Part 1.week 4

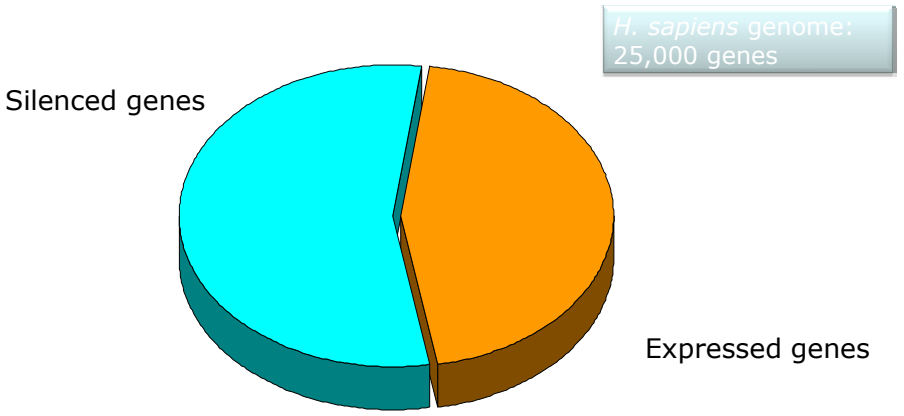
8. Chromatin

9. Epigenetics and imprinting

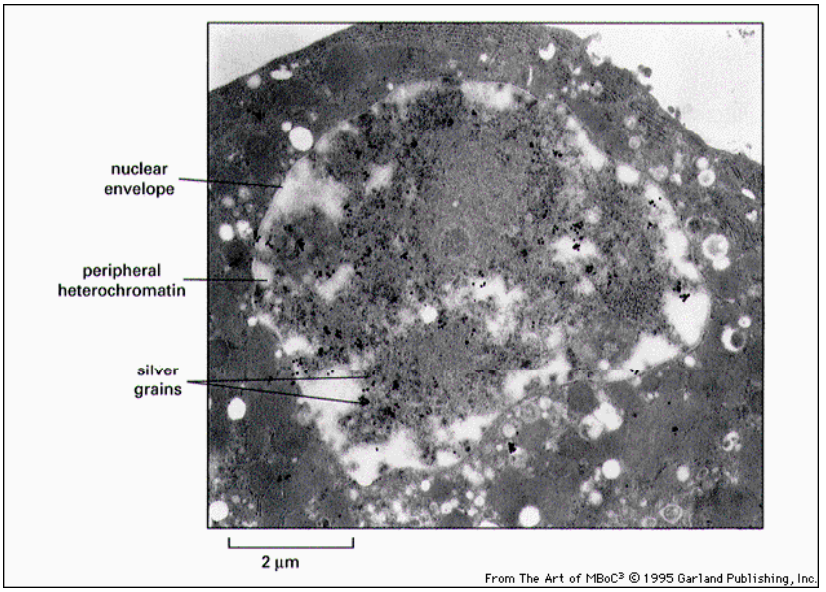
10. Post-transcriptional control and proteomics

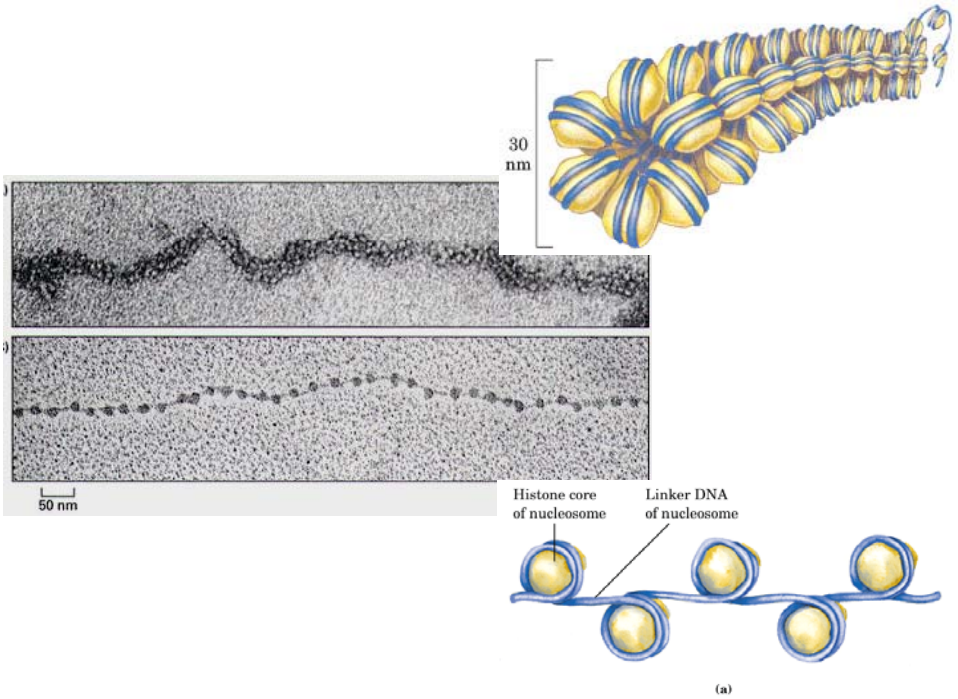
8.1 Nucleosomes
8.2 Covalent modification of histones
8.2 The histone code
8.3 Nucleosome transitions during gene activation/repression

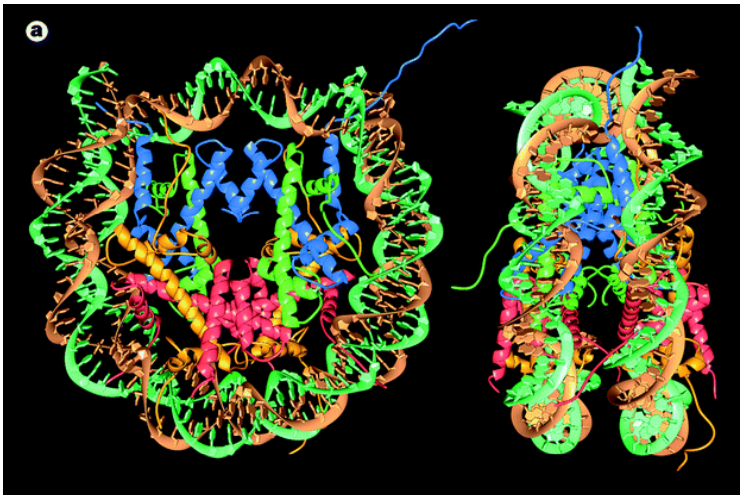
In any cell type, a large part of the genes are kept inactive (not expressed) by gene **silencing**



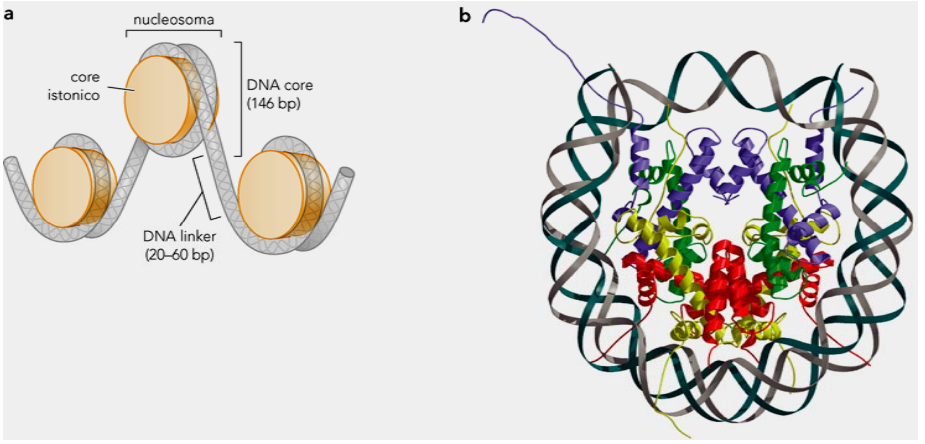
First studies on chromatin structure by E.M. in middle '70. The obtained images led to the hypotesis that the compacted form was typical of nonexpressed genes.



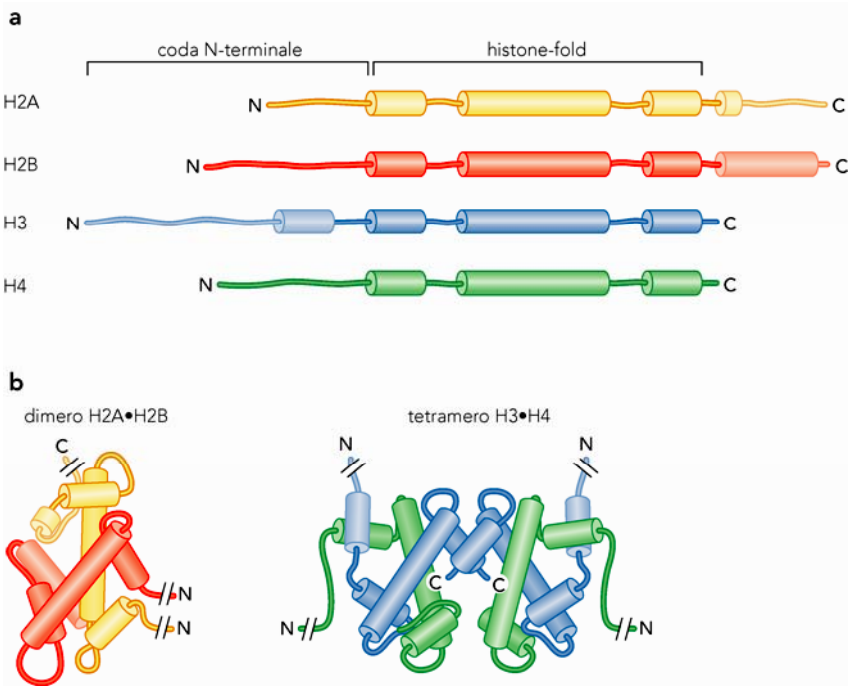


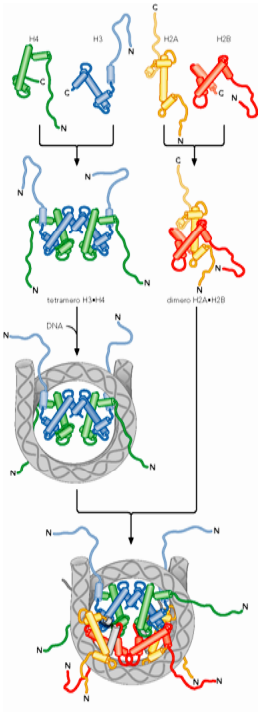


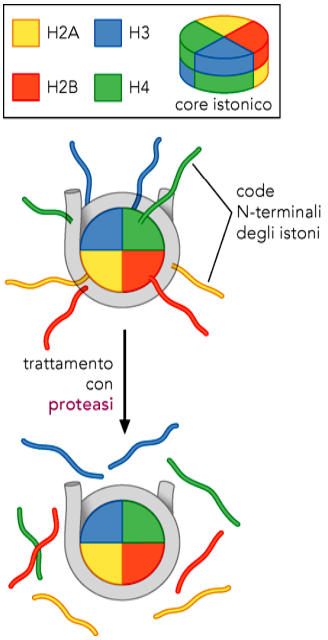
Post-transcriptional modifications, **histone isoform exchange** and **interacting proteins** make the nucleosome an extremely dynamic system whose primary function is to regulate gene transcription.



struttura del DNA nucleosomale
topologia (superavvolgimenti)
topoisomerasi


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


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


H2A
H3
H2B
H4
core istonico
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degli istoni
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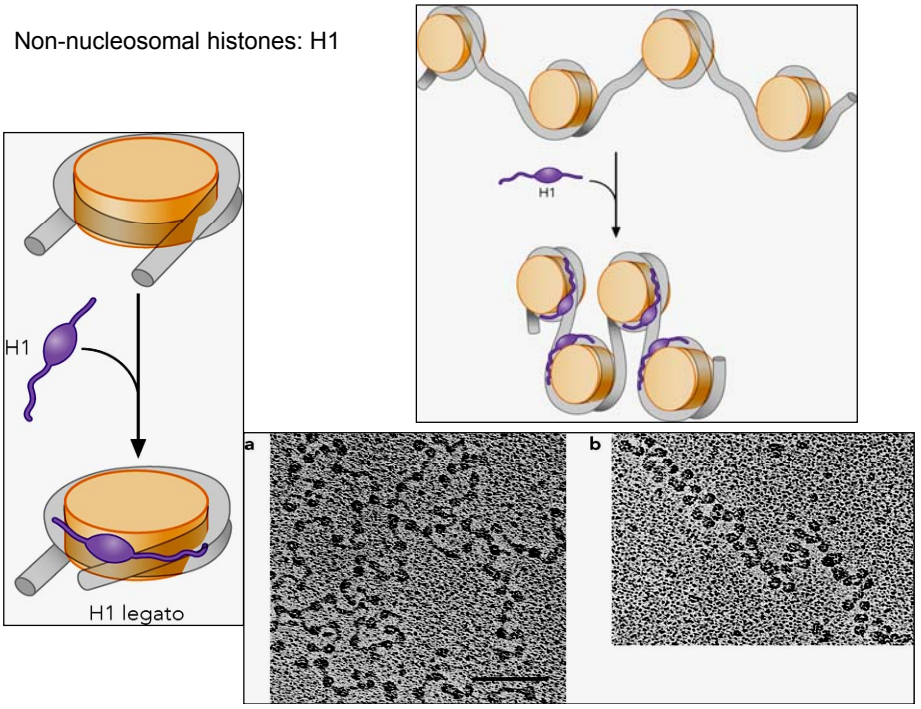
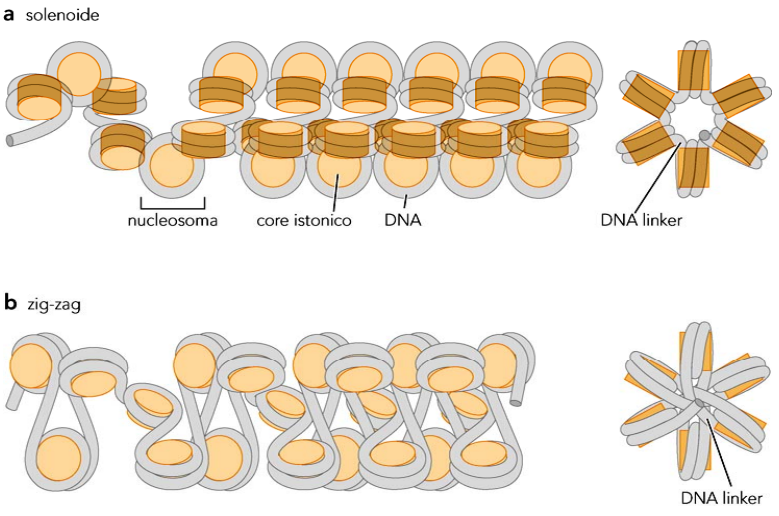
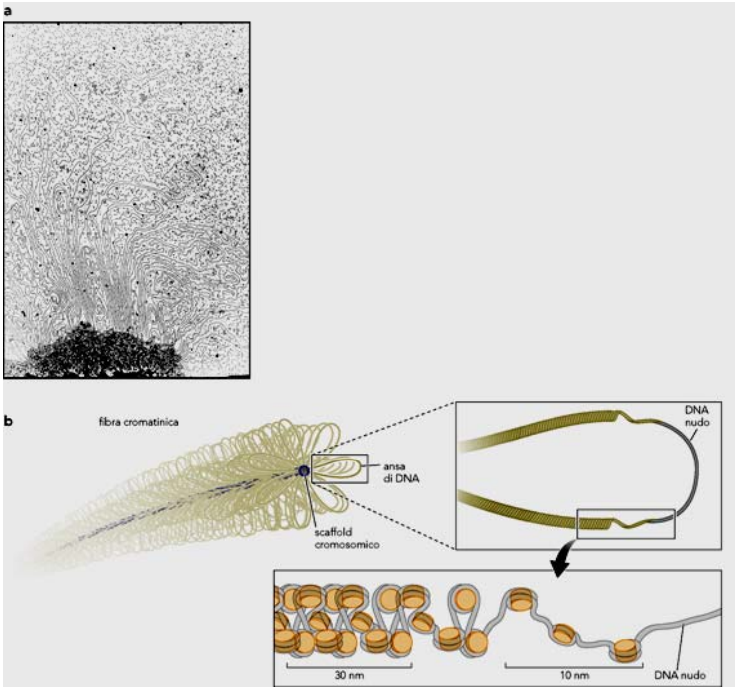
con
proteasi

Non-nucleosomal histones: H1

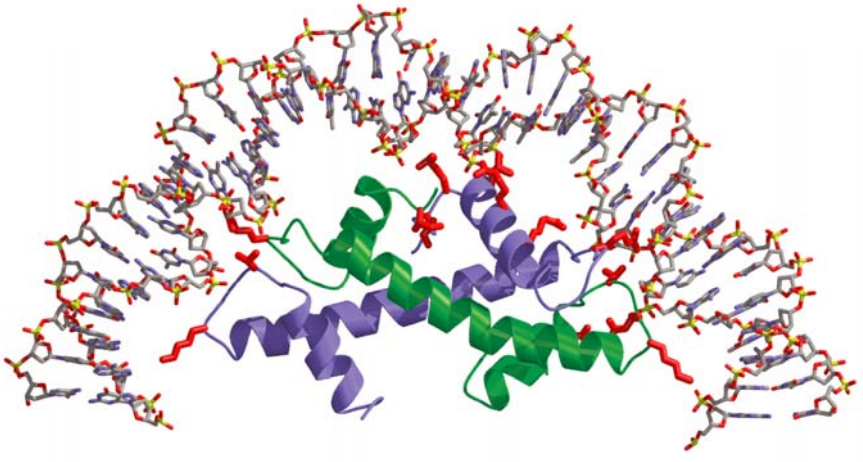


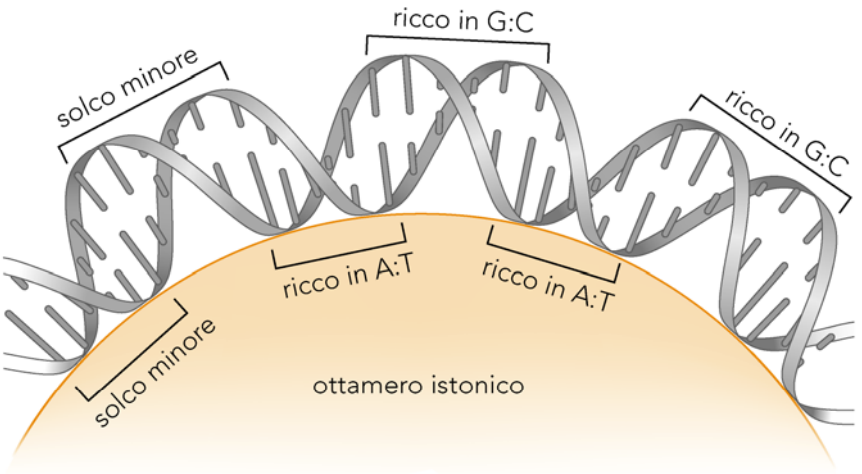
The 30nm fiber: solenoide or zig-zag ?





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

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

nature

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

ARTICLES

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

A genomic code for nucleosome positioning

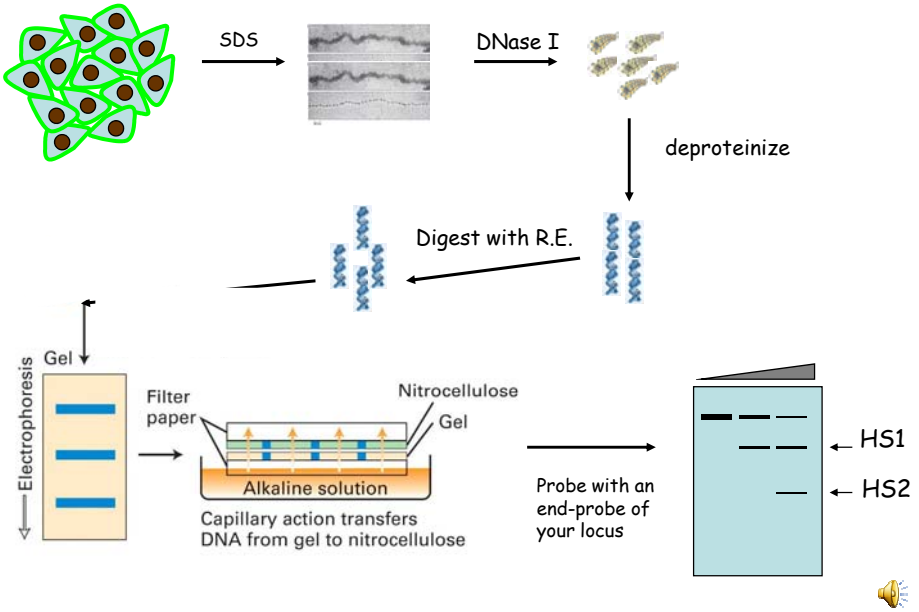
Eran Segal[1], Yvonne Fondufe-Mittendorf[2], Lingyi Chen[2], AnnChristine Thåström[2], Yair Field[1], Irene K. Moore[2], Ji-Ping Z. Wang[3] & Jonathan Widom[2]

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.

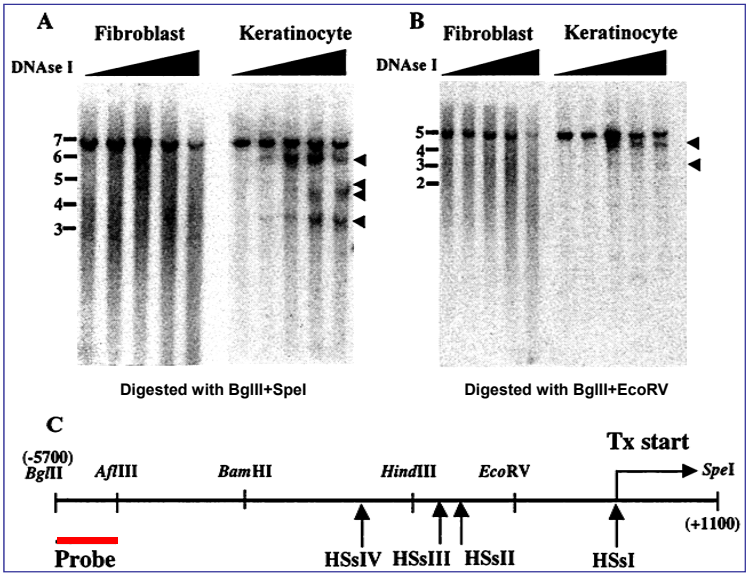
Are condensed (heterochromatic) and noncodensed (euchromatic) chromatin fragments (loci) distinguishable by a simple biochemical assay?

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

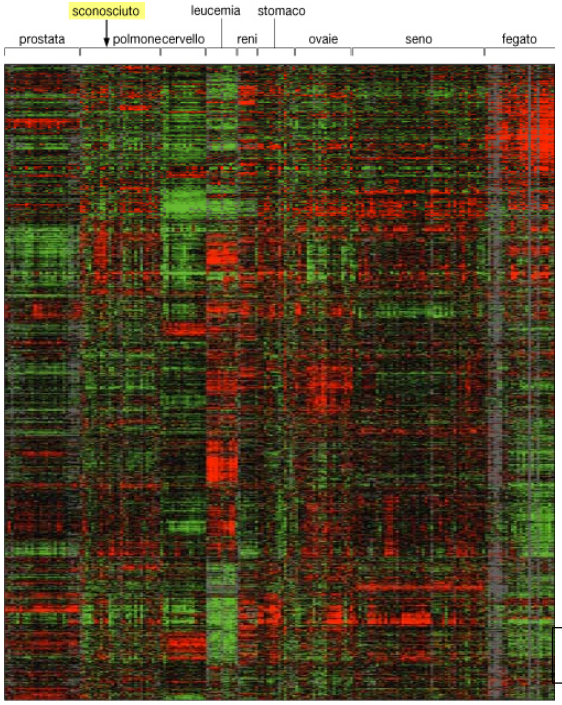


Tratto da: Sinha et al., (2000), Mol Cell Biol, 20: 2543-2555.

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

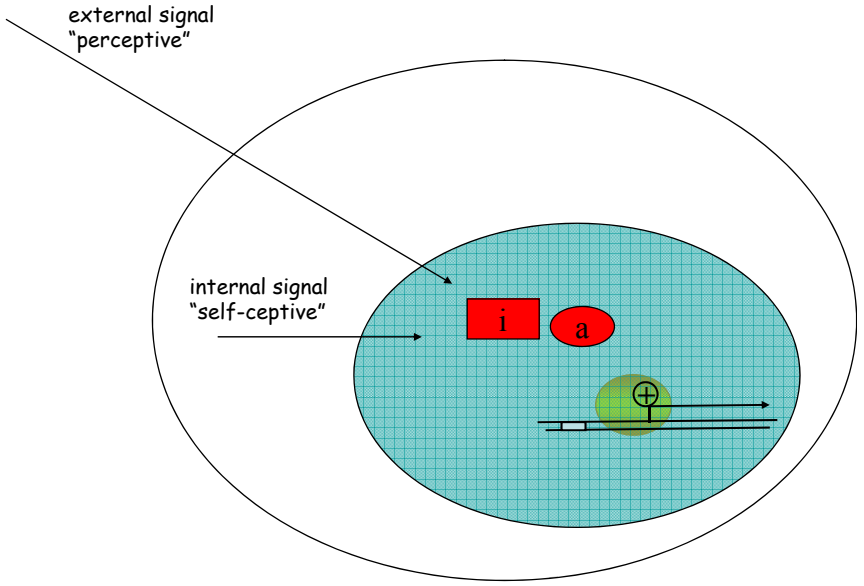
Other loci can switch transiently from one status to the other and back to the original, as a result of signal transduction from either perceptive or proprioceptive stimuli.

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

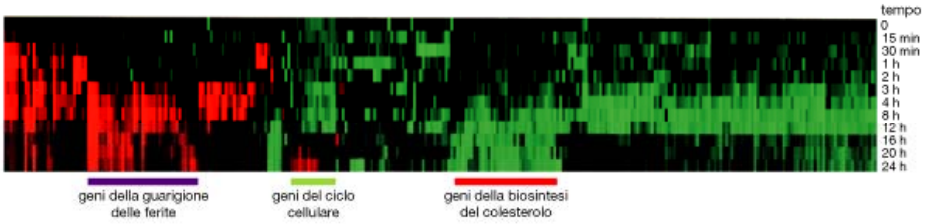


Quite stable, differentiation-linked gene expression profiles

Different expression profiles in human cells of different tissues: 1800 genes probes



1 example:



human fibroblasts: 48 hrs serum starvation, then serum back for the indicated times
RNA extracted at time points labelled with red dye
RNA extracted at time = 0 labelled with green dye

A 2° example:

A genomic view of estrogen actions in human breast cancer cells by expression profiling of the hormone-responsive transcriptome

Luigi Cicatiello[1], Claudio Scafoglio[1], Lucia Altucci[1], Massimo Cancemi[1], Guido Natoli[1], Angelo Facchiano[2], Giovanni Iazzetti[3], Raffaele Calogero[4], Nicoletta Biglia[6], Michele De Bortoli[5,7], Christian Sfiligoi[7], Piero Sismondi[6,7], Francesco Bresciani[1] and **Alessandro Weisz[1]**

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[4]Dipartimento di Scienze cliniche e biologiche, Università degli Studi di Torino, Torino, Italy

[5]Dipartimento di Scienze oncologiche, Università degli Studi di Torino, Torino, Italy

[6]Dipartimento di Discipline ostetriche e ginecologiche, Università degli Studi di Torino, Torino, Italy

[7]Laboratorio di Ginecologia oncologica, Istituto per la Ricerca e la Cura del Cancro, Candiolo, Italy

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Abstract

Estrogen controls key cellular functions of responsive cells including the ability to survive, replicate, communicate and adapt to the extracellular milieu. Changes in the expression of 8400 genes were monitored here by cDNA microarray analysis during the first 32 h of human breast cancer (BC) ZR-75·1 cell stimulation with a mitogenic dose of 17β-estradiol, a timing which corresponds to completion of a full mitotic cycle in hormone-stimulated cells. Hierarchical clustering of 344 genes whose expression either increases or decreases significantly in response to estrogen reveals that the gene expression program activated by the hormone in these cells shows 8 main patterns of gene activation/inhibition. This newly identified estrogen-responsive transcriptome represents more than a simple cell cycle response, as only a few affected genes belong to the transcriptional program of the cell division cycle of eukaryotes, or showed a similar expression profile in other mitogen-stimulated human cells. Indeed, based on the functions assigned to the products of the genes they control, estrogen appears to affect several key features of BC cells, including their metabolic status, proliferation, survival, differentiation and resistance to stress and chemotherapy, as well as RNA and protein synthesis, maturation and turn-over rates. Interestingly, the estrogen-responsive transcriptome does not appear randomly interspersed in the genome. In chromosome 17, for example, a site particularly rich in genes activated by the hormone, physical association of co-regulated genes in clusters is evident in several instances, suggesting the likely existence of estrogen-responsive domains in the human genome.

Journal of Molecular Endocrinology (2004) **32,** 719–775

Human breast cancer cell line in culture (ZR75.1)

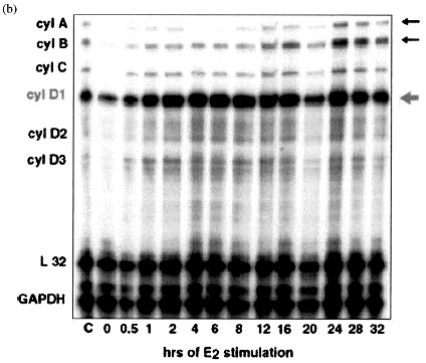
17β-estradiol stimulus

Cells rescued at: 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32 hours

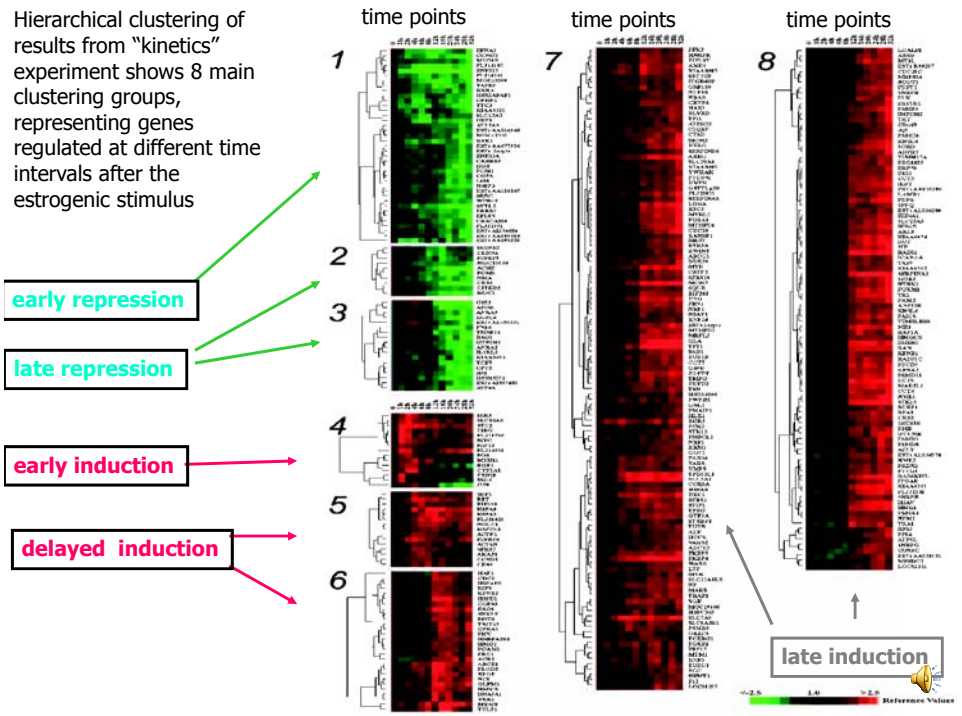
Cy3: time 0 Cy5: time points

9,600-feature cDNA microarrays

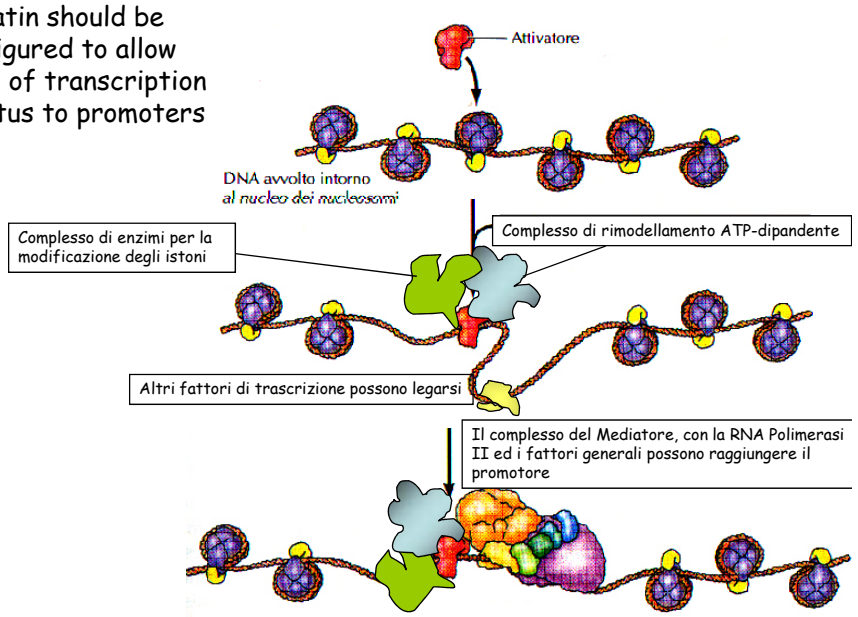
Cyclin mRNA expression by RNase protection assay:



Hierarchical clustering of results from "kinetics" experiment shows 8 main clustering groups, representing genes regulated at different time intervals after the estrogenic stimulus

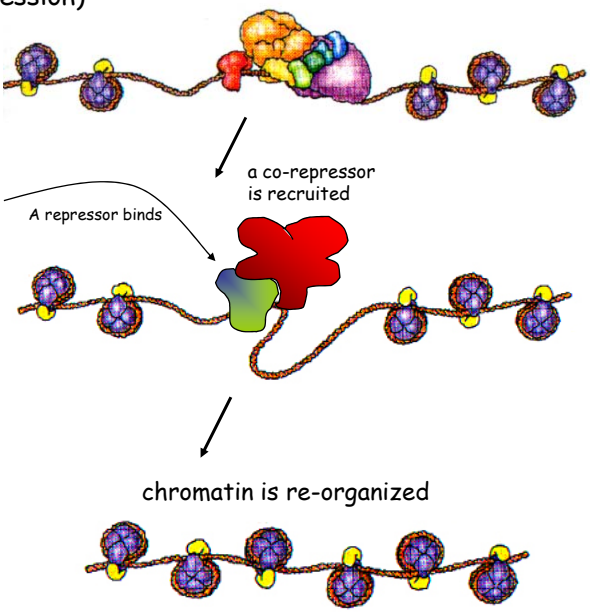


Chromatin should be reconfigured to allow binding of transcription apparatus to promoters



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

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



A quite simple biochemical assay for “activation” at the chromatin level is a **nucleosome positioning assay**

One extremely well studied model system for gene activation (switch from repressed to activated status) is represented by the PHO5 gene in S. cerevisiae.

PHO5 encodes for a protein phosphatase playing a role in phosphate homeostasis

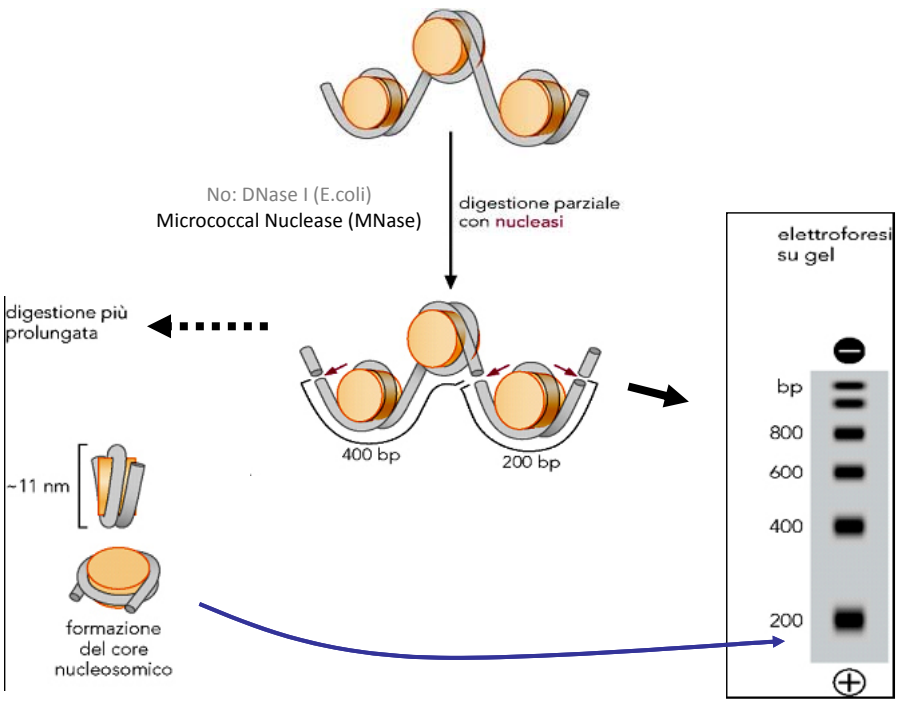
When the ortophosphate level in the cell falls below a treshold value, the PHO5 promoter:

•changes its chromatic status – nucleosomes are **mobilized**

•becomes DNaseI hypersensitive

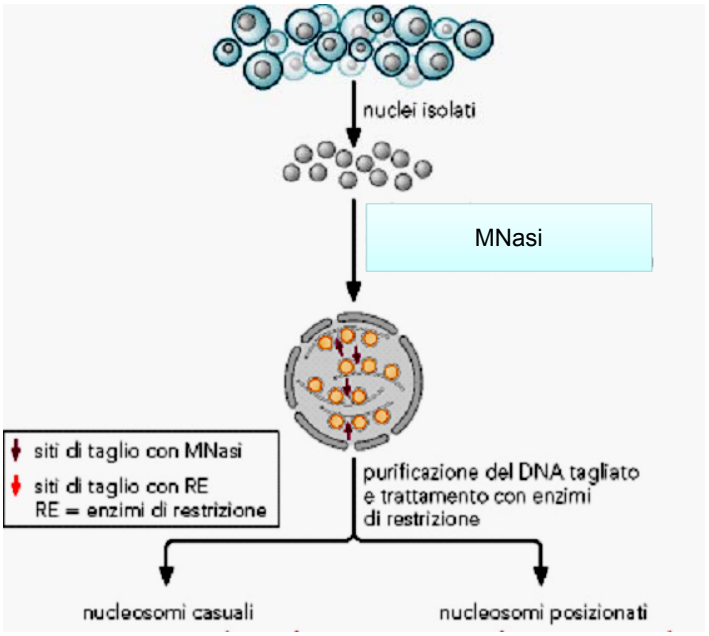
•transcription starts

mRNA and protein is produced → phosphates are removed from storage proteins and the intracellular level is restored

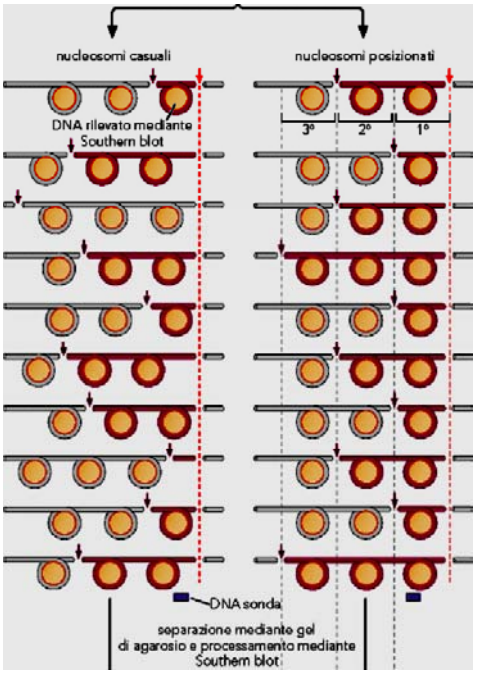


Nucleosome positioning analysis

1st step: digestion

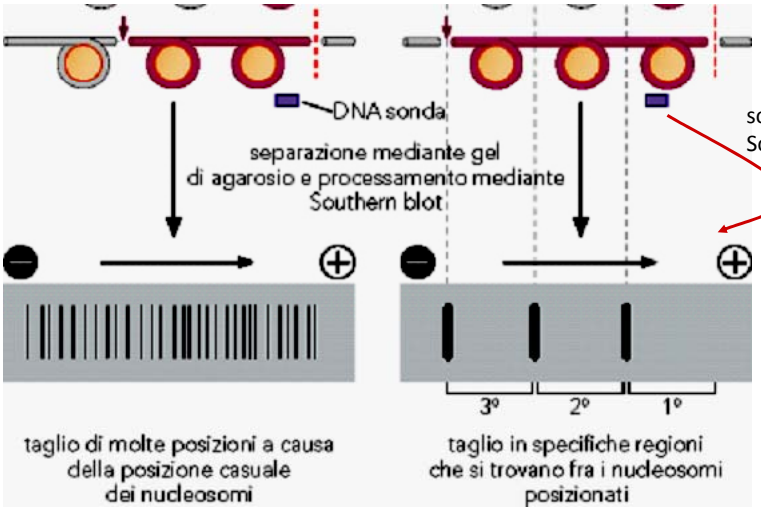


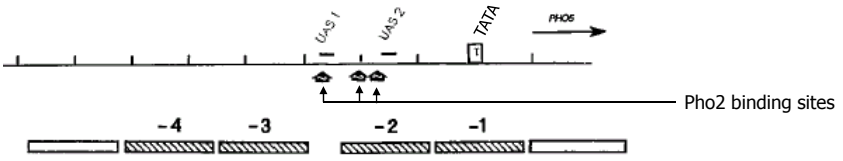
2 different cases:
mobile nucleosomes
versus
positioned nucleosomes



Analysis

mobile versus positioned





Pho4 is the P-sensitive inducer, whereas Pho2 is constitutive

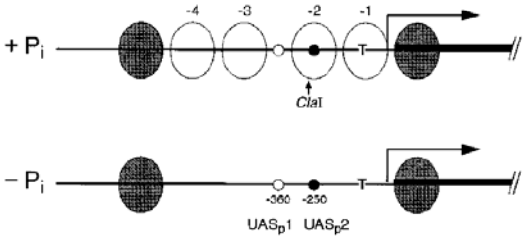


Figure 1. Chromatin Structure at the *PHO5* Promoter

Nucleosomes −1, −2, −3, and −4 are remodeled upon activation of the promoter by phosphate starvation conditions (Almer et al., 1986). The small circles mark UASp1 (open) and UASp2 (solid), which are Pho4-binding sites found by in vitro (Vogel et al., 1989) and in vivo (Venter et al., 1994) footprinting experiments. The positions are listed relative to the coding sequence (solid bar). T denotes the TATA box (Rudolph and Hinnen, 1987). The location of a ClaI site at −275 relative to the coding region is shown.

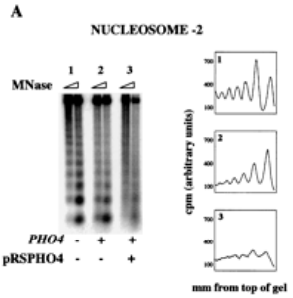
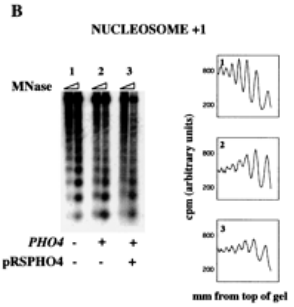


FIG. 5. In vivo chromatin remodeling of episomal *PHO5*. Spheroplasts from the indicated strains were treated with micrococcal nuclease (MNase), and the DNA was purified and Southern blotted. (A) The blot was probed with probe A (Fig. 2). Data from each sample were quantified, and the distance from the top of the gel was graphed against the signal density. (B) The blot shown in panel A was stripped and reprobed with probe B (Fig. 2).



Nucleosome dynamics

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

review

Chromatin Modifications and Their Function

Tony Kouzarides[1,*]
[1]The Gurdon Institute and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, 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.

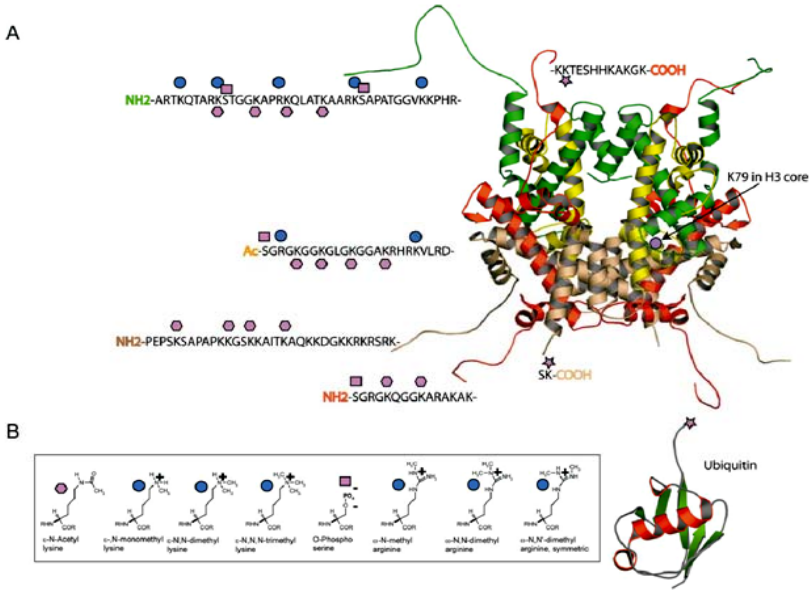
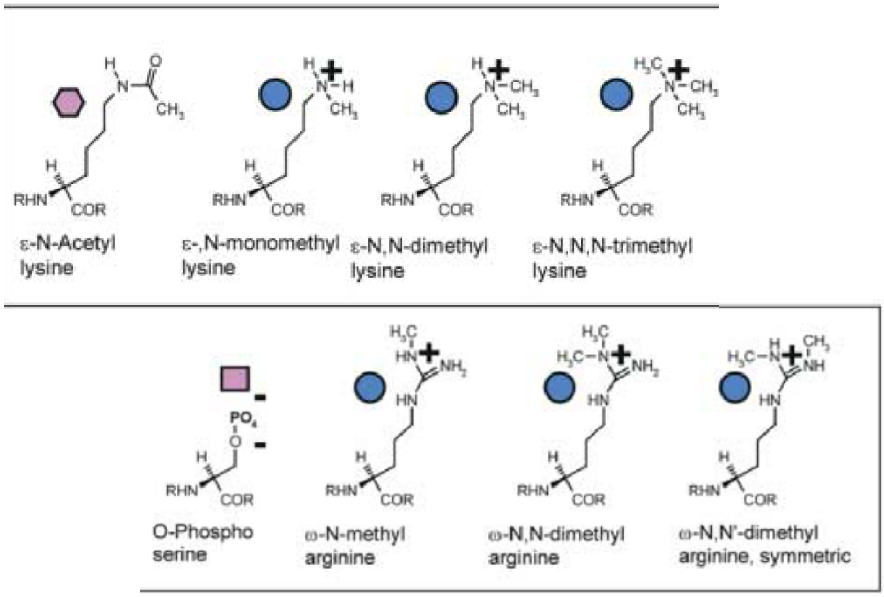


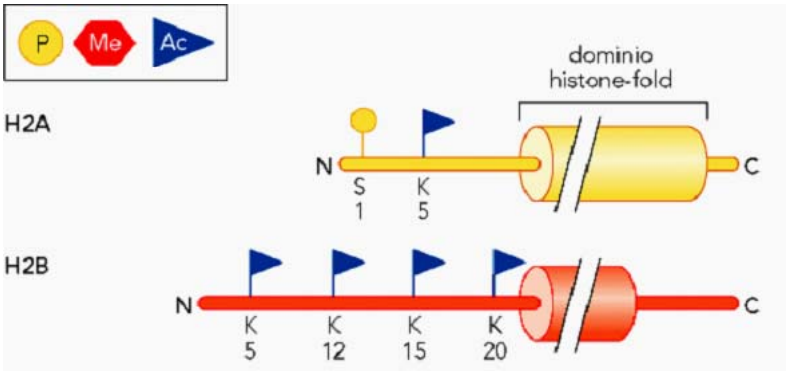
Figure 4. The Types of Posttranslational Modifications Observed on the Core Histones

(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.

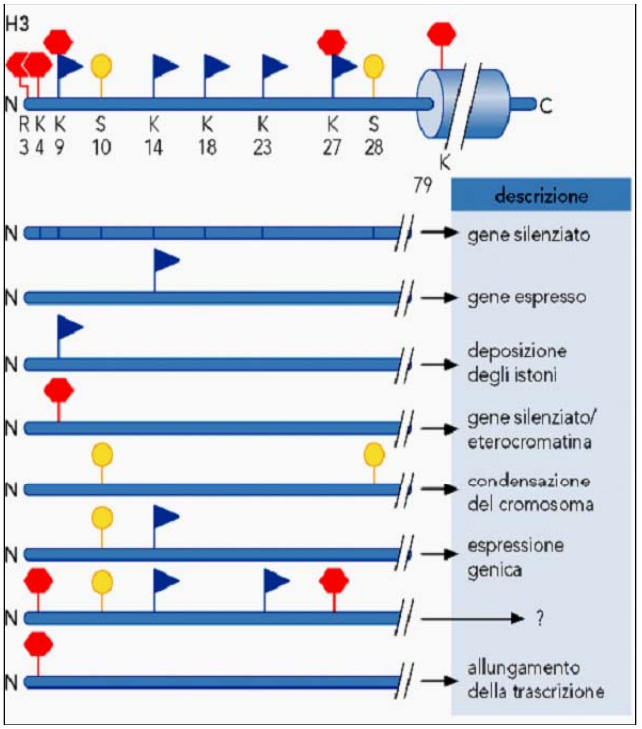


- the histone code

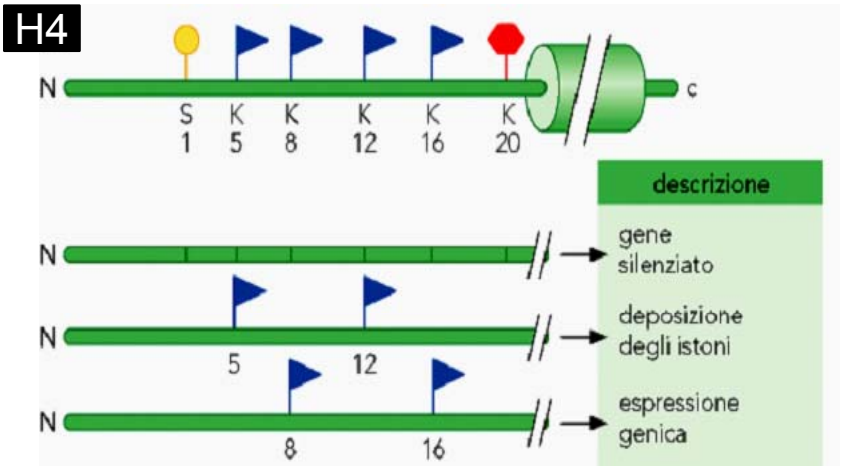


- the histone code

H3



- the histone code

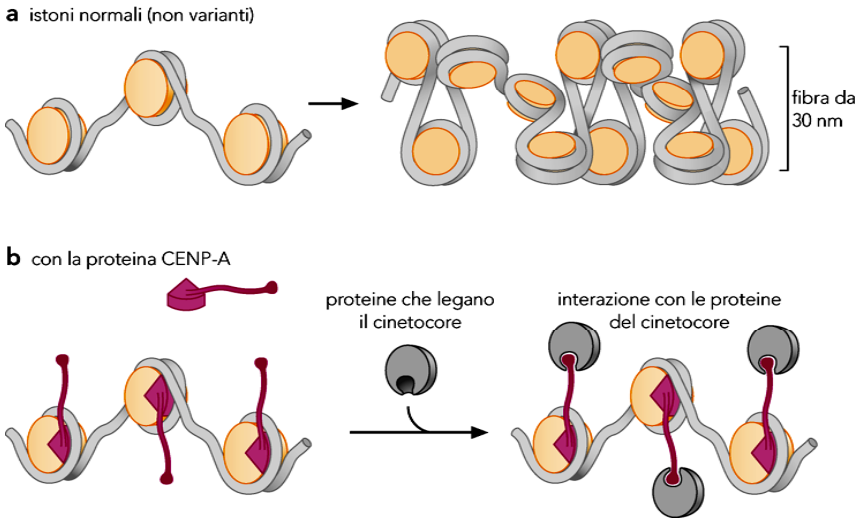


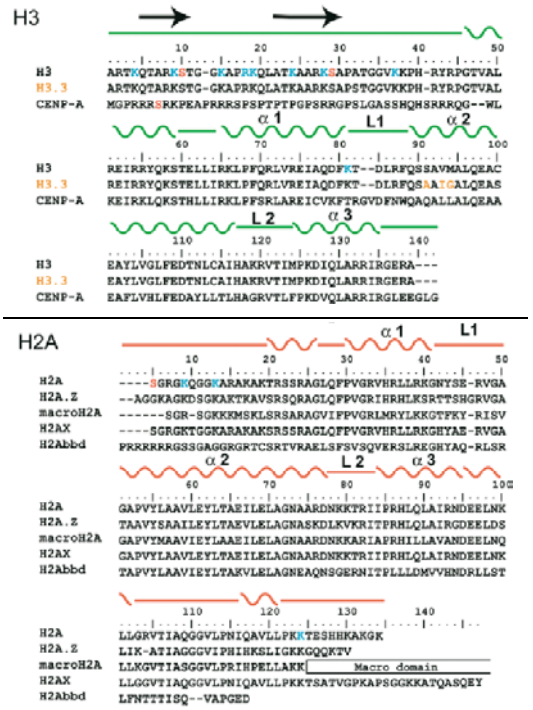
Come possiamo sapere se un locus che ci interessa è occupato da nucleosomi modificati in modo specifico?

Chromatin Immunoprecipitation (ChIP)

- Factors, DNA and histones are cross-linked by formaldehyde in the cells
- Chromatin is extracted and fragmented by sonication
- Hyperacetylated nucleosomes are IMPT with a specific anti-acetyl-lysine(n)-histone(m) antibody
- Cross-linking is reversed, proteins digested and DNA purified
- Specific promoter or enhancer sequences amplified by PCR

2. 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.

histone-modifying enzymes:

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

Histone-modifying enzymes

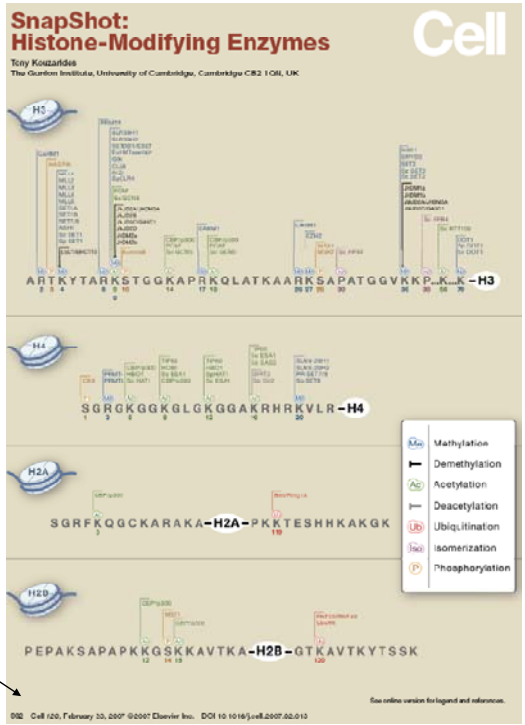
Table 2. Histone-Modifying Enzymes

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

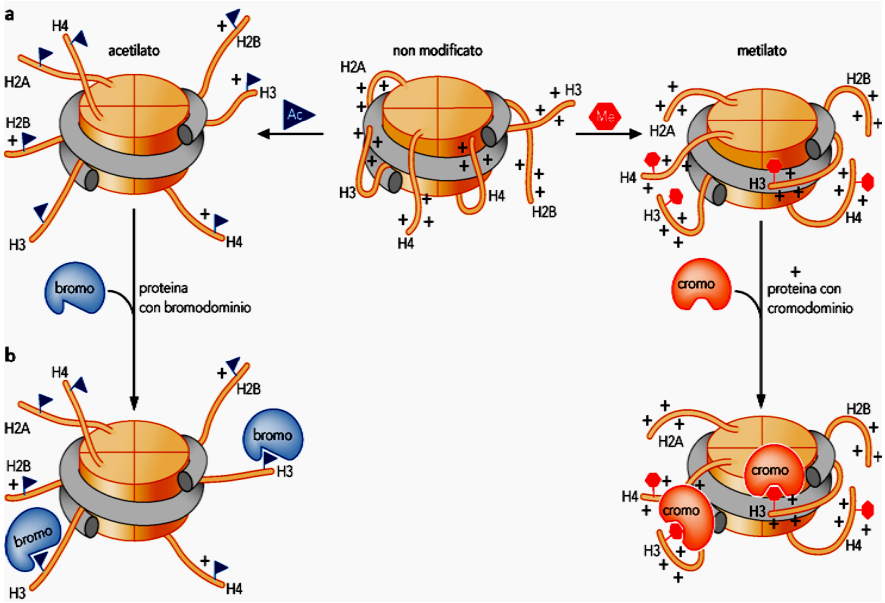
Table 2. *Continued*

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.



3. interacting proteins



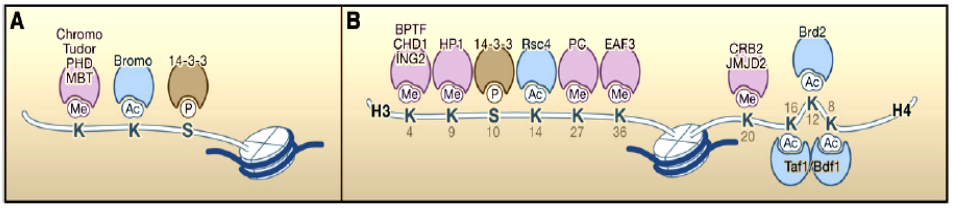


Figure 1. Recruitment of Proteins to Histones

(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.