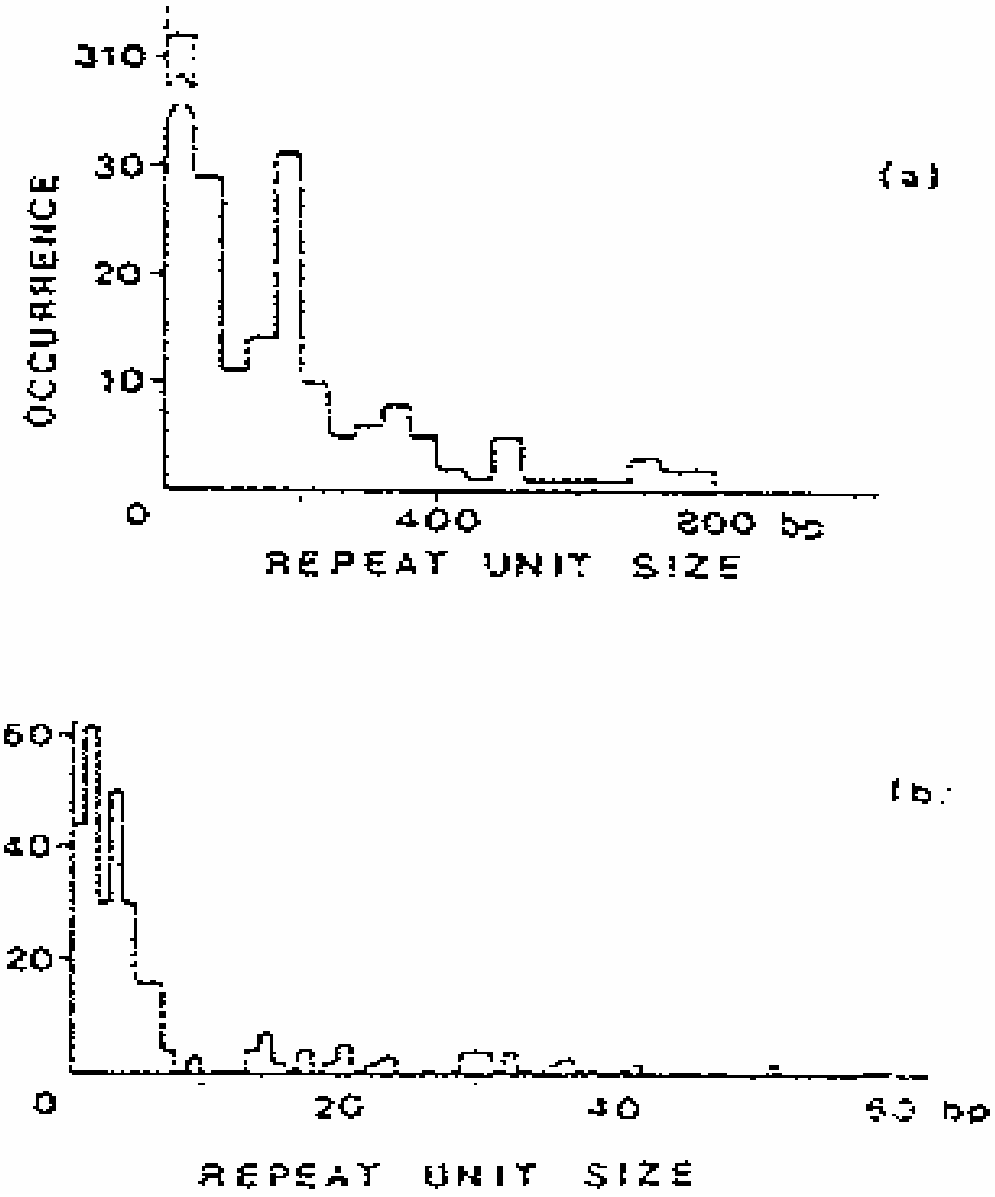
But the bulk of the nucleosomes are only marginally stable.

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

Match of the BamHI nucleosome (typical semistable nucleosome) to the standard nucleosome probe $(GAAAATTTTC)_n$

CGGAAATTTTCCGGAAATTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTCCGGAAATTTTCCGGAAATTTCCGGAAATTTCCGGGAAATTTCCGGAAATTTTCC
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**ctttt**RYYY**gt**RRRR**t**YY**gtgatgcta**YY**g**YY**

Modulation (fast adaptation) code


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

…GCUGCUGCUGCUGCUGCU…

…CGACGACGACGACGACGA…

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.

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
```

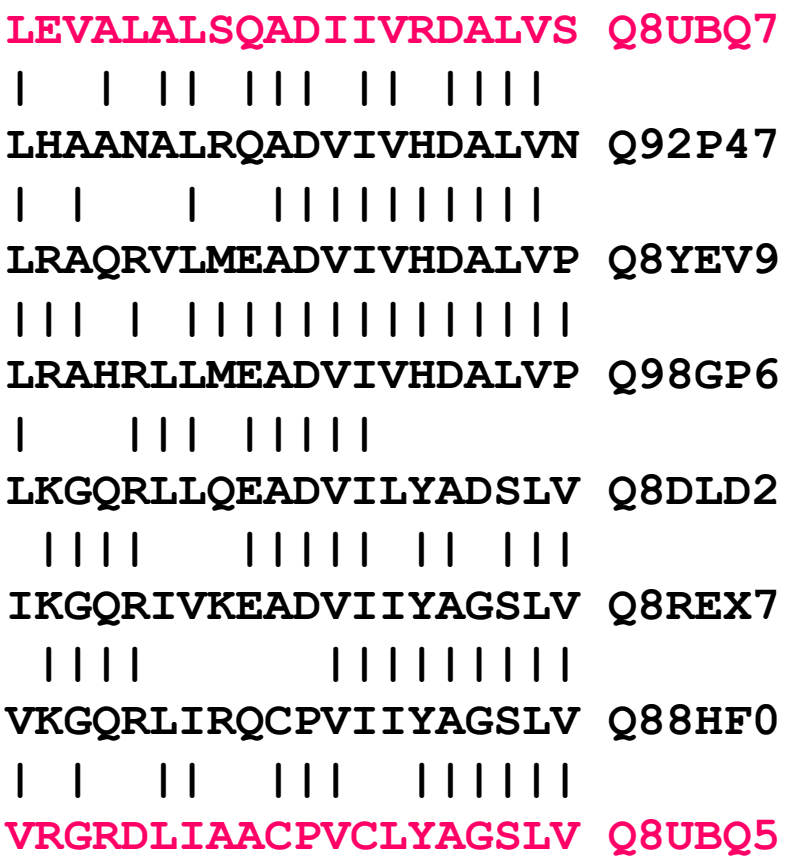
No-match relatives

```
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



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

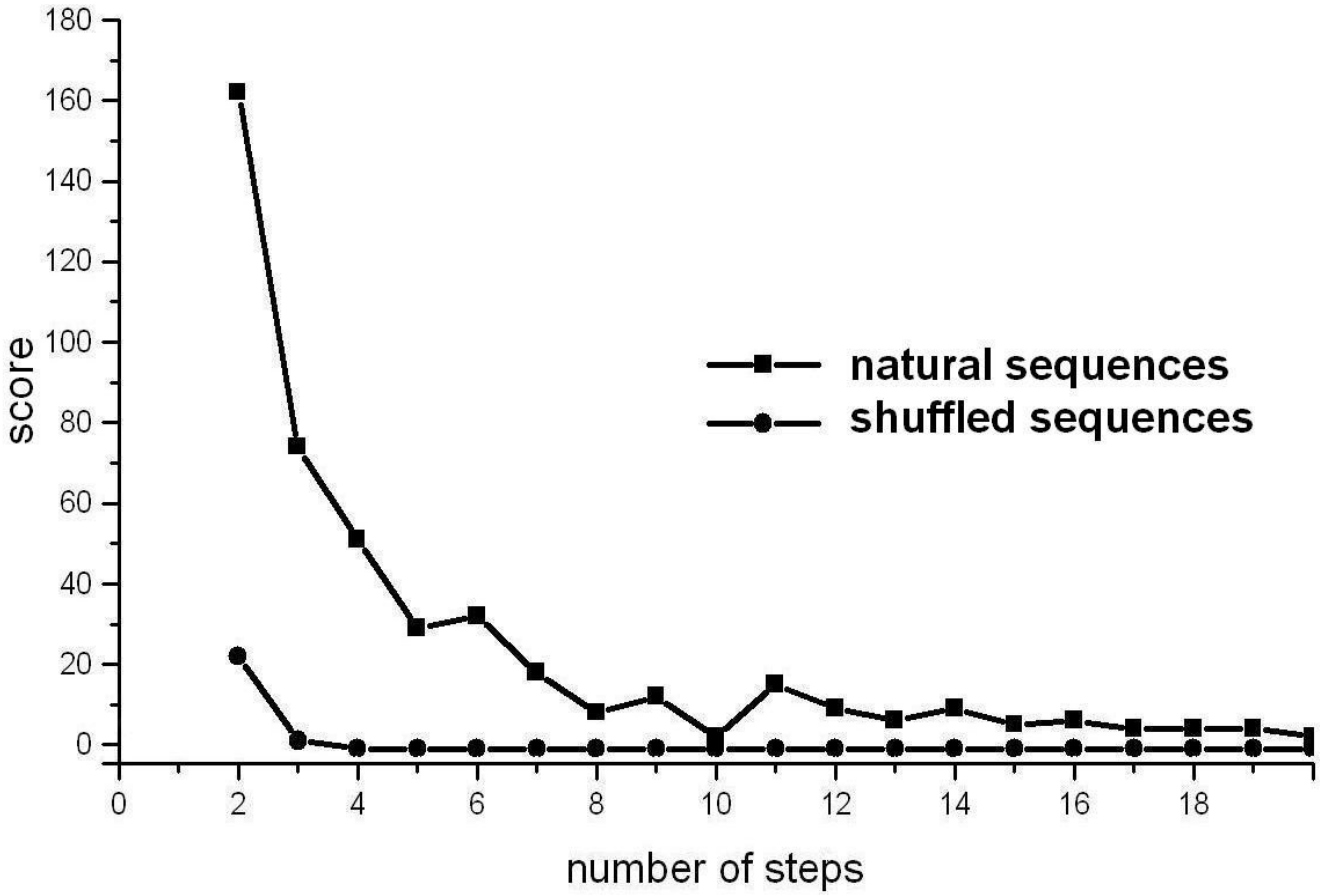
formatted protein sequence space

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

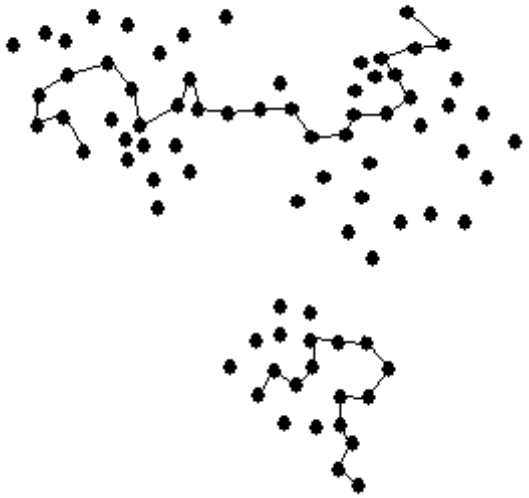
Pair-wise connected matching fragments make also

networks

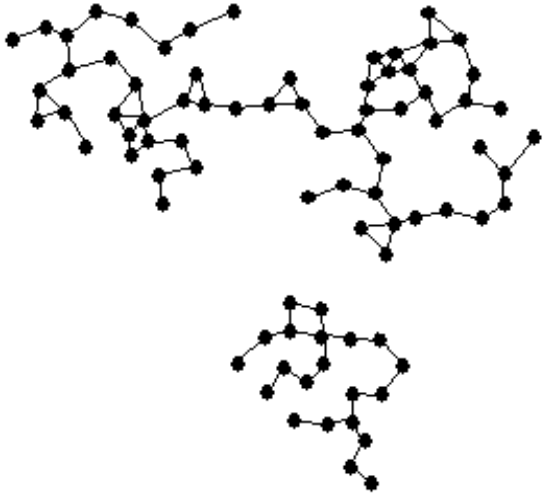
Natural sequence space has longer walks
than random sequence space of the same size



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:

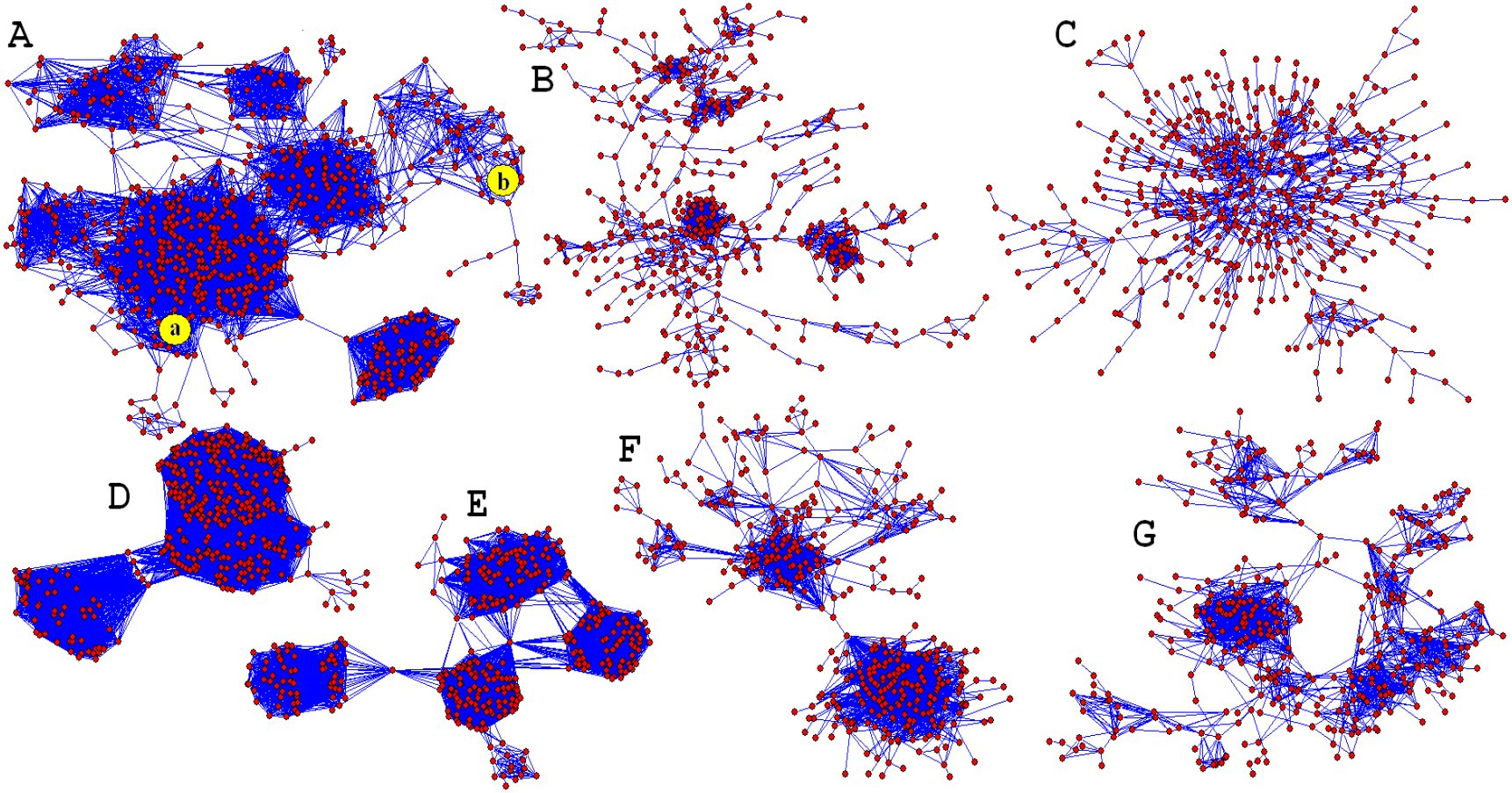
$20^{20} \sim 10^{26}$

Number of fragments in complete natural space:

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

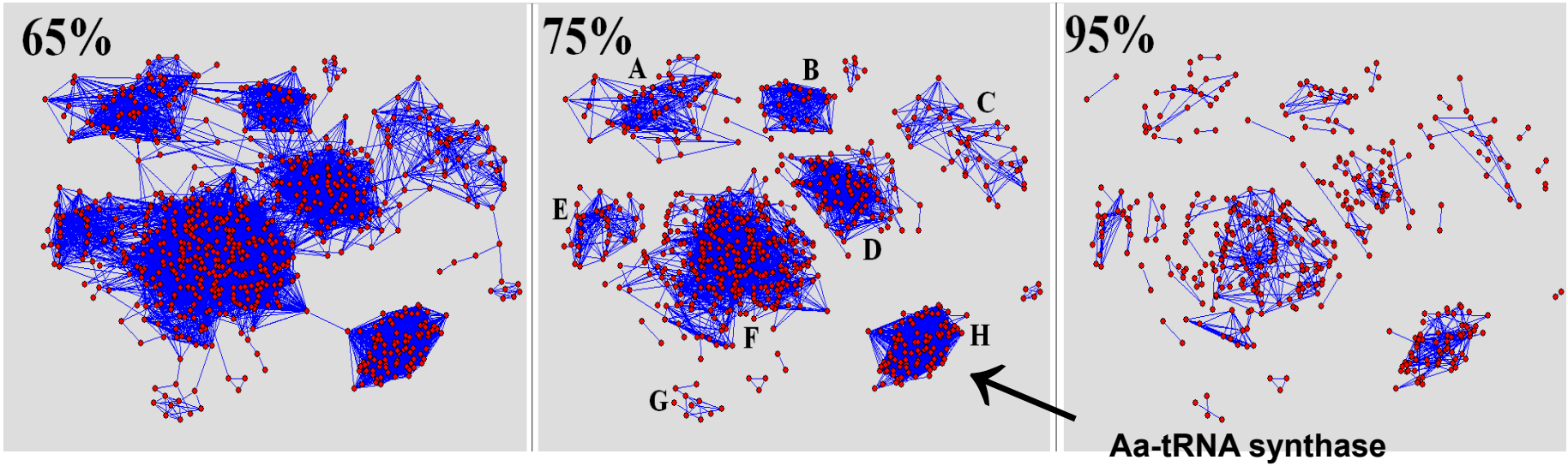
Probability that a given fragment in natural space

is randomly generated is 10^{-12}


A
B
C
D
E
F
G
a
b

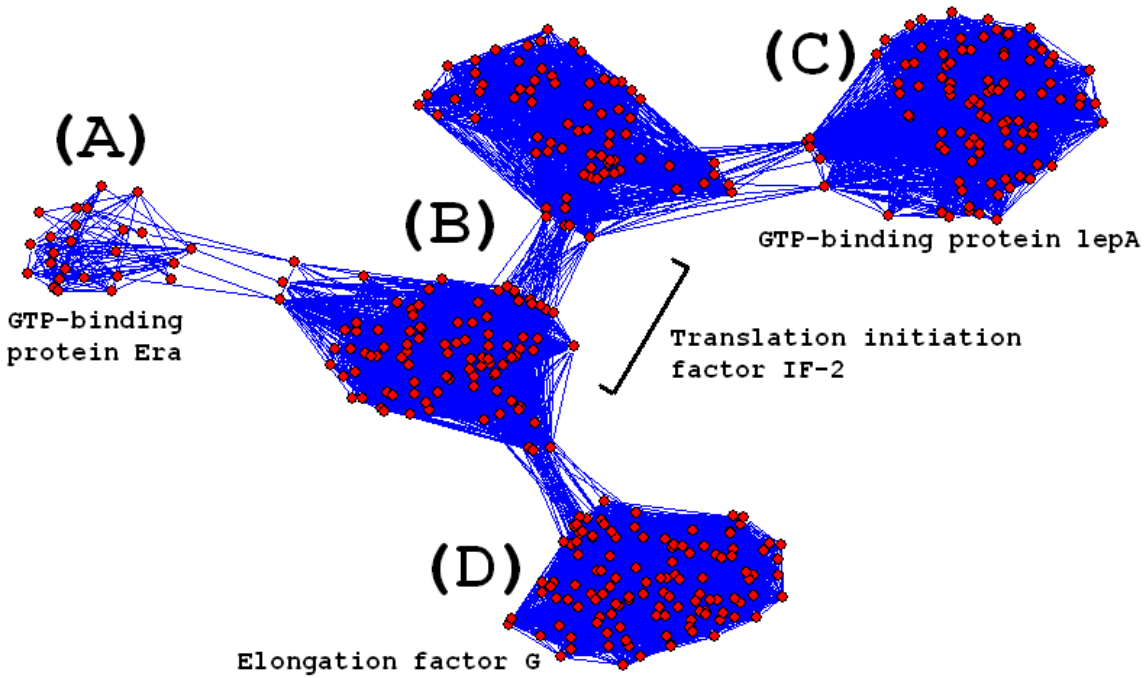
Networks of fragments of aa-tRNA synthetases

at various thresholds of sequence match



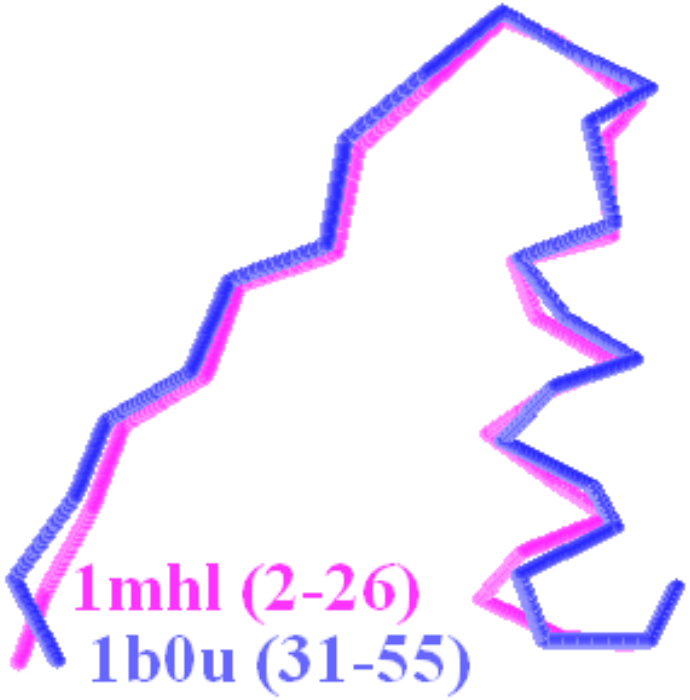
A tyr trp B met C arg trp D cys
E leu F met leu ile val G ile H lepA

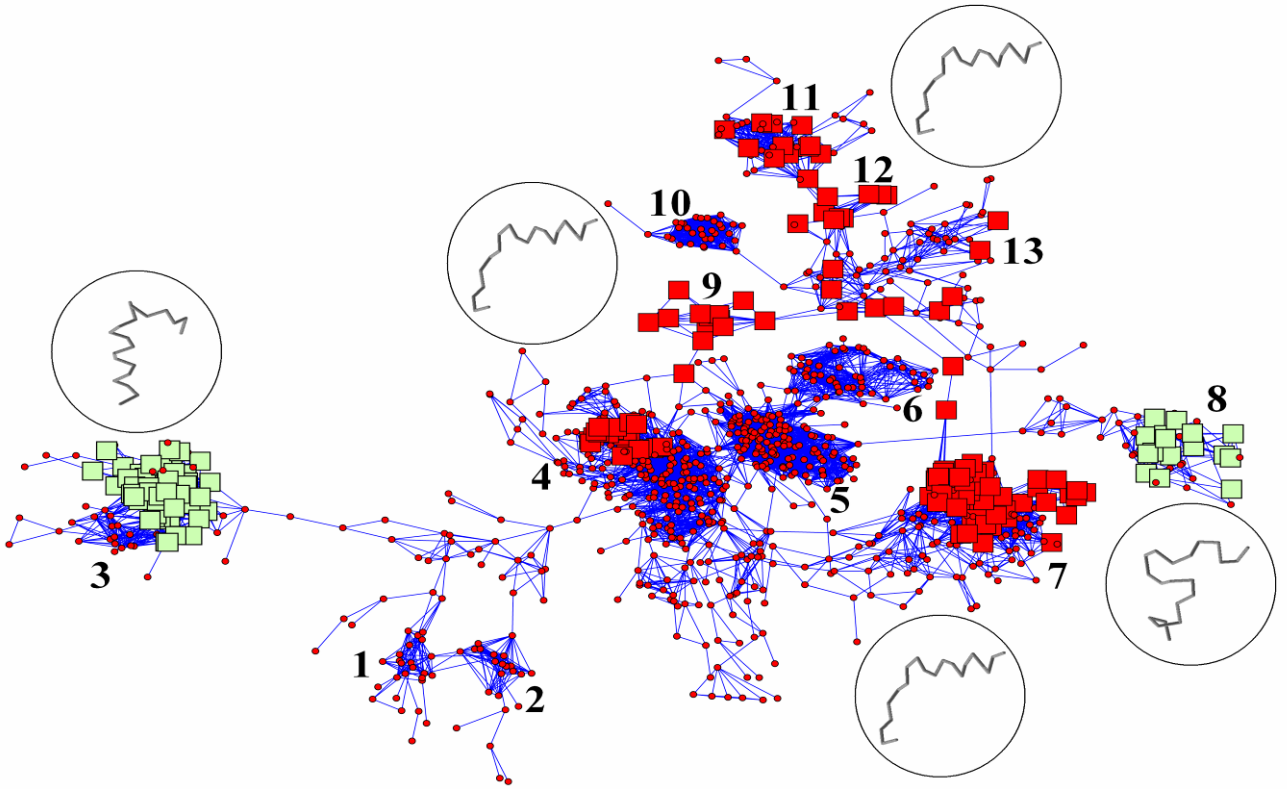
Network of GTP binding proteins



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}
Fig. 2



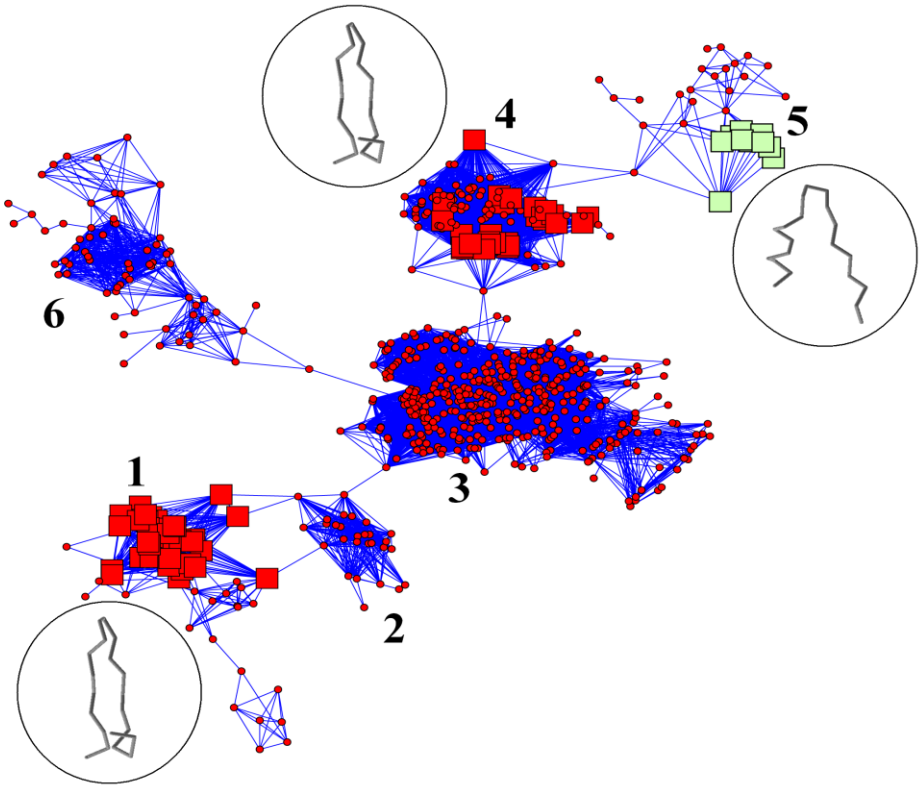


1 Putative peptidoglycan bound protein
2 Collagen adhesion protein
3 Ribosomal protein L11
4 Penicillin-binding protein 2x
5 Penicillin-binding protein 1
6 Penicillin binding protein 2A
7 D-alanyl-D-alanine carboxypeptidase

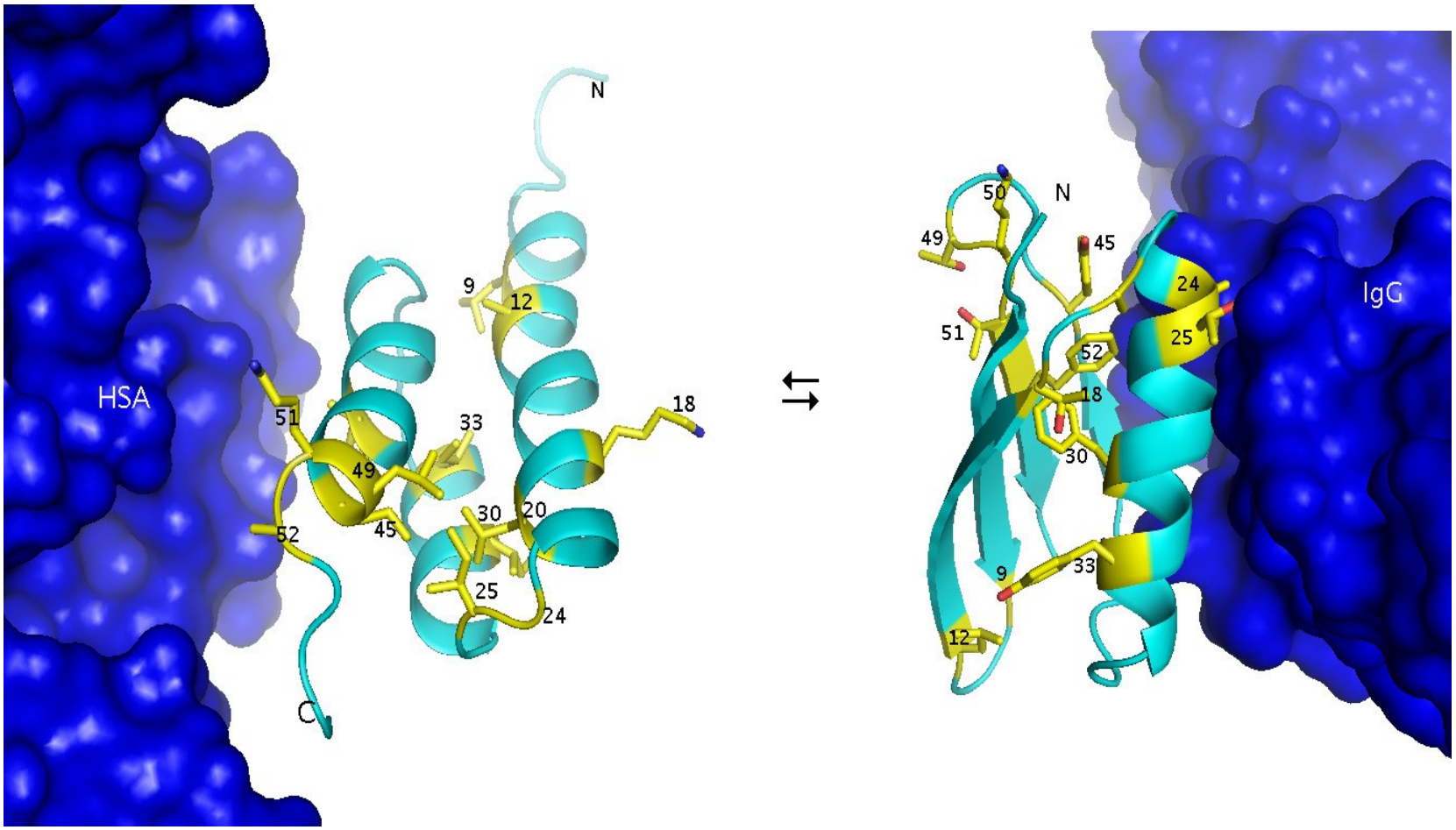
8 cytochrome
9 Beta-Lactamase
10 Mannitol-1-phosphate 5-dehydrogenase
11 glutaminase
12 Beta-lactamase
13 Esterase EstB

Pajek

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

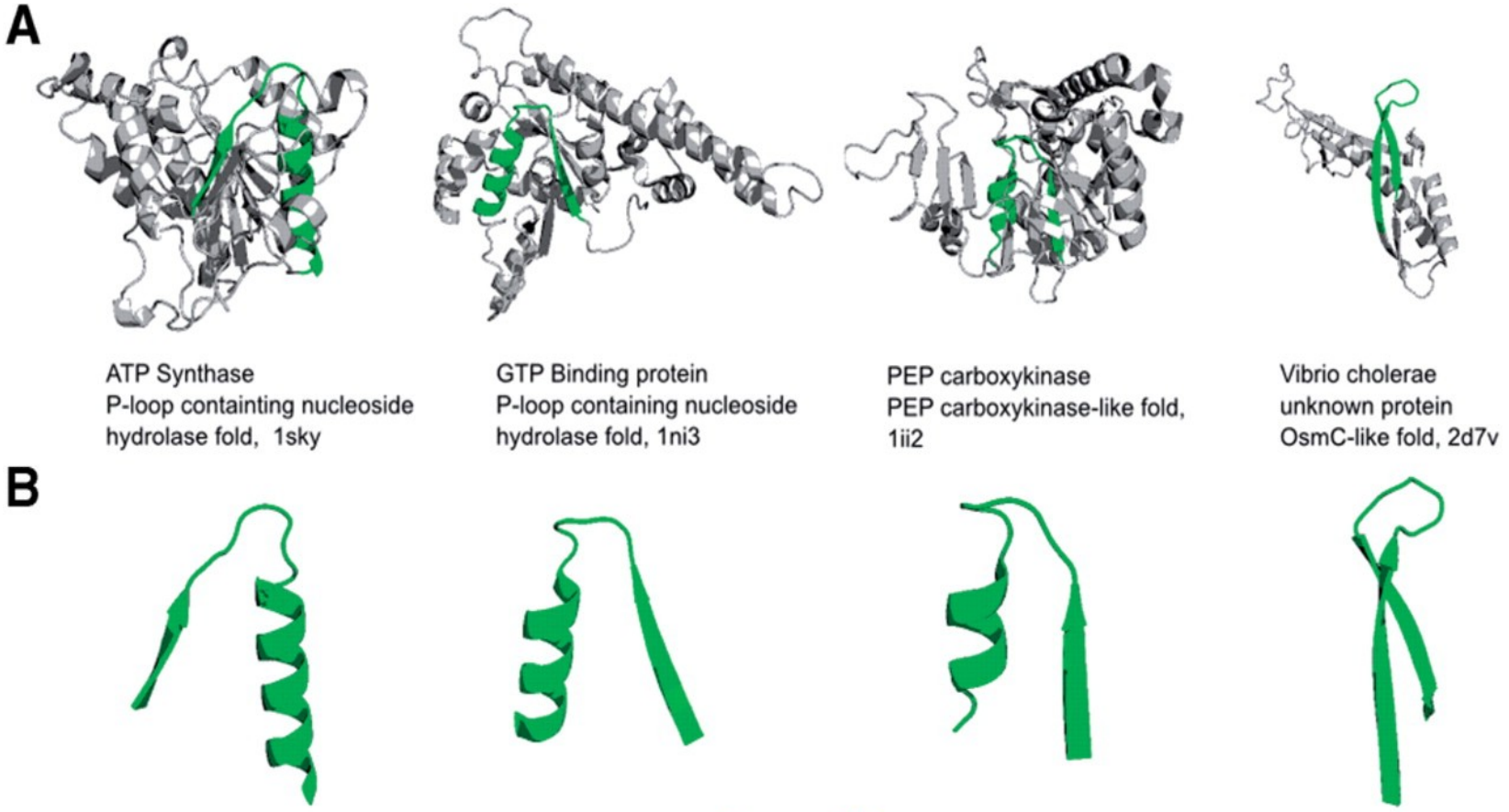

1
2
3
4
5
6
Pajek

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.



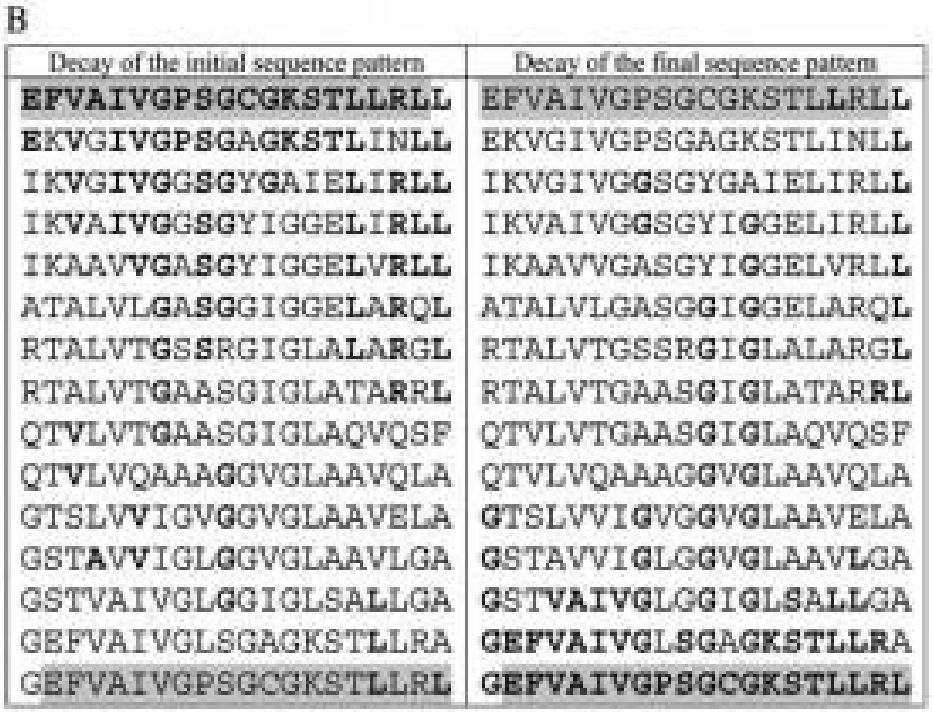
Goncearenco A , Berezovsky I N Bioinformatics 2010;26:i497-i503

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**EAA**ER**AA**AT**GAD**GVG**L**LR**M**	PEAAERAAATGADGVGLLRM
4	PEA**A**RKA**A**ATGA**DG**VGLLRT	P**EAARKAA**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**DI IKA**LVL**GA**KAVG**L**SRT	PLDIIKALVLGAKAVGLSRT
8	GTDIIKALAIGANLVGL**G**RM	GT**DI 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**GR**A	LKARAVLDYT**GAD**AL**M**IGRA	LKARAVLDYTGADALMIGRA
18	KK**AF E**VLQITQA**DG**LMI**GR**A	KKAFEVLQITQ**AD**GL**M**IGRA	KKAFEVLQITQADGLMIGRA
19	Q**NA**K**E**VYKITKC**DG**LMI**GR**A	QNAKEVYKITKC**D**GL**M**IGRA	QNAKEVYKITKCDGLMIGRA
20	Q**NA**K**E**ILGIDSV**DG**L**LI 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

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

Sequence from proteomes	Sequence Position	Swiss-Prot Code
RKLEEGEAAAAAASKPKFPR	590	Q8P7G9
MRKLEDGEAAAAASKPRFPR	580	Q8PIT2
MRKLEEGEAAAAAASKPKFP	589	Q8P7G9

B

Sequence from proteomes	Sequence Position	Swiss-Prot Code
RKLEEGEAAAAAASKPKFPR	590	Q8P7G9
MRKLEDGEAAAAA-SKPRFPR	580	Q8PIT2
MRKLEEGEAAAAAASKPKFP	589	Q8P7G9

Careful with consensus!

The words

COOKY

MANGO

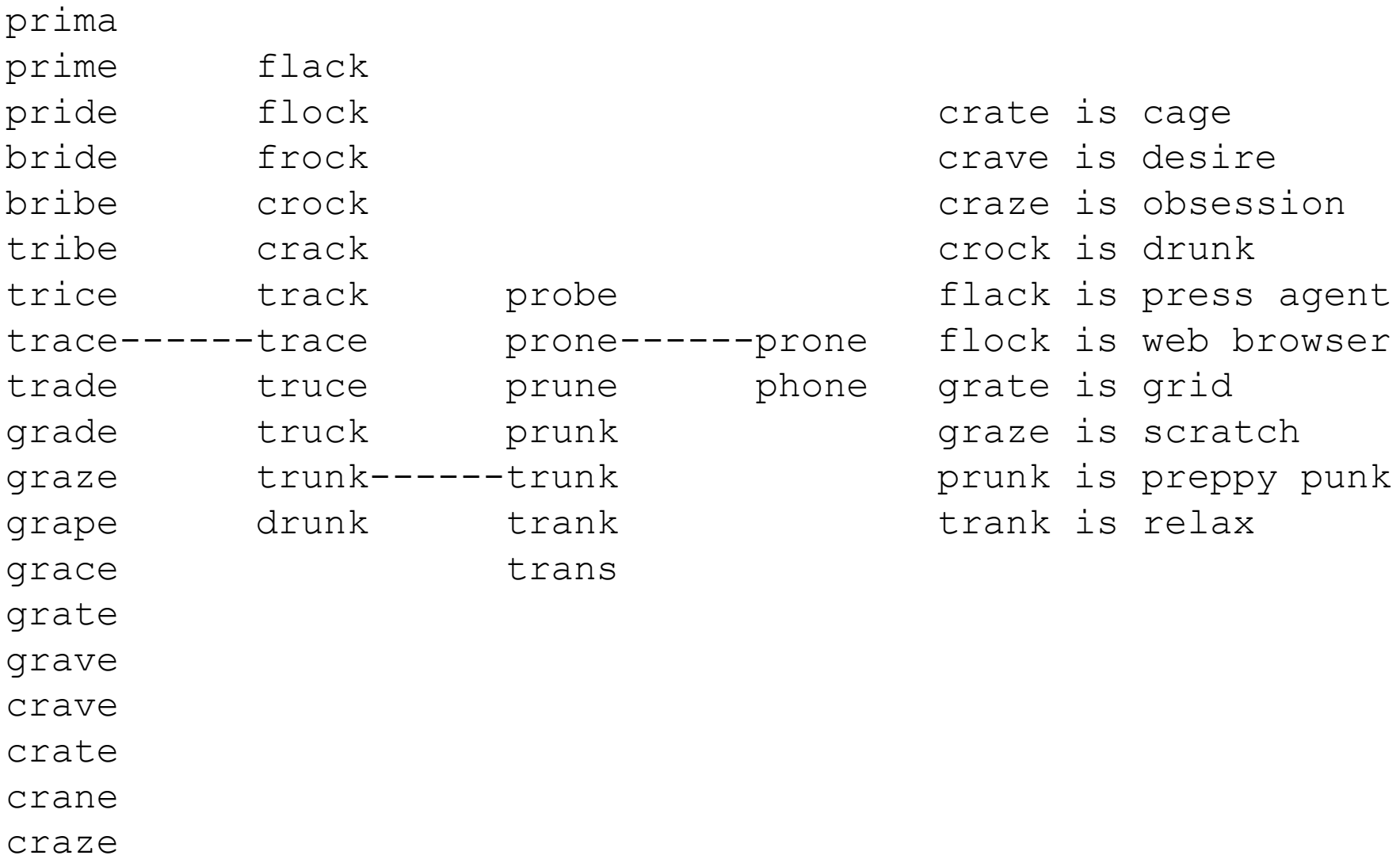
MELON

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

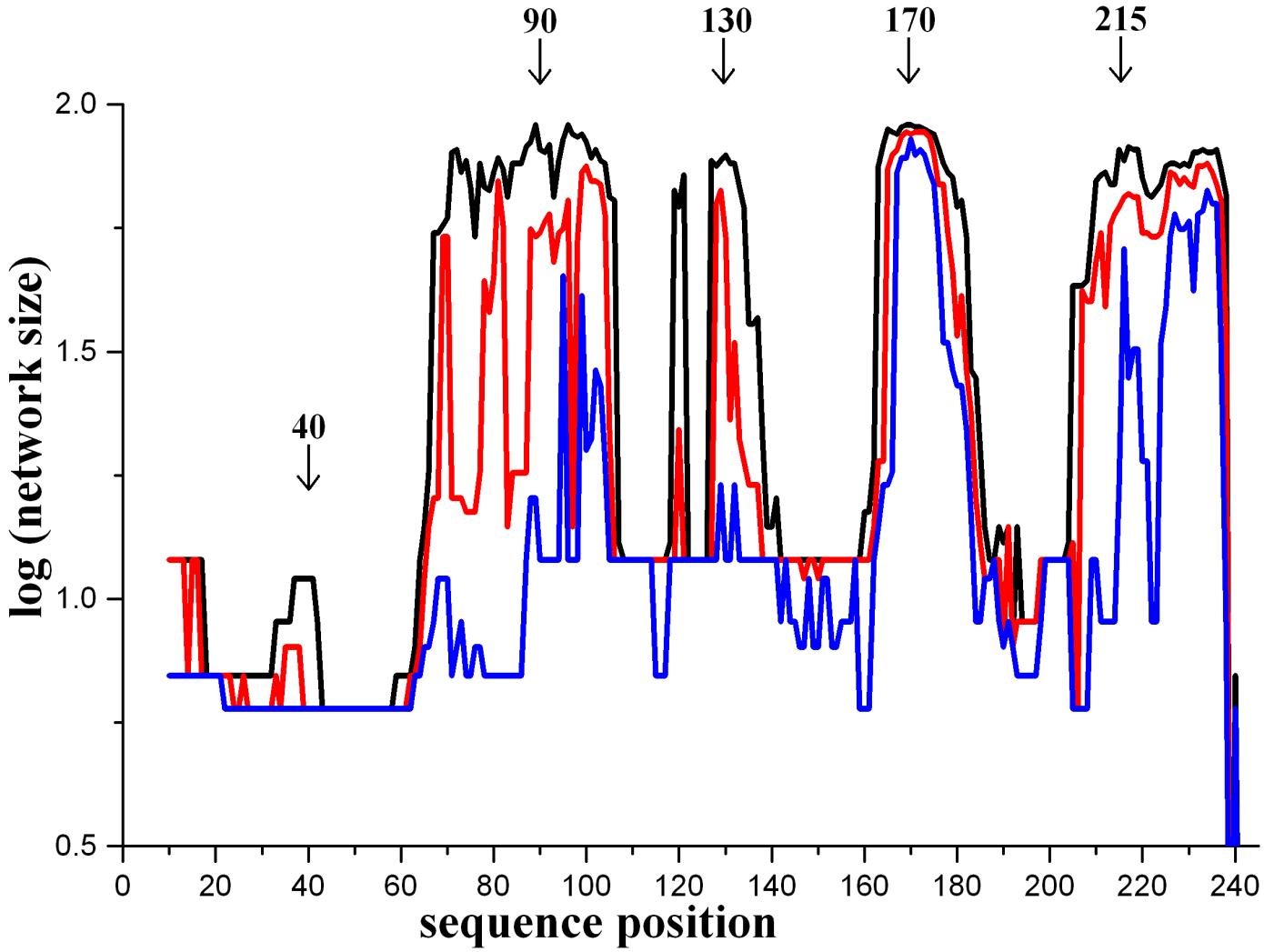
Every fragment
of the precalculated space
is tagged (protein, species)

It is also uniquely located in it s family network.

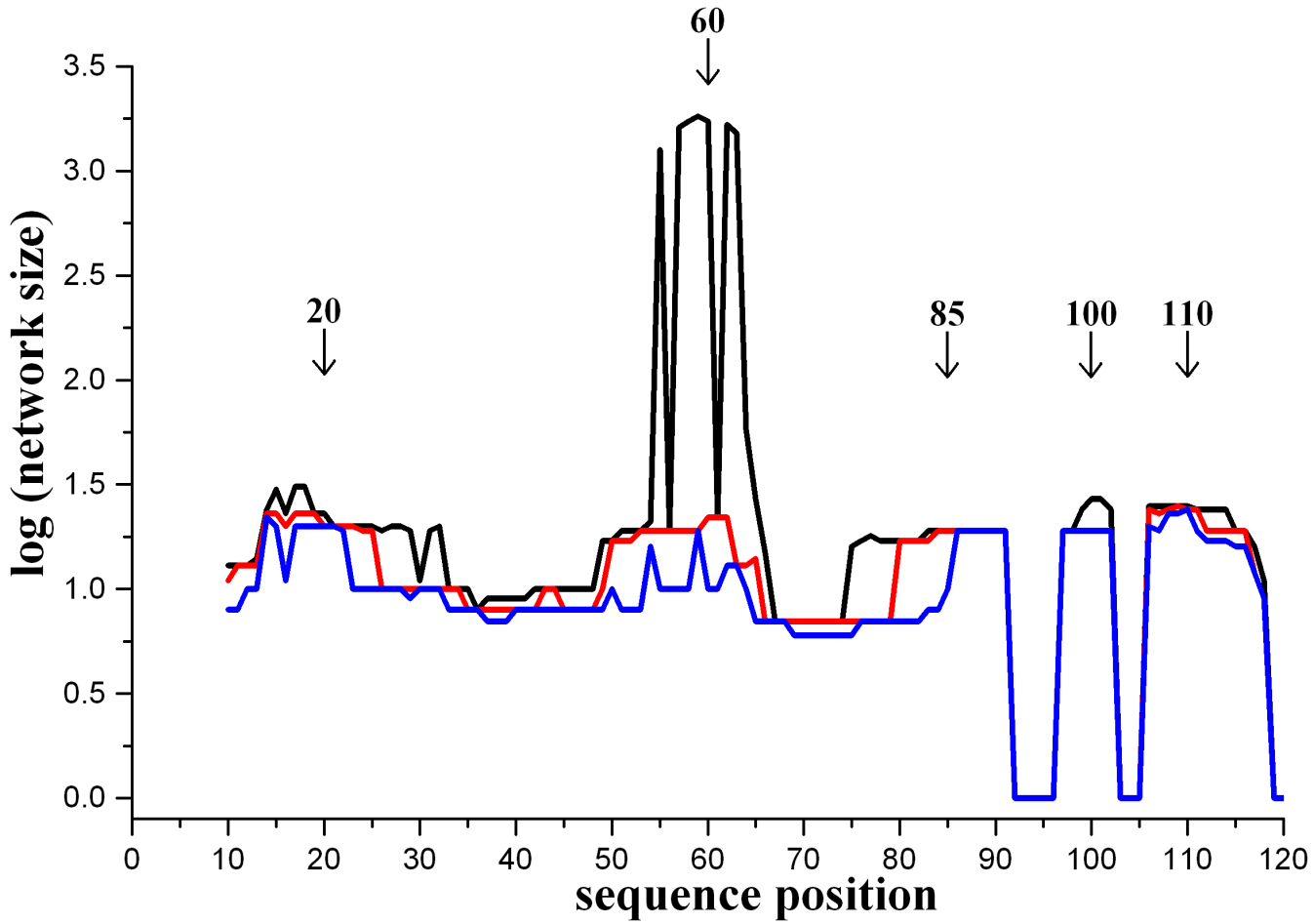
The size of the network says
how many relatives the fragment has

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

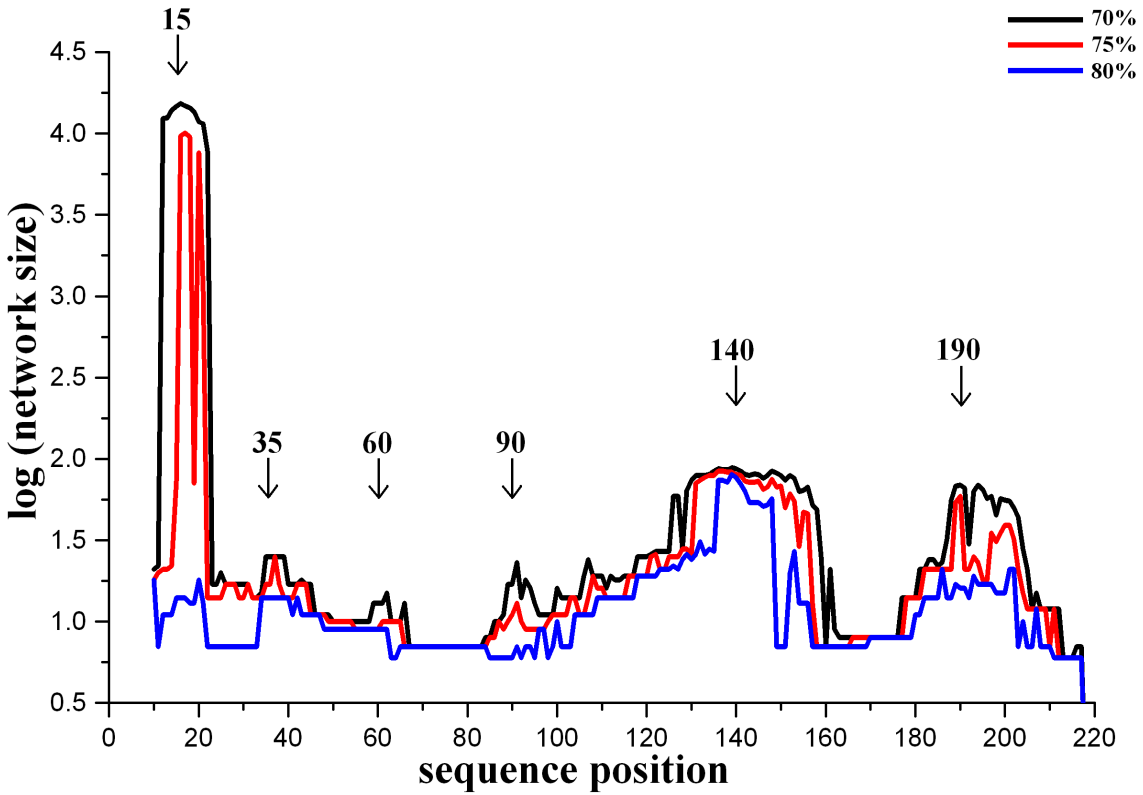
Modules of TIM-barrell protein



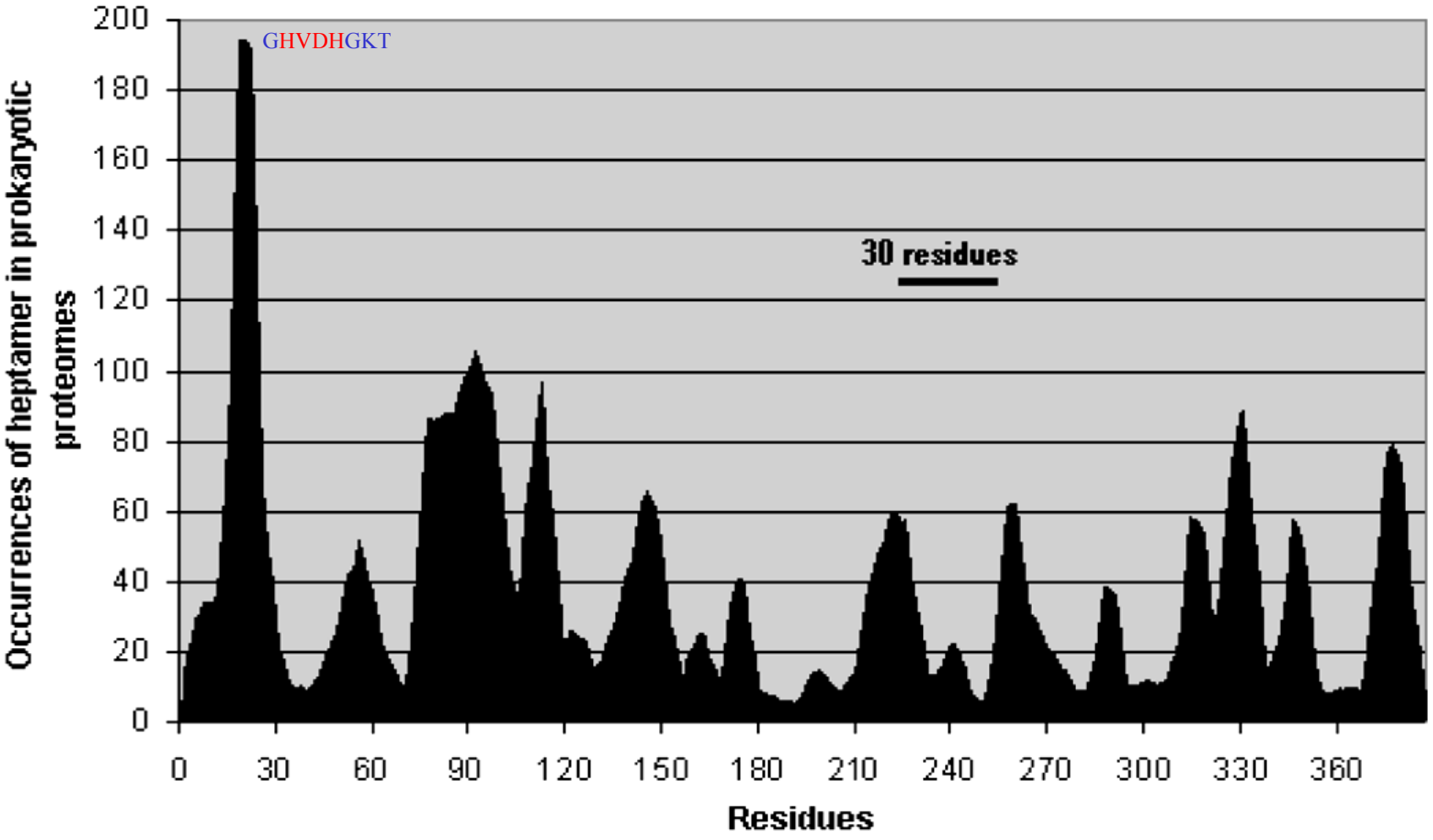
Modules of chemotaxis protein cheY



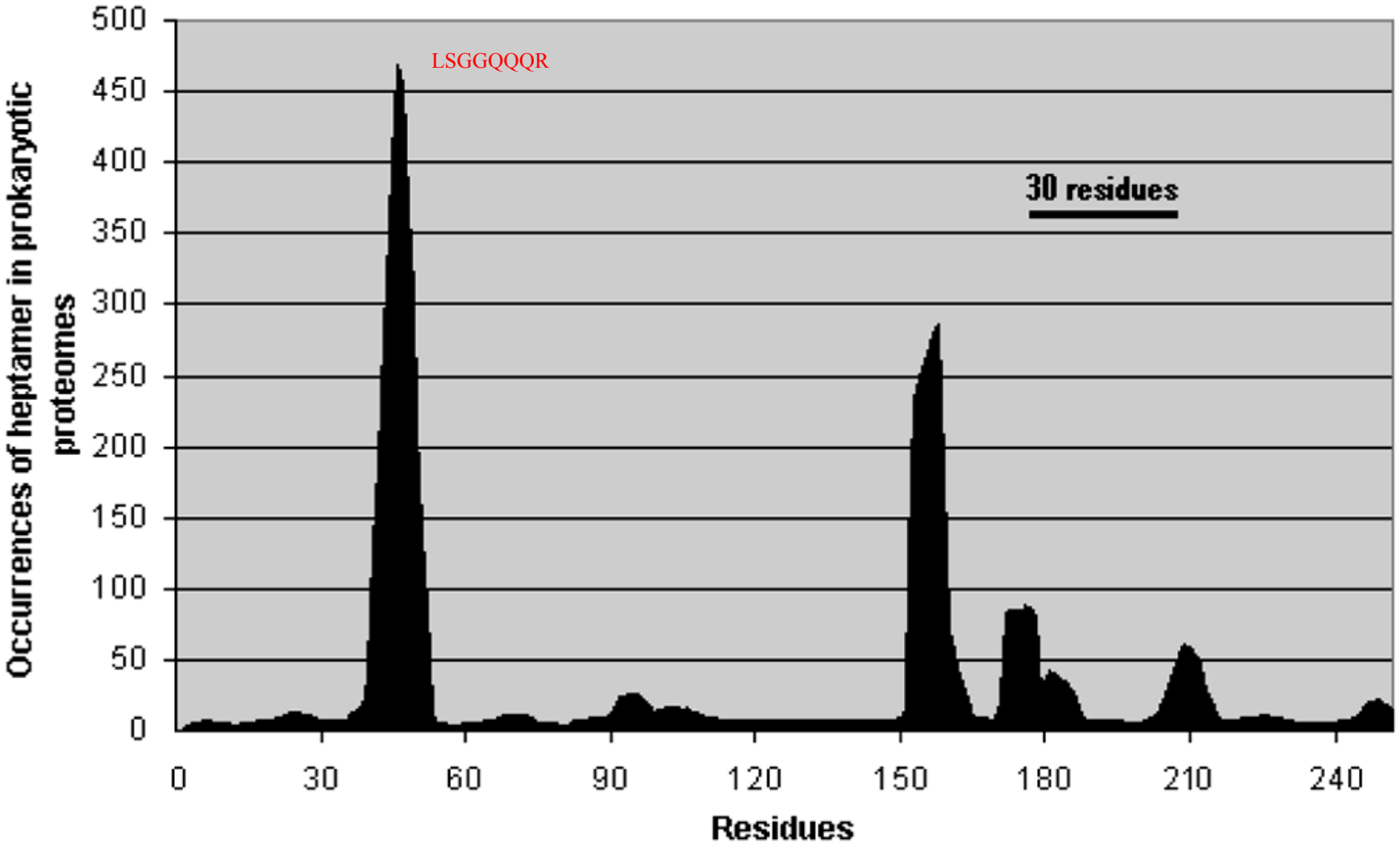
Modules of cytidylate kinase

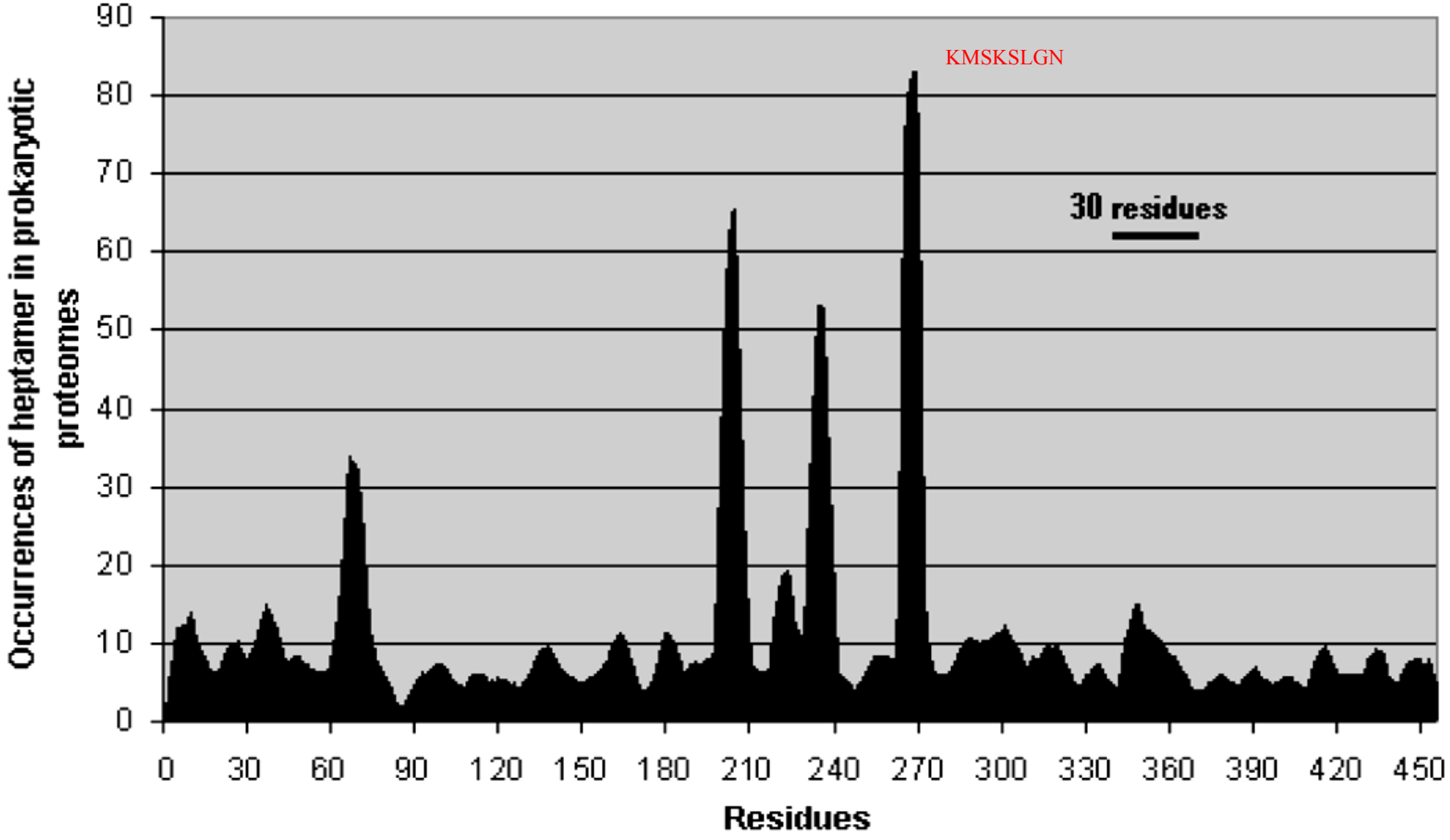


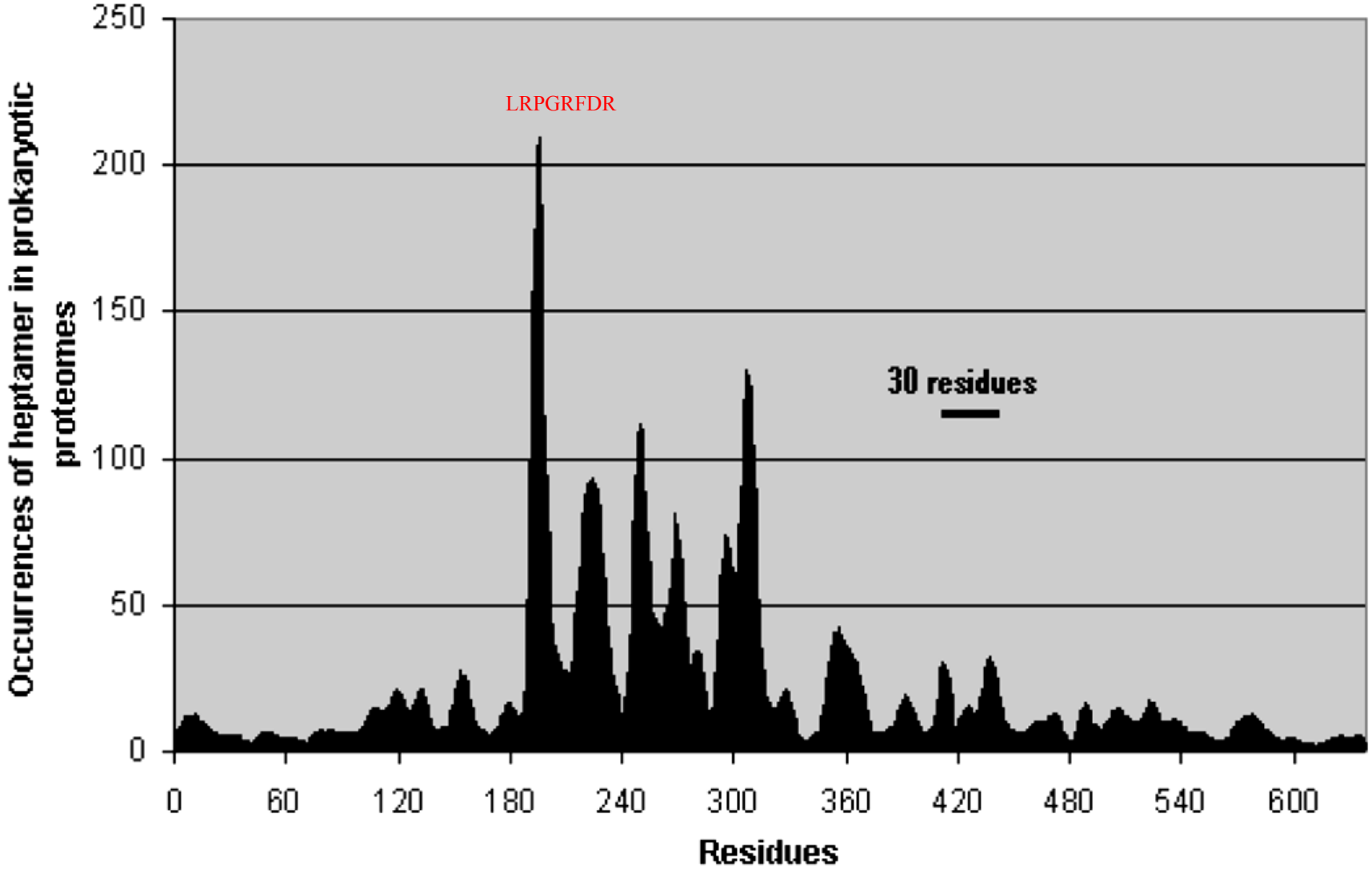
Intact elongation factor, Chain A, *E. Coli*

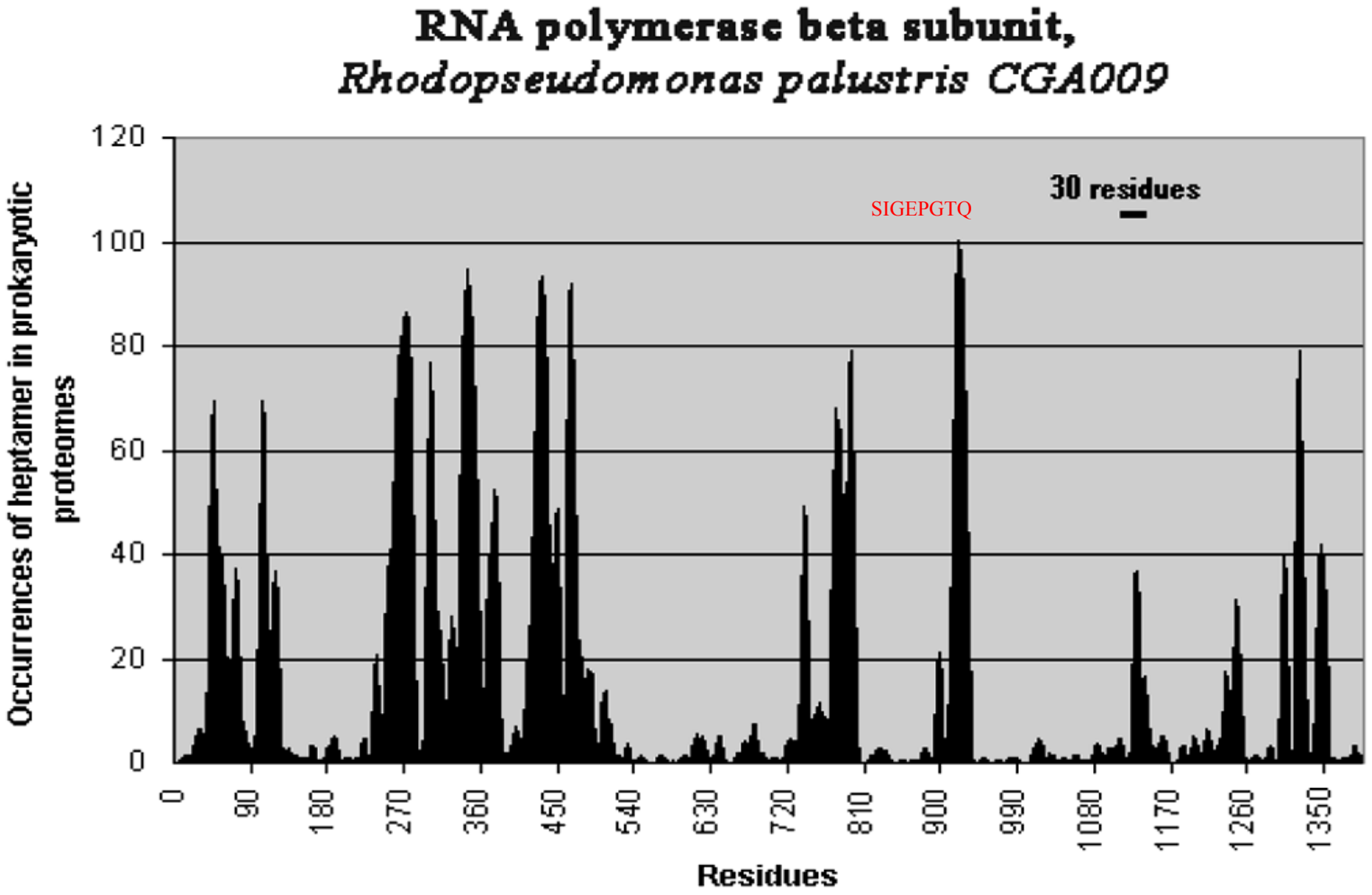


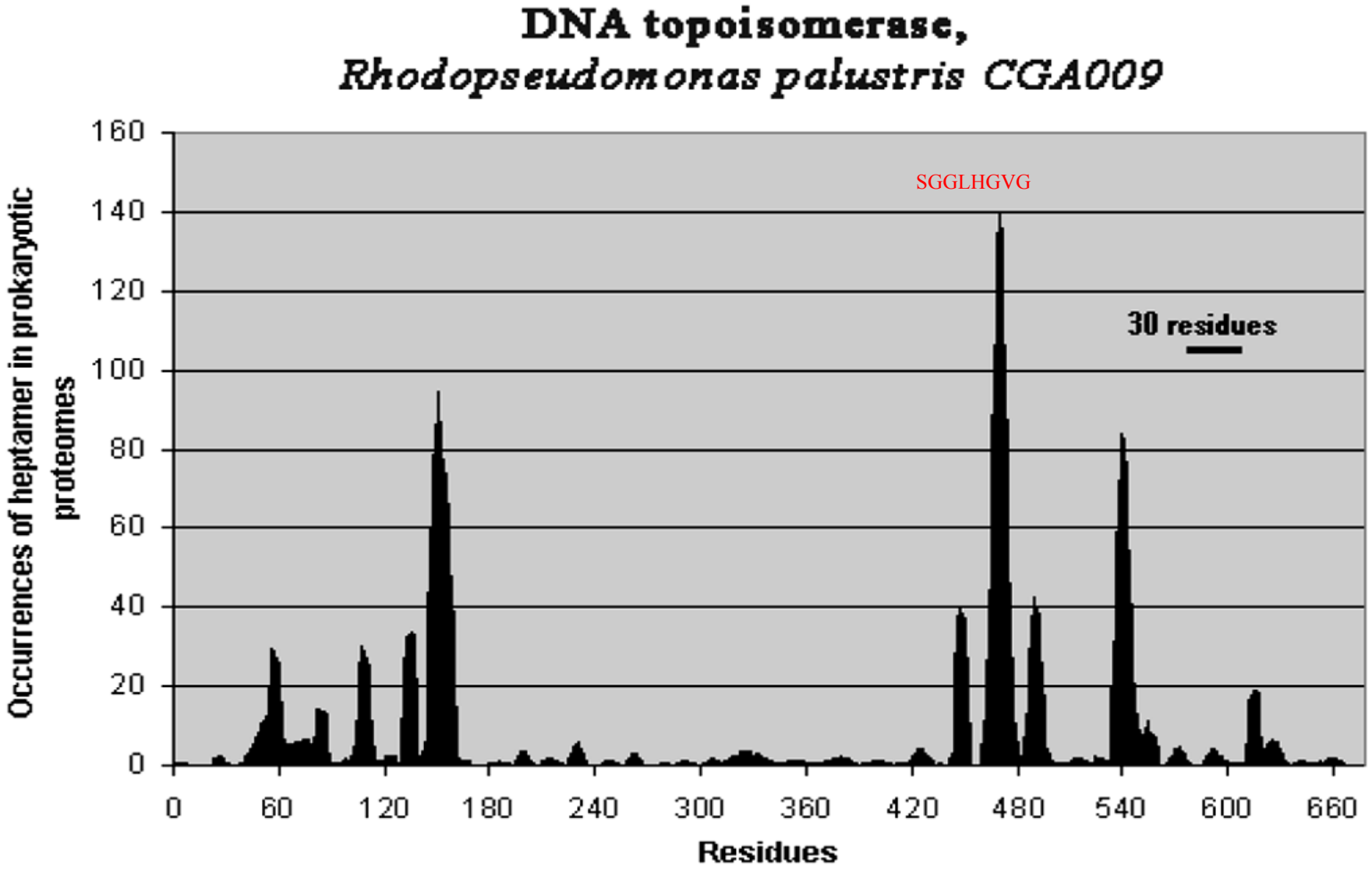
ATP-binding component of high-affinity phosphate-specific transport system, *E. Coli*

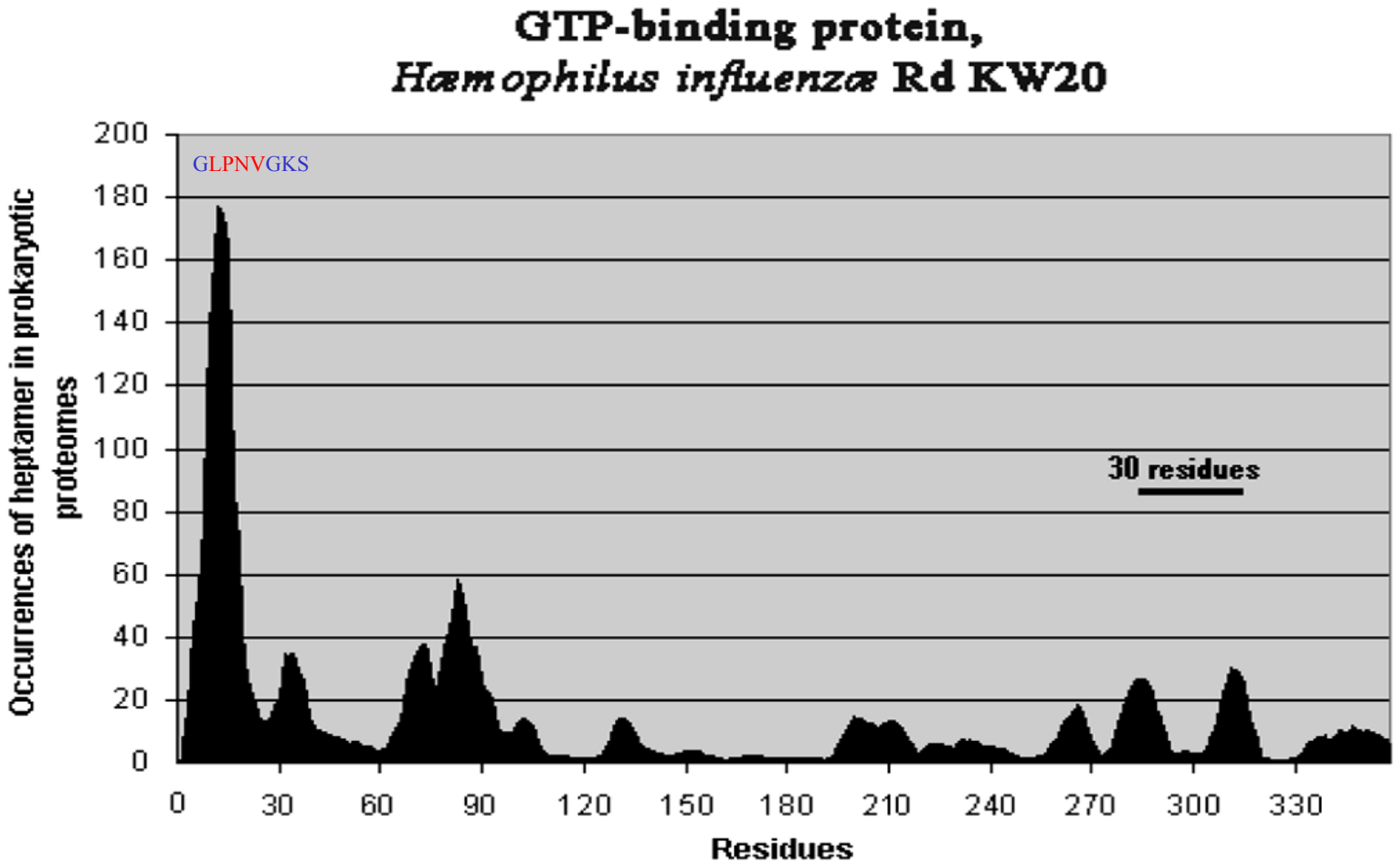


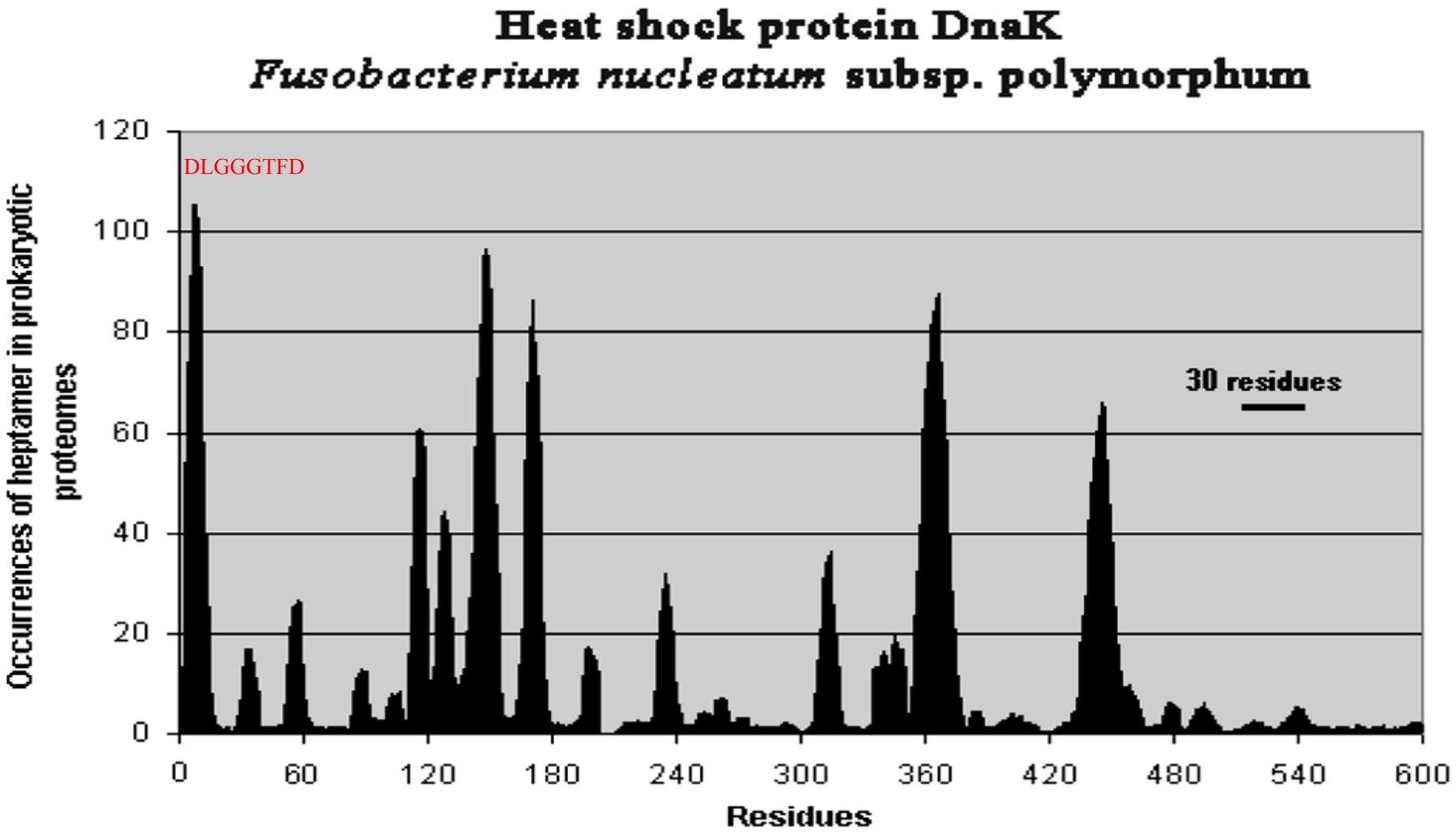

cysteine tRNA synthetase, E. Coli K12
KMSKSLGN
30 residues
Occurrences of heptamer in prokaryotic proteomes
0
10
20
30
40
50
60
70
80
90
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
Residues

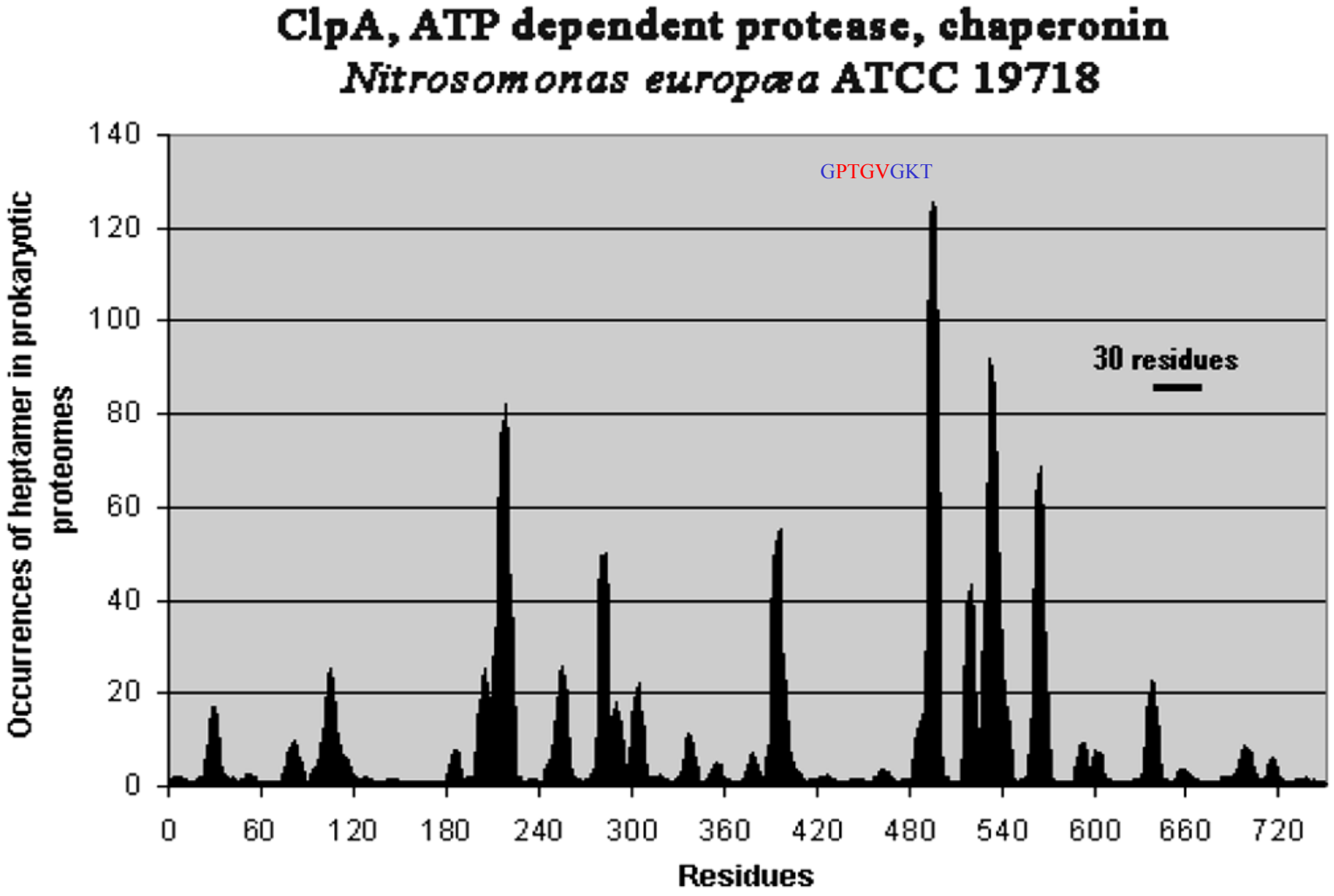

Cell division protein ftsH, E. Coli
LRPGRFDR
30 residues
Occurrences of heptamer in prokaryotic proteomes
Residues
0
50
100
150
200
250
0
60
120
180
240
300
360
420
480
540
600

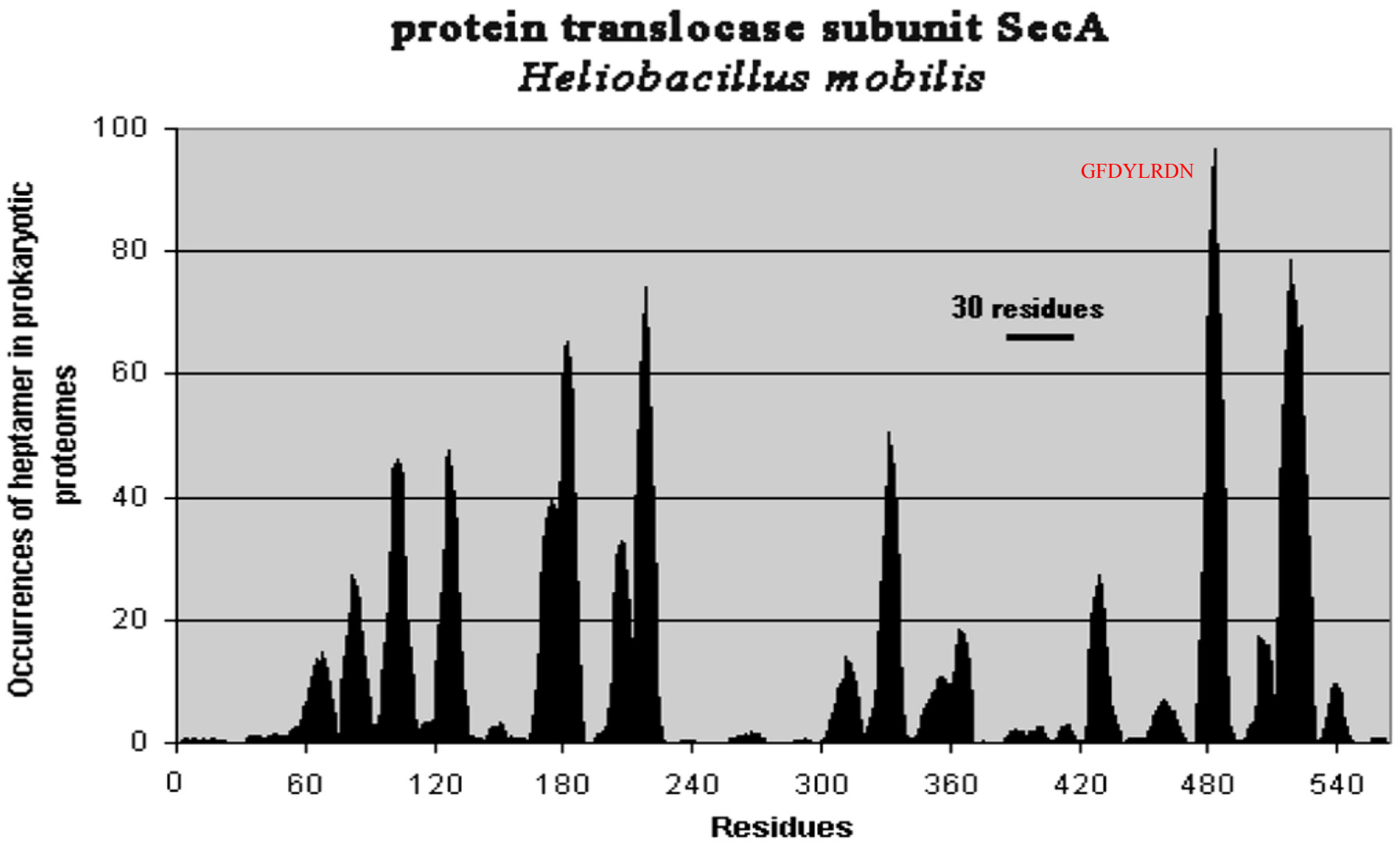

RNA polymerase beta subunit,
Rhodopseudomonas palustris CGA009
30 residues
SIGEPGTQ
Occurrences of heptamer in prokaryotic proteomes
120
100
80
60
40
20
0
0
90
180
270
360
450
540
630
720
810
900
990
1080
1170
1260
1350
Residues

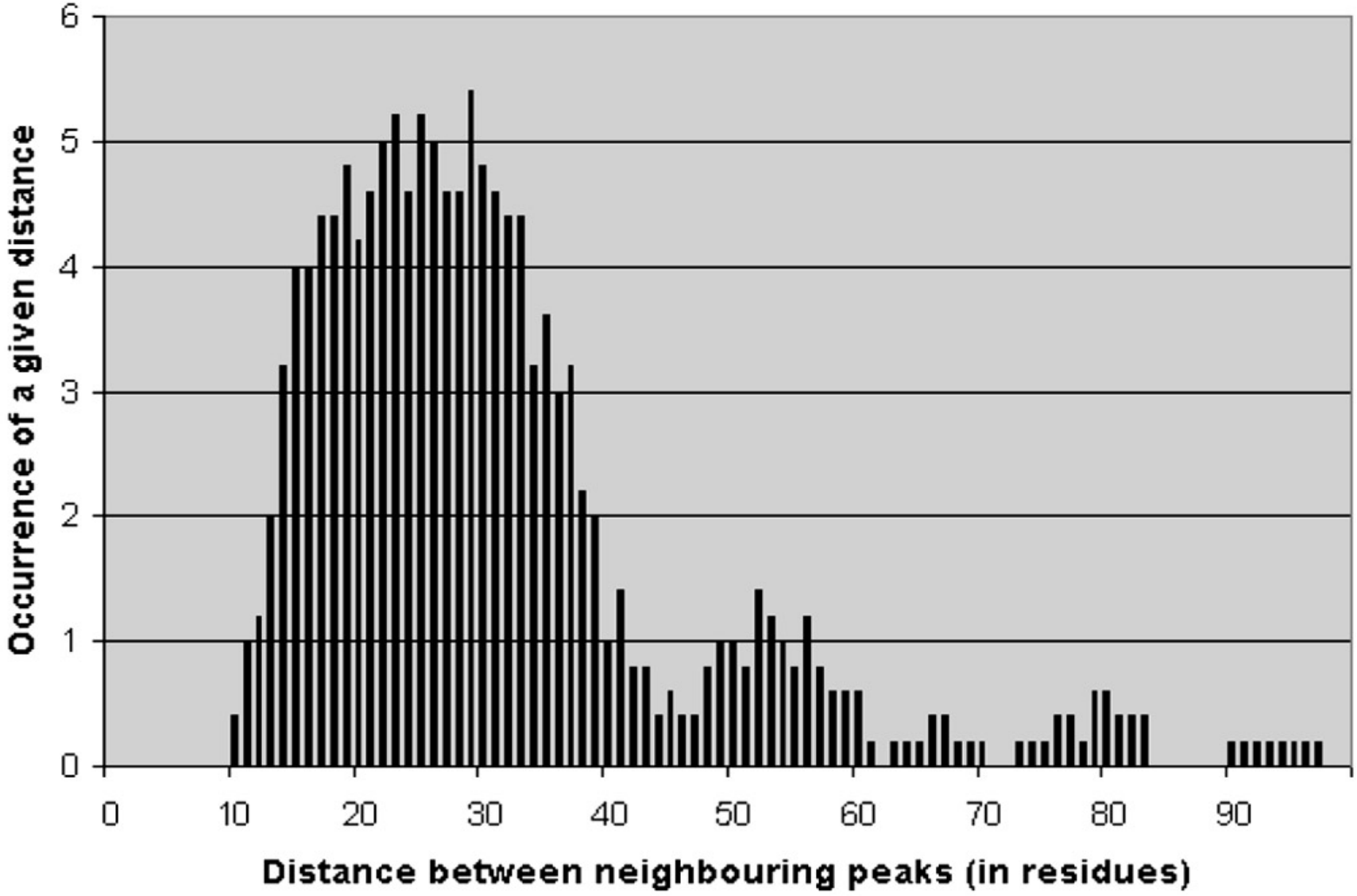

DNA topoisomerase,
Rhodopseudomonas palustris CGA009
SGGLHGVG
30 residues
Occurrences of heptamer in prokaryotic proteomes
Residues
0
20
40
60
80
100
120
140
160
0
60
120
180
240
300
360
420
480
540
600
660


GTP-binding protein,
Hæmophilus influenzæ Rd KW20
GLPNVGKS
30 residues
Occurrences of heptamer in prokaryotic proteomes
Residues
0
20
40
60
80
100
120
140
160
180
200
0
30
60
90
120
150
180
210
240
270
300
330


Heat shock protein DnaK
Fusobacterium nucleatum subsp. polymorphum
DLGGGTFD
30 residues
Occurrences of heptamer in prokaryotic proteomes
Residues
0
20
40
60
80
100
120
0
60
120
180
240
300
360
420
480
540
600


ClpA, ATP dependent protease, chaperonin
Nitrosomonas europæa ATCC 19718
GPTGVGKT
30 residues
Occurrences of heptamer in prokaryotic proteomes
Residues
0
20
40
60
80
100
120
140
0
60
120
180
240
300
360
420
480
540
600
660
720


protein translocase subunit SecA
Heliobacillus mobilis
GFDYLRDN
30 residues
Occurrences of heptamer in prokaryotic proteomes
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
540
Residues


Occurrence of a given distance
0
1
2
3
4
5
6
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

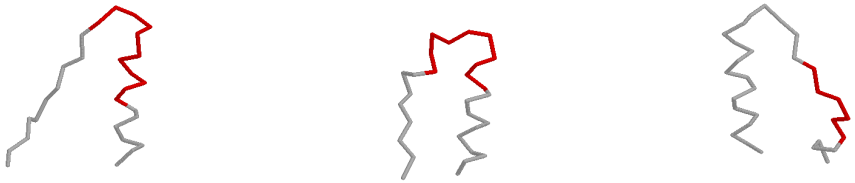
(32-72)GPSGSGKTTLL(29-41)MVFQNYALFPHLTALENV(31-42)QLSGGQQQRVAIARAL(6 LLADEPTSALD(21-22)IYVTHDQ(28-263) **consensus**

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**

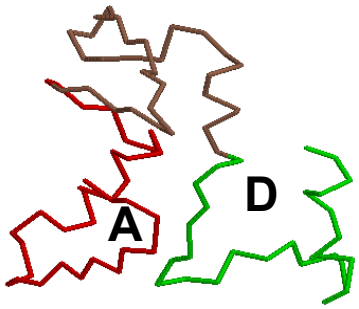
(62) KVVLLNRAPTLHRLGIQAF (18) AFNADFDGDQMAVH (776) 2A6E

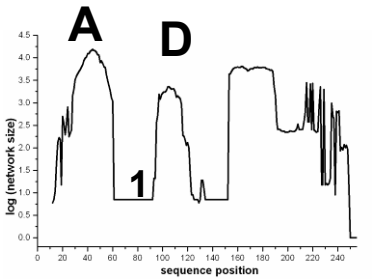
(59-84)HPVLLNRAPTLHRLGIQAF (18) AFNADFDGDQMAVH (131-961) consensus

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

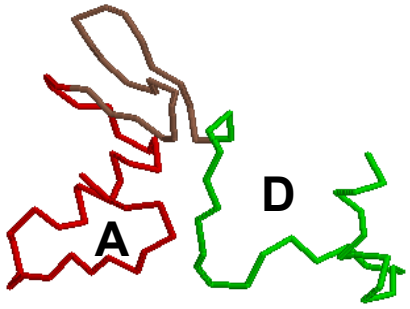
These would be of not much interest
for the sequence alignment community

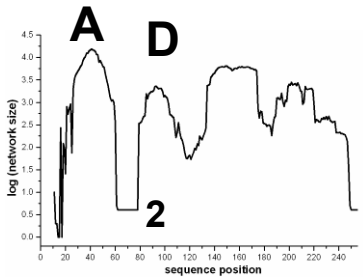
silent module 1



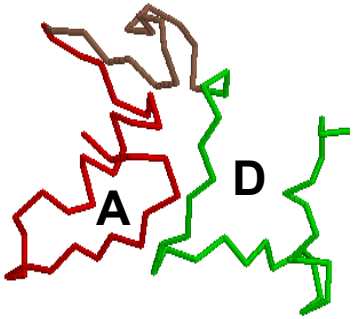


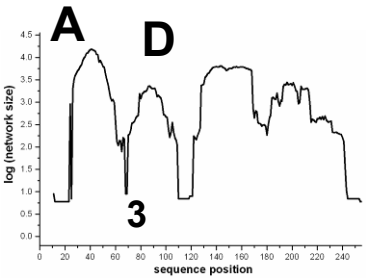
silent module 2





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:

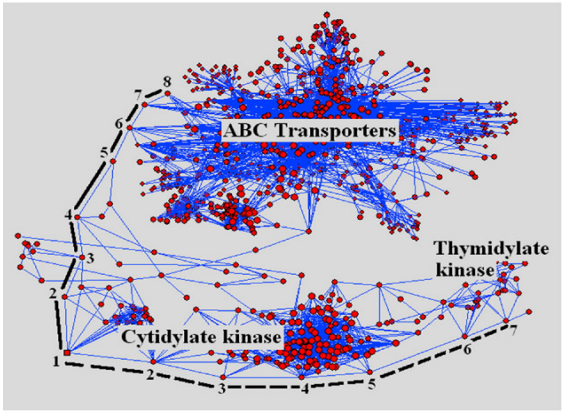
Protein world is world of modules

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

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)		
1	VITIDGPSGAGKGTLCKAMA	P23863
2	IITIDGPSGTGKSTLAKALA	O84458
3	NIAIDGPSGVGKSTIAKKLA	Q98RC0
4	KIAIDGPAGAGKSTVAKKLA	Q8RA78
5	TIAIDGPAGAGKGTLARRLA	Q98CC2
6	LIAIEGIDGAGKTTLARRLA	Q8PFG7
7	FIAVEGIDGAGKTTLAKSLS	Q97CC8

Examples of evolutionary paths

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
```

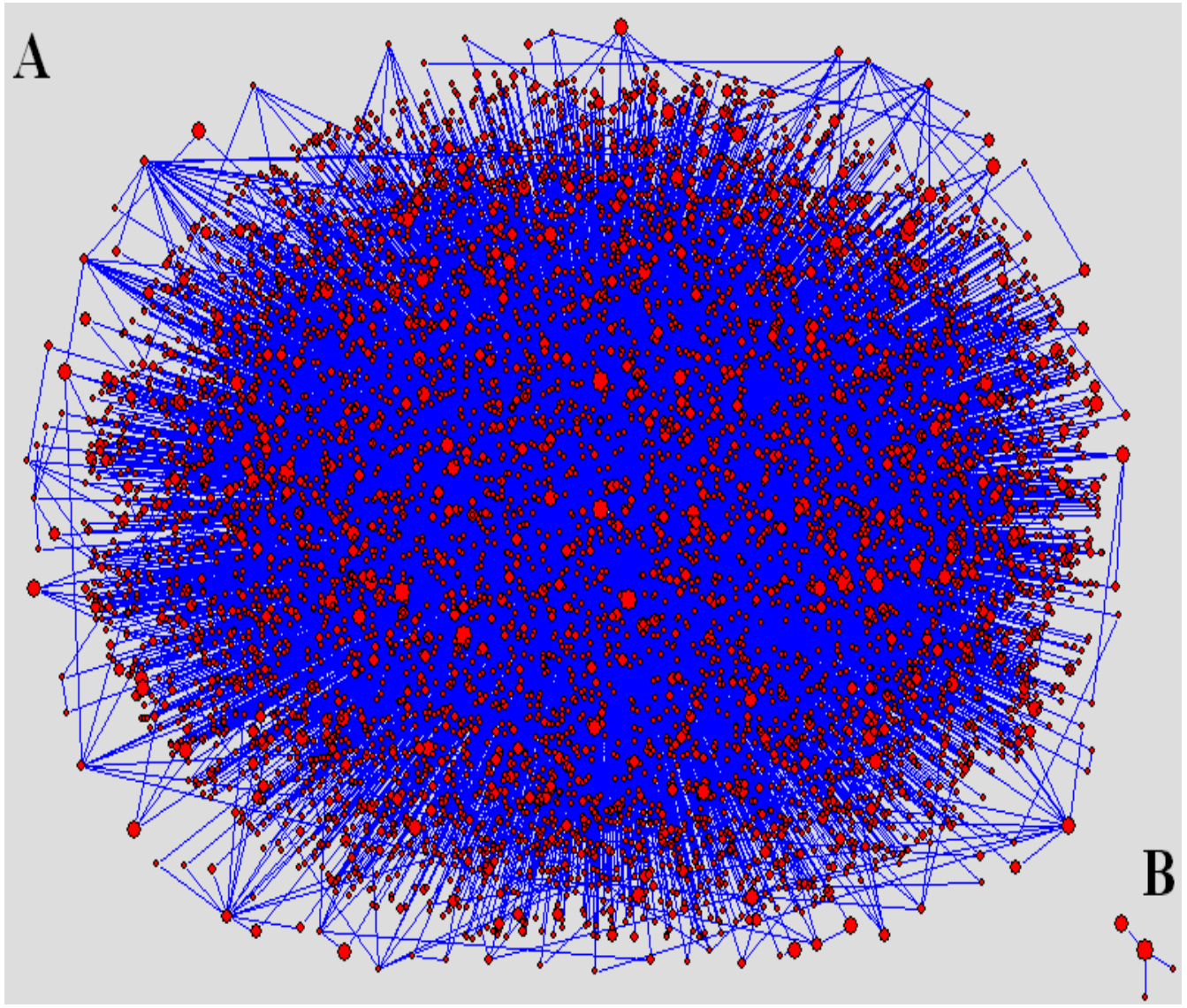
Omnipresence is a new measure of sequence conservation. These elements are the most conserved ones, coming, presumably from last common ancestor

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

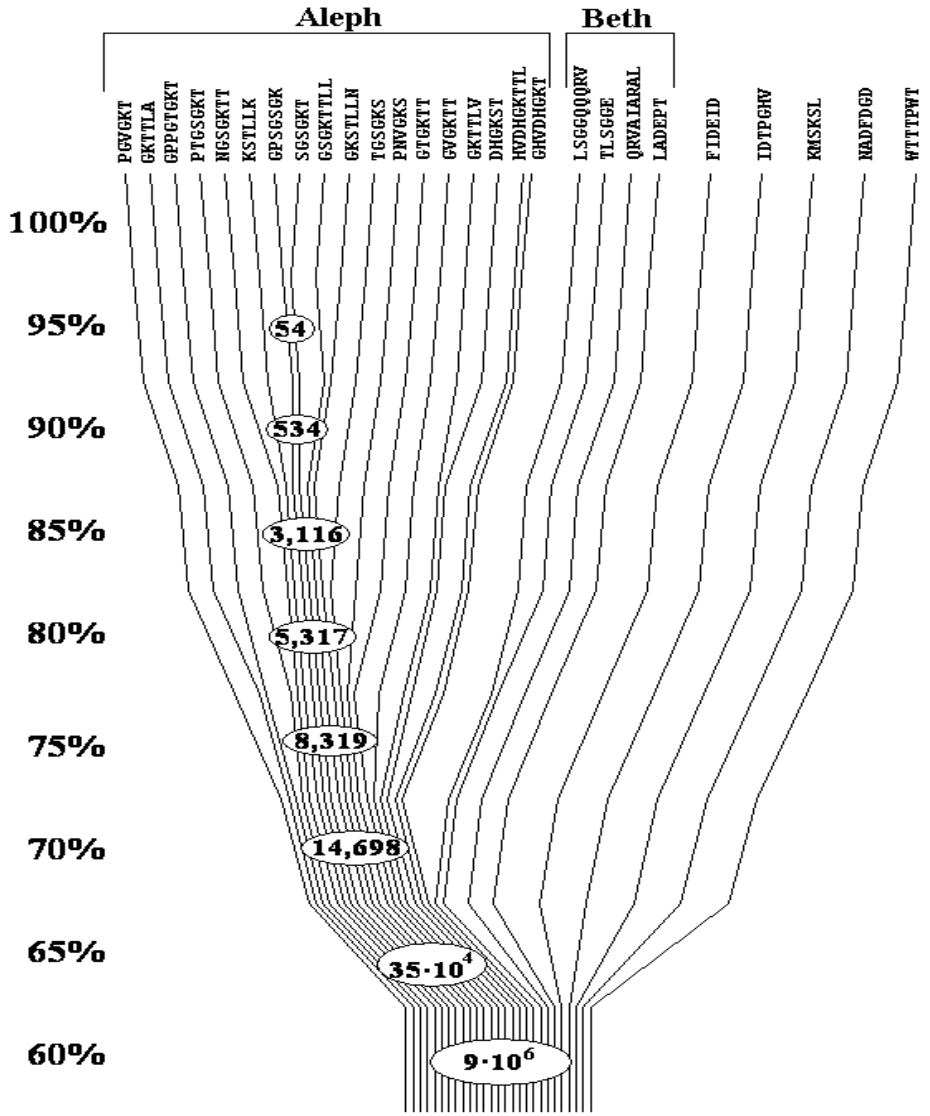
```
IDTPGHVDHGKTTLLN      ALEPH
  |
  TLSGGQQQRVAIARAL    BETH
```

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	AQTIALIGESGSGKSTLLGI	Q8ZCB4	QVVVVGAGPSGSTVSALLKS	Q87R97
4	ATLAALIGAGGLGKLILLGI	Q813M6	DVVVVGAGPSGSSAARYLSE	O66509
5	AVIAALIGAGGFGALVFQGL	Q8X670	DVVVIGAGPGGYVAAIRASQ	Q9A7J2
6	VVLAGLVGAGGLGAEVTRGL	Q8U8Y4	DAVIIGGGPGGYVCAIKLAQ	Q9WYL2
7	VVGGGVVGAGTALDAVTRGL	Q82DH4	FAVITGGGPGAMEAANKGAQ	Q8KC62
8	VVGGGSTGAGVARDLAMRGL	Q9HNS4	LTVATGGGPGAMEAANLGAY	O86748
9	VVGGGFTGQSAALHLAEGGL	Q8UCD8	LDVGTGSGVLAMAAAKLGAA	Q9RU72
10	LCGGGFTGQSQALRLAIARA	Q8A0Z5	LDLGTGSGALAVHAARLGAR	Q826J9
11	LSGGERIALSIALRLAIAKA	Q97WH0	LDTGIMSGADIVAAIALGAR	Q9CBF2
12	LSGGQRRALGIALALASNPE	Q9YBQ1	MDGGIRSGQDVLKAVALGAR	Q8UD10
13	LSGGQRQRVAIARALALDPD	Q82BU6	VSGGIRSGADVAKALALGAD	Q8U870
14	ASGGMRDGVMMAKALAMGAS	O58893		
15	LSGGMRQRVMIAIALACGPD	Q89KL2		
16	LSGGQRQRVAIARALALDPD	Q82BU6		
	C		D	
1	GEFVAIVGPSGCGKSTLLRL	Q825G5	GEFVAIVGPSGCGKSTLLRL	Q825G5
2	GQVVVVLGPSGSGKSTLCRT	Q8RQL7	GKLVALLGPSGSGKSTLLRL	Q8Z0H0
3	GQVVMVTGAGGSIGSELCRQ	Q9HZ86	NKLVLLTGPSGSGKSTLALD	Q9KEY5
4	RKVAFVTGGAGGIGSETCRQ	Q9KCM1	IHLVNLSGPAGSGKTILALA	Q887P5
5	GRVAFVTGGAGGIGRATAER	Q8UA89	GHLQSASGPLGLMKTILALR	O50436
6	GKTAFITGGGQGIGLACAEA	Q89QA5	GHMDAAAGIGGLIKTVLALR	Q8U9Q4
7	LVTGANTGLGQGIALALAEA	Q8PE31	GHTGGAAGIAGLLKAVLAIE	O06586
8	LVTGANKGIGLAIARQLGAA	Q7CP30	GRTGGWAAIAGLLAAIGATV	Q98BE5
9	LVTGSSQGIGAAIAAGLARA	Q9RK29	GSRGIGAAIARRLAADGAHV	Q8XT12
10	SACGSSSGSGAAVAAGLAPL	Q9A5H4	ASRGIGKAIAEVAARDGAPV	Q92PY2
11	LPGGSSSGAGVVVAAGLVPV	Q8UAX4	SSGKMGYAIAEVAANLGADV	Q819T8
12	ISGGSSGGSAVAVALGLVDV	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…"

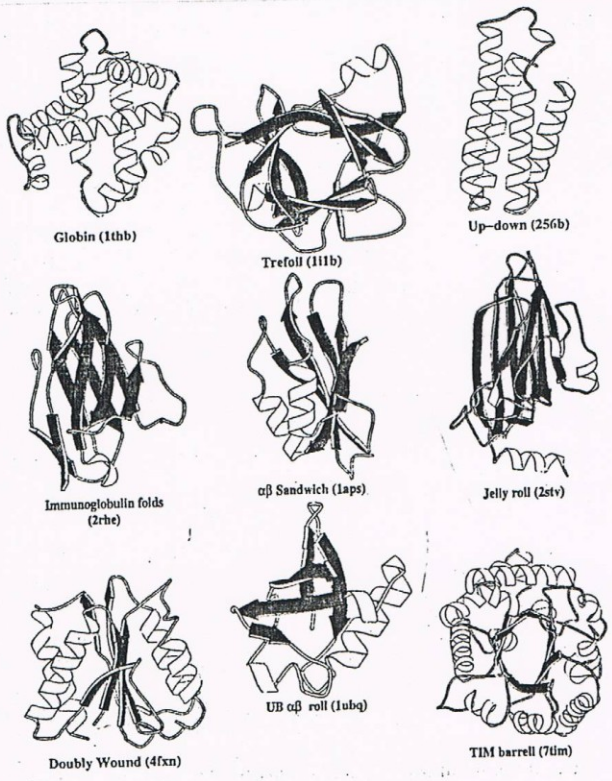
The Svedberg
Mass and size of protein molecules
Nature 123, 871 (1929)

~ 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



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)

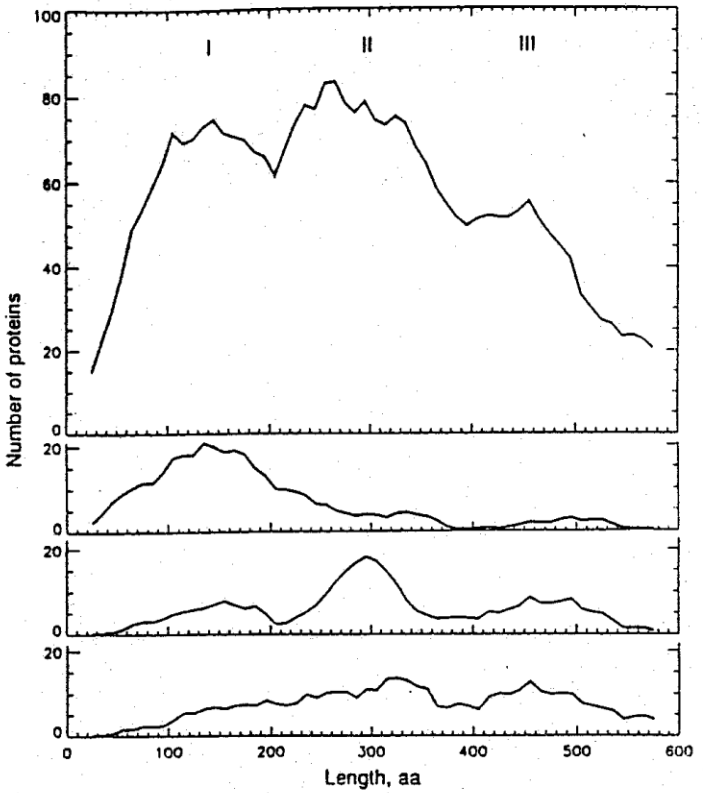


FIG. 4. Components of prokaryotic protein length distribution. Smoothed distributions (running window of 50 aa) are shown for groups of proteins that are major contributors to the peaks indicated (I–III).

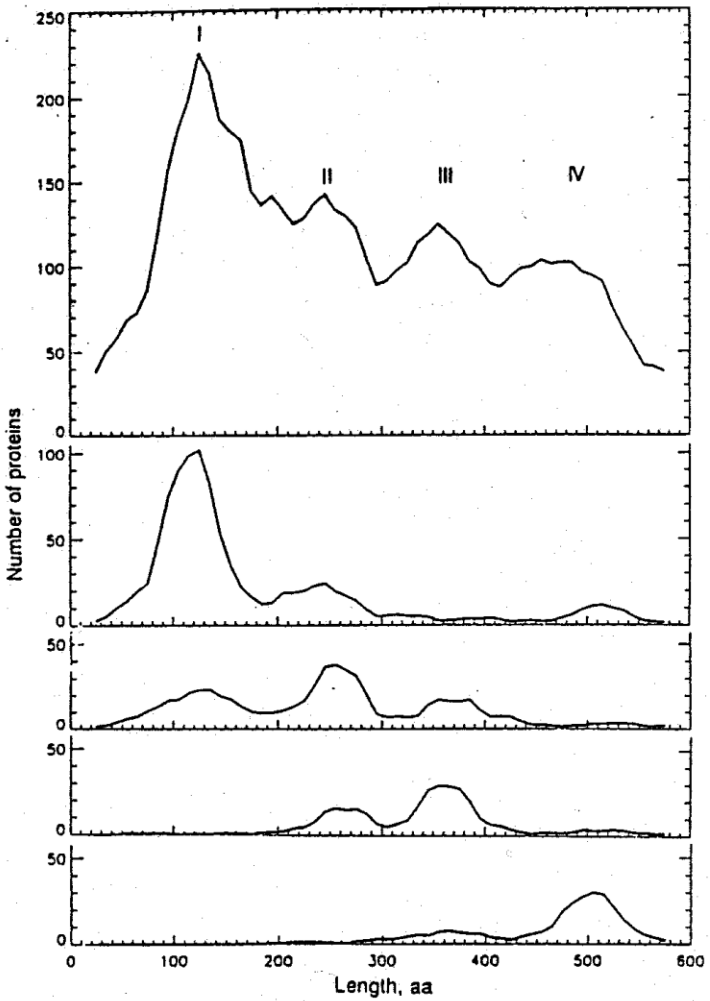
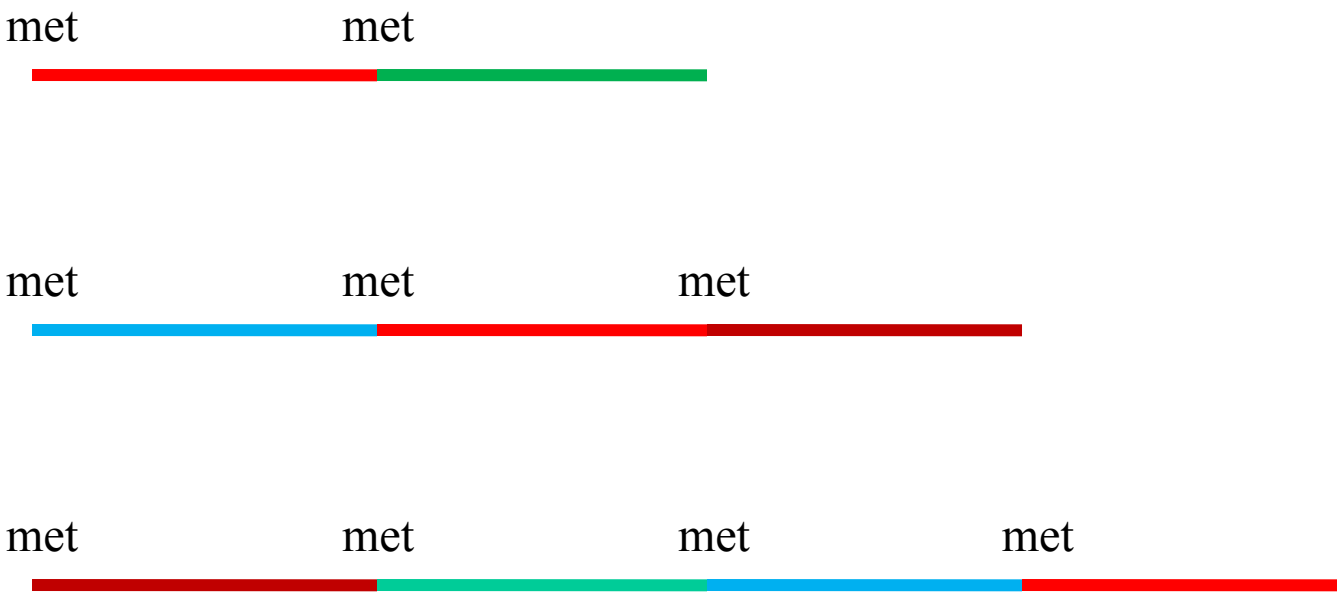
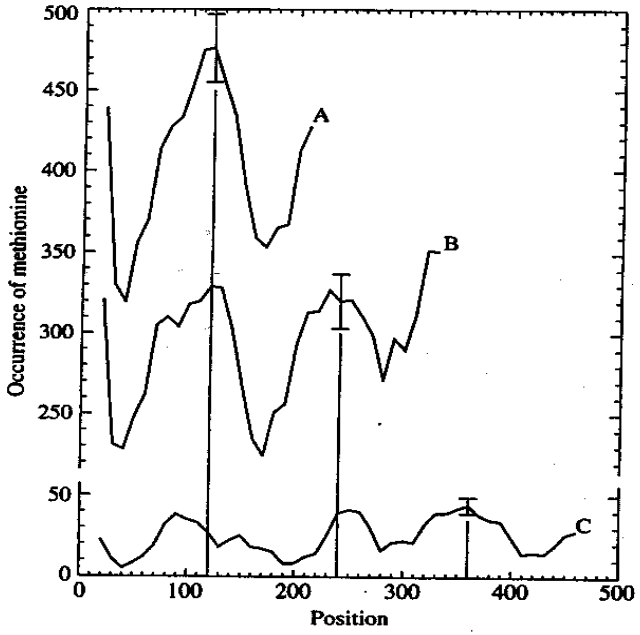
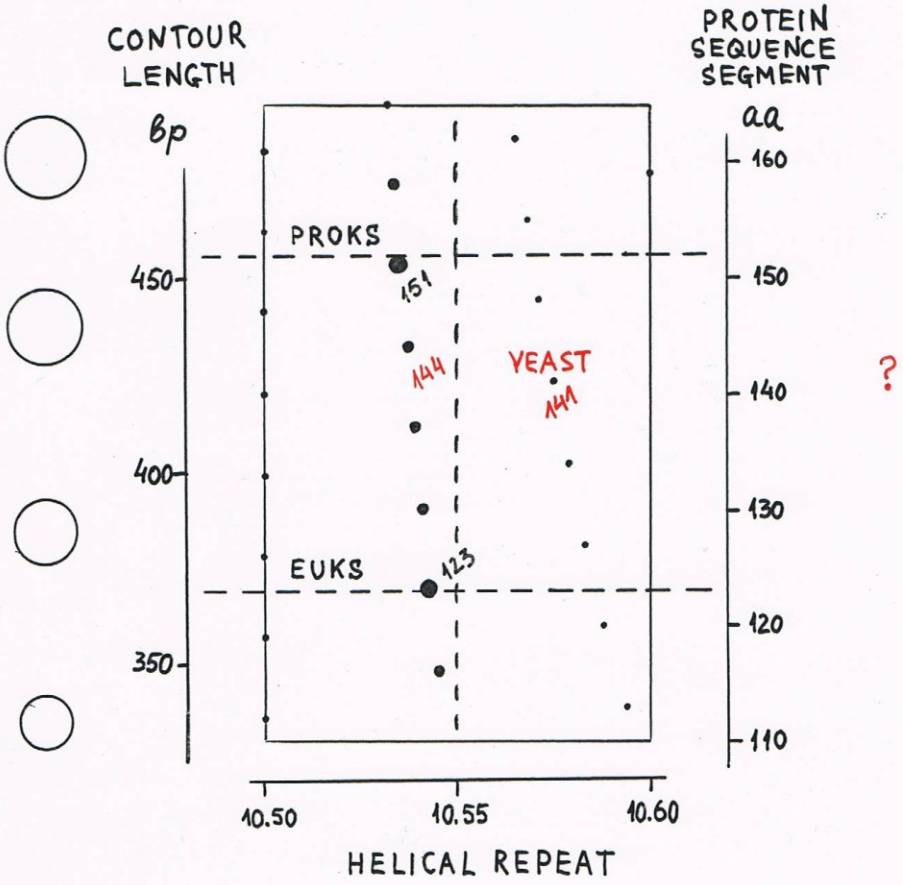


FIG. 2. Components of eukaryotic protein length distribution. Smoothed distributions (running window of 50 aa) are shown for groups of proteins that are major contributors to the peaks indicated (I–IV).


met
met
met
met
met
met
met


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


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

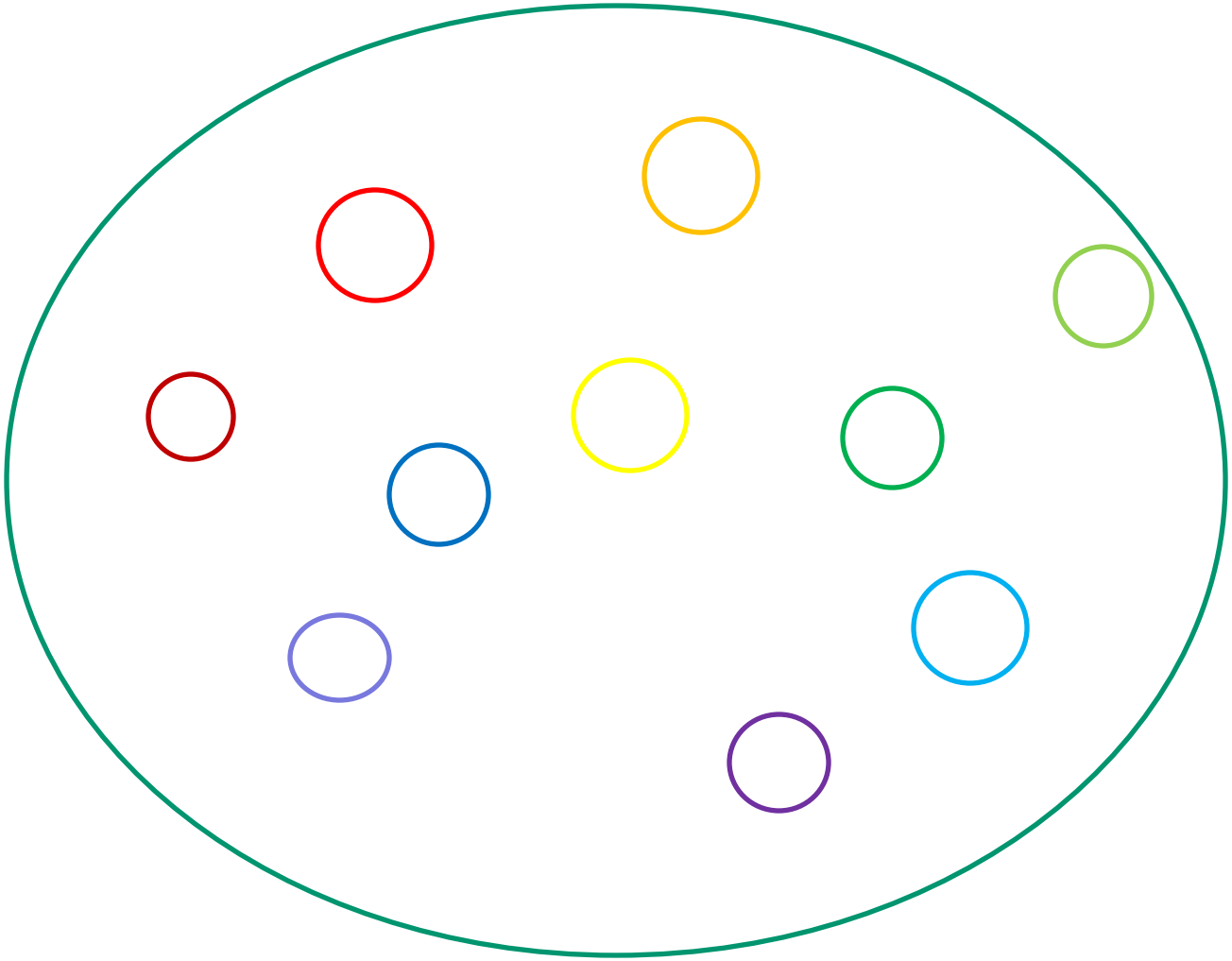
?

The Lord Of The Rings

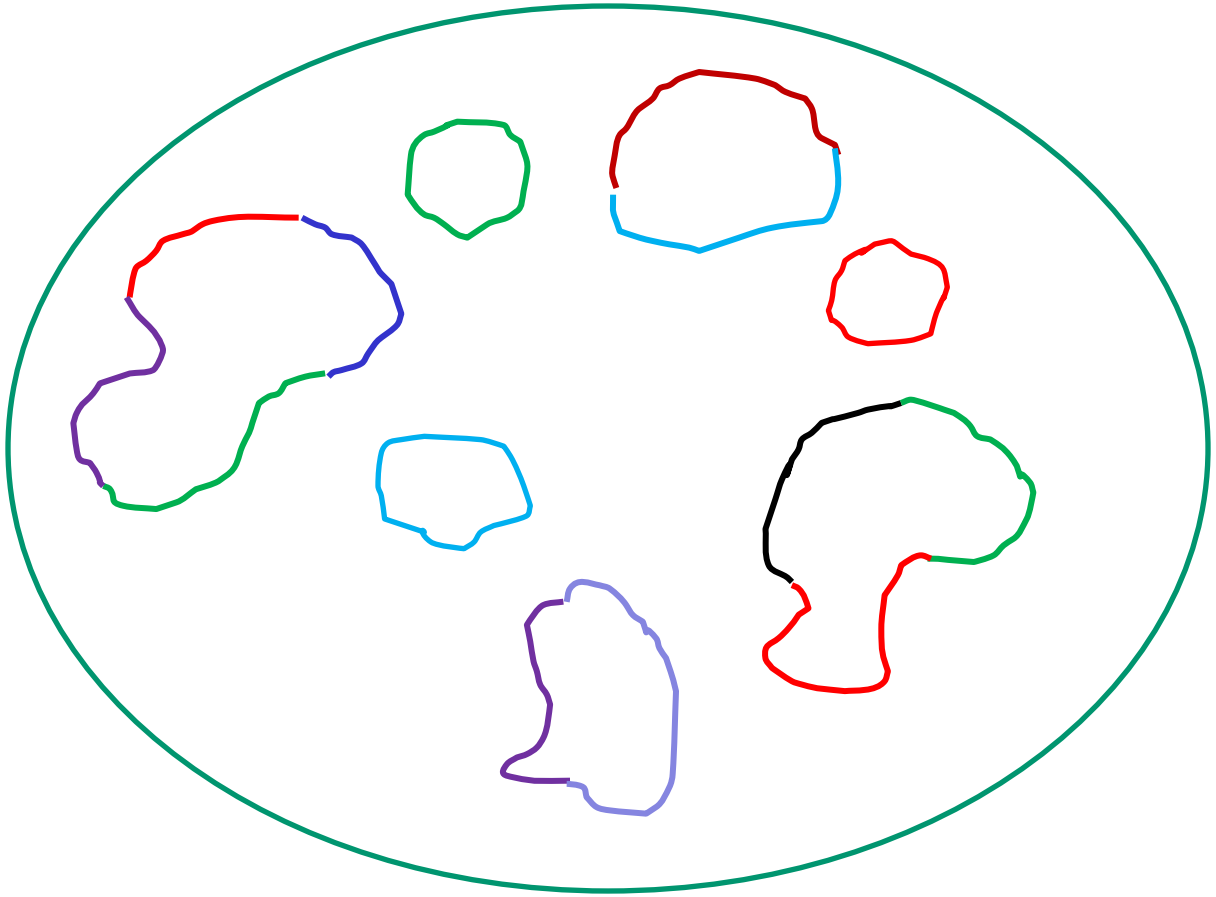
Three rings for the Elven-kings under the sky,
Seven for the Dwarf-lords in their halls of stone,
Nine for Mortal Men doomed to die,
One for the Dark Lord on his dark throne.

J. R. R. Tolkien

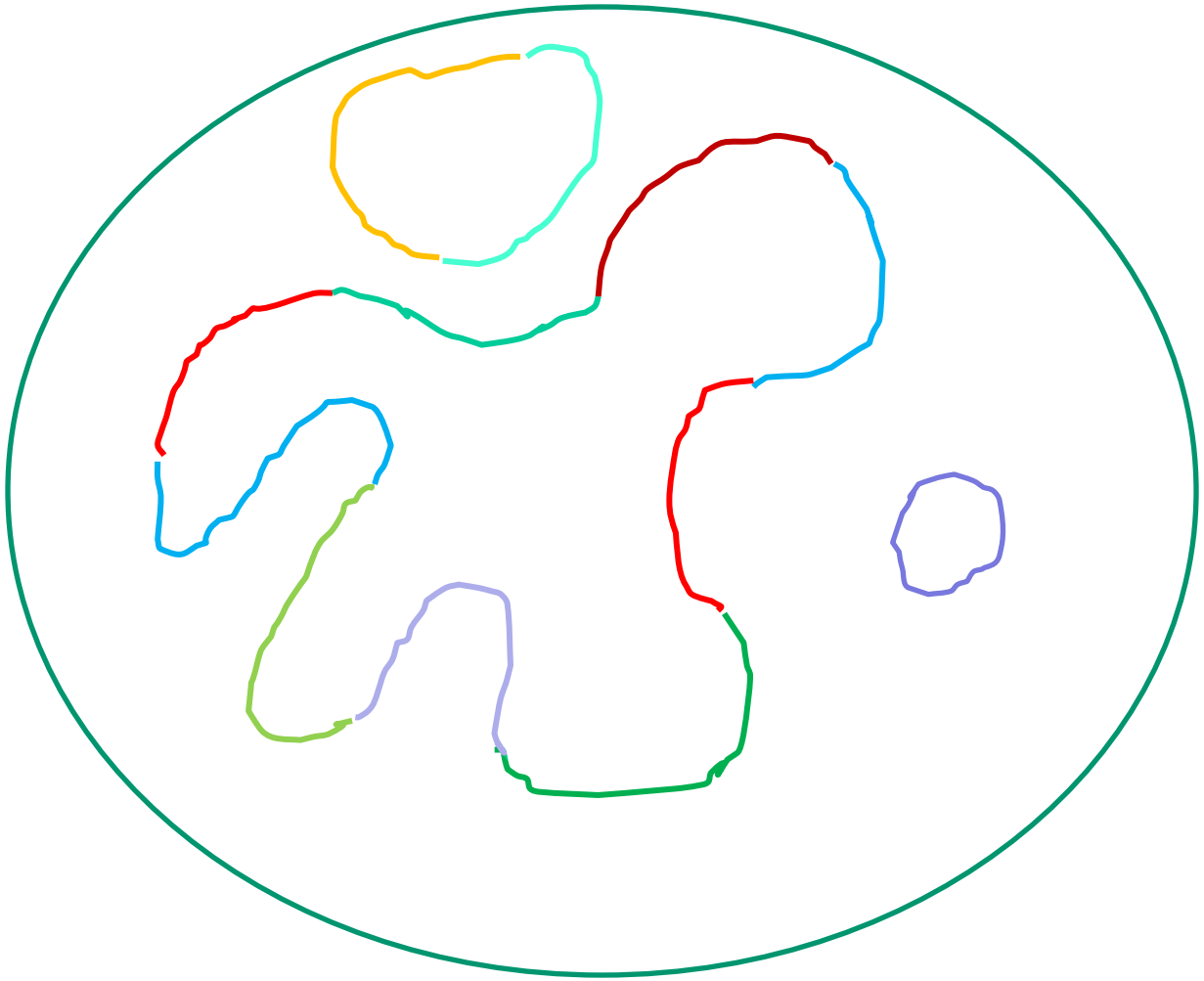
Pre-genomic, pre-recombination stage



Pre-genomic, recombination stage



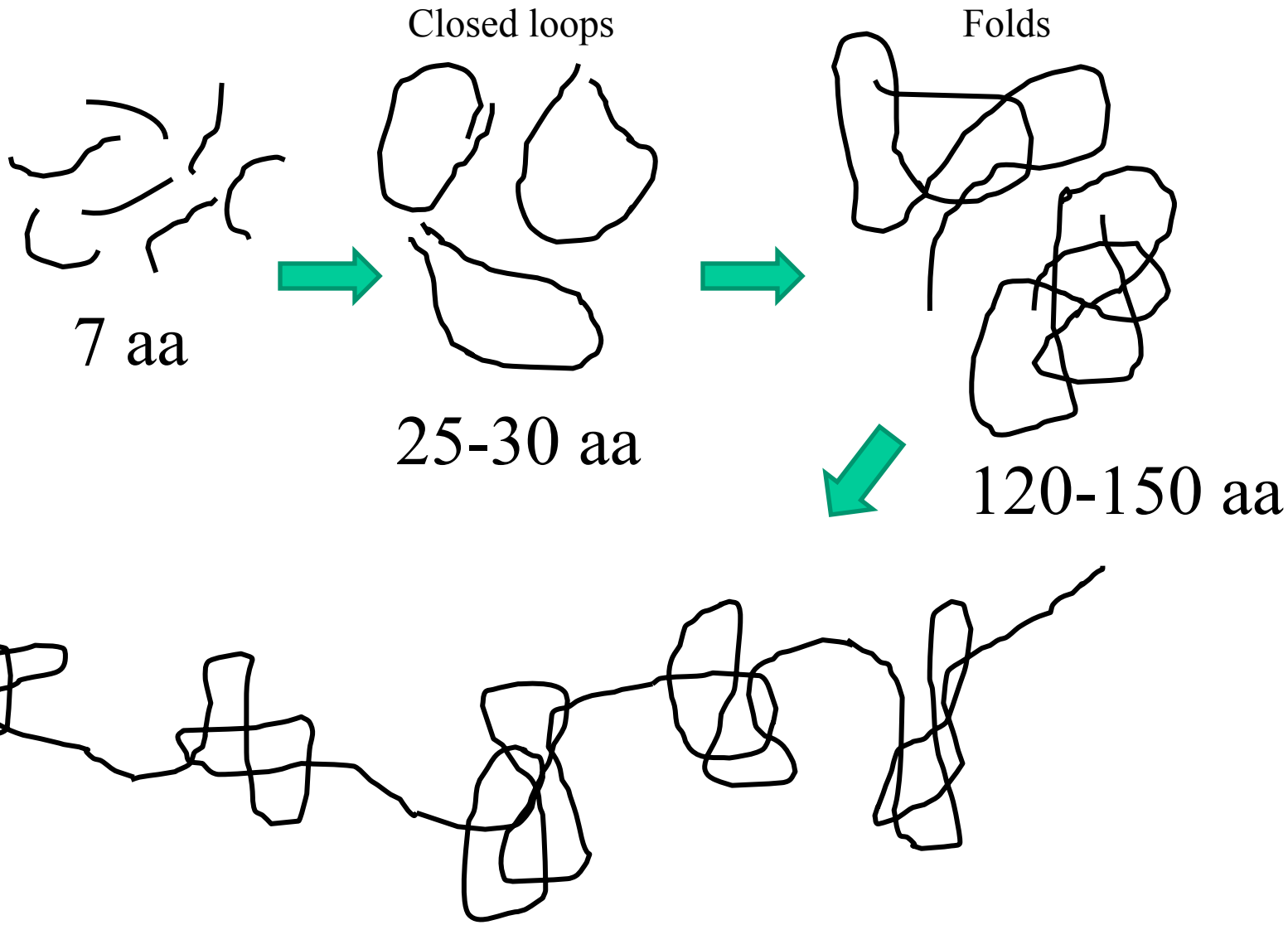
Early genomic stage



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

D. Reanney

Bact. Rev. 40, 552, 1976



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	polar amino acids
ala	**arg**
gly	**asn**
ile	asp
leu	cys
met	**glu**
phe	gln
pro	his
val	**lys**
	ser
	thr
	trp
	tyr

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

(glu asn phe arg lys phe ser lys ile phe)glu asn phe

period 3.5
period 3.5

α-helices

10-15 aa long
(30-45 bases in DNA)

are often amphipathic
(alternating polar/non-polar aa)

with period ~3.5 residues
(~10.5 bases in DNA)

That keeps polar and non-polar residues on opposite sides of the helix

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 !

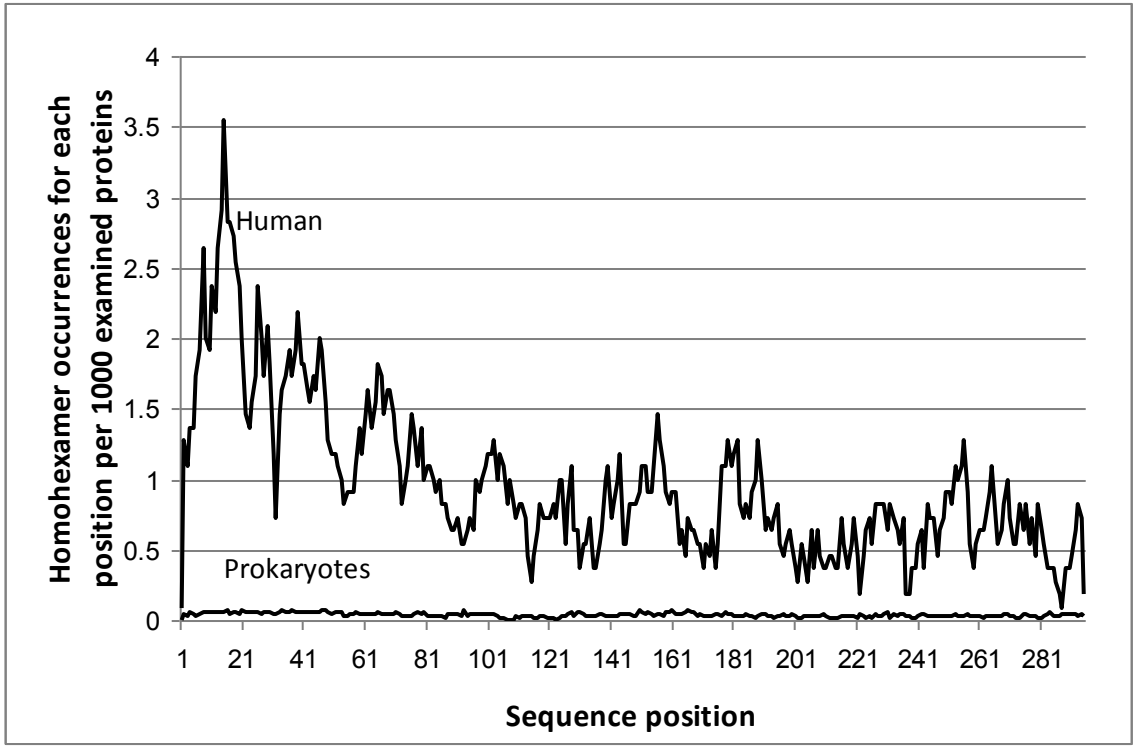
Not only there are many different codes
in the sequences,

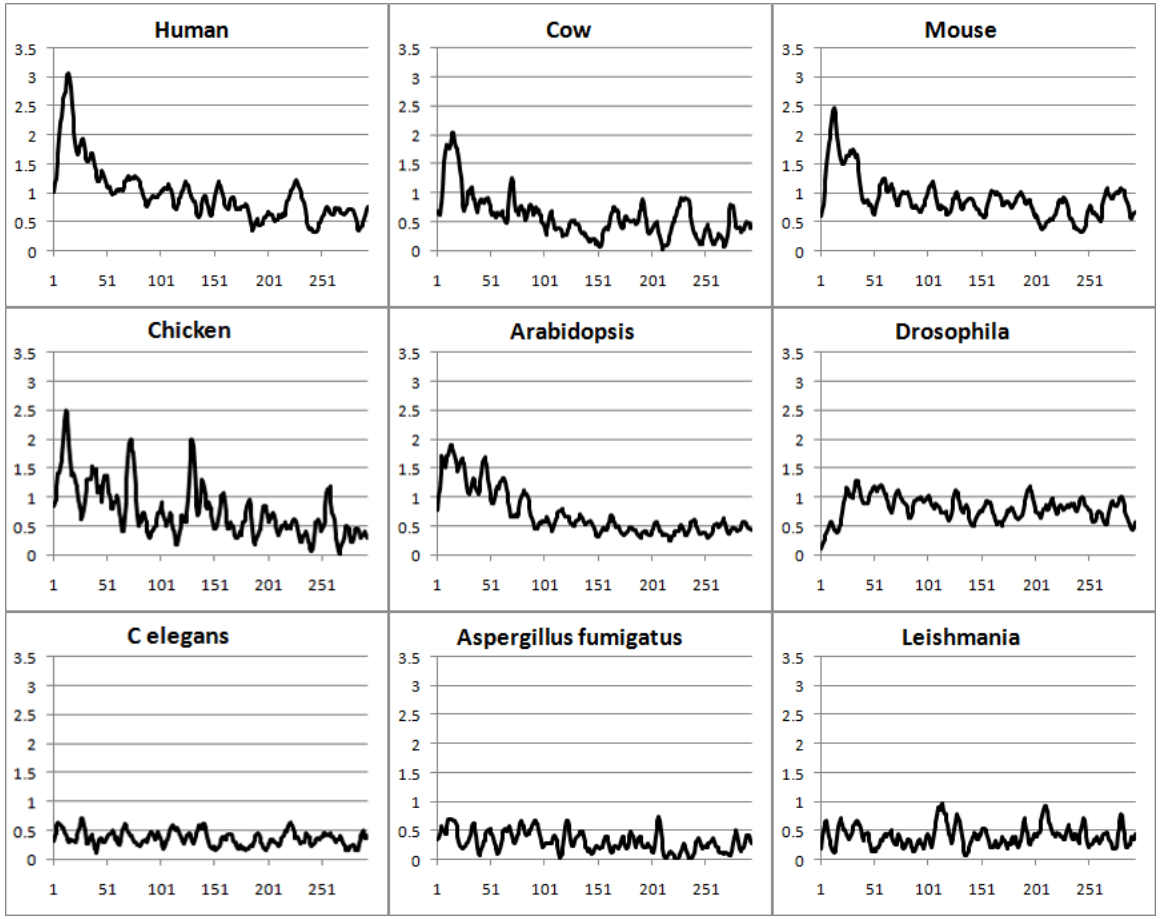
but also they overlap,

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

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

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%**

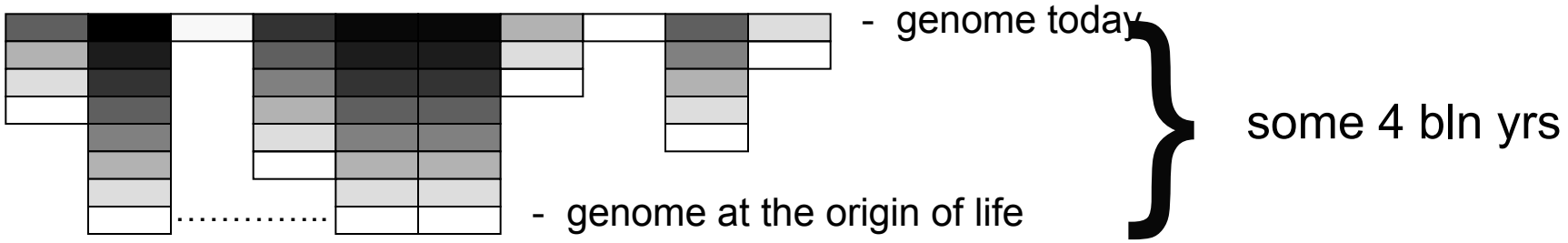
Codons, preferentially used for repeating amino acids in various eukaryotes

	G+C%	**E**	**G**	**K**	**L**	**P**	**Q**	**R**	**S**
A.gambiae	55.8	GAG/**GAA**	GGU	AAA	-	CCA	**CAG**	**-**	**AGC**
D.melan.	53.9	GAG	GGA	AAA/**AAG**	-	CCA	**CAG**	AGG	**AGC**
T.rubrip.	53.5	GAG	-	-	-	-	**CAG**	**-**	**-**
R.norveg.	52.6	GAG	**GGC**	AAA/**AAG**	**CUG**	**CCG**	**CAG**	**AGA**	**AGC**
H.sapiens	52.3	GAG	**GGC**	AAA/**AAG**	**CUG**	CCA/**CCG**/CCU	**CAG**	**CGG**	**AGC**
M.musc.	52.0	GAG	**GGC**	AAA/**AAG**	**CUG**	CCA/CCU	**CAG**	**CGG**	**AGC**
G.gallus	51.4	GAG	**GGC**	**AAG**	**CUG**	**-**	**CAG**	**CGC**	**AGC**
D.rerio	50.2	GAG	-	**AAG**	**CUG**	CCU	**CAG**	**AGA**	UCC
A.thal.	44.6	**GAA**	GGU	**AAG**	**CUU**	CCU	CAA	-	**UCU**
A.mellif.	43.5	-	GGA	AAA/**AAG**	-	-	CAA	AGG	**AGC**
C.elegans	42.9	**GAA**	GGA	**AAG**	**CUU**	CCA	CAA	CGA	UCA
S.cerev.	39.8	**GAA**	**-**	**AAG**	-	CCA	CAA/**CAG**	**-**	**AGC**
P.falcip.	23.8	**GAA**	GGA/GGU	AAA	UUA	CCA	CAA	**AGA**	AGU
Dominant codons:		GAG	**GGC**	**AAG**	**CUG**	CCA	**CAG**	**AGA**	**AGC**

Codons most frequently used by aggressive amino acids

		G+C%	**F**	**L**	**S**	**P**	**Q**	**K**	**E**	**C**	**R**
A.gambiae	55.8	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	**CGG**	**GGC**
D. melan	53.9	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	**CGC**	**GGC**
T. rubrip	53.5	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	AGG	**GGC**
R. norveg	52.6	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	**GAA**	**UGC**	AGG	**GGC**
H. sapiens	52.3	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	**CGG**	**GGC**
M. muscul	52.0	**UUC**	**CUG**	**AGC**	CCU	**CAG**	**AAG**	GAG	**UGC**	AGG	**GGC**
G. gallus	51.4	**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	**AGA**	**GGC**
D. rerio	50.2	**UUC**	**CUG**	**AGC**	CCU	**CAG**	**AAG**	GAG	UGU	**AGA**	GGA
A. thal	44.6	UUU	**CUU**	**UCU**	CCU	CAA	**AAG**	**GAA**	UGU	**AGA**	GGA
A. mellif	43.5	**UUC**	UUG	**UCU**	CCA	CAA	AAA	**GAA**	**UGC**	**AGA**	GGA
C. eleg	42.9	**UUC**	**CUU**	UCA	CCA	CAA	AAA	**GAA**	UGU	**AGA**	GGA
S. cerev	39.8	UUU	UUG	**UCU**	CCA	CAA	AAA	**GAA**	UGU	**AGA**	GGU
P. falcip	23.8	UUU	UUA	AGU	CCA	CAA	AAA	**GAA**	UGU	AGU	GGA
dominant codon:		**UUC**	**CUG**	**AGC**	CCC	**CAG**	**AAG**	GAG	**UGC**	**AGA**	**GGC**

Protein sequences evolve as a mosaic of expanding amino acids,
homopeptides at the moment of expansion event,
gradually mutating to their modern sequence appearance
not recognizable as repeats anymore



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

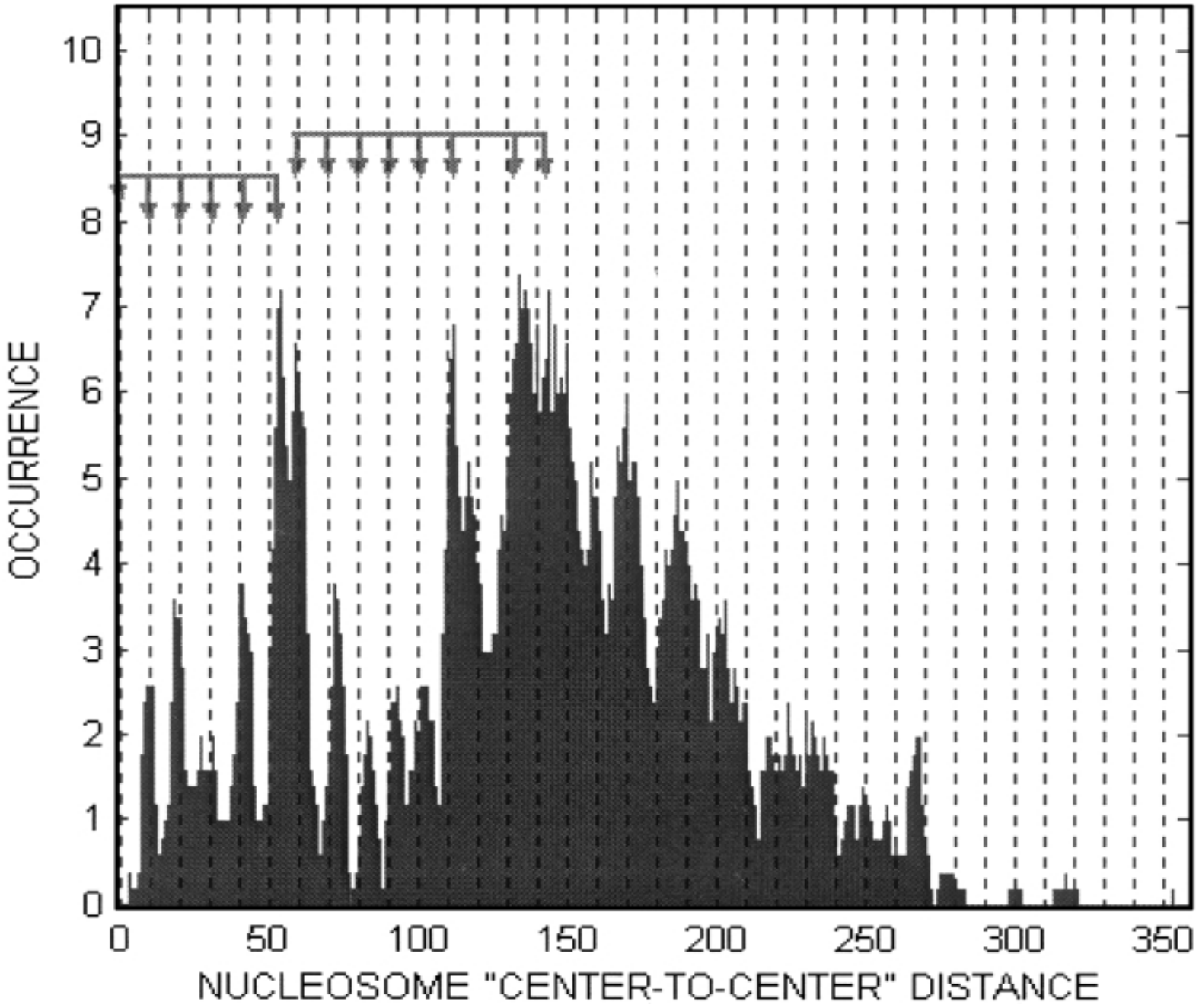
Total 388 slides (2013)

10 2-hour lectures, 40 slides each.

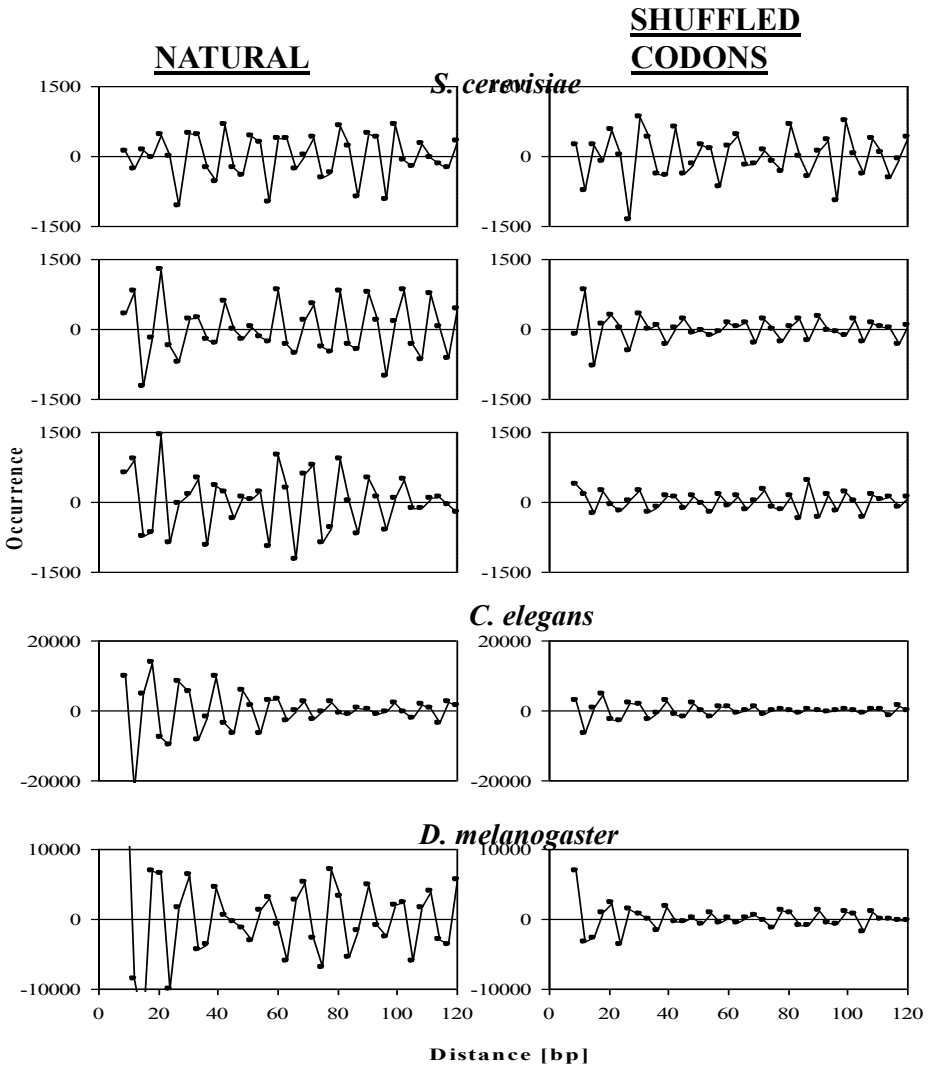
5-lecture course, 200 slides

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