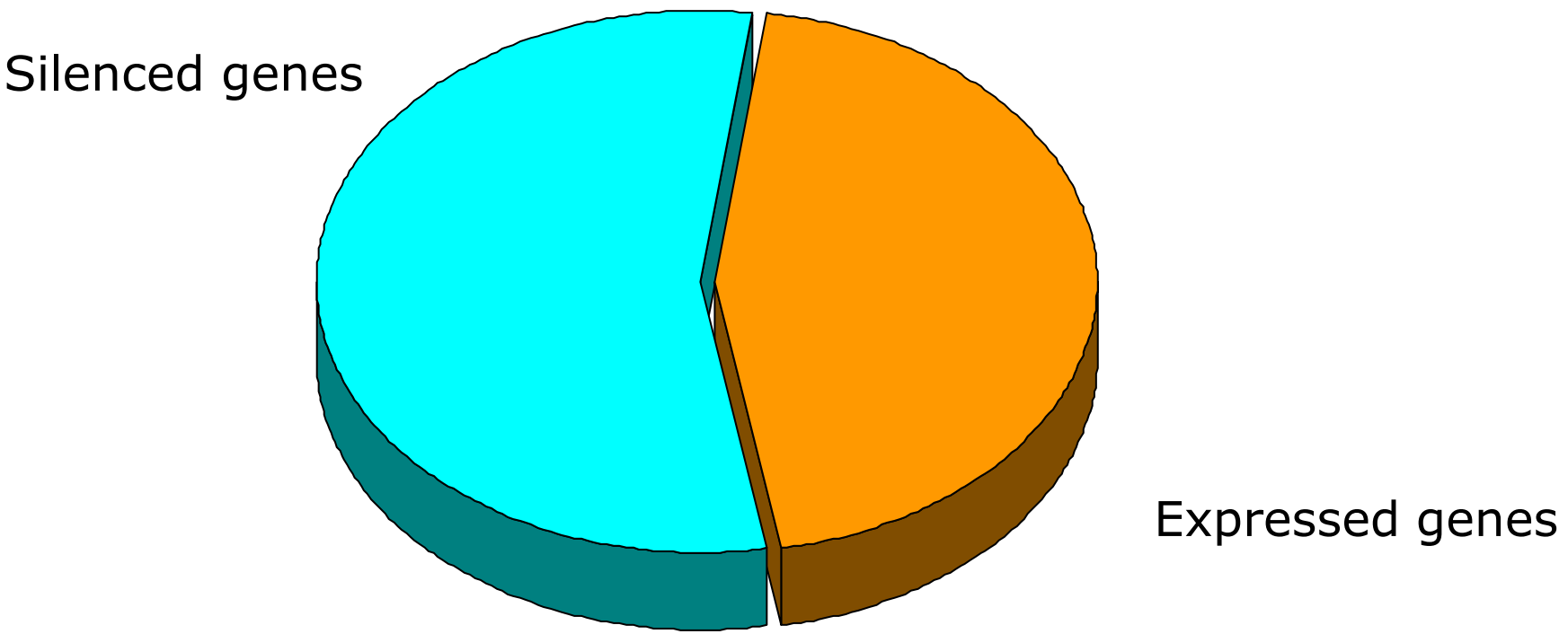
8th / 9th

Chromatin - silencing – epigenetics - imprinting

8.1 Nucleosomes
8.2 Covalent modification of histones
8.3 The histone code
8.4 Decoding proteins
8.5 DNA CpG methylation

In any cell type, a large part of the genes are kept inactive (not expressed) by gene **silencing**

H. sapiens genome:
25,000 genes



22-10-10 Lecture (BC)

The nuclear envelope and transcriptional control

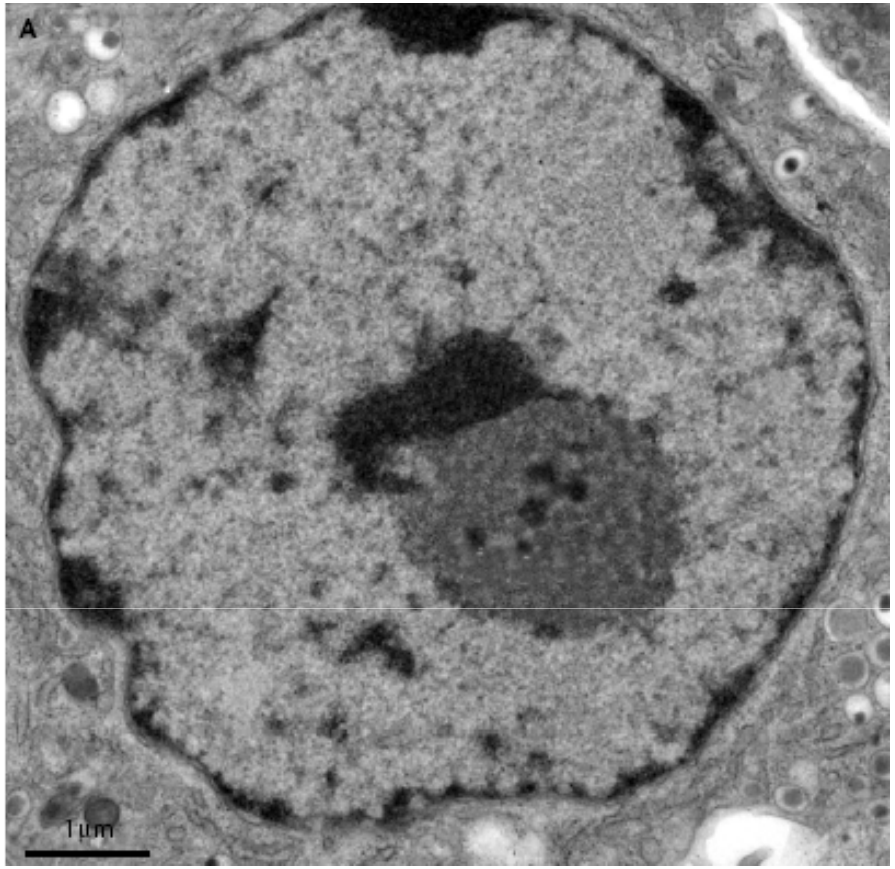
Asifa Akhtar and Susan M. Gasser‡*

Abstract | Cells have evolved sophisticated multi-protein complexes that can regulate gene activity at various steps of the transcription process. Recent advances highlight the role of nuclear positioning in the control of gene expression and have put nuclear envelope components at centre stage. On the inner face of the nuclear envelope, active genes localize to nuclear-pore structures whereas silent chromatin localizes to non-pore sites. Nuclear-pore components seem to not only recruit the RNA-processing and RNA-export machinery, but contribute a level of regulation that might enhance gene expression in a heritable manner.

2007

22-10-10 Lecture (BC)

A



B

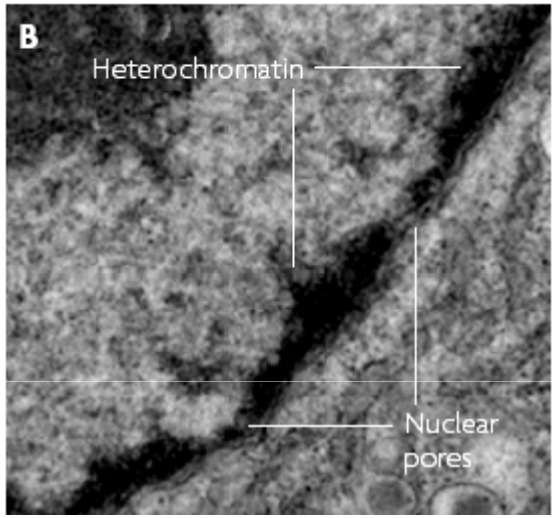


Figure 1 | **Heterochromatin in mammalian and yeast cells is distinct from nuclear pores. A** | An electron micrograph of the mammalian liver nucleus (with an enlarged section shown in part **B**), showing dense-staining heterochromatin located around the nucleolus and against the nuclear envelope. Nuclear pores open onto lighterstaining open chromatin.

22-10-10 Lecture (BC)

Research

Tissue-specific spatial organization of genomes

Luis A Parada*, Philip G McQueen† and Tom Misteli*

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Correspondence: Tom Misteli. E-mail: mistelit@mail.nih.gov

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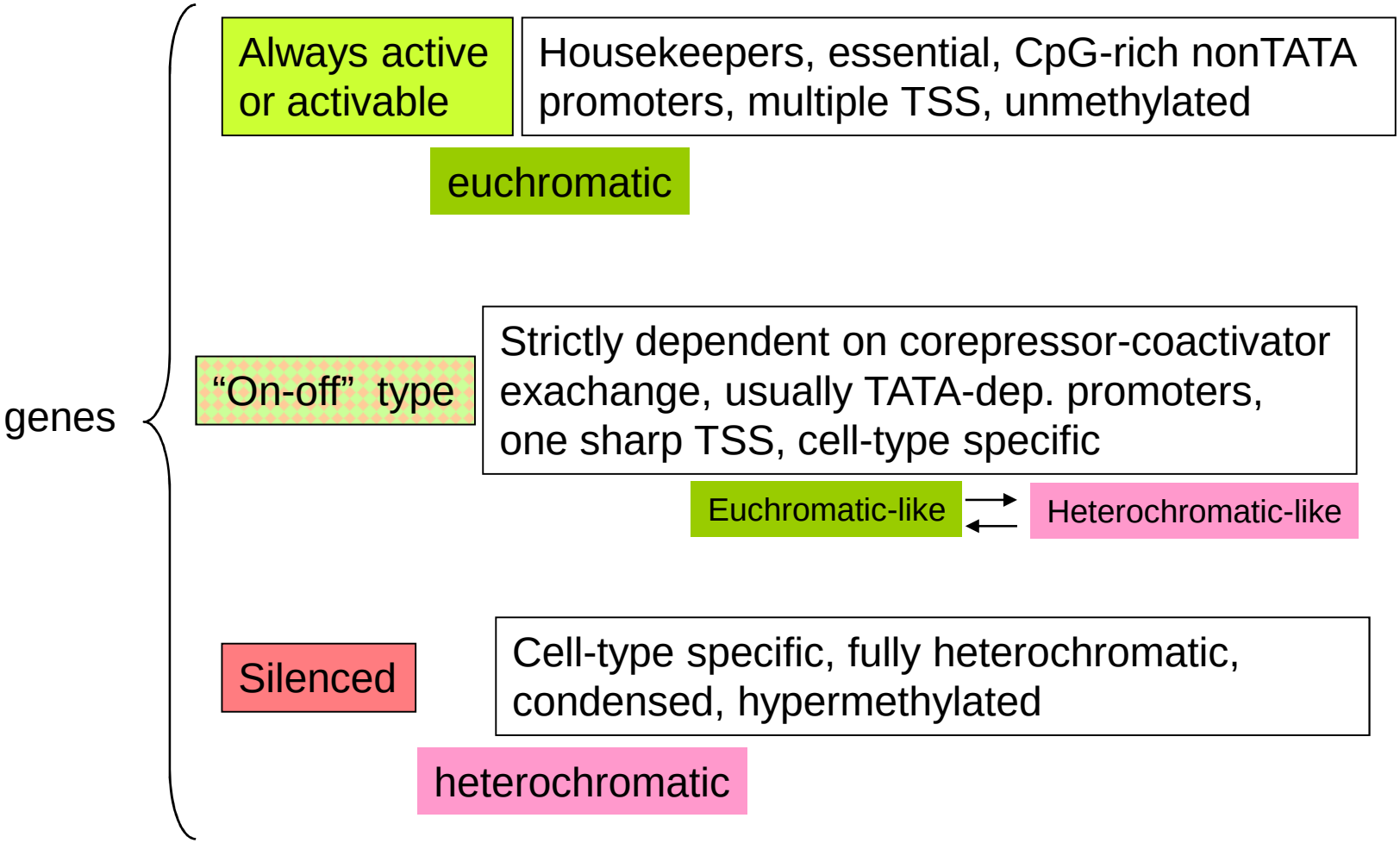
22-10-10 Lecture (BC)

Vol 452|13 March 2008|doi:10.1038/nature06727 nature

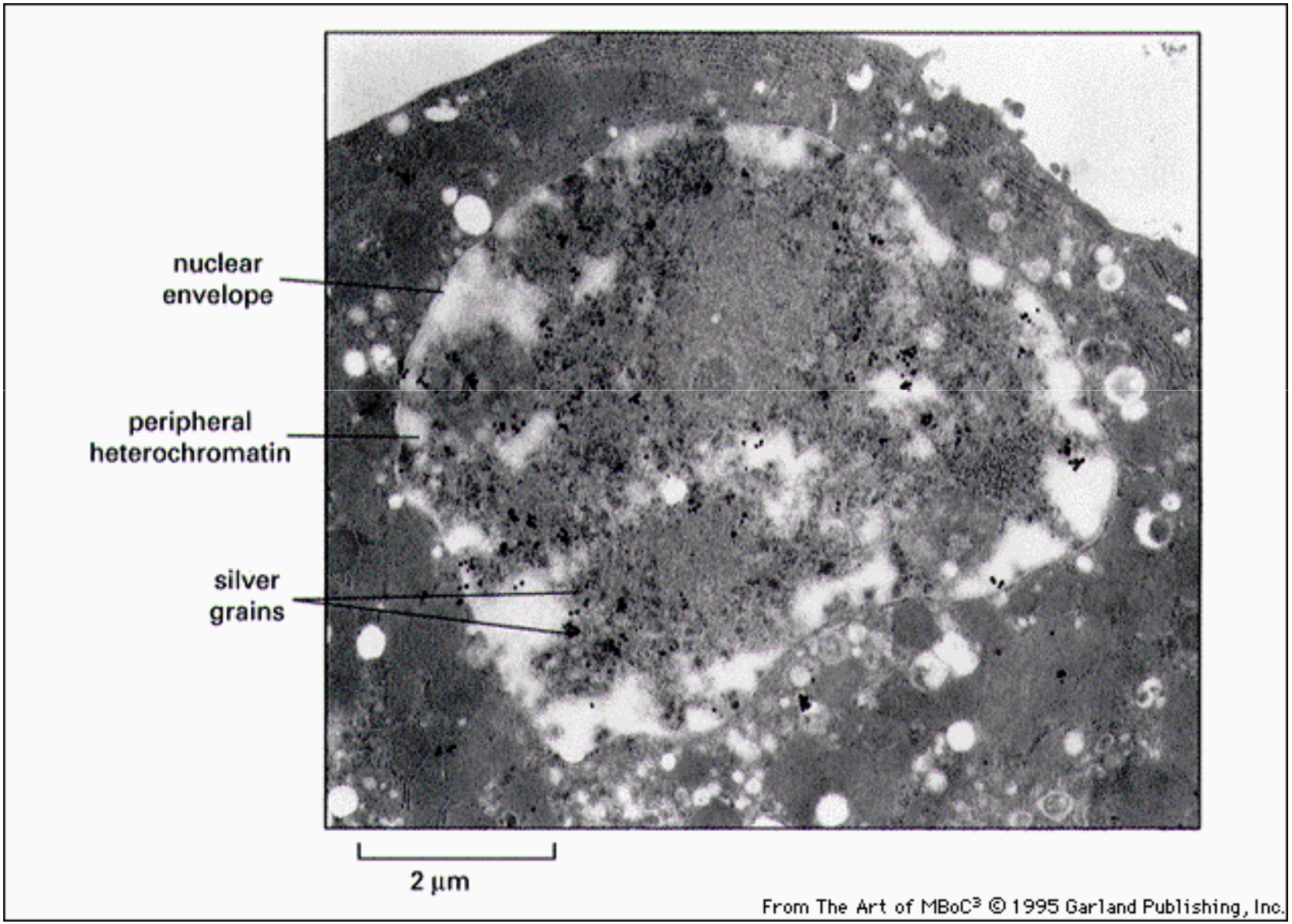
LETTERS

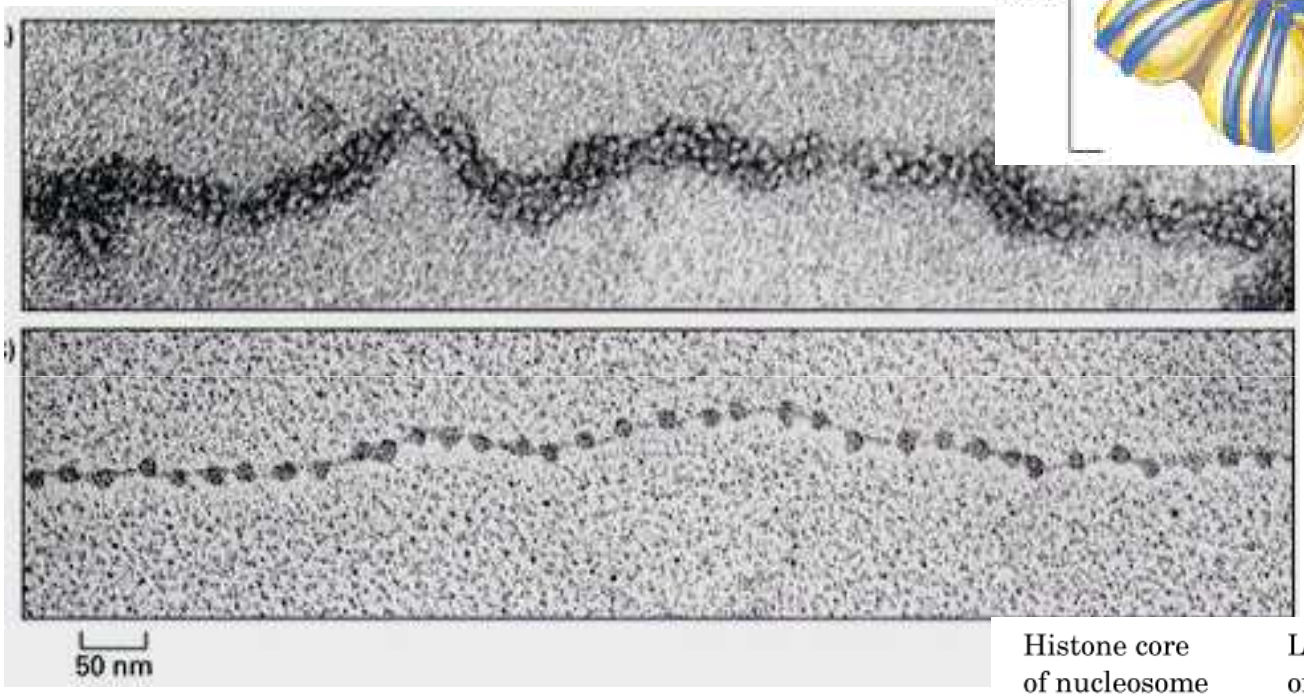
Transcriptional repression mediated by repositioning of genes to the nuclear lamina

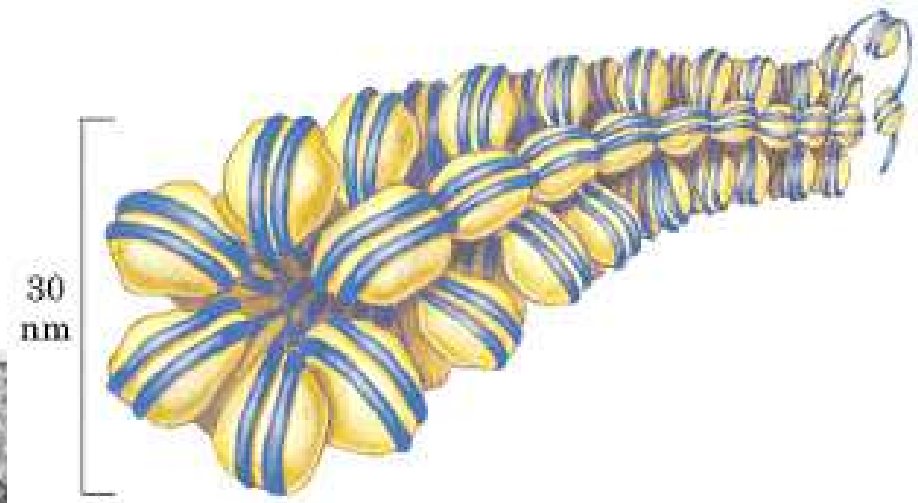
K. L. Reddy[1,2], J. M. Zullo[1,2], E. Bertolino[2] & H. Singh[1,2]

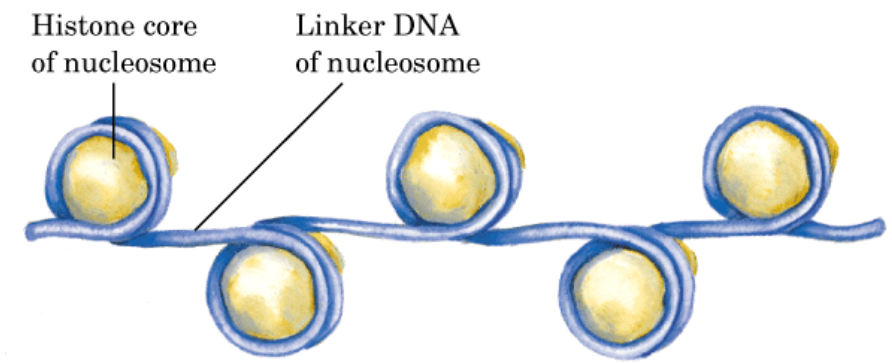

genes
Always active or activable
Housekeepers, essential, CpG-rich nonTATA promoters, multiple TSS, unmethylated
euchromatic
“On-off” type
Strictly dependent on corepressor-coactivator exachange, usually TATA-dep. promoters, one sharp TSS, cell-type specific
Euchromatic-like
Heterochromatic-like
Silenced
Cell-type specific, fully heterochromatic, condensed, hypermethylated
heterochromatic

First studies on chromatin structure by E.M. in middle '70. The obtained images led to the hypotesis that the compacted form was typical of nonexpressed genes.

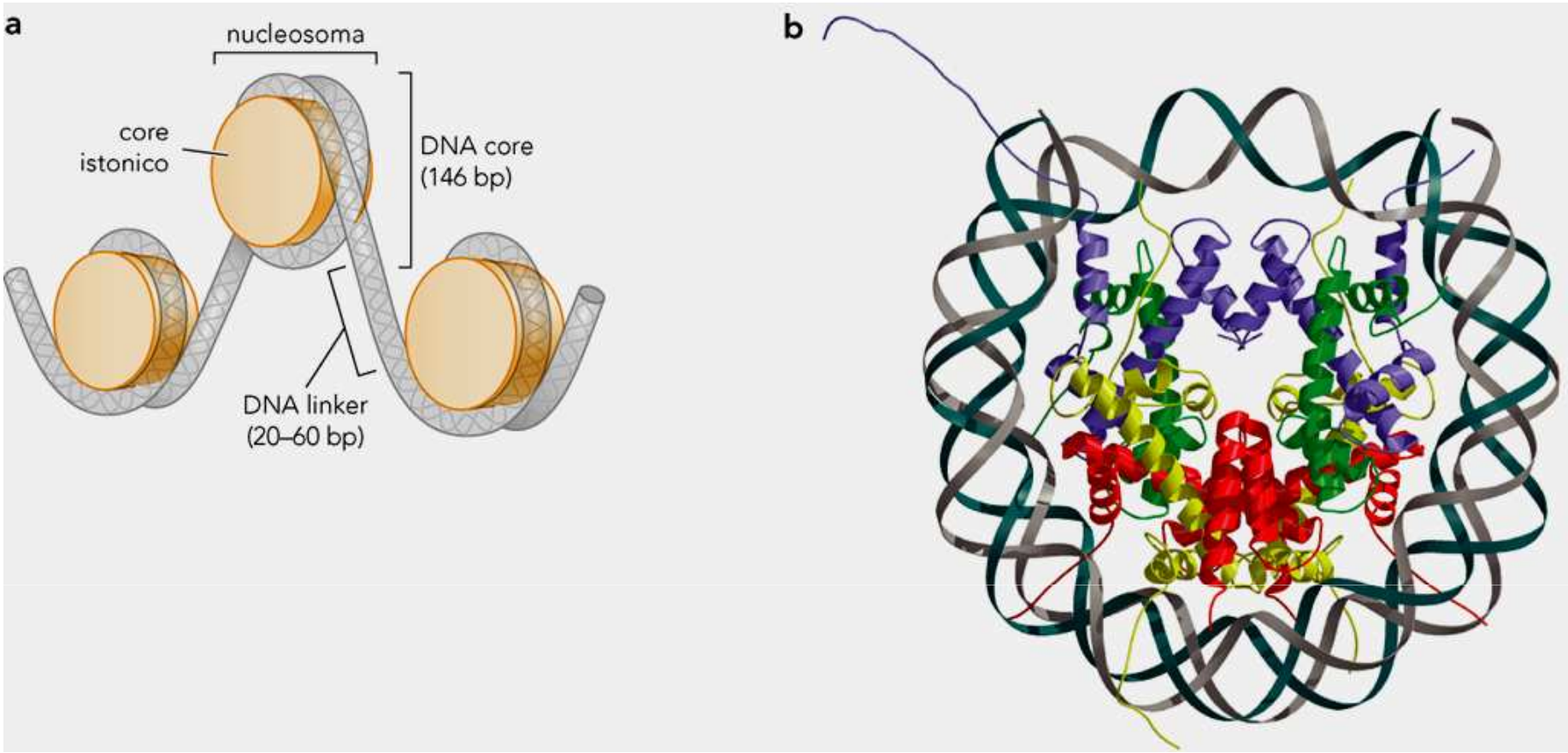



50 nm


30
nm


Histone core
of nucleosome
Linker DNA
of nucleosome

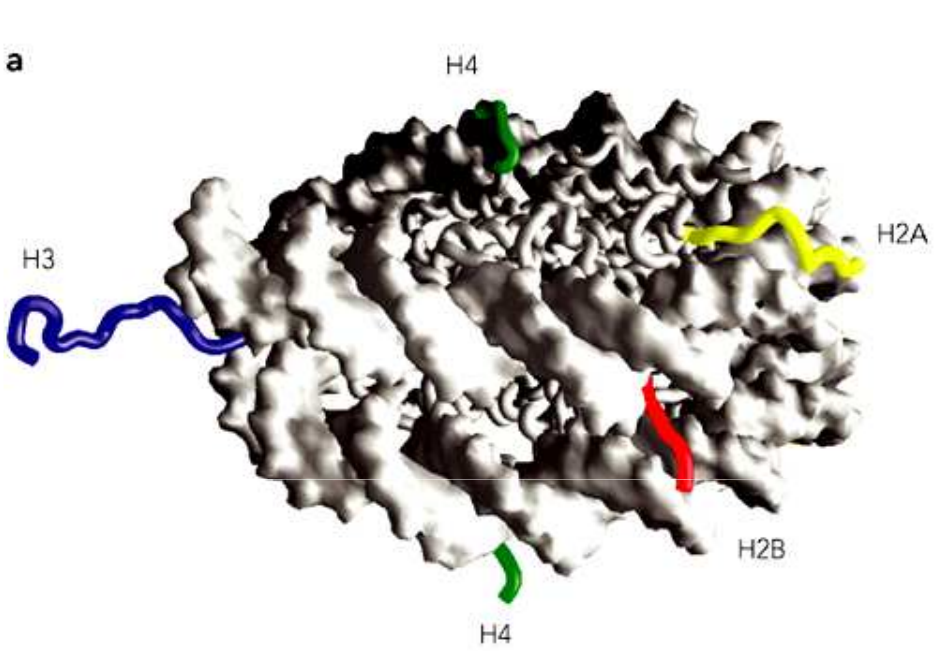
(a)

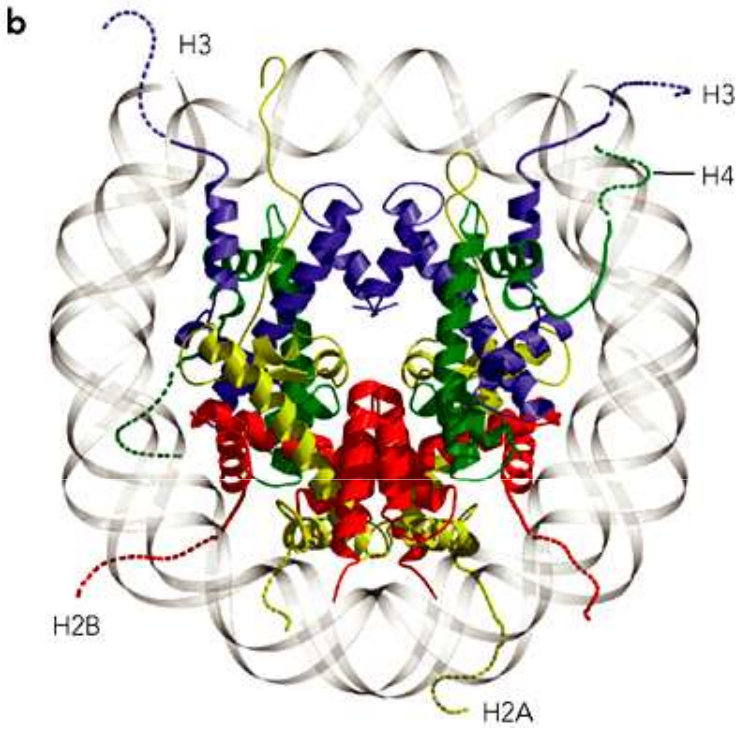


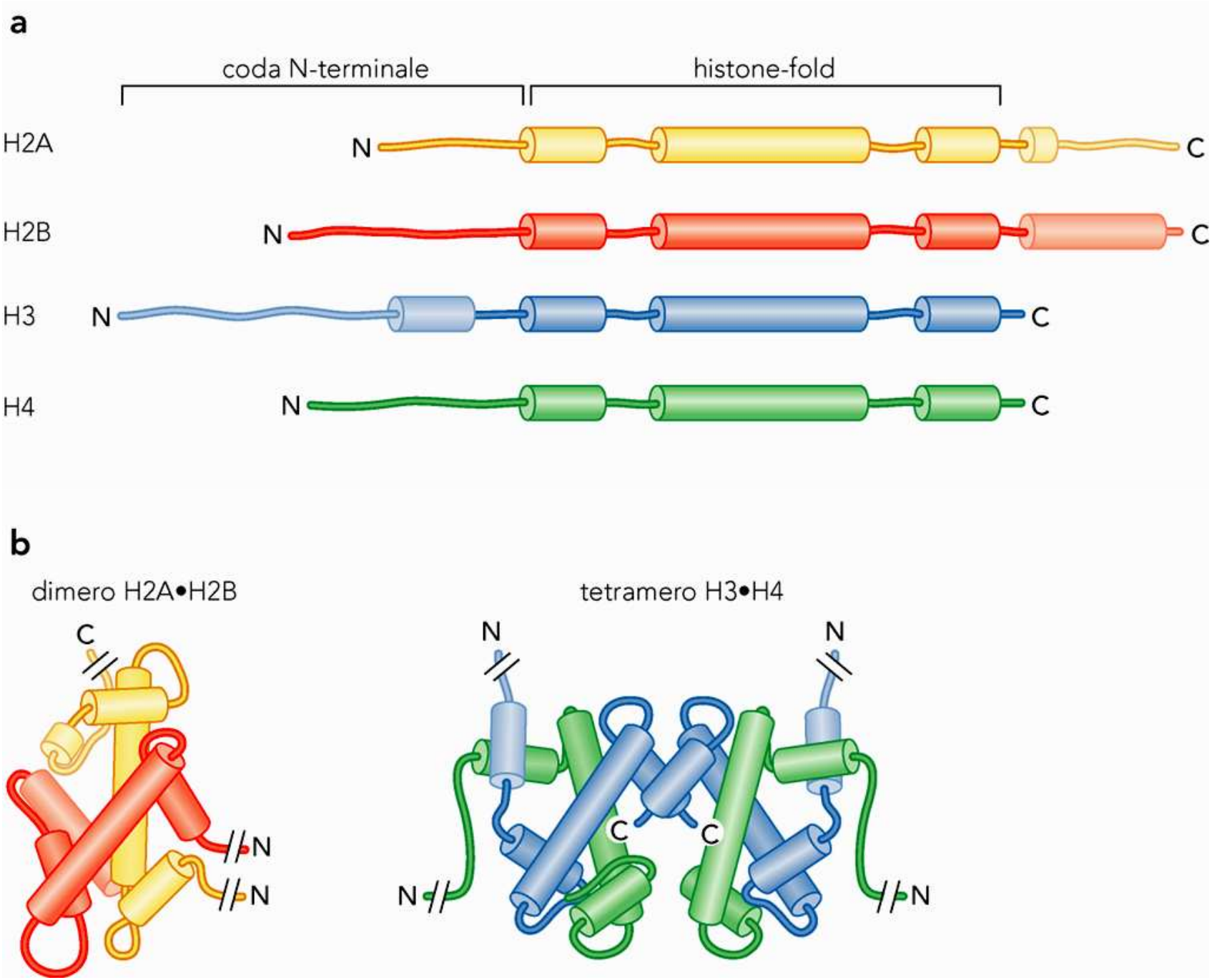
struttura del DNA nucleosomale

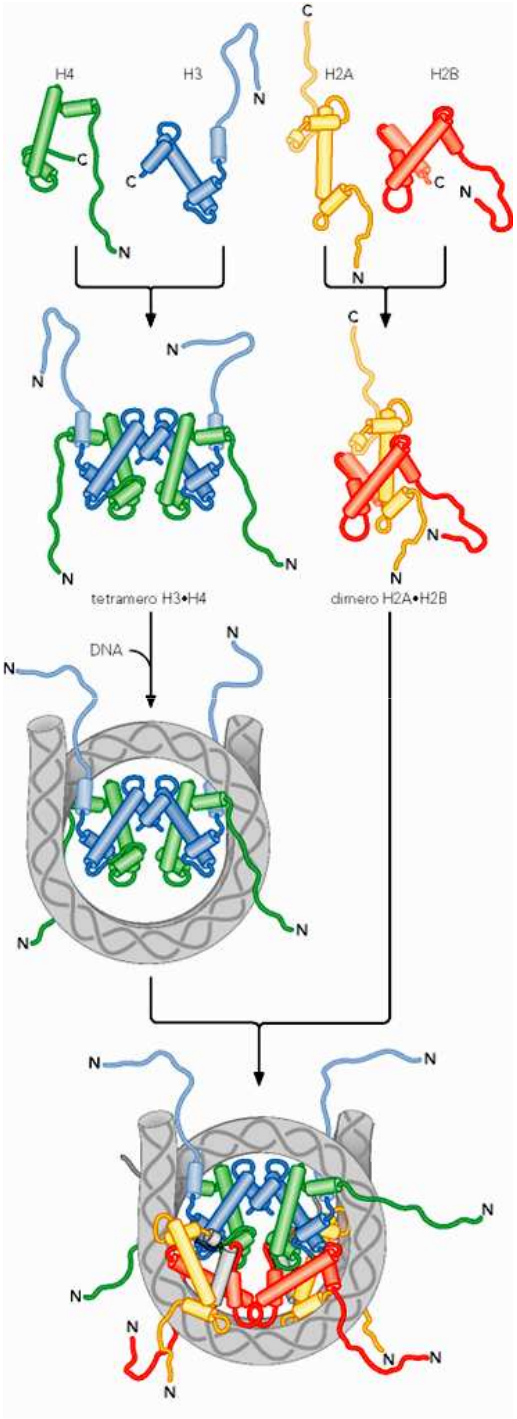
topologia (superavvolgimenti)

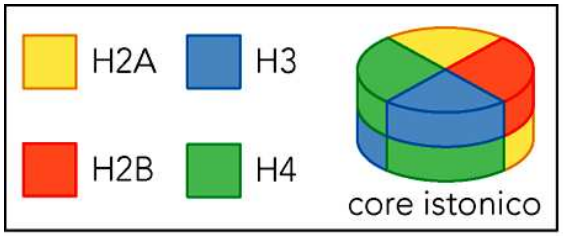
topoisomerasi

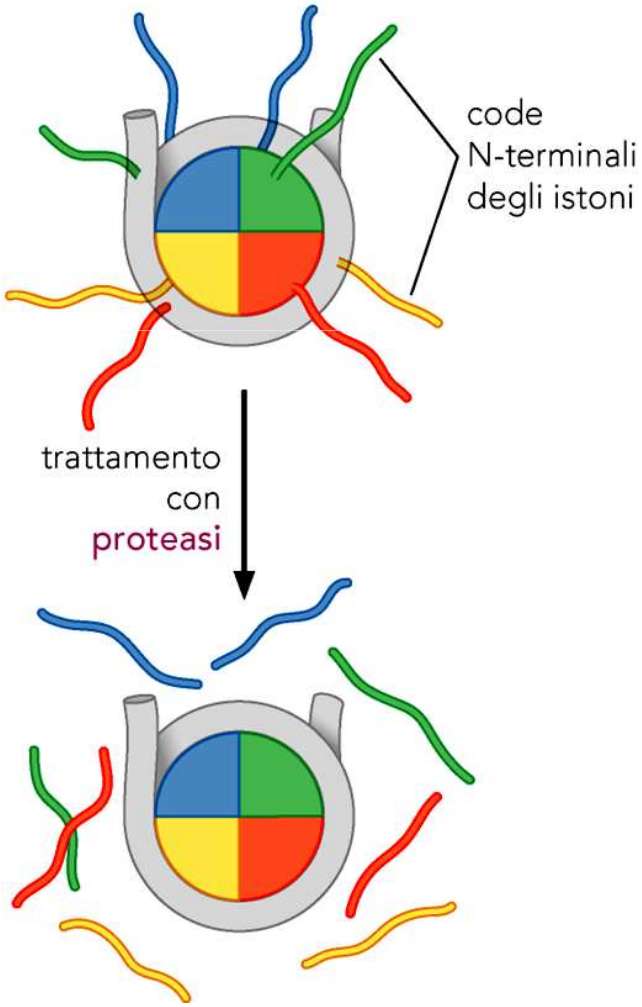

a
H4
H3
H2A
H2B
H4


b
H3
H3
H4
H2B
H2A

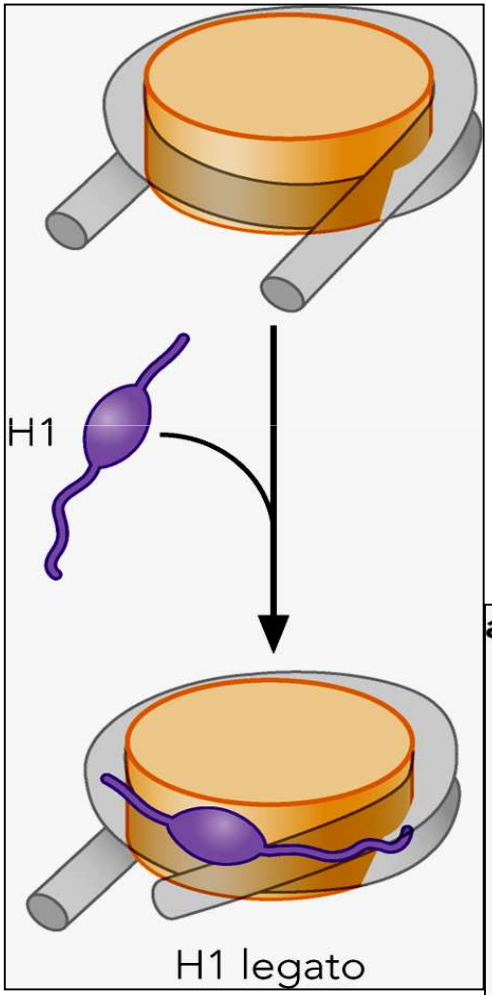

a
coda N-terminale
histone-fold
H2A
H2B
H3
H4
N
C
b
dimero H2A•H2B
tetramero H3•H4
C
N

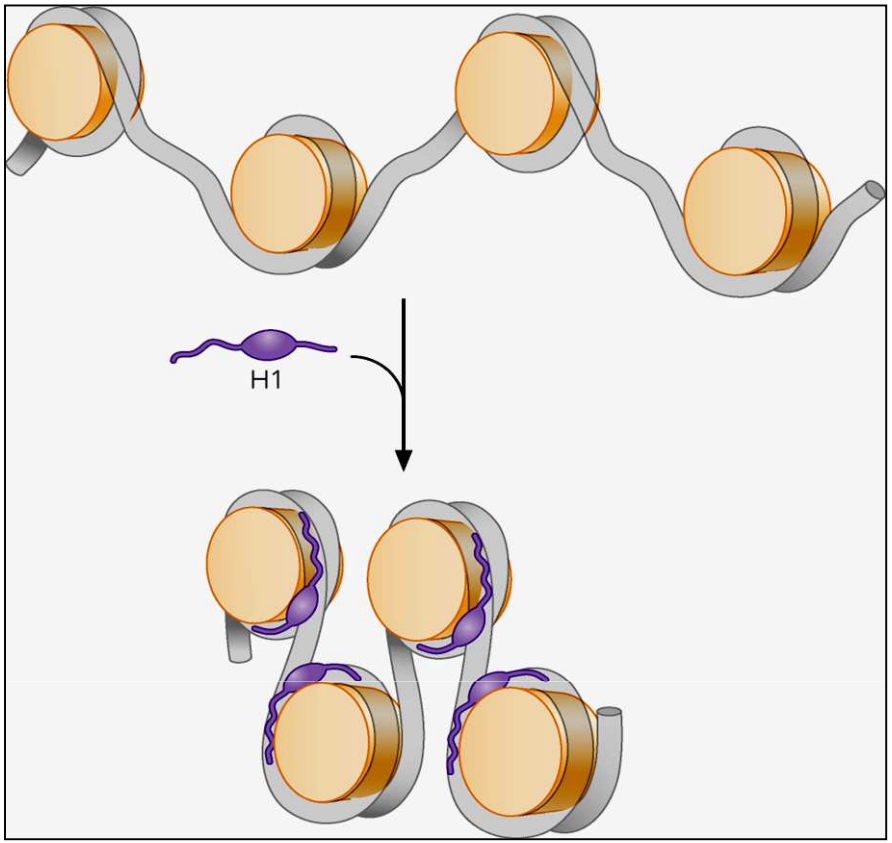

H4
H3
H2A
H2B
C
N
tetramero H3•H4
dimero H2A•H2B
DNA

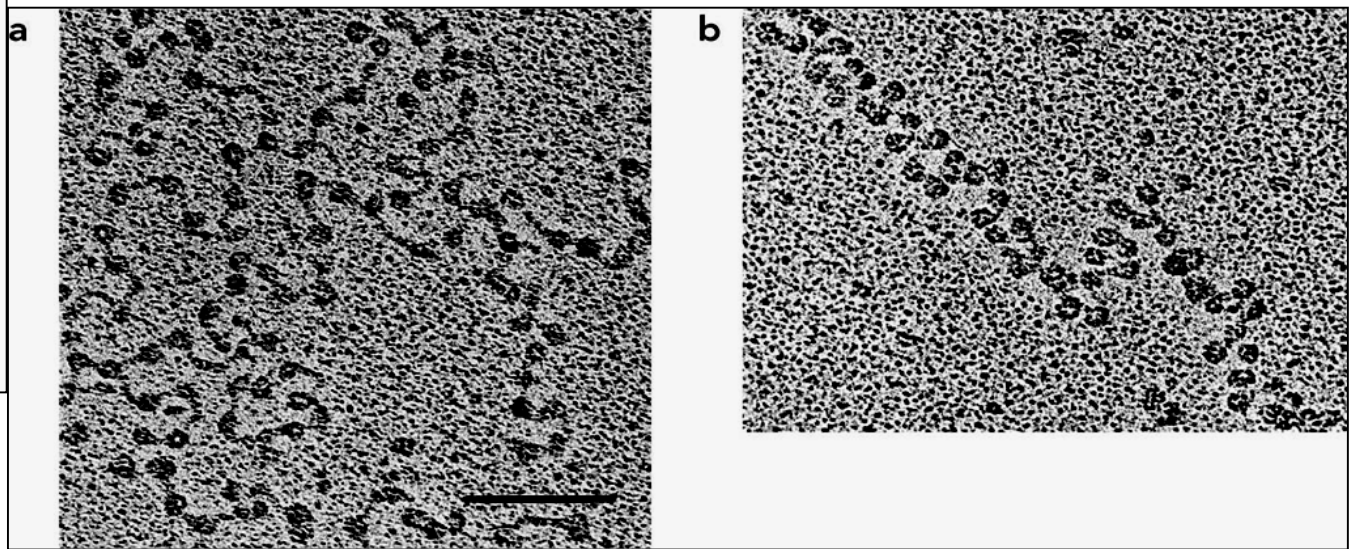

H2A
H3
H2B
H4
core istonico


code
N-terminali
degli istoni
trattamento
con
proteasi

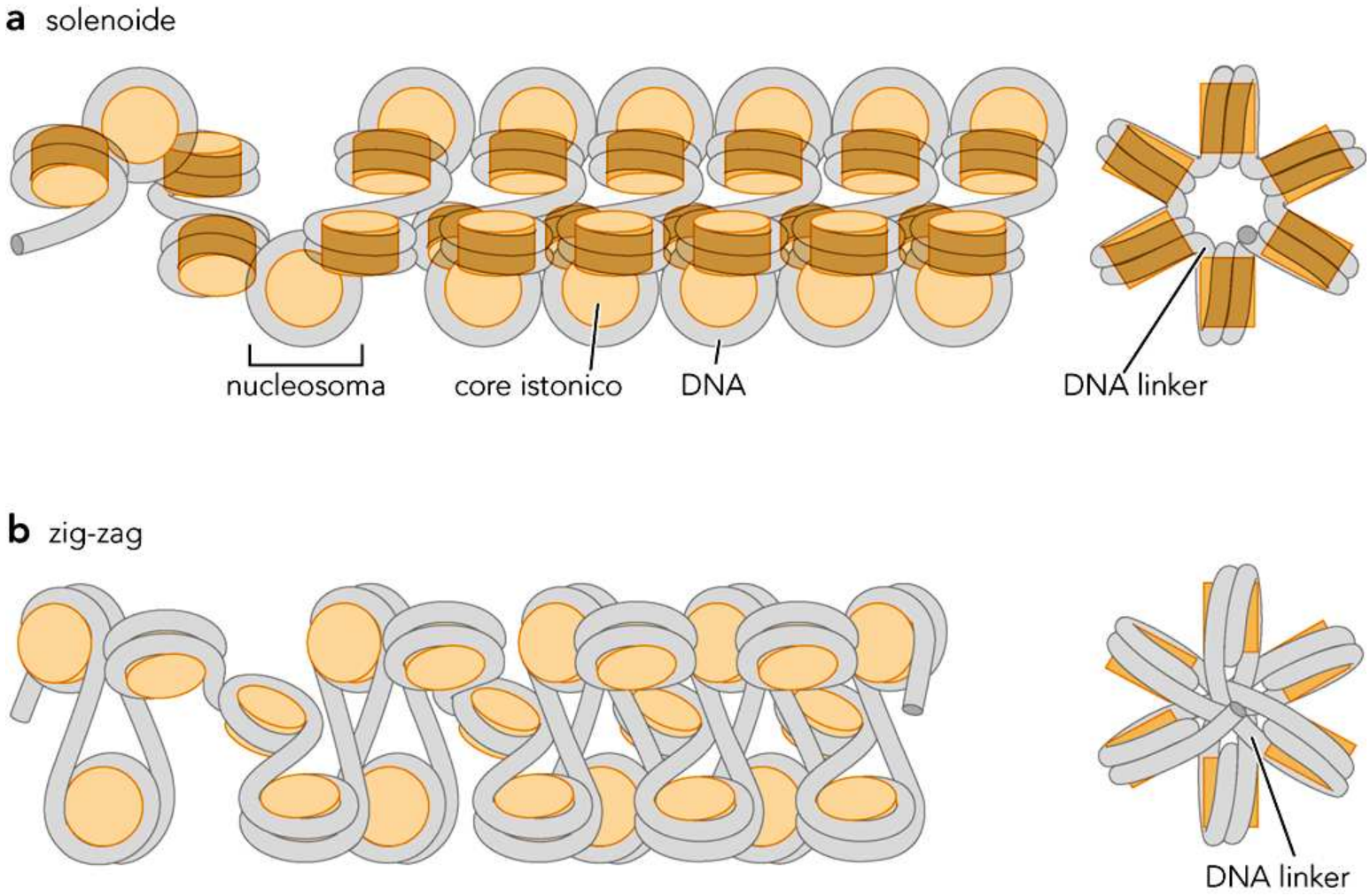
Non-nucleosomal histones: H1

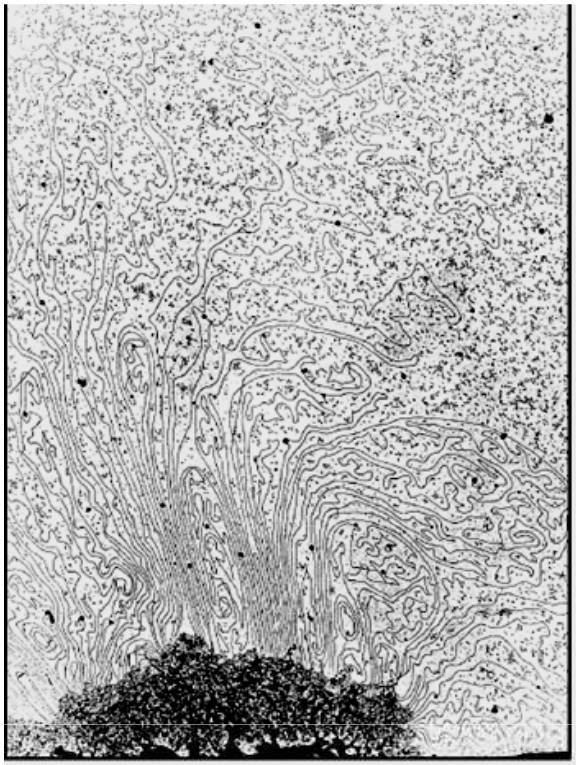


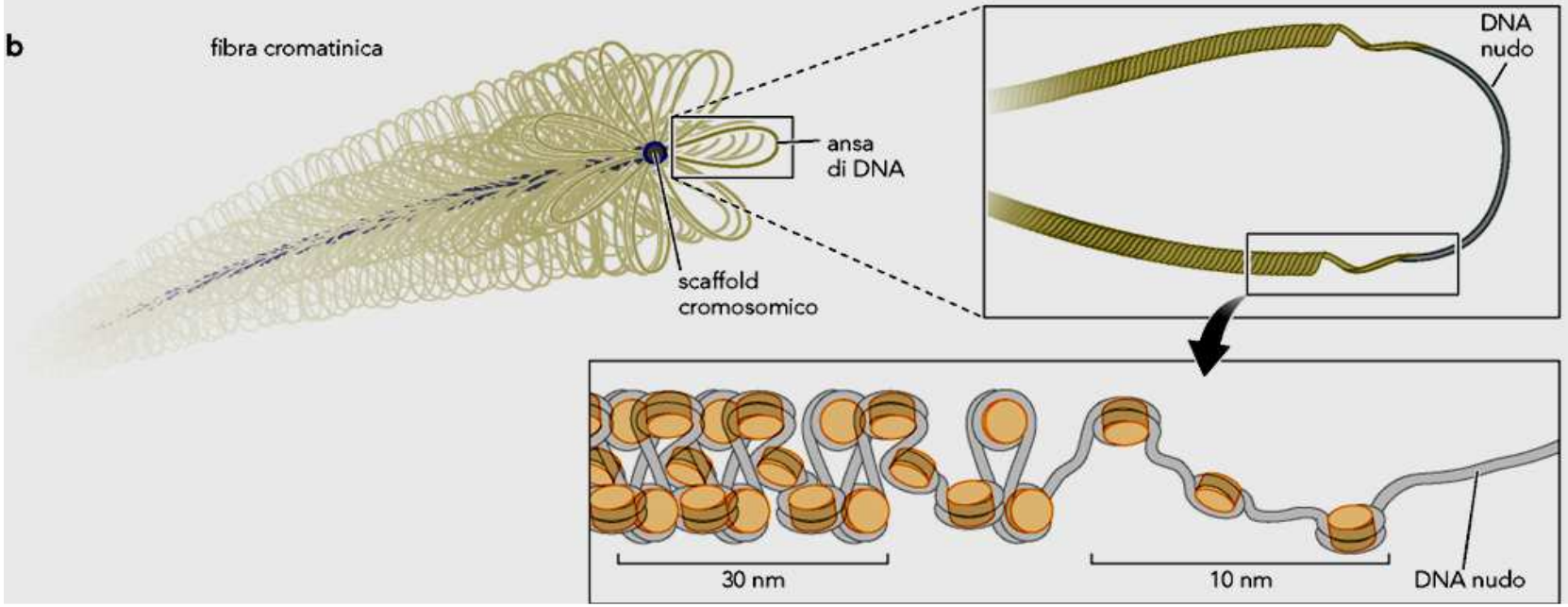




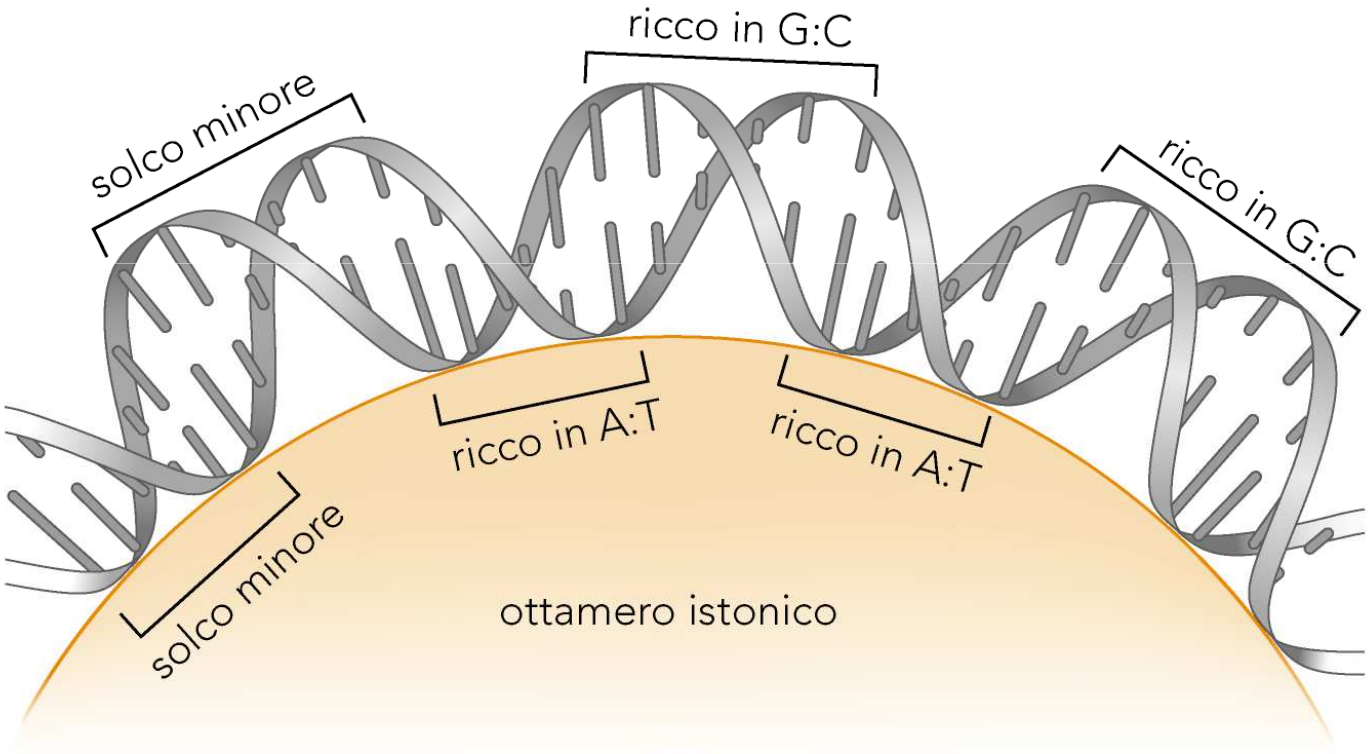
The 30nm fiber: solenoide or zig-zag ?






b
fibra cromatinica
ansa
di DNA
scaffold
cromosomico
DNA
nudo
30 nm
10 nm
DNA nudo

Do nucleosomes display any kind of **specificity** for nucleotide sequence?



Nucleosome positions predicted through comparative genomics

Ilya P Ioshikhes[1], Istvan Albert[2], Sara J Zanton[3] & B Franklin Pugh[3]

DNA sequence has long been recognized as an important contributor to nucleosome positioning, which has the potential to regulate access to genes. The extent to which the nucleosomal architecture at promoters is delineated by the underlying sequence is now being worked out. Here we use comparative genomics to report a genome-wide map of nucleosome positioning sequences (NPSs) located in the vicinity of all *Saccharomyces cerevisiae* genes. We find that the underlying DNA sequence provides a very good predictor of nucleosome locations that have been experimentally mapped to a small fraction of the genome. Notably, distinct classes of genes possess characteristic arrangements of NPSs that may be important for their regulation. In particular, genes that have a relatively compact NPS arrangement over the promoter r tend to have a TATA box buried in an NPS and tend to b highly regulated by chromatin modifying and remodeling

A nucleosome is composed of DNA wrapped around a hist complex[1]. The underlying DNA sequence can facilitate DNA w

collections of similarly regulate reinforced and noise was s genes that were either most p by histones[9], rationalizing tha might arise from distinct chr collection of ~900 genes betwe ATG translational start codo derived 139-bp AA/TT nuc **Figure 1a**. We averaged the re Peaks and valleys in the profile region. Peaks correspond to th sequences. Valleys correspond positioning (NPS anticorrelat

nature Vol 442|17 August 2006|doi:10.1038/nature04979

ARTICLES

A genomic code for nucleosome positioning

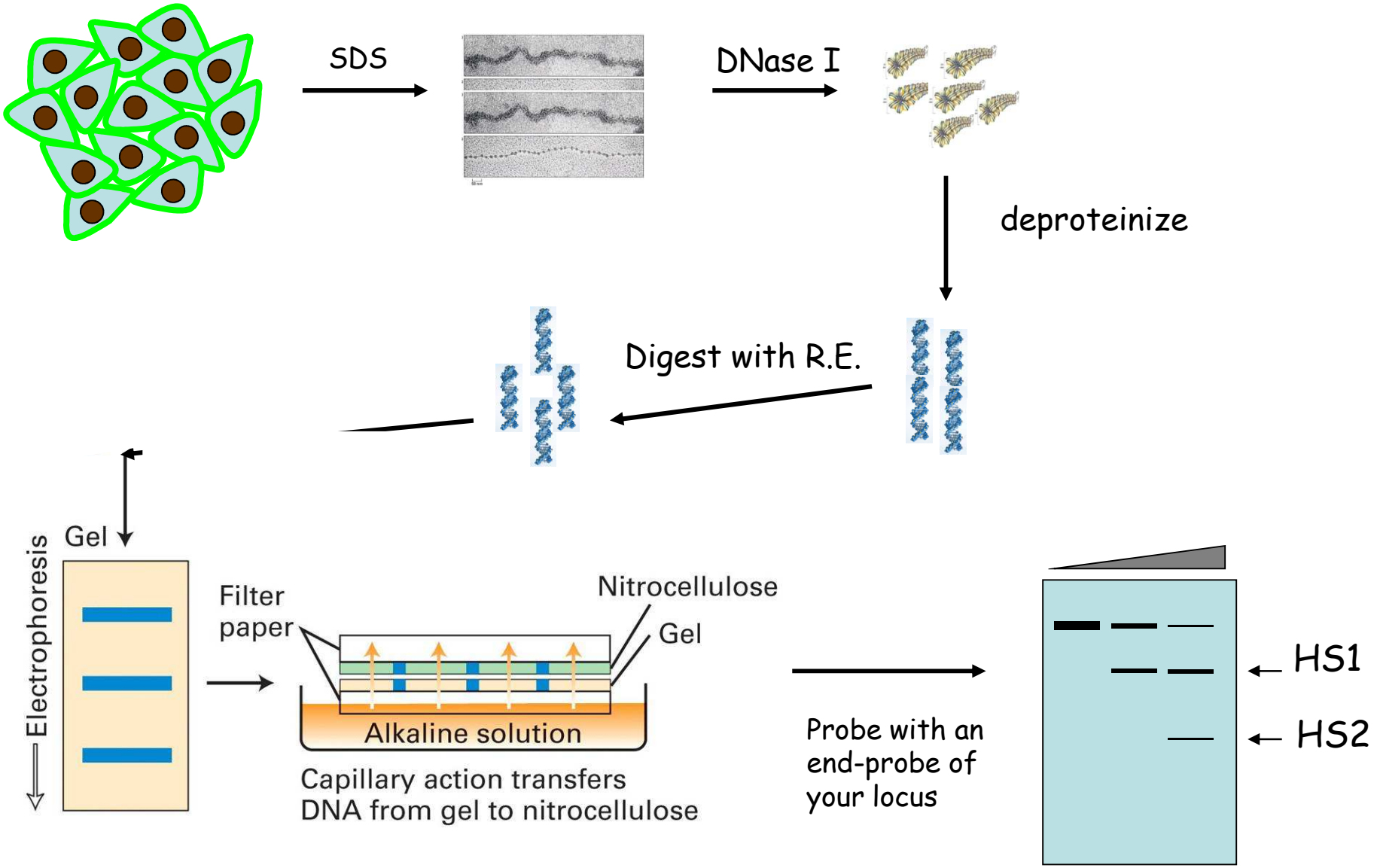
Eran Segal[1], Yvonne Fondufe-Mittendorf[2], Lingyi Chen[2], AnnChristine Thåström[2], Yair Field[1], Irene K. Moore[2], Ji-Ping Z. Wang[3] & Jonathan Widom[2]

Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. Nucleosomes have higher affinity for particular DNA sequences, reflecting the ability of the sequence to bend sharply, as required by the nucleosome structure. However, it is not known whether these sequence preferences have a significant influence on nucleosome position *in vivo*, and thus regulate the access of other proteins to DNA. Here we isolated nucleosome-bound sequences at high resolution from yeast and used these sequences in a new computational approach to construct and validate experimentally a nucleosome–DNA interaction model, and to predict the genome-wide organization of nucleosomes. Our results demonstrate that genomes encode an intrinsic nucleosome organization and that this intrinsic organization can explain ~50% of the *in vivo* nucleosome positions. This nucleosome positioning code may facilitate specific chromosome functions including transcription factor binding, transcription initiation, and even remodelling of the nucleosomes themselves.

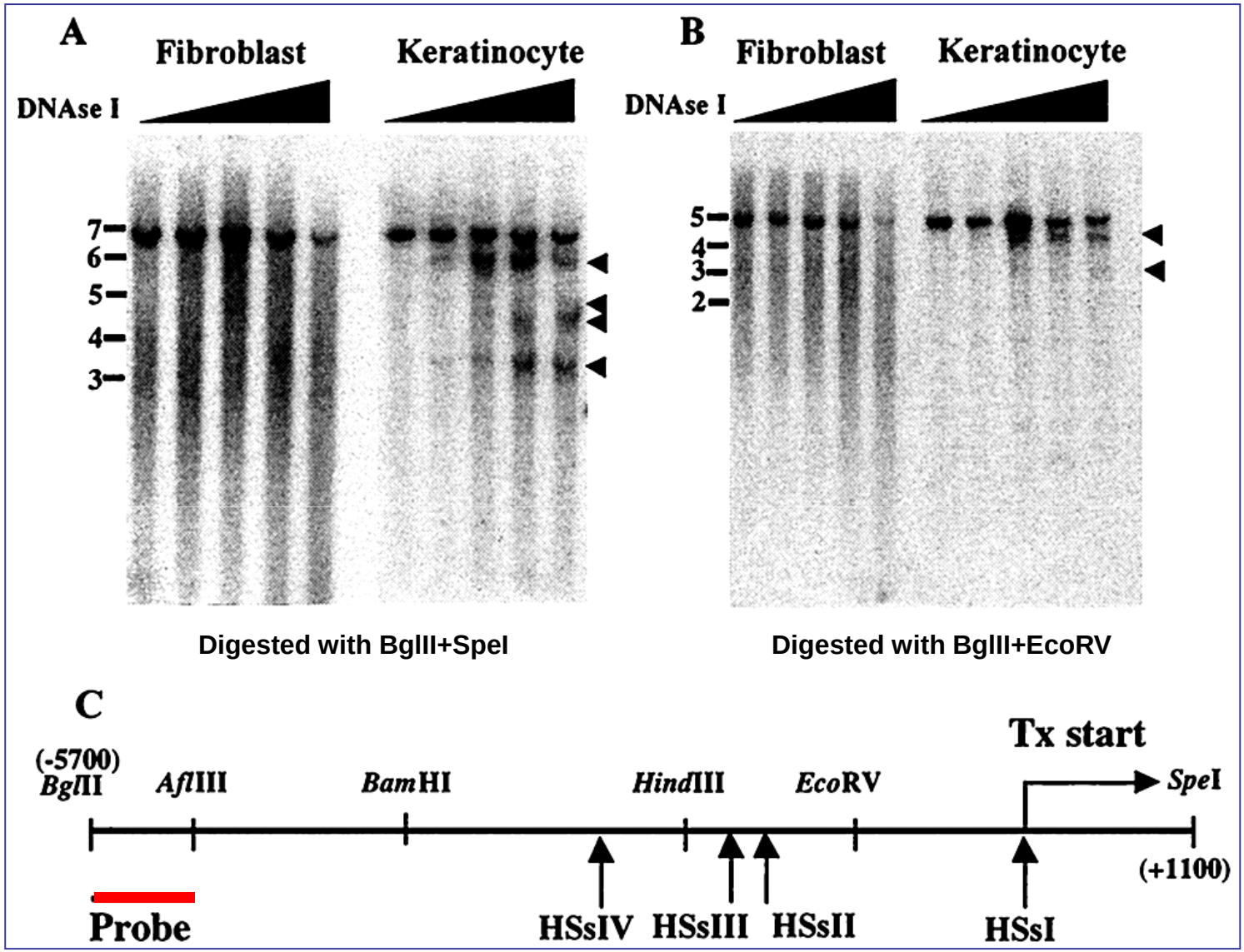
Are condensed (heterochromatic) and noncodensed (euchromatic) chromatin fragments (loci) distinguishable by a simple biochemical assay?

Primordial: **enzyme accessibility**.

The classical assay to detect the gross organization of chromatin at a specified locus: the DNase I Hypersensitivity Assay



Keratin K14 gene, analysis of the 5'-flanking region



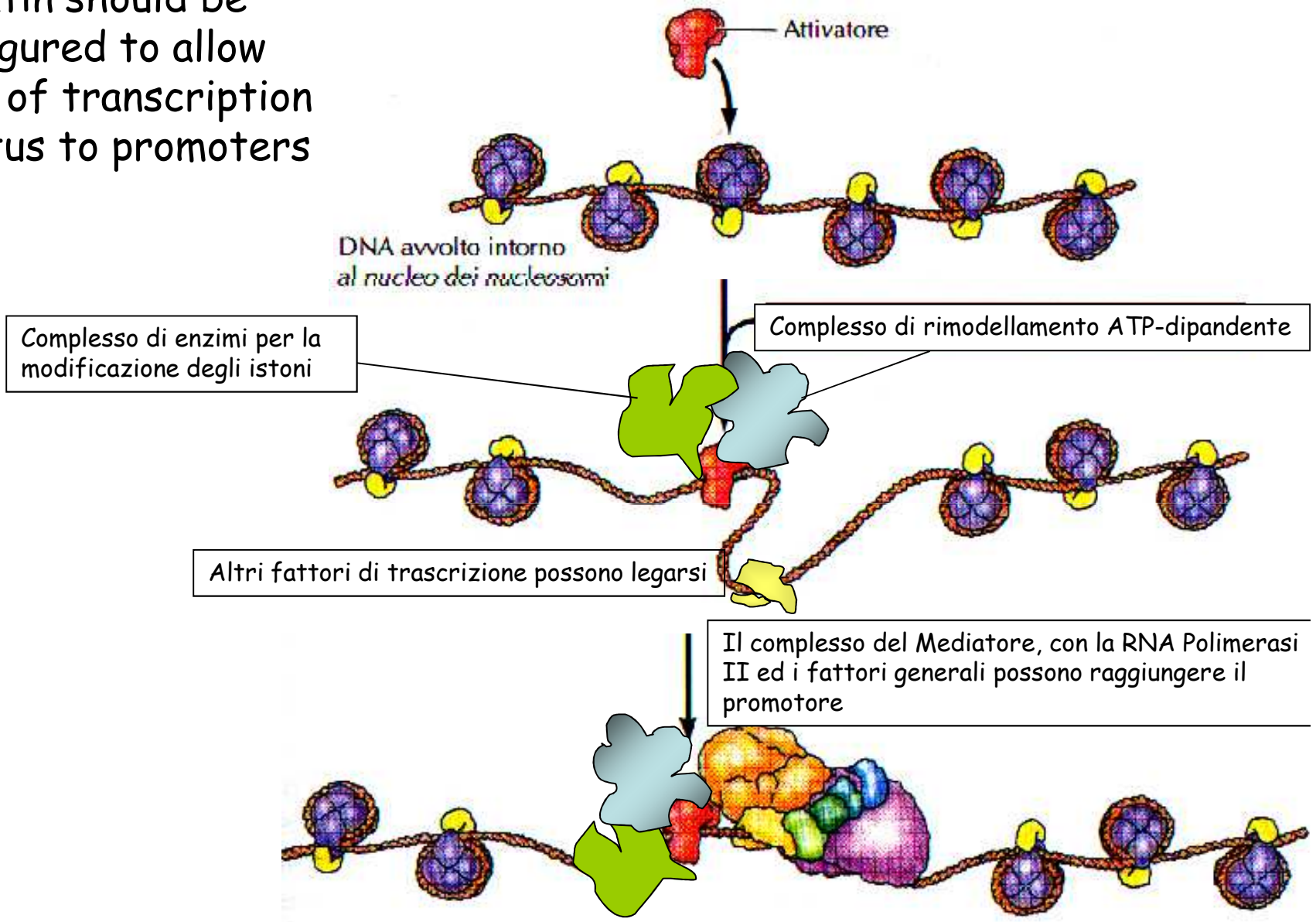
Tratto da: Sinha et al., (2000), Mol Cell Biol, 20: 2543-2555.

A number of differentiation-related loci are quite stably heterochromatic, depending on cell types.

Other loci can switch transiently from one status to the other and back to the original, as a result of signal transduction from either perceptive or proprioceptive stimuli.

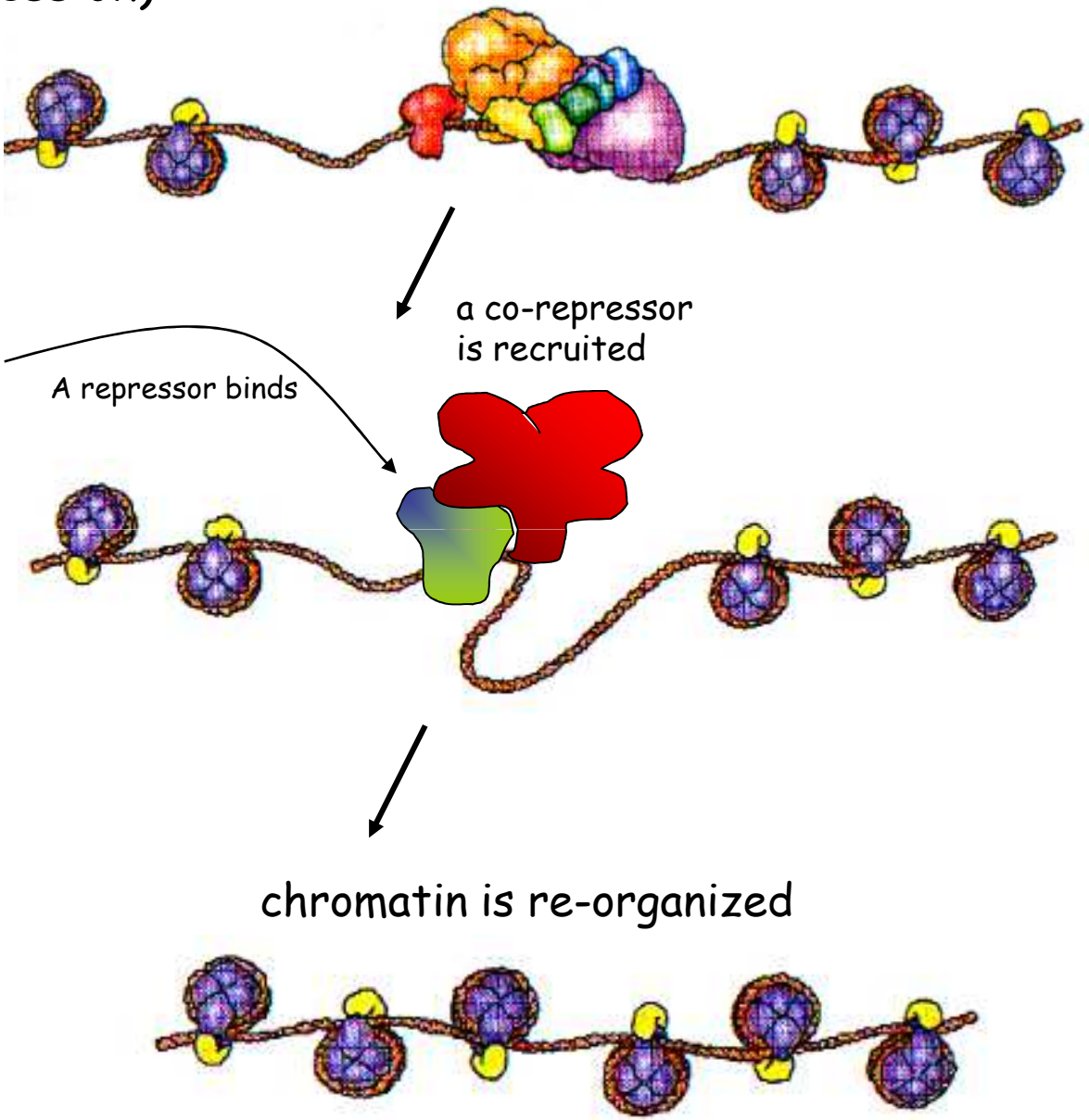
Of course, this means transcriptional **activation** or **repression**

Chromatin should be reconfigured to allow binding of transcription apparatus to promoters



modified from Cooper "The Cell", Sinauer Ed. 1997

Chromatin should be re-organized to stop transcription (repression)



Nucleosome dynamics

1. post-transcriptional modifications
2. histone isoform exchange
3. interacting proteins

review 1

Chromatin Modifications and Their Function

Tony Kouzarides[1,*]
[1]The Gurdon Institute and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB21QN, UK
*Correspondence: t.kouzarides@gurdon.cam.ac.uk
DOI 10.1016/j.cell.2007.02.005

The surface of nucleosomes is studded with a multiplicity of modifications. At least eight different classes have been characterized to date and many different sites have been identified for each class. Operationally, modifications function either by disrupting chromatin contacts or by affecting the recruitment of nonhistone proteins to chromatin. Their presence on histones can dictate the higher-order chromatin structure in which DNA is packaged and can orchestrate the ordered recruitment of enzyme complexes to manipulate DNA. In this way, histone modifications have the potential to influence many fundamental biological processes, some of which may be epigenetically inherited.

Cell 128, 693–705, February 23, 2007

Table 1. Different Classes of Modifications Identified on Histones

Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

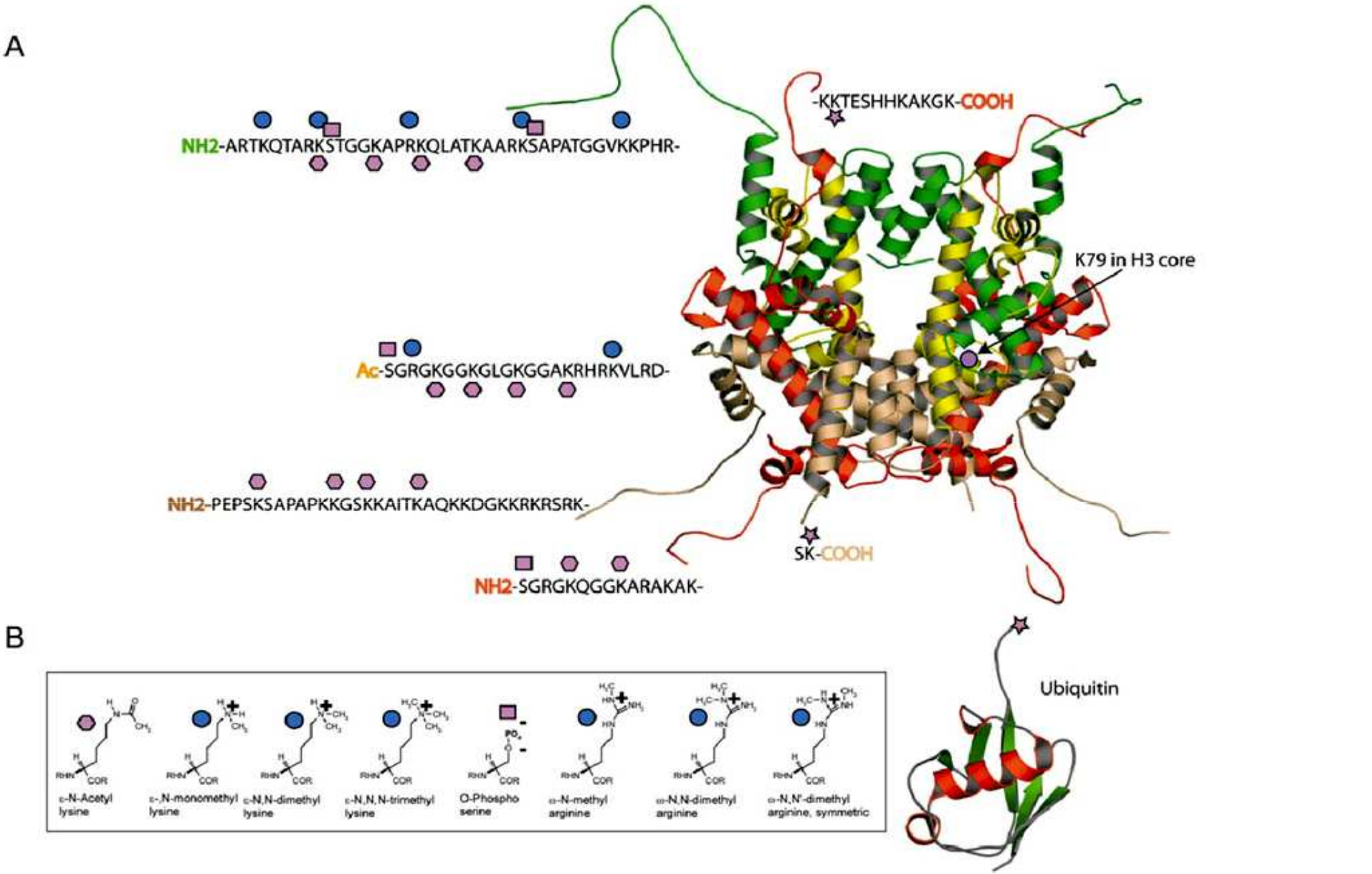
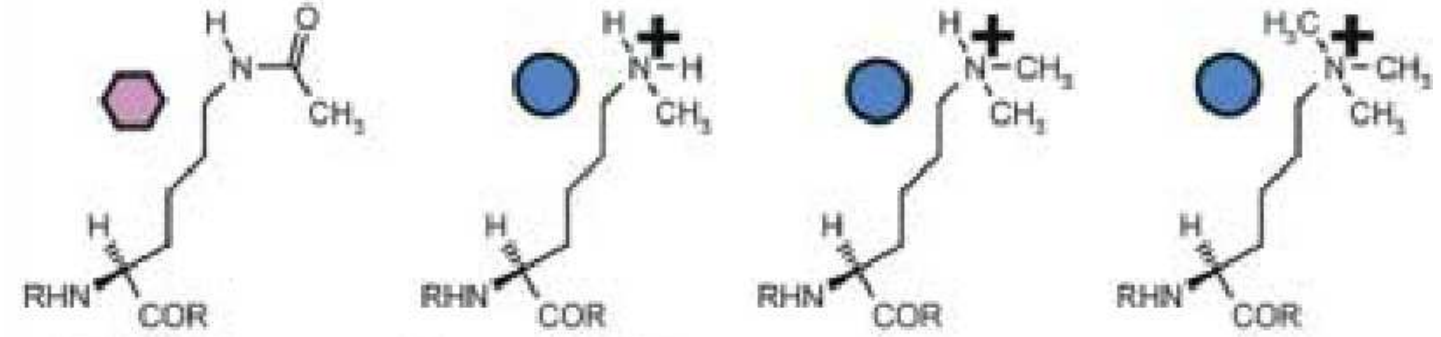
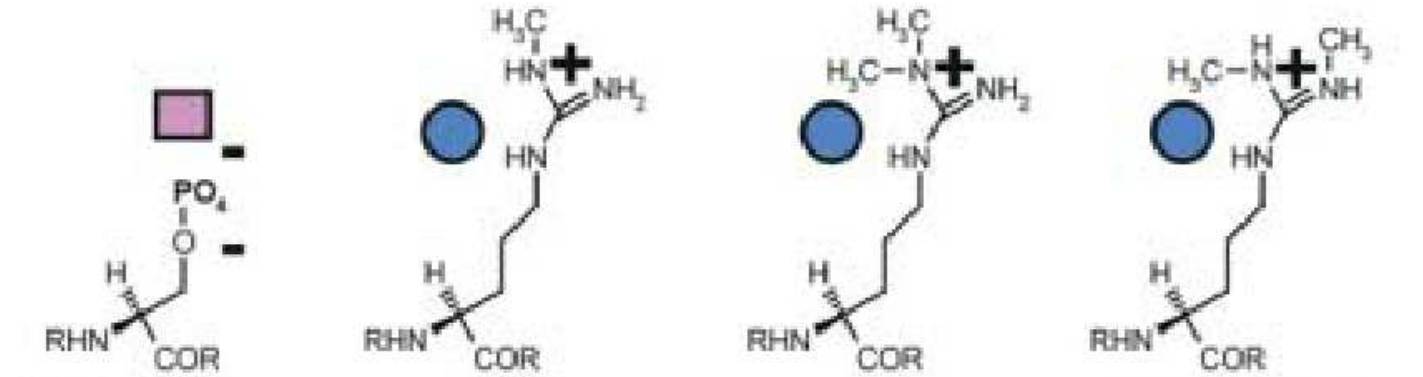


Figure 4. The Types of Posttranslational Modifications Observed on the Core Histones

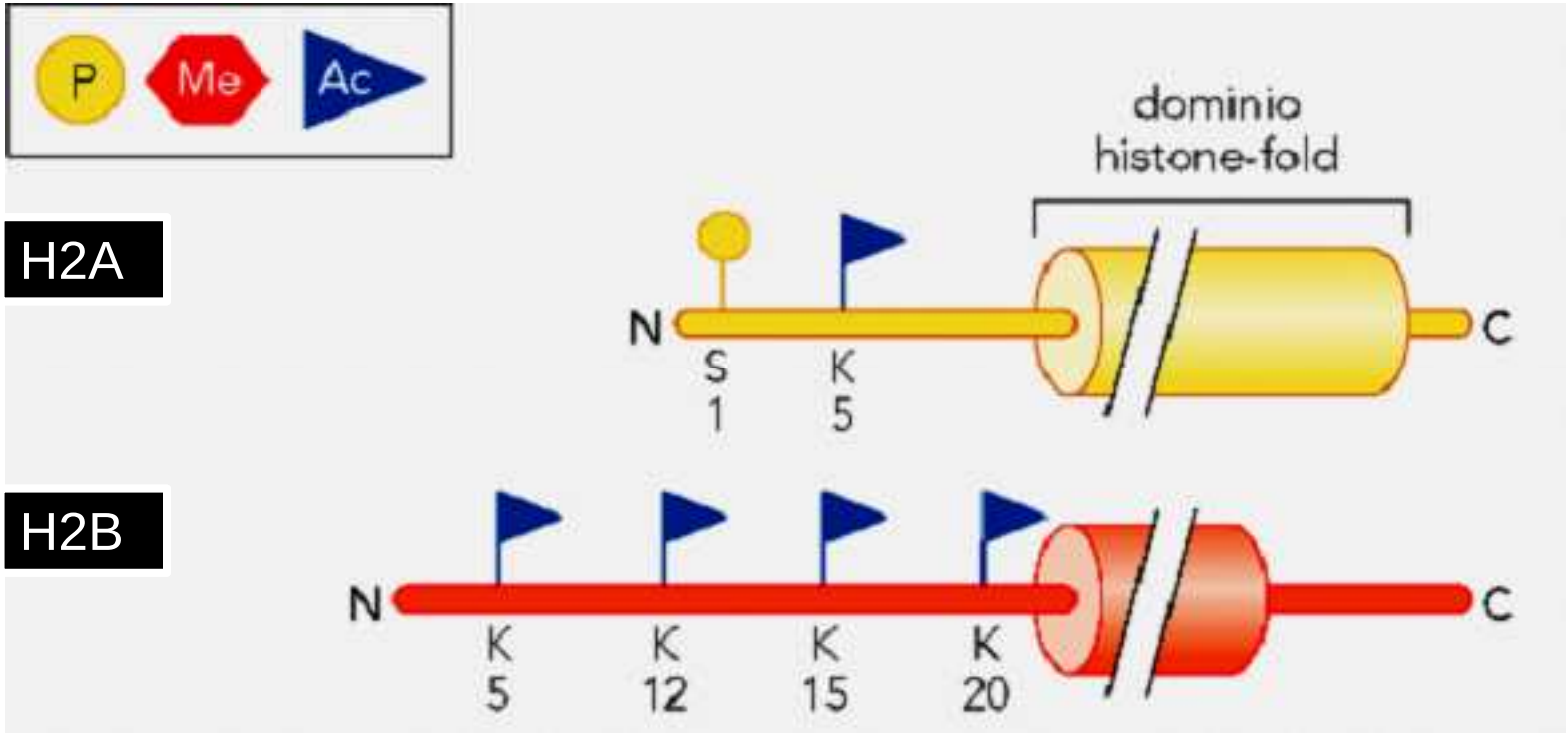
(A) The histone octamer portion of the nucleosome core particle is shown. The sites of modifications on marked. For clarity, the modifications are shown on one copy of each protein.

(B) The covalent modifications of the amino acids are shown.


ε-N-Acetyl lysine
ε-,N-monomethyl lysine
ε-N,N-dimethyl lysine
ε-N,N,N-trimethyl lysine

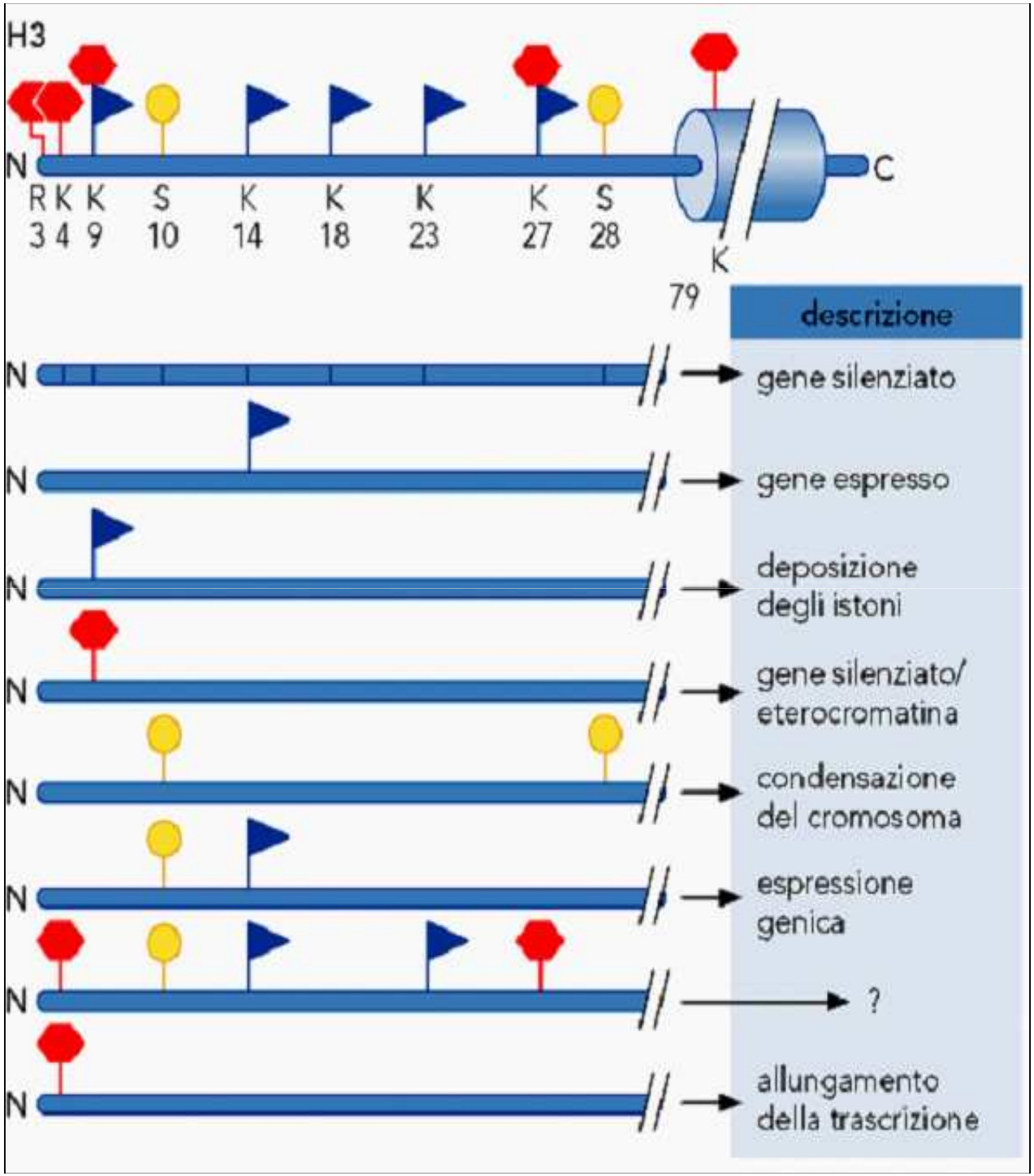

O-Phospho serine
ω-N-methyl arginine
ω-N,N-dimethyl arginine
ω-N,N'-dimethyl arginine, symmetric

- the histone code

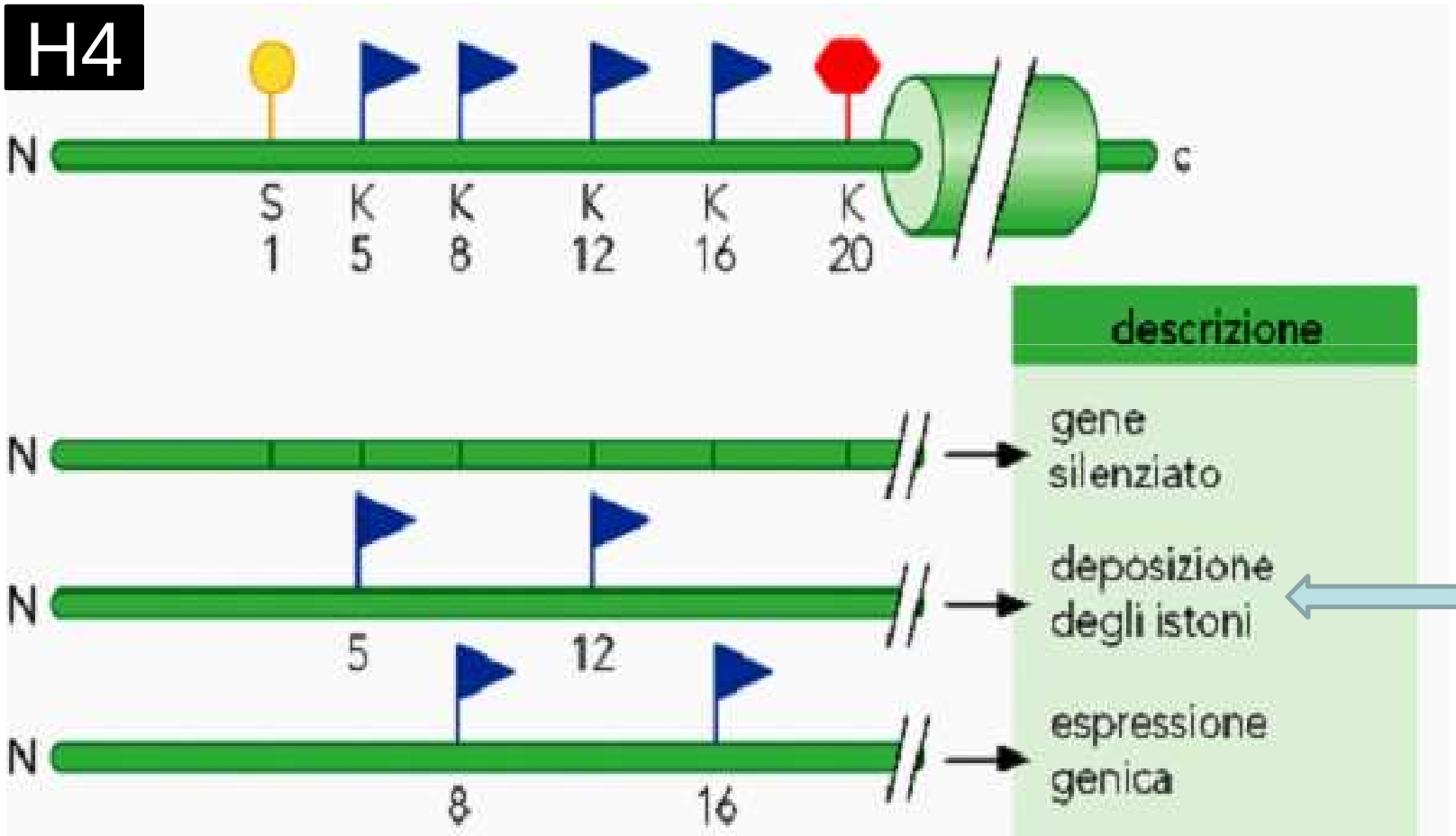


- the histone code

H3

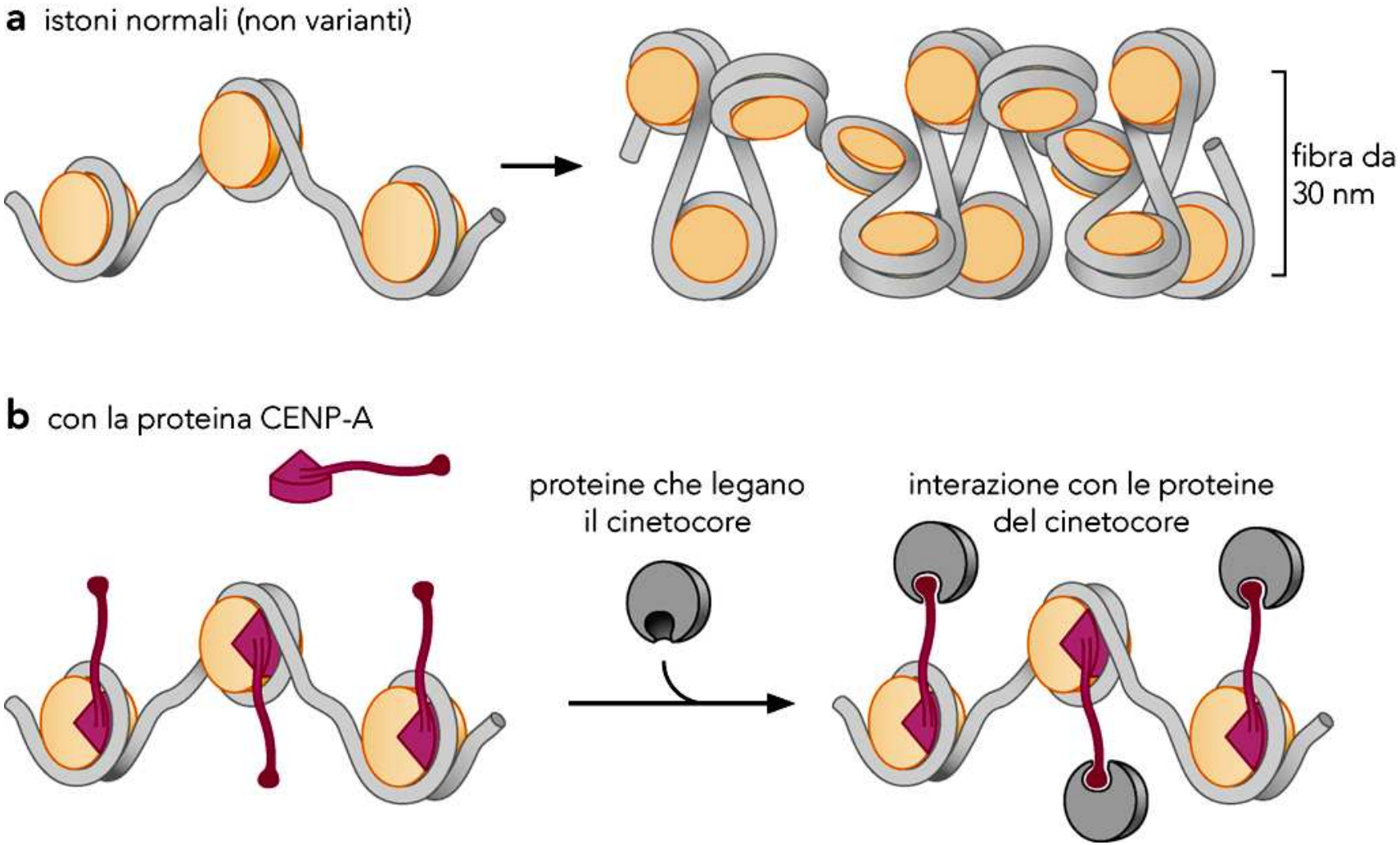


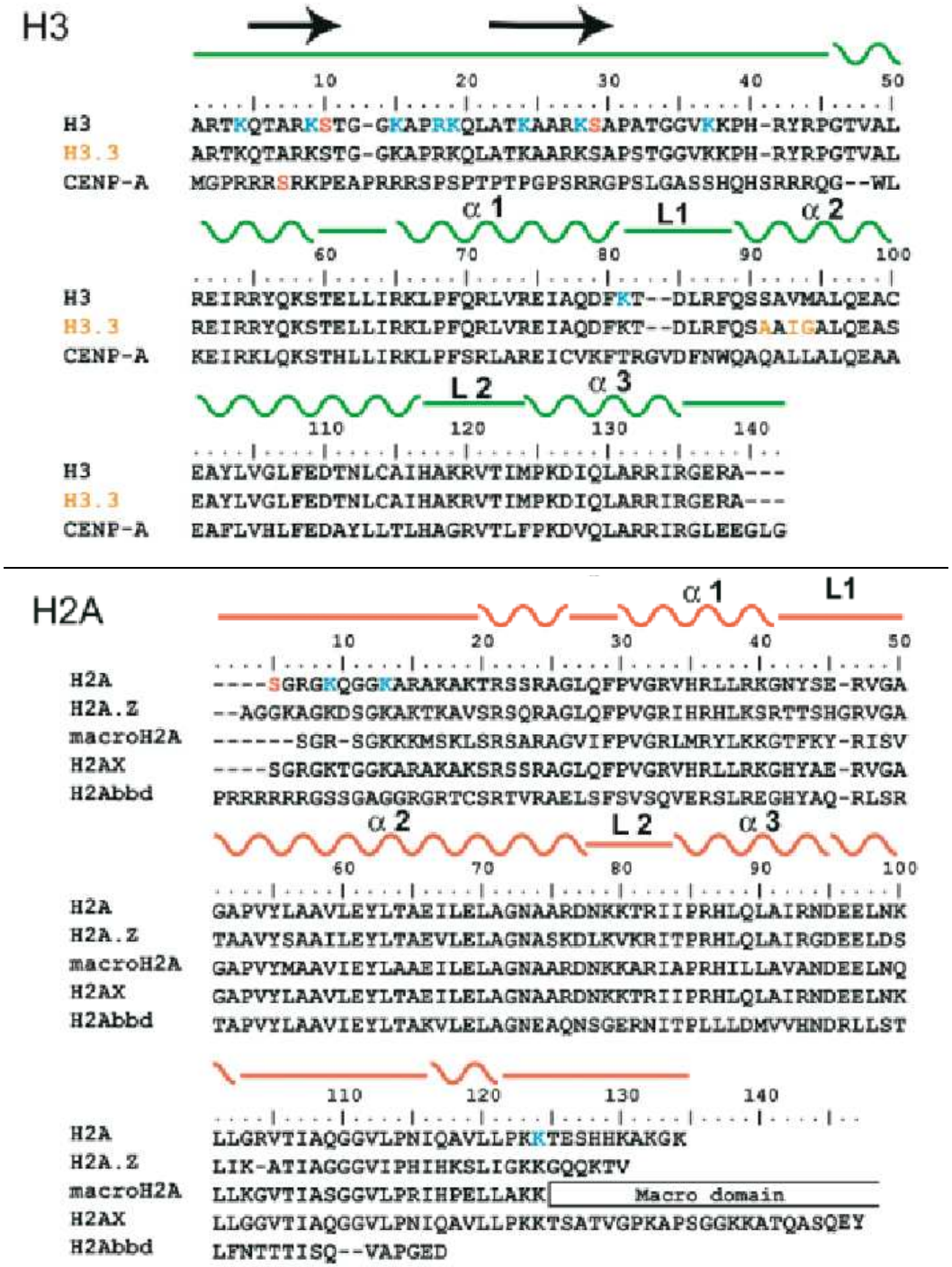
- the histone code



1. post-transcriptional modifications
2. histone isoform exchange
3. interacting proteins

isoform exchange





Sequence Alignment of Variants of Histones H3 and H2A with the Known Secondary Structures of H3 and H2A Depicted on Top.

Upper:
The sequences of the conserved H3.3 and CENP-A variants. H3.3 differs by only a few residues. The arrows above the H3 N-terminal tail indicate the sites that form strands upon binding to chromodomains.

Lower
The sequences of the conserved H2A.Z, macroH2A, H2AX, and H2ABbd variants of H2A. The sequence of H2ABbd is most divergent, while others are closely related with some changes in the turn regions connecting the helices.

CENP-A is an H3-like histone and is found only at centromeres over a stretch of 300-500 Kbp

CENP-A, unlike all other histones, is not replaced by protamines in sperm: chromatin status inheritance.

H3.3 is a variant of H3 showing only 4 aminoacids variation.

H3.3 is deposited in chromatin also outside S-phase

H3.3 replaces H3 carrying H3K9me in re-activated genes

H2A.Z in S. cerevisiae is incorporated near silenced regions and inhibits the spread of heterochromatin.

3. interacting proteins

Two cathegories: modifying enzymes and binding proteins

histone-modifying enzymes:

HAT- histone acetyltransferases
HDAC – histone deacetylases
HMT – histone methyltransferases
histone demethylases
histone kinases
histone ribosyltrabsferases
ubiquitin-transferases
ATP-dependent remodelling enzymes

Histone-modifying enzymes

Suppressor of variegation

Table 2. Histone-Modifying Enzymes

Enzymes that Modify Histones	Residues Modified
Acetyltransferase	
HAT1	H4 (K5, K12)
CBP/P300	H3 (K14, K18) H4 (K5, K8) H2A (K5) H2B (K12, K15)
PCAF/GCN5	H3 (K9, K14, K18)
TIP60	H4 (K5, K8, K12, K16) H3 K14
HB01 (ScESA1, SpMST1)	H4 (K5, K8, K12)
ScSAS3	H3 (K14, K23)
ScSAS2 (SpMST2)	H4 K16
ScRTT109	H3 K56
Deacetylases	
SirT2 (ScSir2)	H4 K16
Lysine Methyltransferase	
SUV39H1	H3K9
SUV39H2	H3K9
G9a	H3K9
ESET/SETDB1	H3K9
EuHMTase/GLP	H3K9
CLL8	H3K9
SpClr4	H3K9
MLL1	H3K4
MLL2	H3K4
MLL3	H3K4
MLL4	H3K4
MLL5	H3K4
SET1A	H3K4
SET1B	H3K4
ASH1	H3K4

Table 2. *Continued*

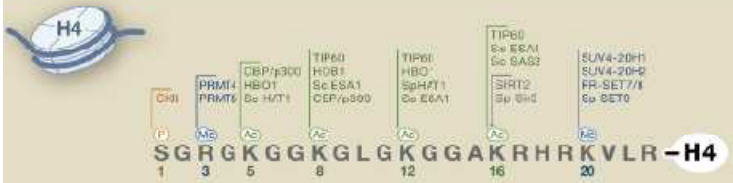
Enzymes that Modify Histones	Residues Modified
Lysine Demethylases	
LSD1/BHC110	H3K4
JHDM1a	H3K36
JHDM1b	H3K36
JHDM2a	H3K9
JHDM2b	H3K9
JMJD2A/JHDM3A	H3K9, H3K36
JMJD2B	H3K9
JMJD2C/GASC1	H3K9, H3K36
JMJD2D	H3K9
Arginine Methlytransferases	
CARM1	H3 (R2, R17, R26)
PRMT4	H4R3
PRMT5	H3R8, H4R3
Serine/Thrionine Kinases	
Haspin	H3T3
MSK1	H3S28
MSK2	H3S28
CKII	H4S1
Mst1	H2BS14
Ubiquitilases	
Bmi/Ring1A	H2AK119
RNF20/RNF40	H2BK120
Proline Isomerases	
ScFPR4	H3P30, H3P38

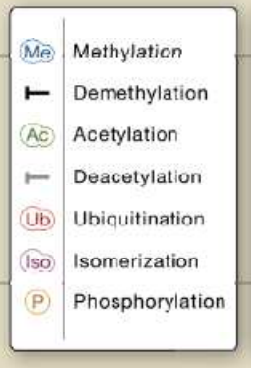
Only enzymes with specificity for one or a few sites have been included, along with the sites they modify. Human and yeast enzymes are shown. The yeast enzymes are distinguished by a prefix: Sc (*Saccharomyces cerevisiae*) or Sp (*Saccharomyces pombe*). Enzymes that fall within the same family are grouped.

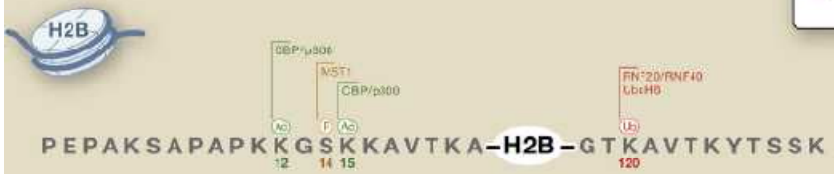
SnapShot: Histone-Modifying Enzymes

Cell

Tony Kouzarides
The Gurdon Institute, University of Cambridge, Cambridge CB2 1QN, UK

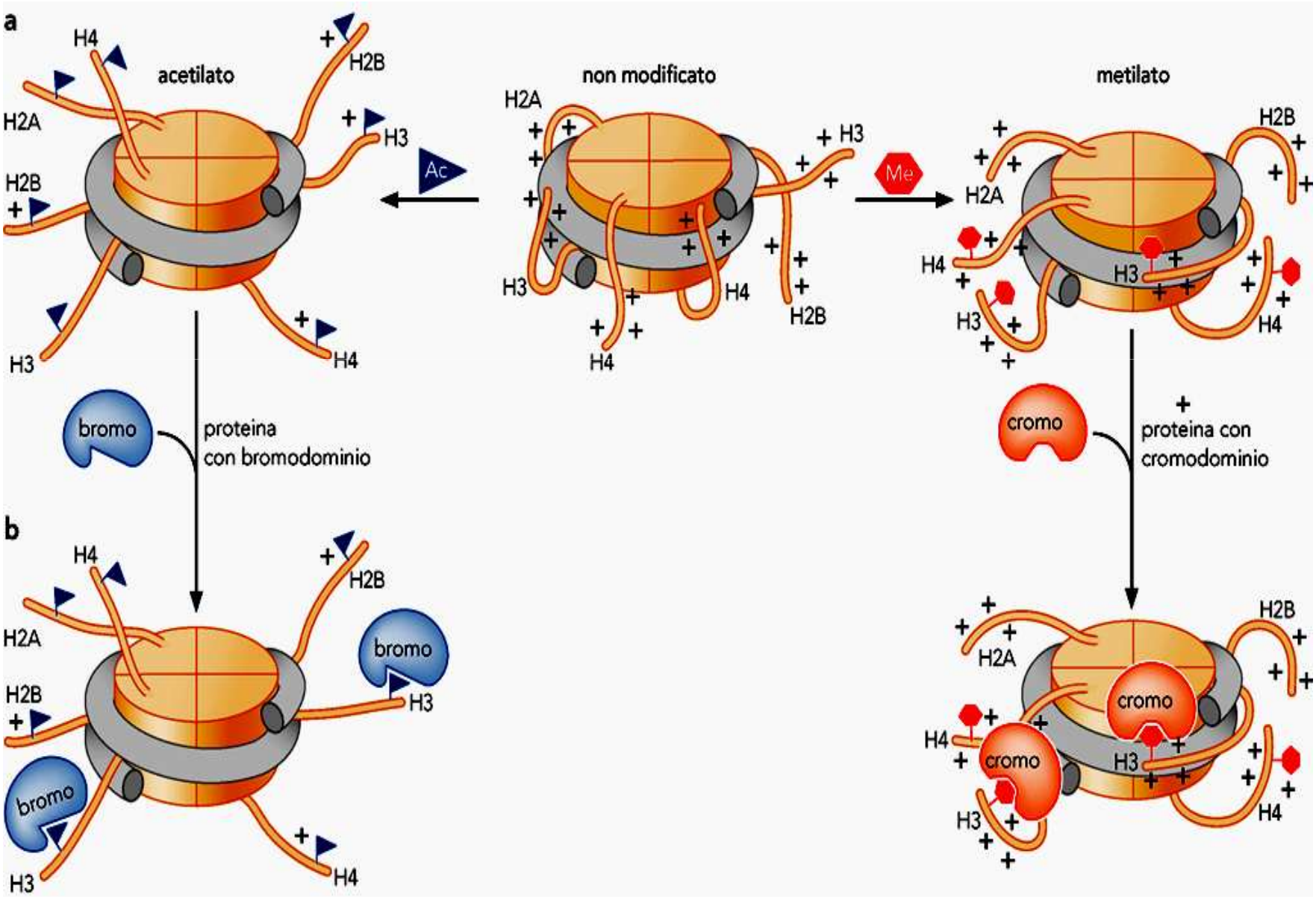






See online version for legend and references.

3. interacting proteins



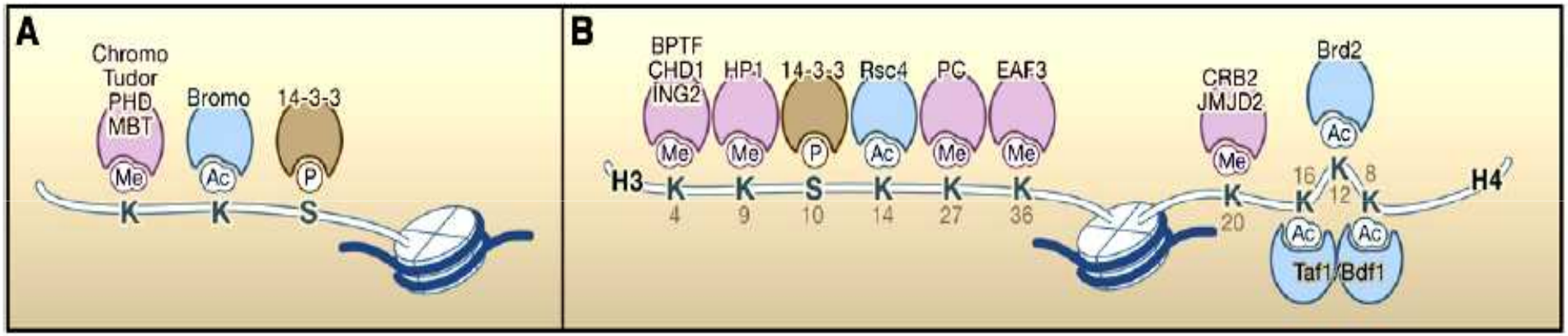


Figure 1. Recruitment of Proteins to Histones

(A) Domains used for the recognition of methylated lysines, acetylated lysines, or phosphorylated serines. (B) Proteins found that associate preferentially with modified versions of histone H3 and histone H4.

REVIEW

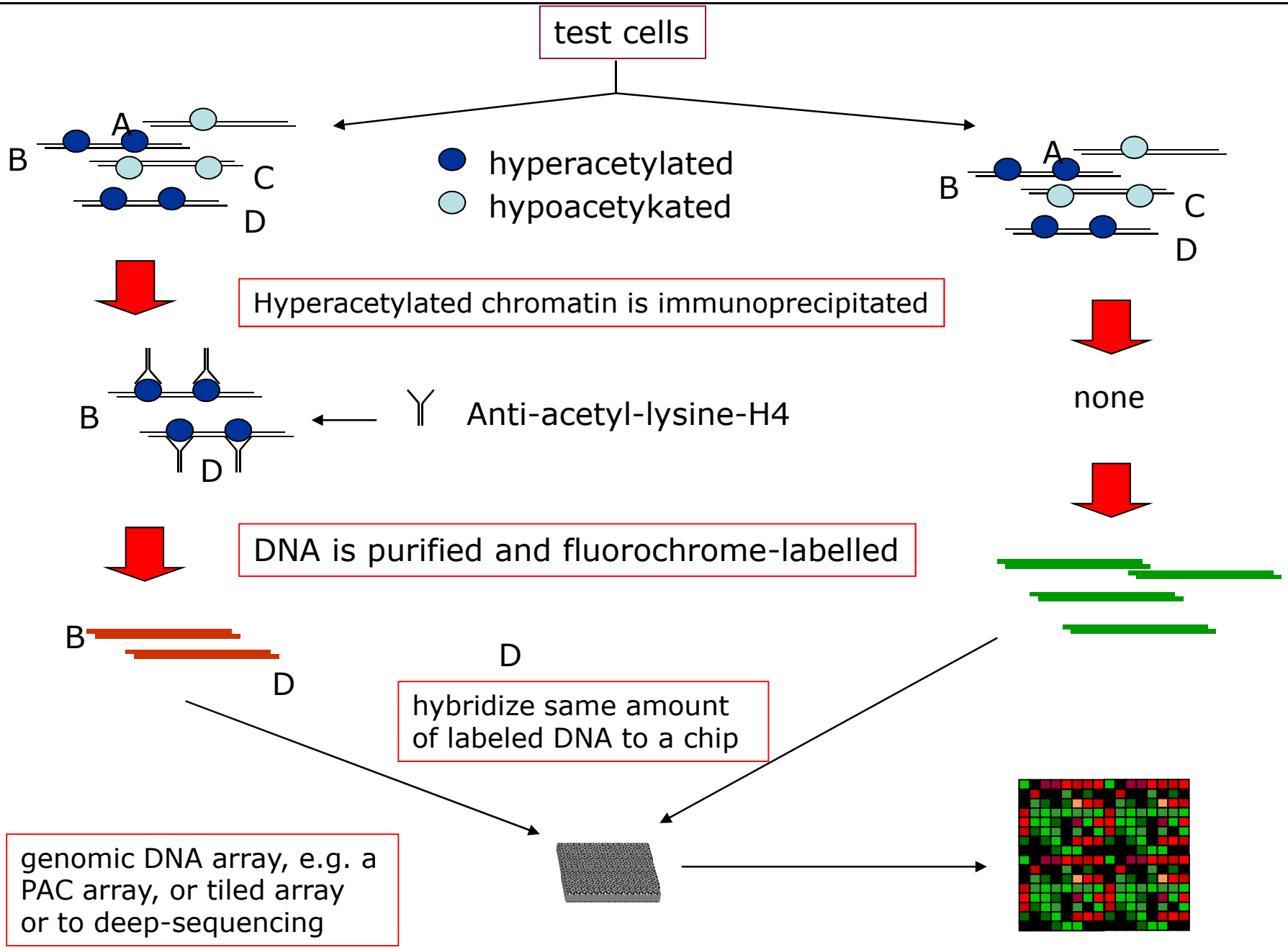
Methods and technologies used to study chromatin status at the genome-wide level are reviewed here

Microarray technology: beyond transcript profiling and genotype analysis

Jörg D. Hoheisel

Abstract | Understanding complex functional mechanisms requires the global and parallel analysis of different cellular processes. DNA microarrays have become synonymous with this kind of study and, in many cases, are the obvious platform to achieve this aim. They have already made important contributions, most notably to gene-expression studies, although the true potential of this technology is far greater. Whereas some assays, such as transcript profiling and genotyping, are becoming routine, others are still in the early phases of development, and new areas of application, such as genome-wide epigenetic analysis and on-chip synthesis, continue to emerge.

Identification of hyperacetylated nucleosomes by genome-wide chromatin immunoprecipitation



Direct isolation and identification of promoters in the human genome

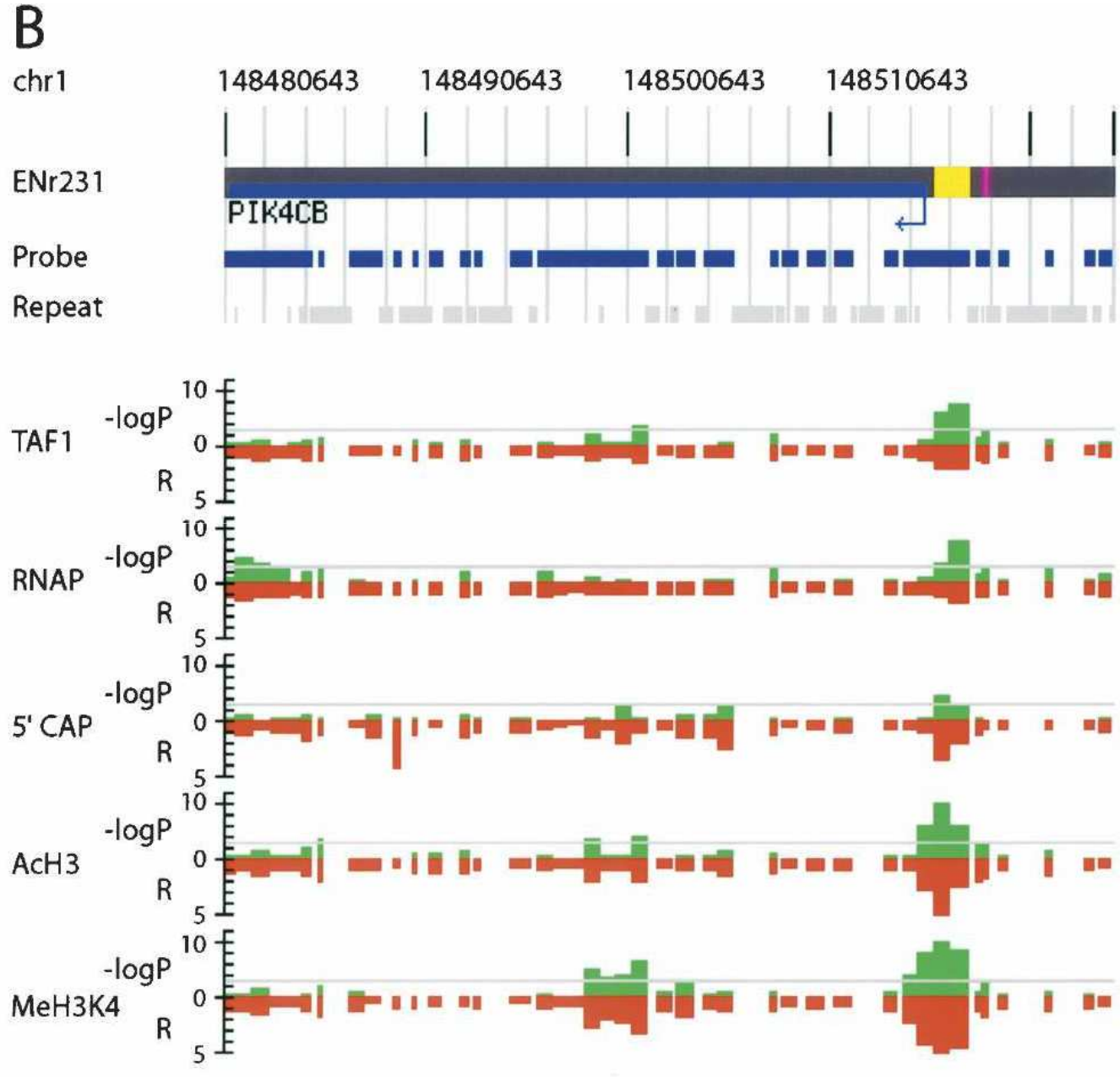
Tae Hoon Kim,[1] Leah O. Barrera,[1] Chunxu Qu,[1] Sara Van Calcar,[1] Nathan D. Trinklein,[4] Sara J. Cooper,[4] Rosa M. Luna,[2] Christopher K. Glass,[2] Michael G. Rosenfeld,[3] Richard M. Myers,[4] and Bing Ren[1,2,5]

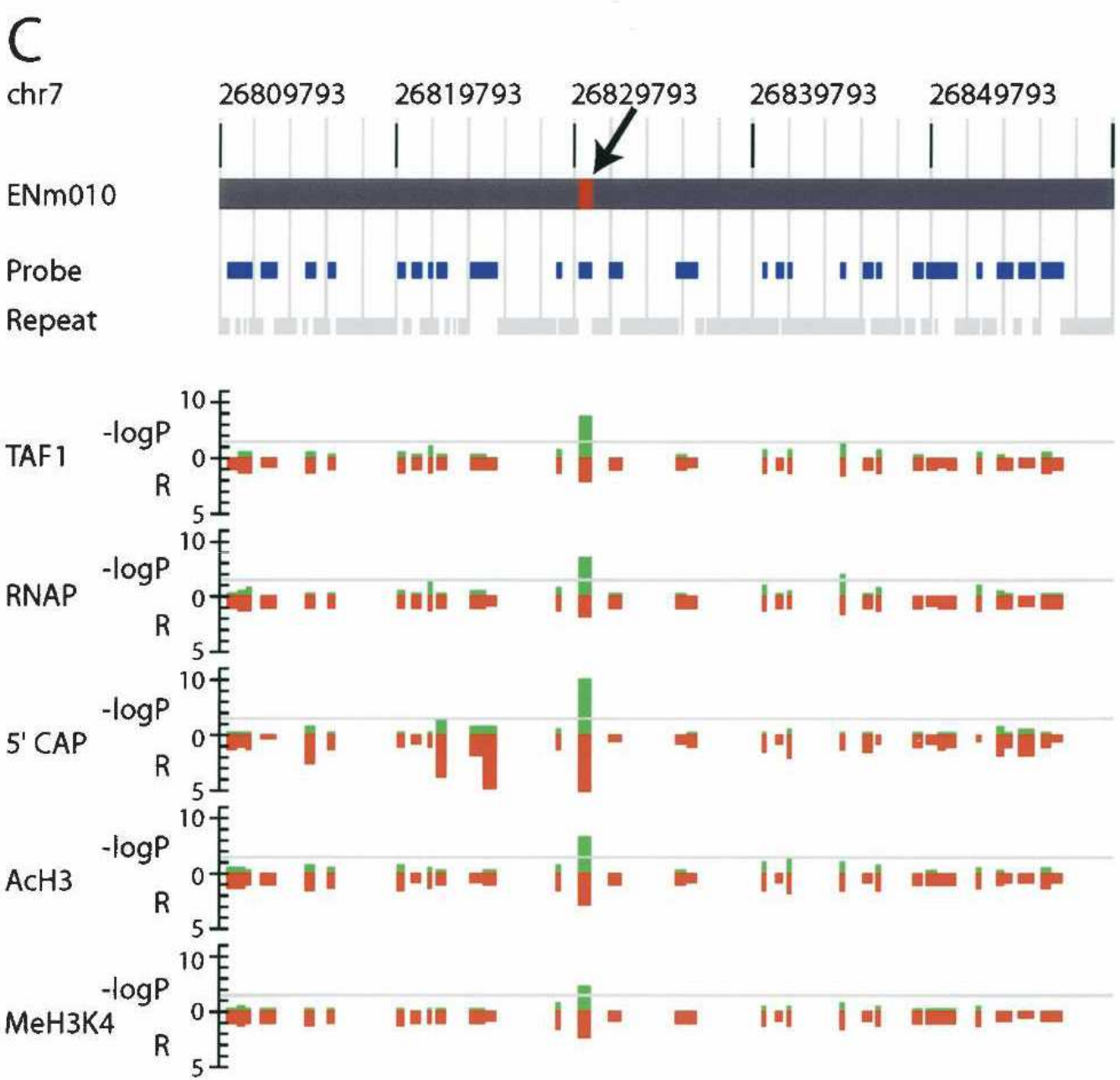
[1]Ludwig Institute for Cancer Research, [2]Department of Cellular and Molecular Medicine, and [3]Howard Hughes Medical Institu University of California, San Diego, La Jolla, California 92093, USA; [4]Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA

Transcriptional regulatory elements play essential roles in gene expression during animal development and cellular response to environmental signals, but our knowledge of these regions in the human genome is limited despite the availability of the complete genome sequence. Promoters mark the start of every transcript and are an important class of regulatory elements. A large, complex protein structure known as the pre-initiation complex (PIC) is assembled on all active promoters, and the presence of these proteins distinguishes promoters from other sequences in the genome. Using components of the PIC as tags, we isolated promoters directly from human cells as protein–DNA complexes and identified the resulting DNA sequences using genomic tiling microarrays. Our experiments in four human cell lines uncovered 252 PIC-binding sites in 44 semirandomly selected human genomic regions comprising 1% (30 megabase pairs) of the human genome. Nearly 72% of the identified fragments overlap or immediately flank 5′ ends of known cDNA sequences, while the remainder is found in other genomic regions that likely harbor putative promoters of unannotated transcripts. Indeed, molecular analysis of the RNA isolated from one cell line uncovered transcripts initiated from over half of the putative promoter fragments, and transient transfection assays revealed promoter activity for a significant proportion of fragments when they were fused to a luciferase reporter gene. These results demonstrate the specificity of a genome-wide analysis method for mapping transcriptional regulatory elements and also indicate that a small, yet significant number of human genes remains to be discovered.

[Supplemental material is available online at www.genome.org.]

15:830–839 ©2005 by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/05; www.genome.org

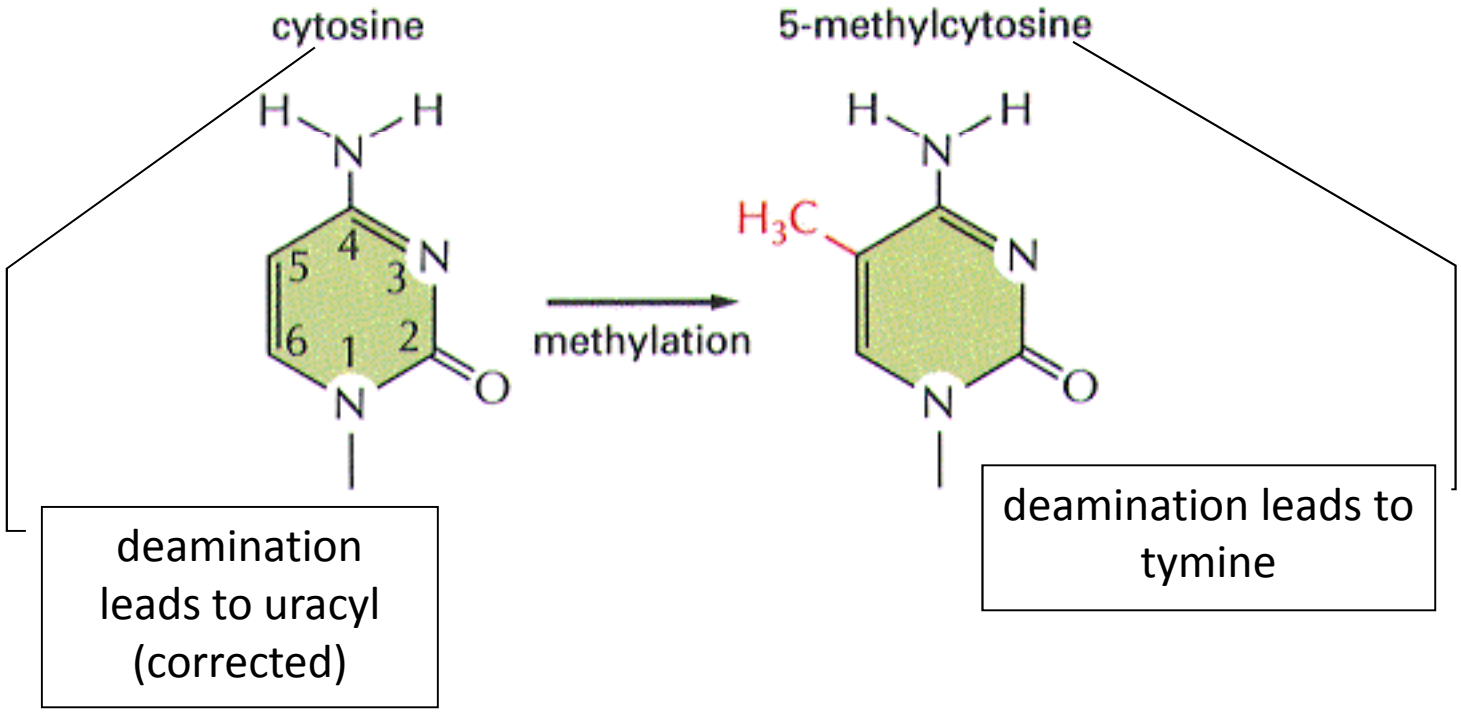

B
chr1
148480643
148490643
148500643
148510643
ENr231
PIK4CB
Probe
Repeat
TAF1
RNAP
5' CAP
AcH3
MeH3K4
-logP
10
0
5
R


C
chr7
26809793
26819793
26829793
26839793
26849793
ENm010
Probe
Repeat
TAF1
RNAP
5' CAP
AcH3
MeH3K4
-logP
R
10
0
5

Heterochromatic	Euchromatic
more packed	less packed
silenced	active or poised
rich in HP1α	poor in HP1α
Histones hypoacetylated	Histones hyperacetylated
H3K9me H3K27me other H metylations	H3K4me
CpG islands methylated	CpG islands un-methylated

CpG methylation and DNA replication, DNMT1

Analysis of heterochromatic regions and of silenced gene promoters has shown that a modification of 5'-CpG-3' sequences with methylation of the C-5 of cytosine is very frequent



CpG methylation is the only epigenetic modification concerning the DNA

Among experimental evidences obtained:

a. Methylation of CpG is observed at regulatory regions of silenced genes

b. Hypermethylation is observed through the inactive X chromosome

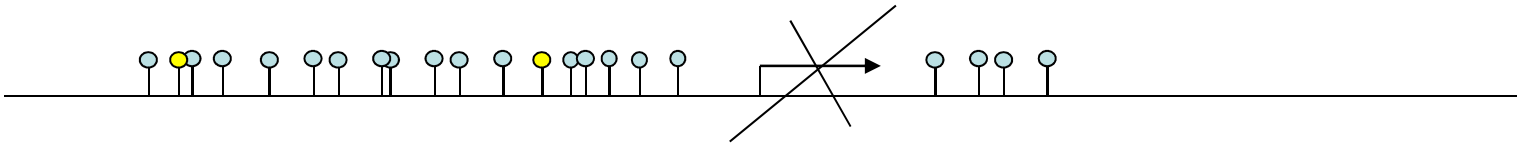
c. Methylated DNA can not usually be expressed as a transgene

d. Housekeeping genes (constitutively expressed) show unmethylated CpG islands

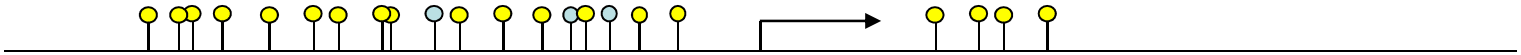
e. CpG methylation is observed at imprinted genes

f. CpG methylation profiles can be reproduced with fidelity during DNA replication and cell division

Important: CpG methylation is common in Mammals and Plants, but is not used in the same way in *S. cerevisiae* and *C. elegans*



gene in cell A (heterochromatic)

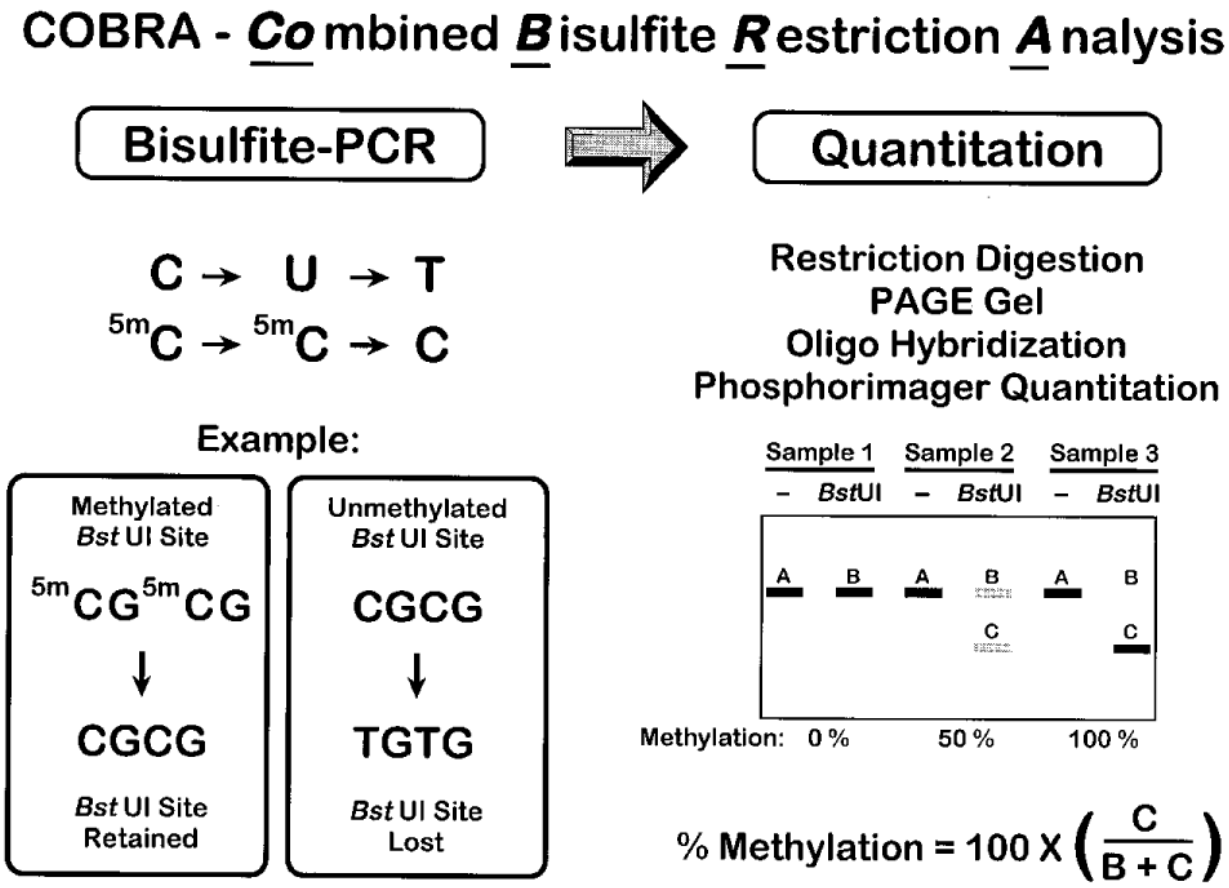


gene in cell B (euchromatic)

unmethylated CpG

methylated CpG

Most common methods to detect CpC methylation are based on bisulfite, that converts C (but not methyl-C) to Uridine. Uridine in DNA is then replaced by T in the following PCR.
Conservation of C (in the case of methyl-C) or change in T are subsequently detected (simplest) by restriction site analysis, as in the **example** following:



CpG methylation of a given DNA fragment is different in each cell

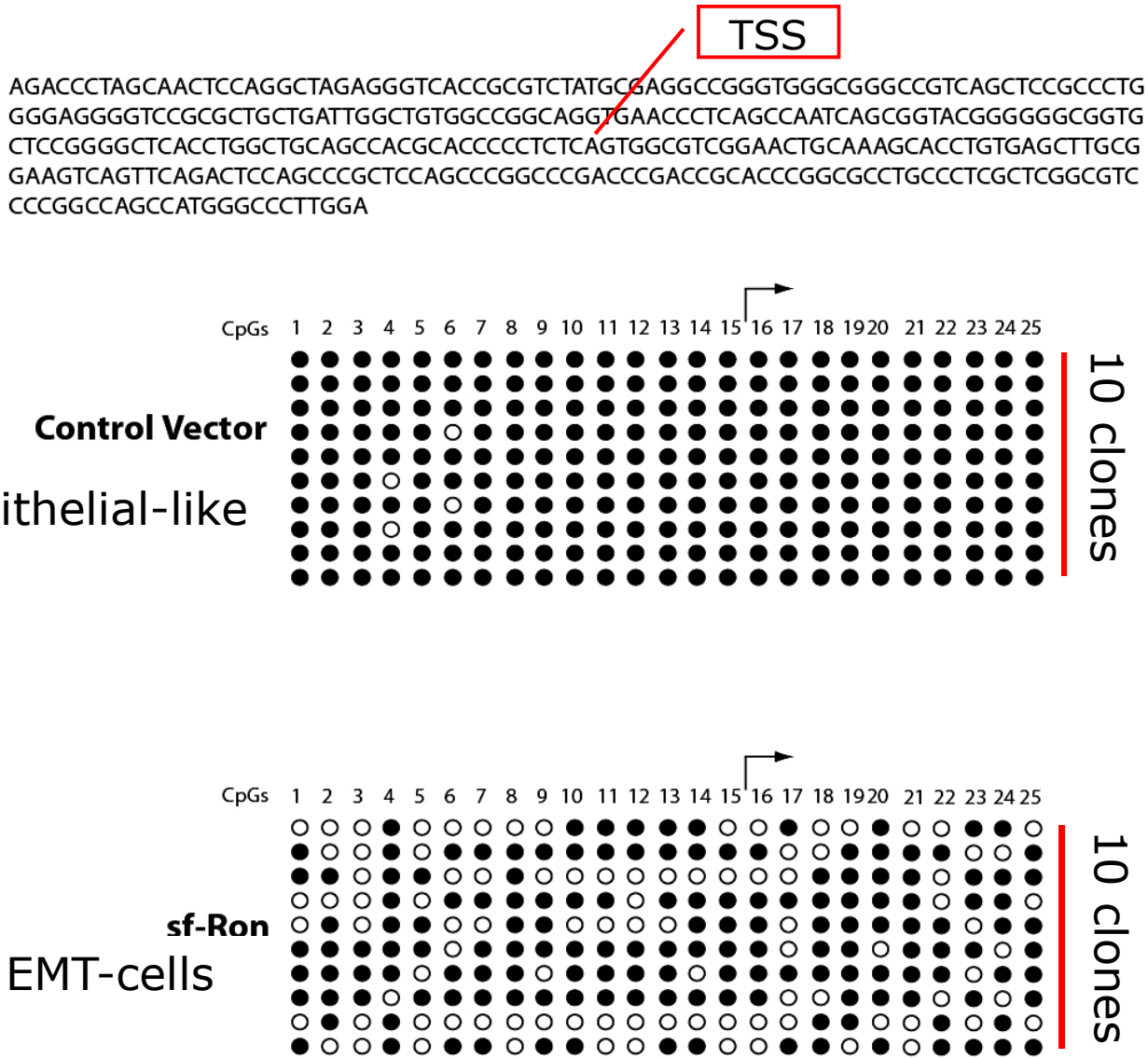
Therefore, to determine the methylation profile:

1) extract DNA from cells
2) bisulfite treatment → 3) deep sequencing

or

3) PCR the fragment
4) clone individual fragment in a vector
5) sequence a representative number of clones

E-Cadherin gene



From: Cardamone et al., 2009, PNAS 106(18):7420-5

Is it possible to study DNA CpG methylation genome-wide ?

Yes:

Approach I -

1) DNA immunoprecipitation using an Ab agaist 5'-methyl-cytosine
2) DNA precipitation using tagged-MBD

followed by hybridization to microarrays or deep-sequencing

Approach II –

Bisulfite conversion of the whole genomic DNA, followed by deep-sequencing

Very difficult! bisulfite changes the sequence so that a very high "sequencing depth" is required to allow mapping of the reads.

nature genetics

Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome

Michael Weber[1], Ines Hellmann[2,3], Michael B Stadler[1], Liliana Ramos[4], Svante Pääbo[2], Michael Rebhan[1] & Dirk Schübeler[1]

To gain insight into the function of DNA methylation at *cis*-regulatory regions and its impact on gene expression, we measured methylation, RNA polymerase occupancy and histone modifications at 16,000 promoters in primary human somatic and germline cells. We find CpG-poor promoters hypermethylated in somatic cells, which does not preclude their activity. This methylation is present in male gametes and results in evolutionary loss of CpG dinucleotides, as measured by divergence between humans and primates. In contrast, strong CpG island promoters are mostly unmethylated, even when inactive. Weak CpG island promoters are distinct, as they are preferential targets for *de novo* methylation in somatic cells. Notably, most germline-specific genes are methylated in somatic cells, suggesting additional functional selection. These results show that promoter sequence and gene function are major predictors of promoter methylation states. Moreover, we observe that inactive unmethylated CpG island promoters show elevated levels of dimethylation of Lys4 of histone H3, suggesting that this chromatin mark may protect DNA from methylation.

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Human DNA methylomes at base resolution show widespread epigenomic differences

Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

DNA cytosine methylation is a central epigenetic modification that has essential roles in cellular processes including genome regulation, development and disease. Here we present the first genome-wide, single-base-resolution maps of methylated cytosines in a mammalian genome, from both human embryonic stem cells and fetal fibroblasts, along with comparative analysis of messenger RNA and small RNA components of the transcriptome, several histone modifications, and sites of DNA–protein interaction for several key regulatory factors. Widespread differences were identified in the composition and patterning of cytosine methylation between the two genomes. Nearly one-quarter of all methylation identified in embryonic stem cells was in a non-CG context, suggesting that embryonic stem cells may use different methylation mechanisms to affect gene regulation. Methylation in non-CG contexts showed enrichment in gene bodies and depletion in protein binding sites and enhancers. Non-CG methylation disappeared upon induced differentiation of the embryonic stem cells, and was restored in induced pluripotent stem cells. We identified hundreds of differentially methylated regions proximal to genes involved in pluripotency and differentiation, and widespread reduced methylation levels in fibroblasts associated with lower transcriptional activity. These reference epigenomes provide a foundation for future studies exploring this key epigenetic modification in human disease and development.

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