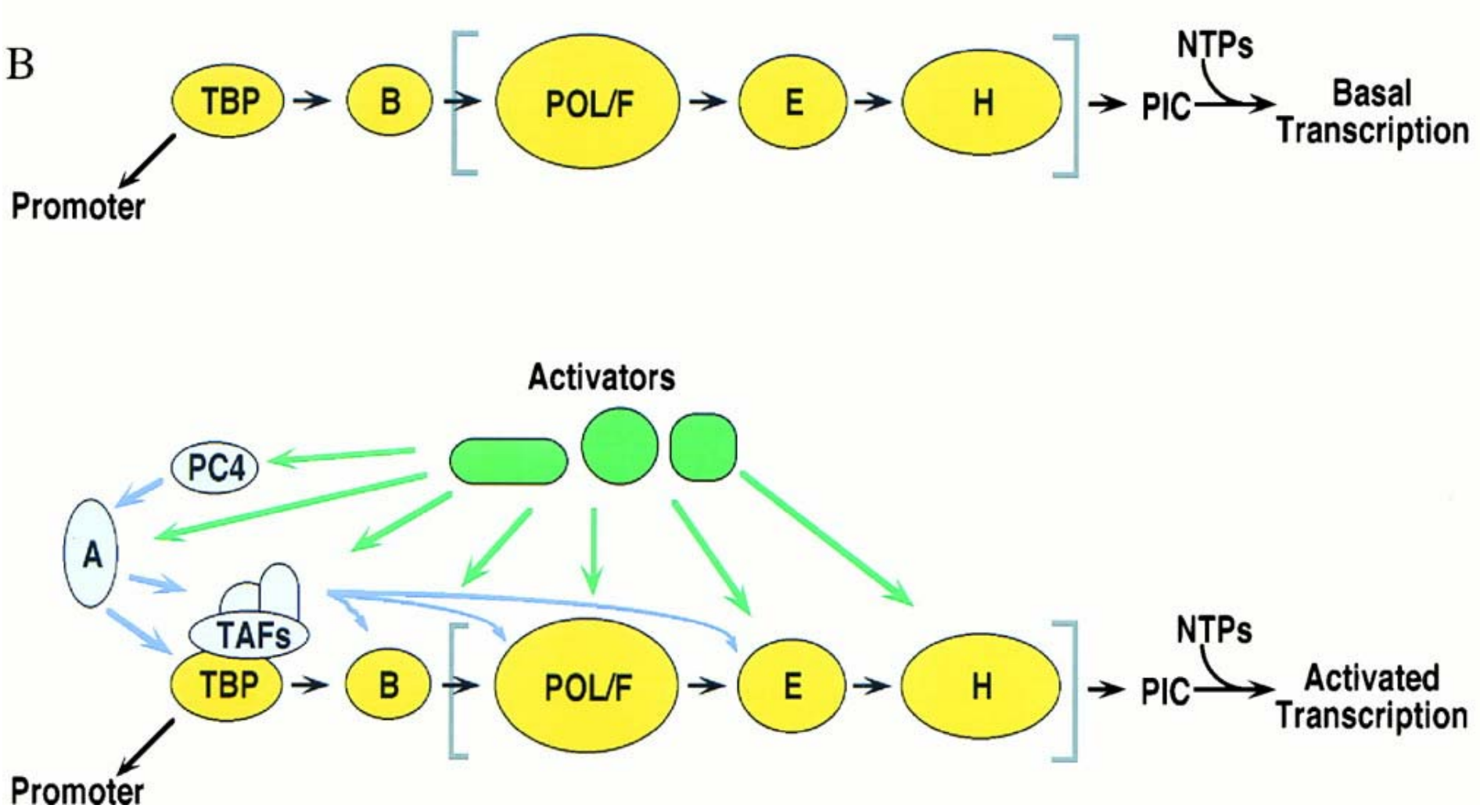
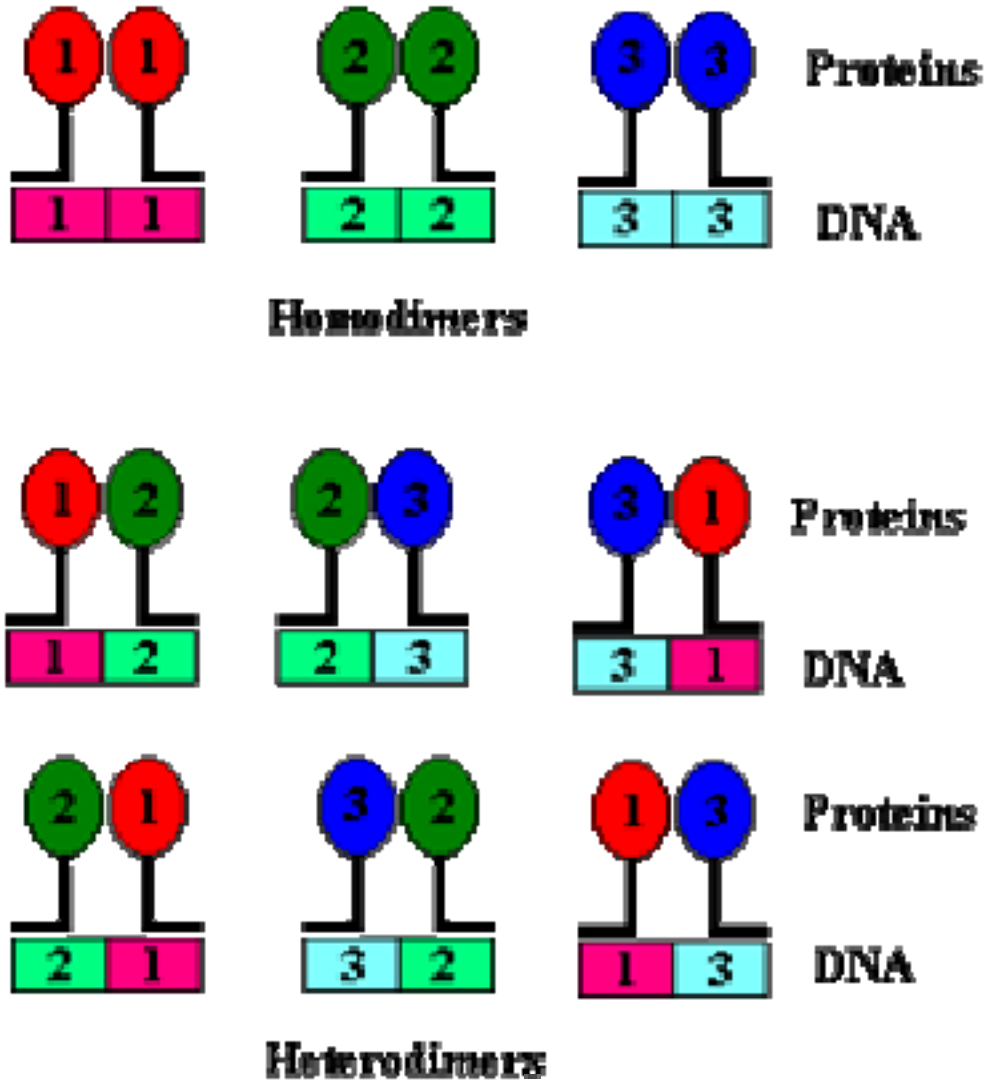
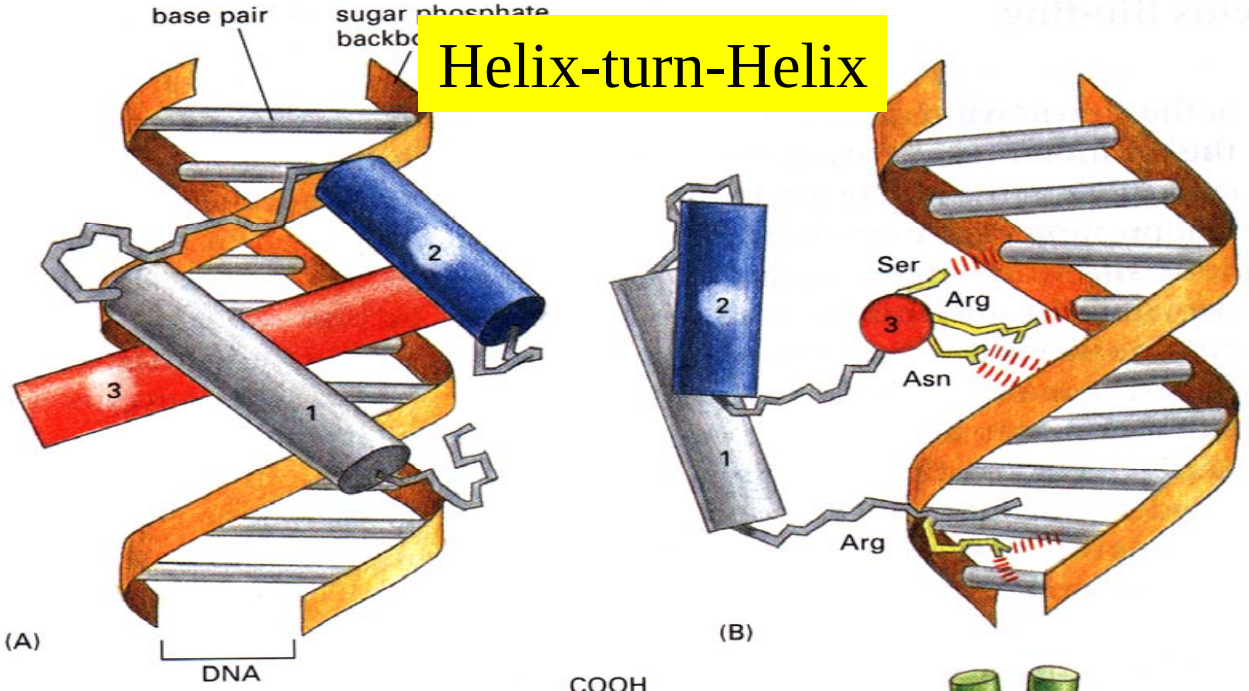

B
TBP
B
POL/F
E
H
NTPs
PIC
Basal
Transcription
Promoter
Activators
PC4
A
TAFs
TBP
B
POL/F
E
H
NTPs
PIC
Activated
Transcription
Promoter

DNA bindende Proteine haben eine DNA-Bindedomäne und binden oft als Dimere

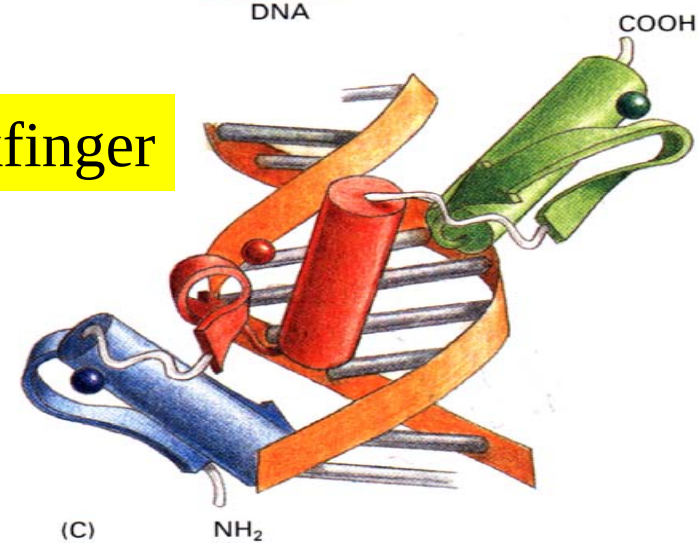


Die wichtigsten DNA-binde-Proteine

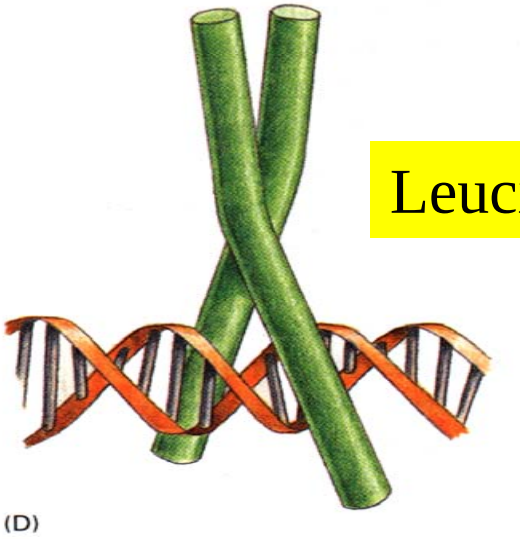
Helix-turn-Helix



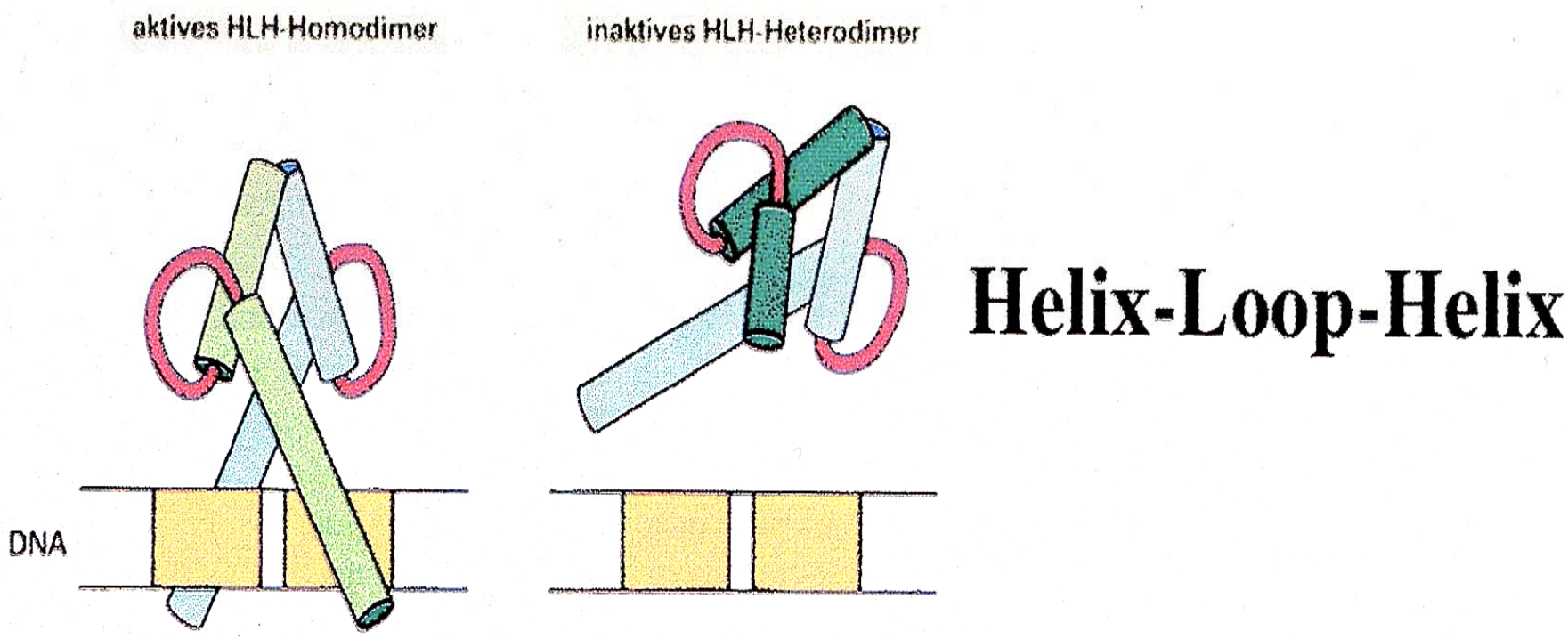
Zinkfinger



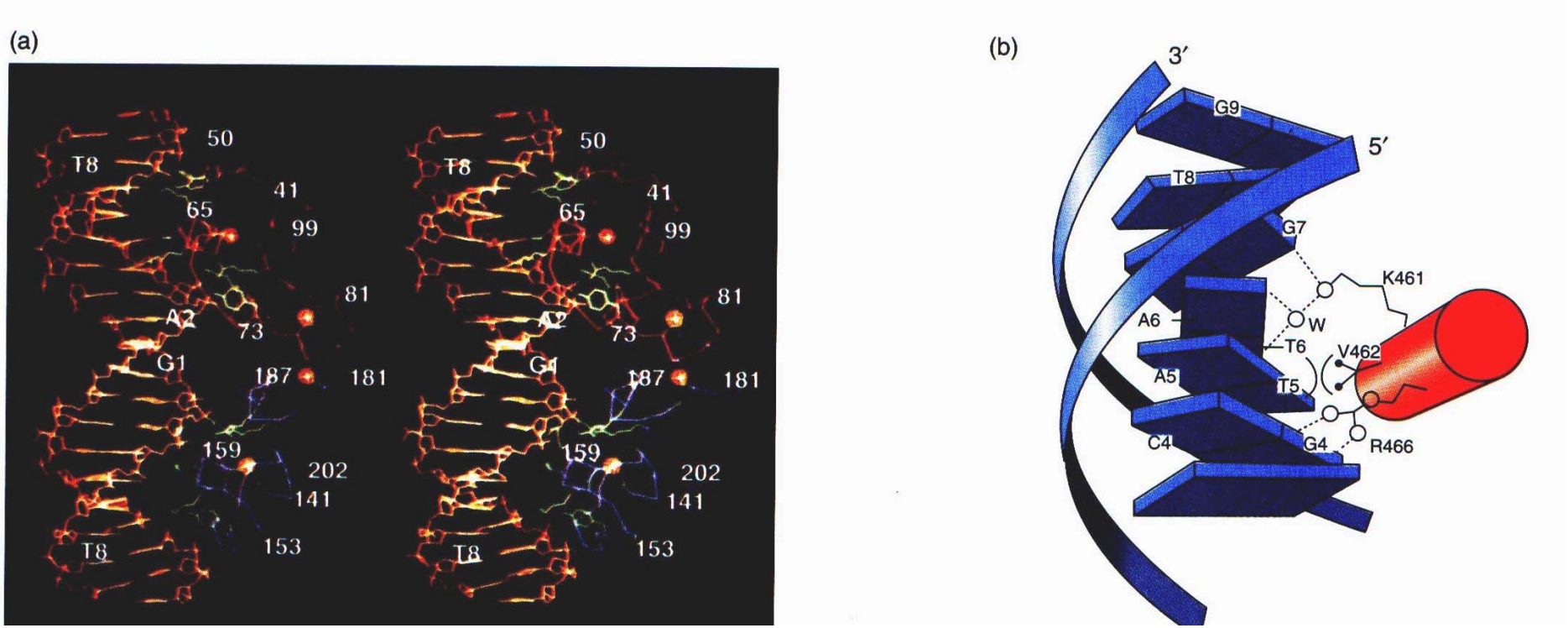
Leucin Zipper



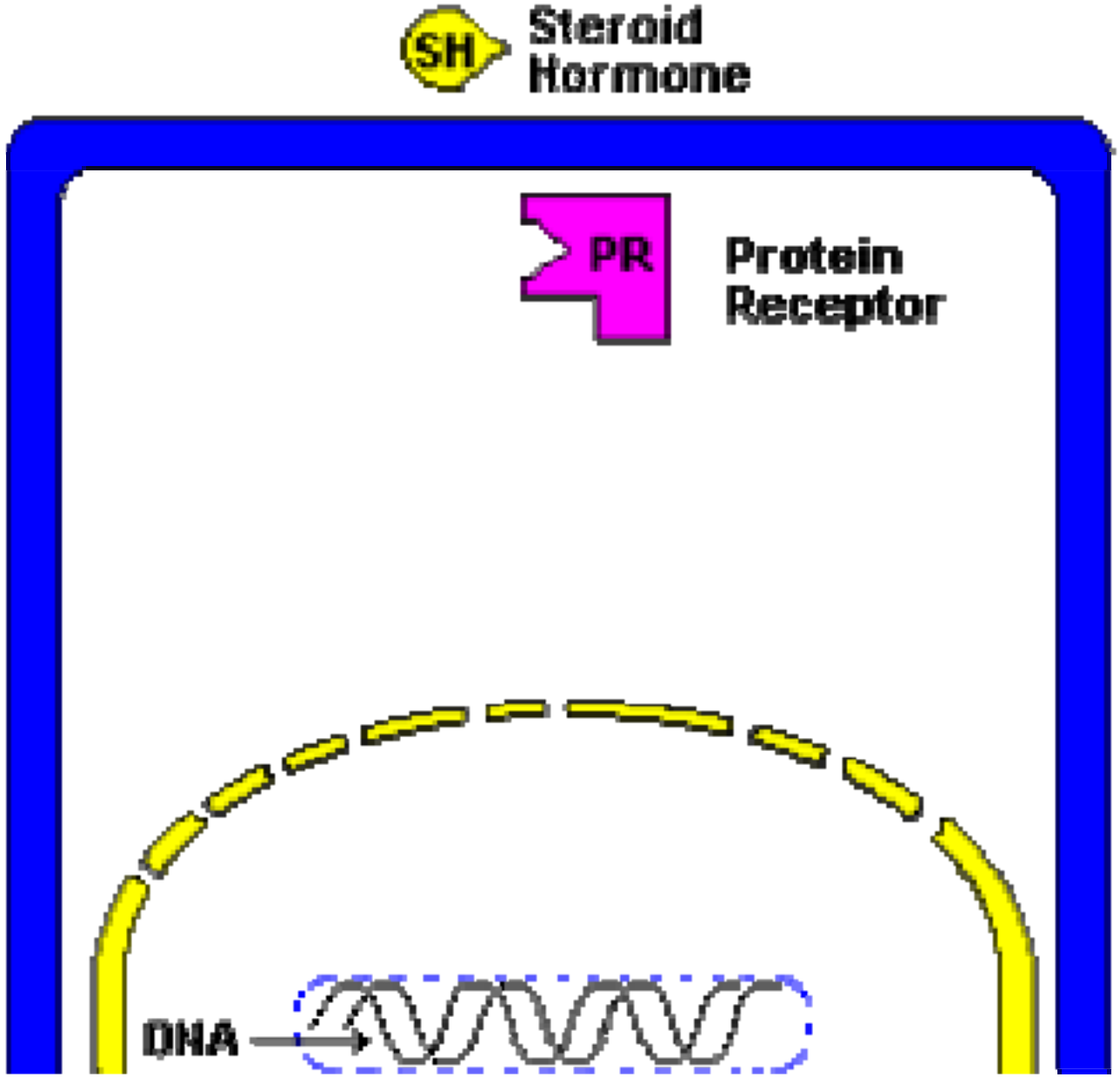
Basisches Helix-Loop-Helix



H-Brückenbindungen zwischen Aminosäuren des Proteins und Basen der DNA über die große Grube stellen die sequenzspezifische Bindung sicher

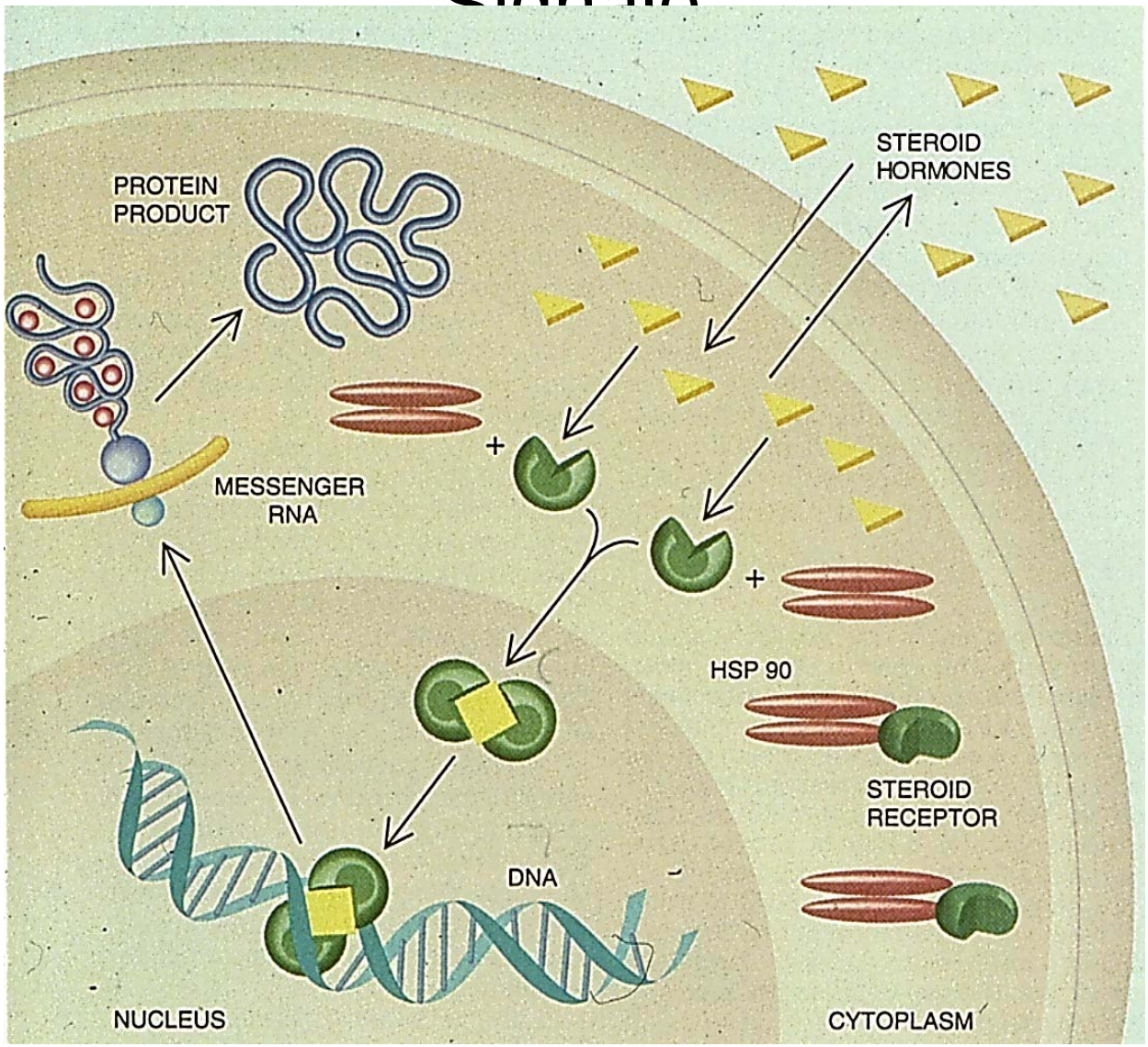


Genregulation durch Steroidhormon



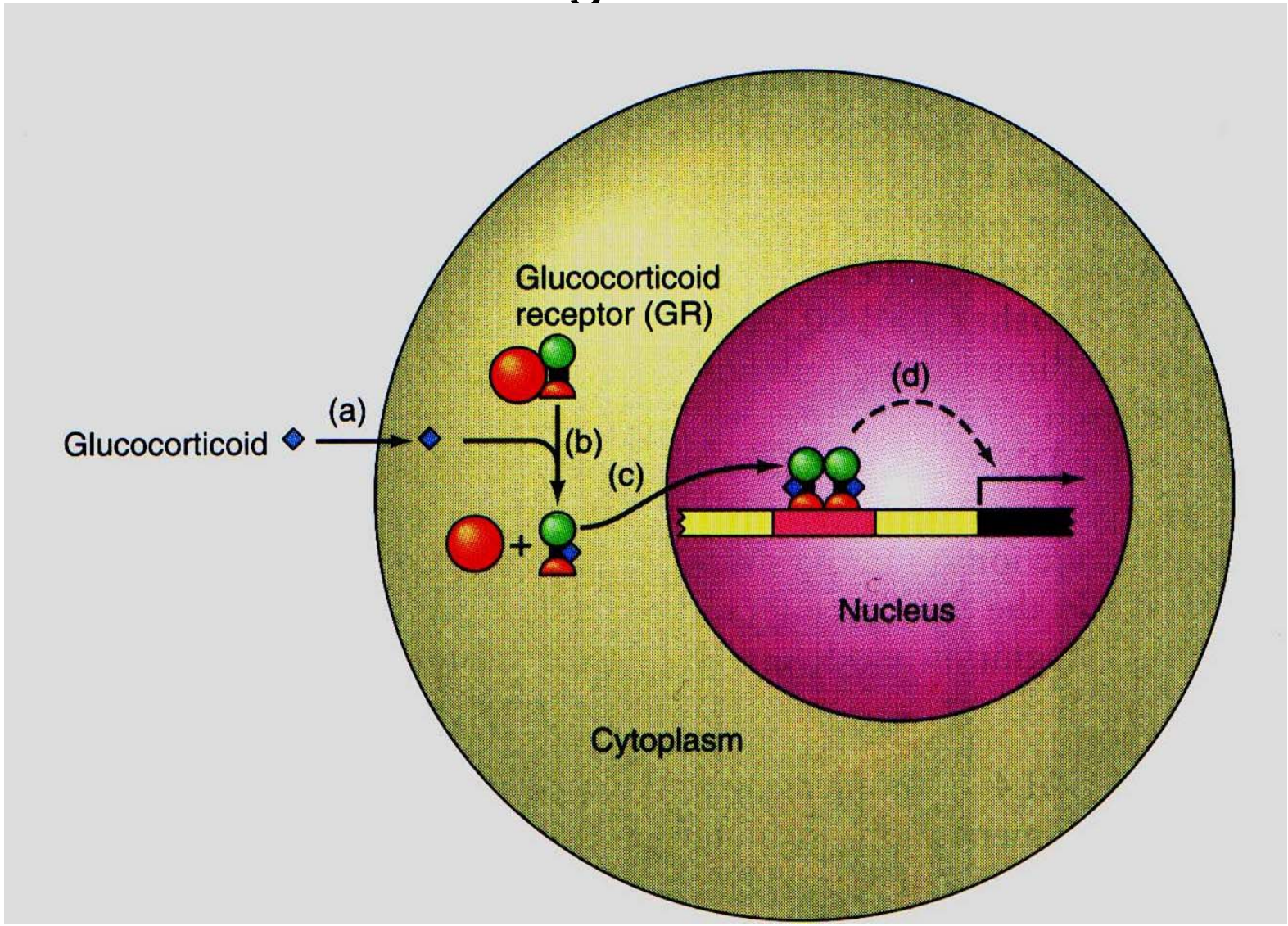
Hormon induzierte Genaktivität

Beispiel für Genaktivierung durch externe Signale



Hormon induzierte Genaktivität

Beispiel für Genaktivierung durch externe Signale



Nuclear Receptors

Palindromic Repeats

Glucocorticoid	RGRACANNNTGTYCY
Oestrogen	RGGTCANNNTGACCY
Thyroid	RGGTCA------TGACCY

Direct Repeats

6-cis retinoic acid	$AGGTCAN_1AGGTCA$
All trans retinoic acid	$AGGTCAN_2AGGTCA$
Thyroid hormone	$AGGTCAN_4AGGTCA$

N indicates any nucleotide
R indicates a purine ie. A or G
Y indicates a pyrimidine ie. C or T

Nuclear Receptors

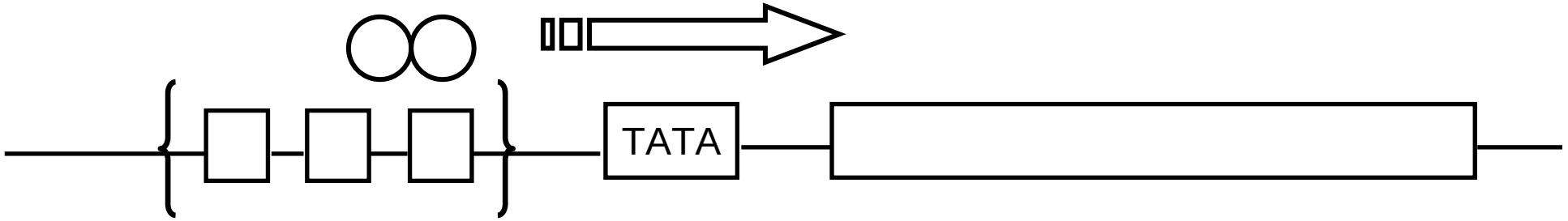
Binding of hormone

Dissociation from hsp90

Dimerisation

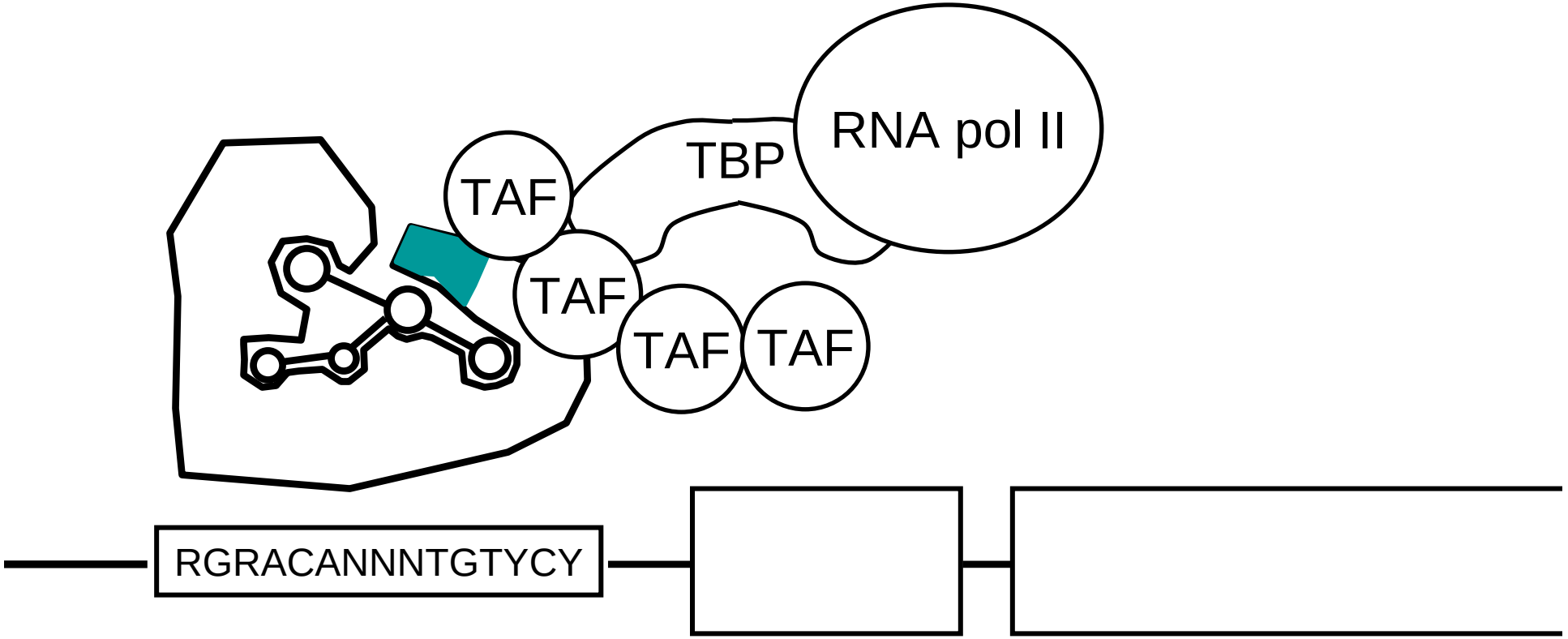
Migration to nucleus

Binding to URE

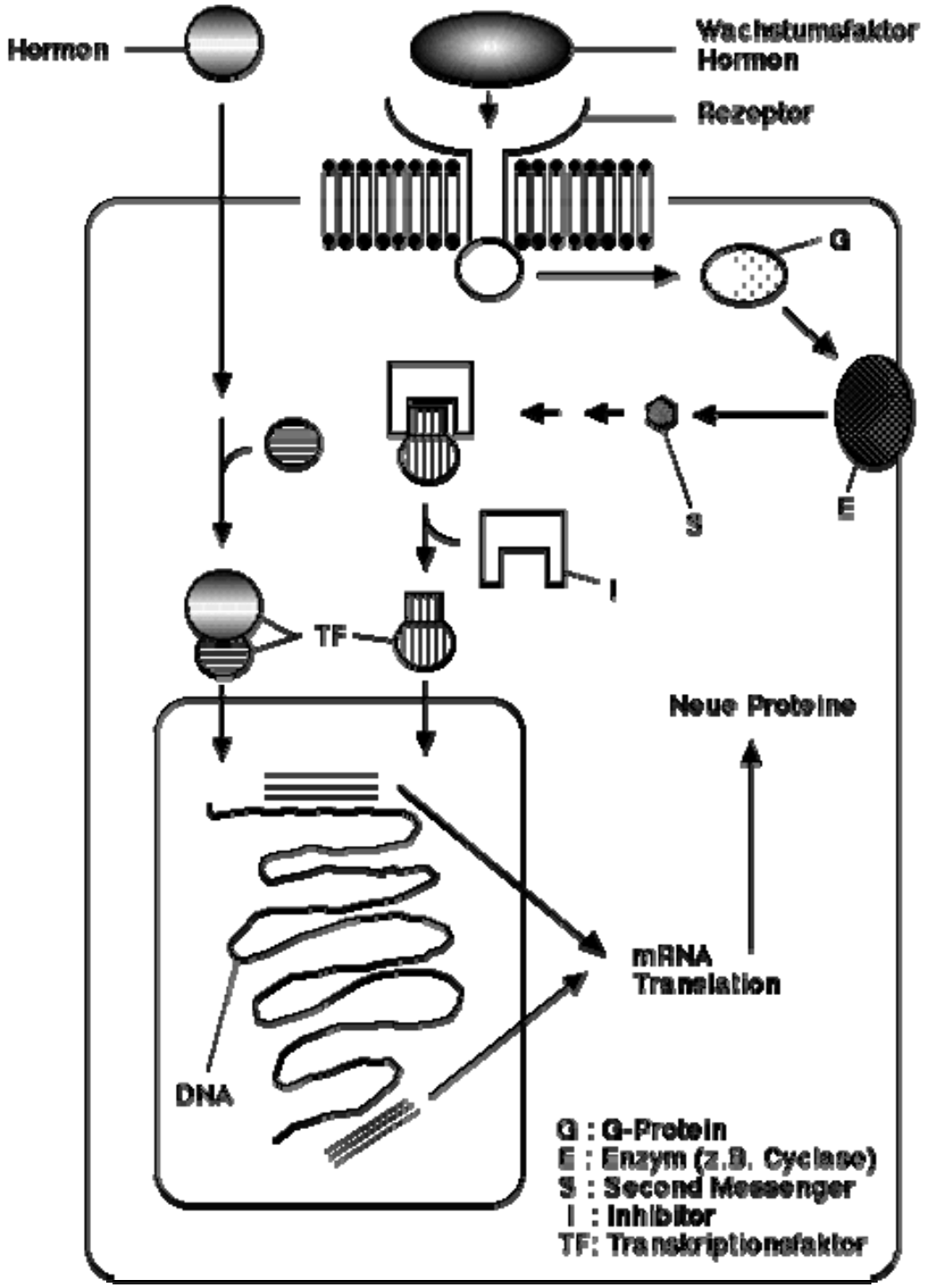


Nuclear Receptors

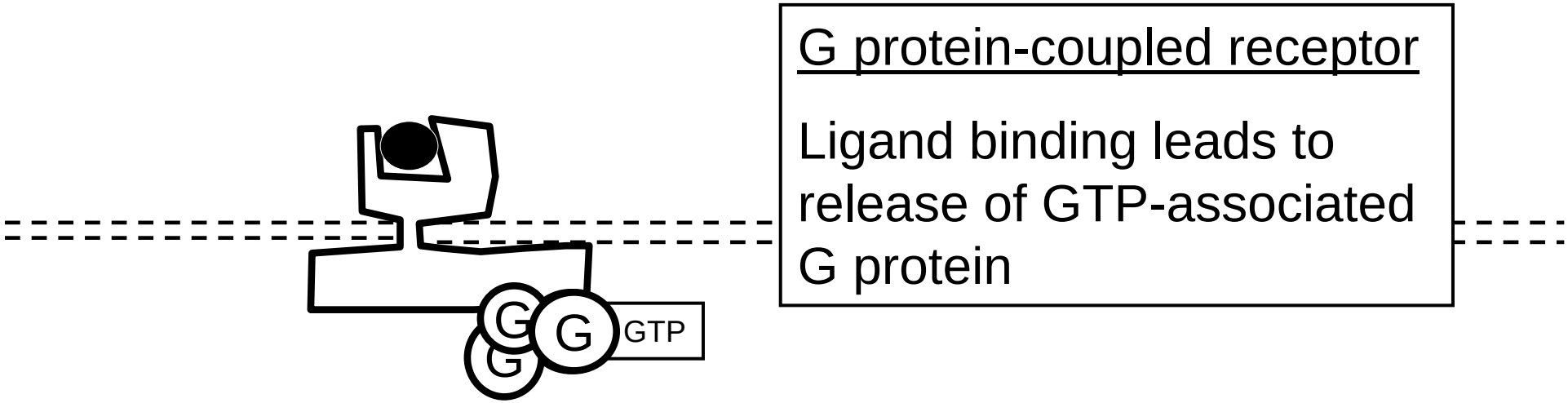
Binding of ligand causes conformational change allowing transactivation domain to interact with transcriptional machinery



Hormon-regulation von Genen



cAMP signalling

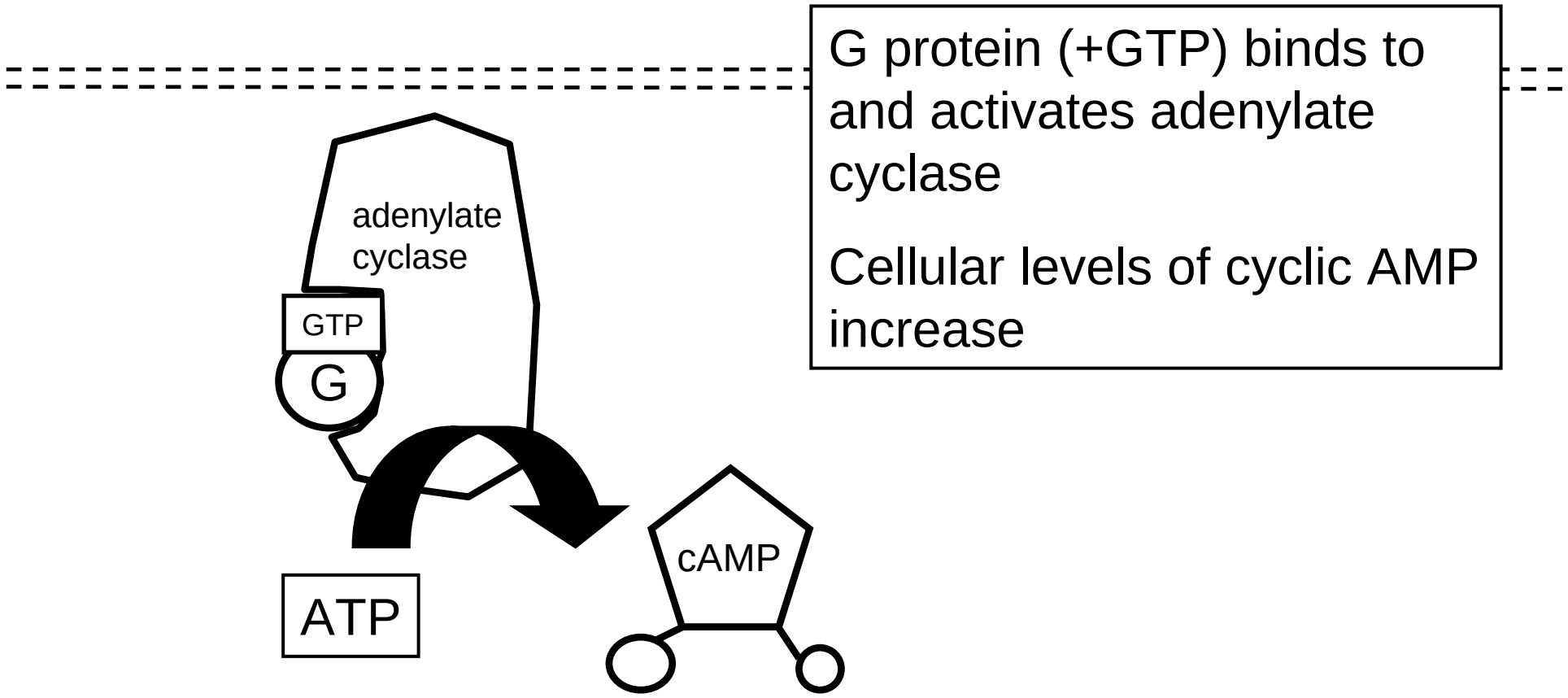


Small GTP-binding proteins

Example: ras – covered in earlier lecture

Alternate between inactive GDP and active GTP bound forms

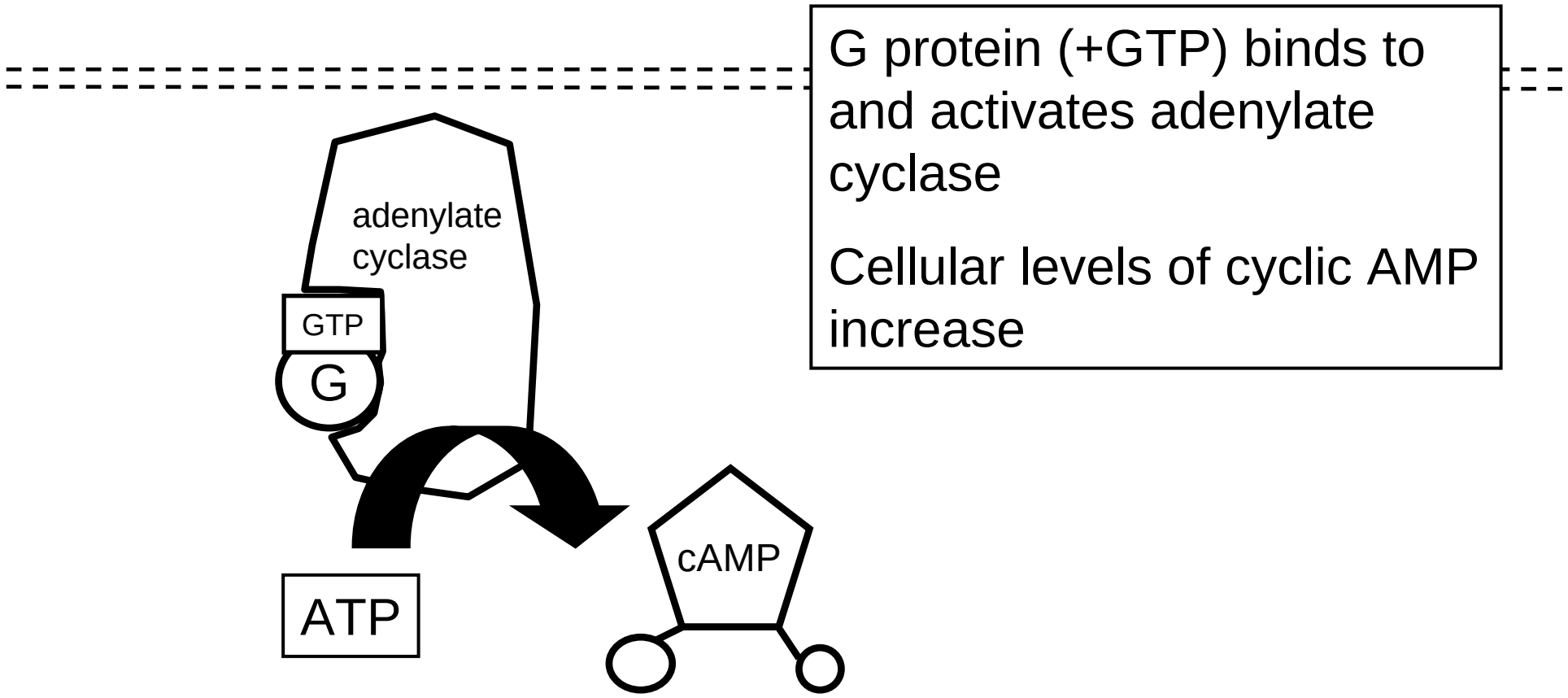
cAMP signalling



G protein (+GTP) binds to and activates adenylate cyclase

Cellular levels of cyclic AMP increase

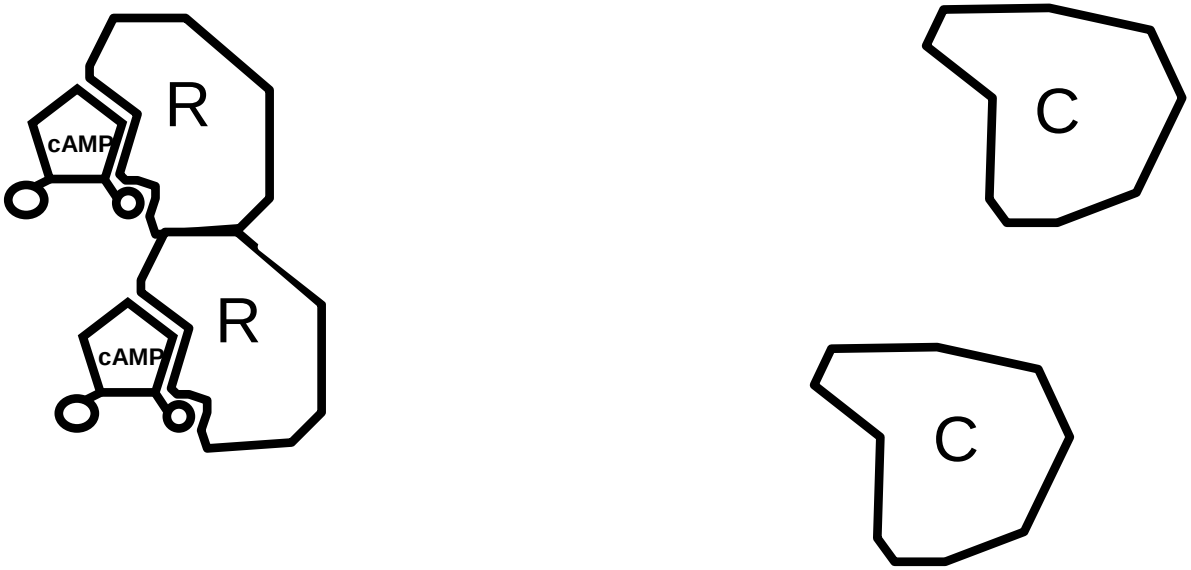
cAMP signalling



G protein (+GTP) binds to and activates adenylate cyclase

Cellular levels of cyclic AMP increase

cAMP signalling

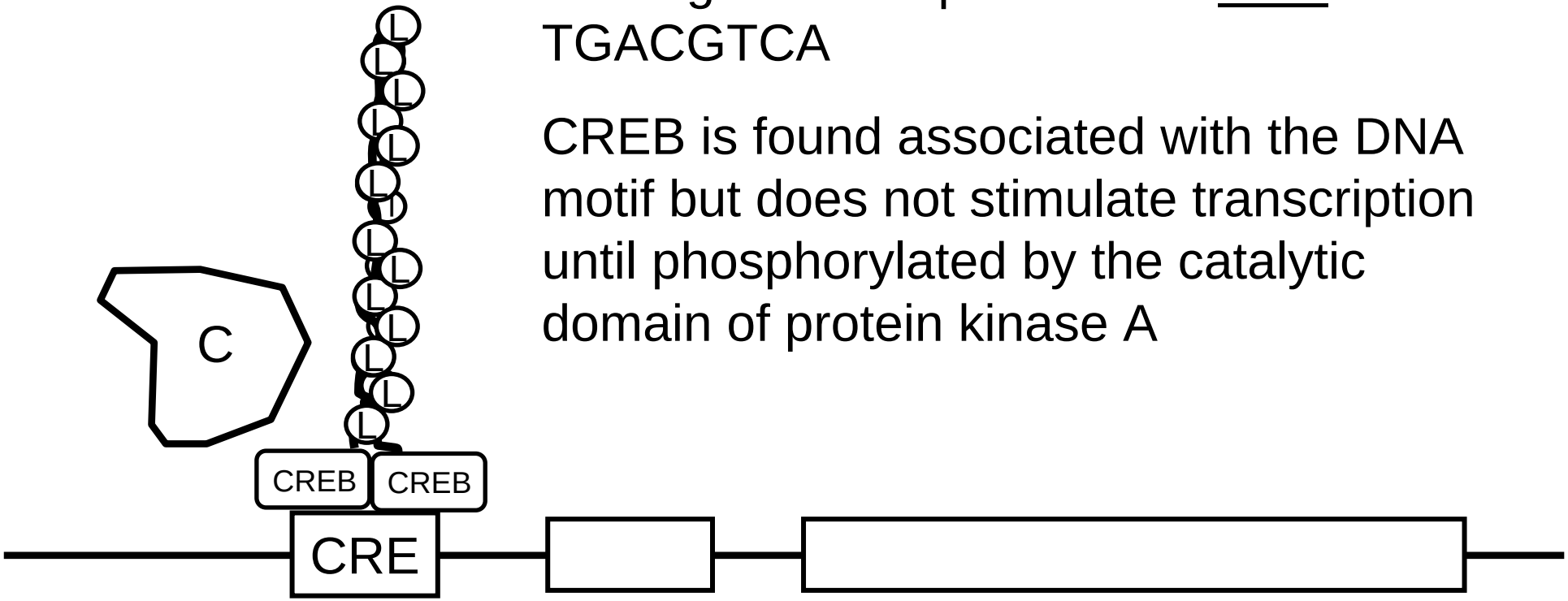


Cyclic AMP Response Element Binding protein

CREB is a member of the bZIP family of transcription factors

It recognizes the palindromic CRE motif TGACGTCA

CREB is found associated with the DNA motif but does not stimulate transcription until phosphorylated by the catalytic domain of protein kinase A

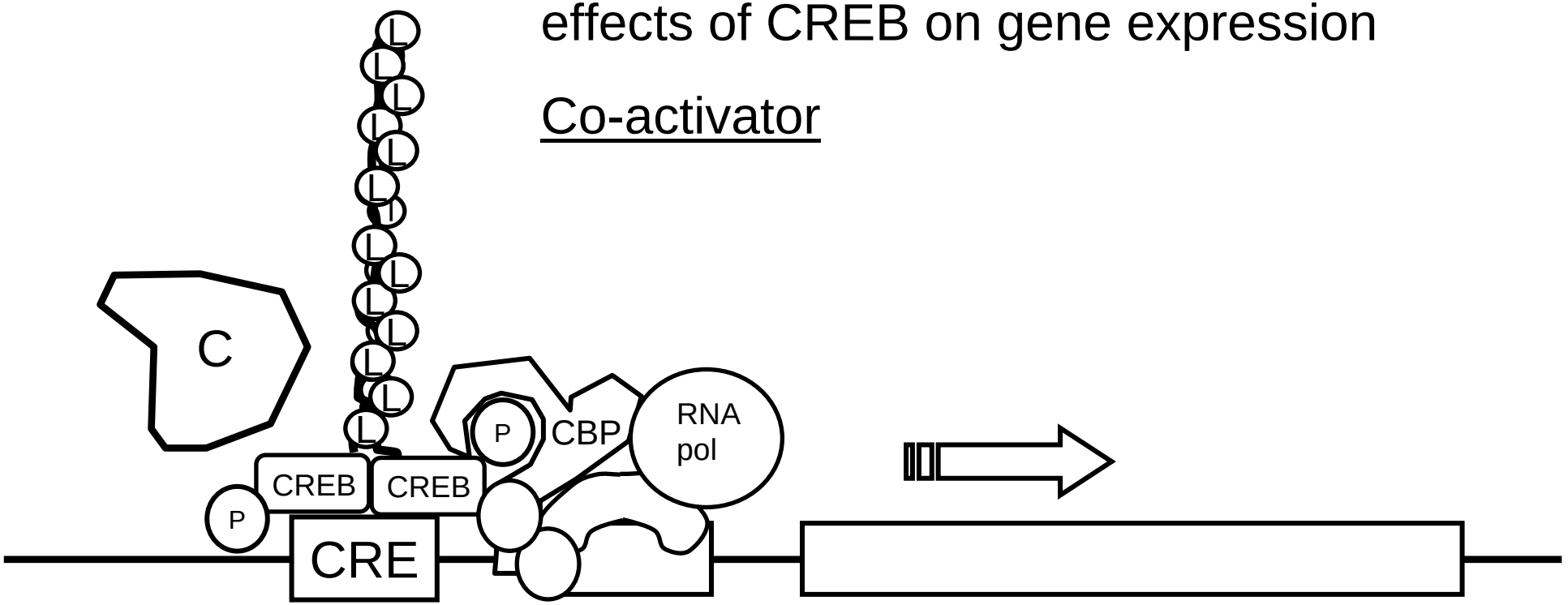


CREB binding protein

CBP recognises and binds the phosphorylated form of CREB

CBP interacts with the basal transcription complex to mediate the effects of CREB on gene expression

Co-activator

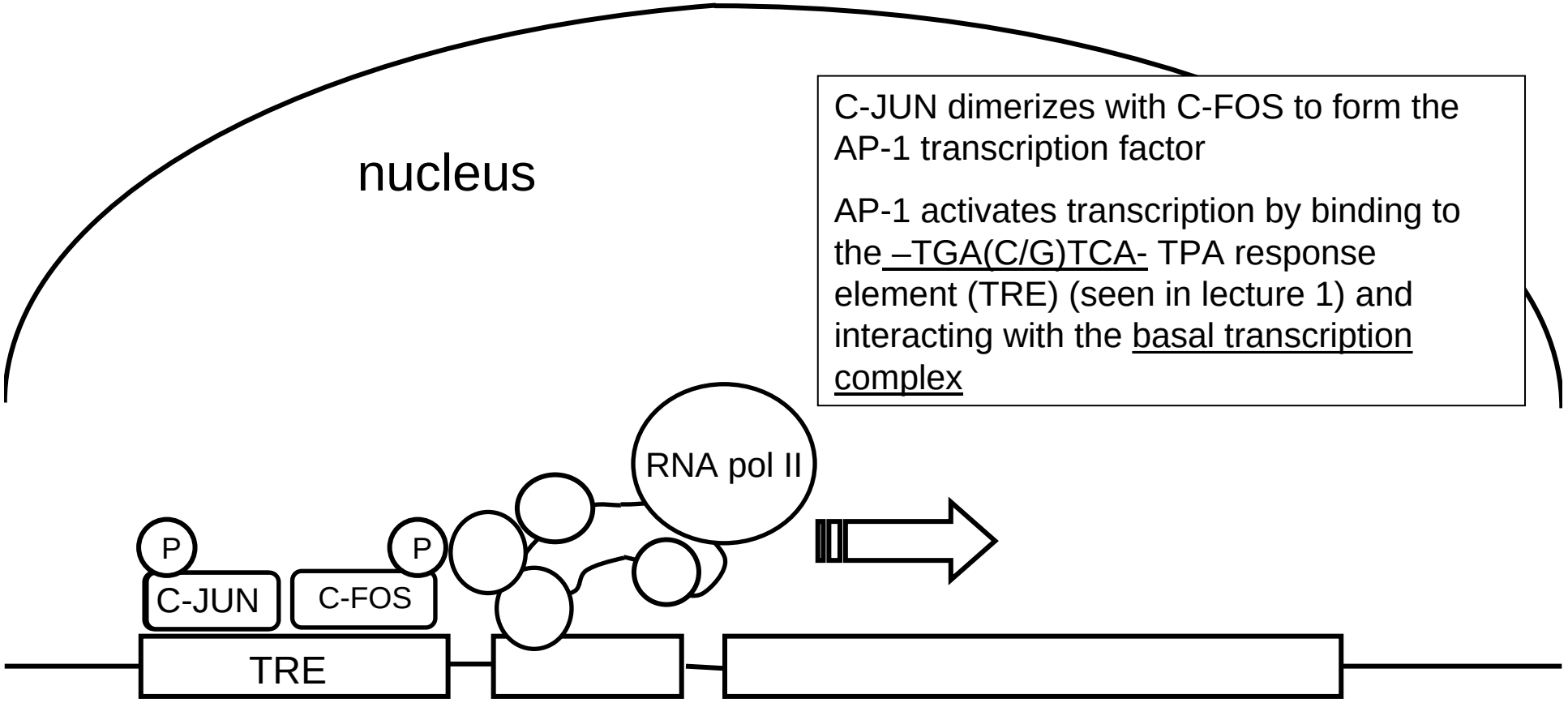


Kinase Cascades

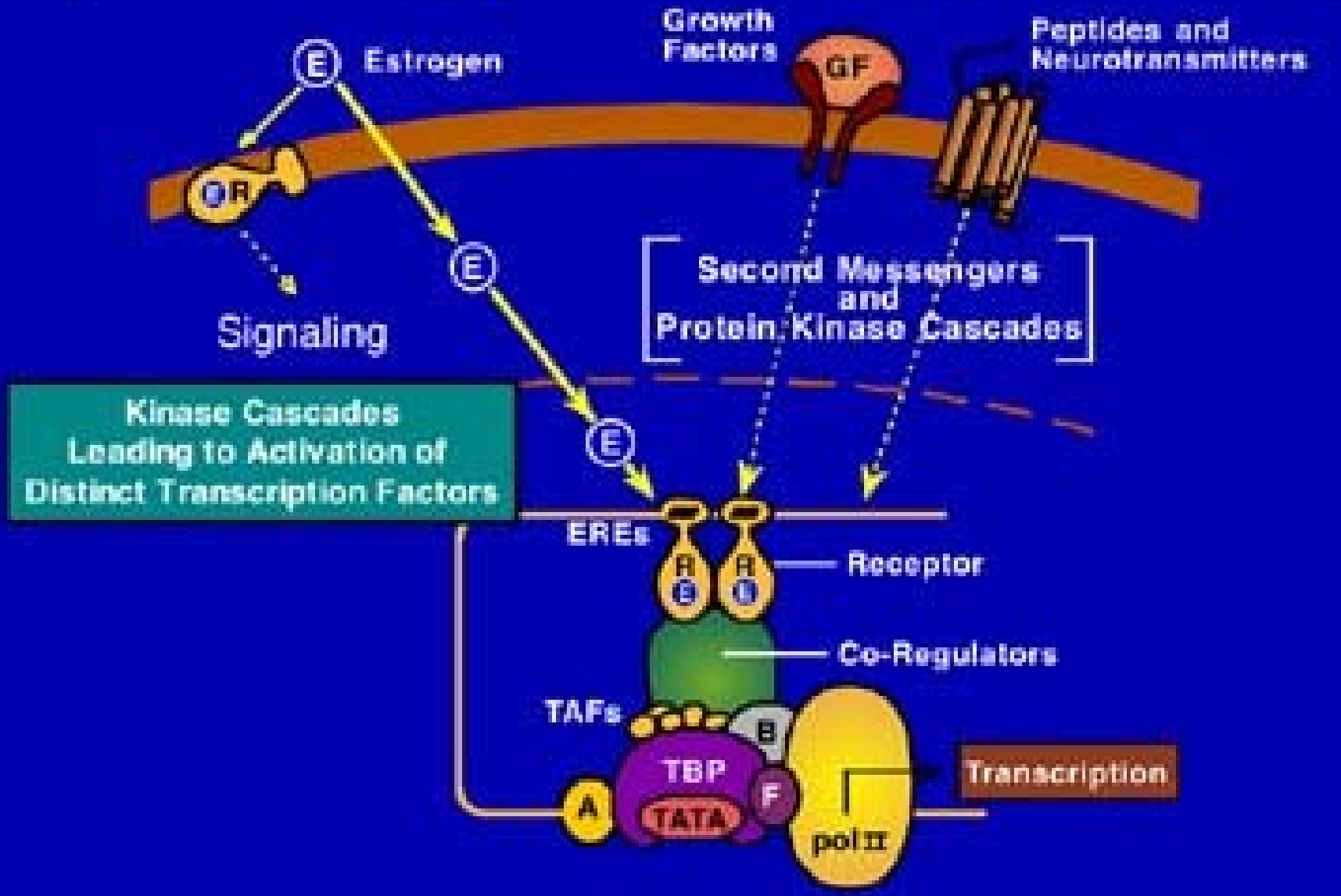
Active JNK travels to the nucleus and phosphorylates the bZIP transcription factor C-JUN

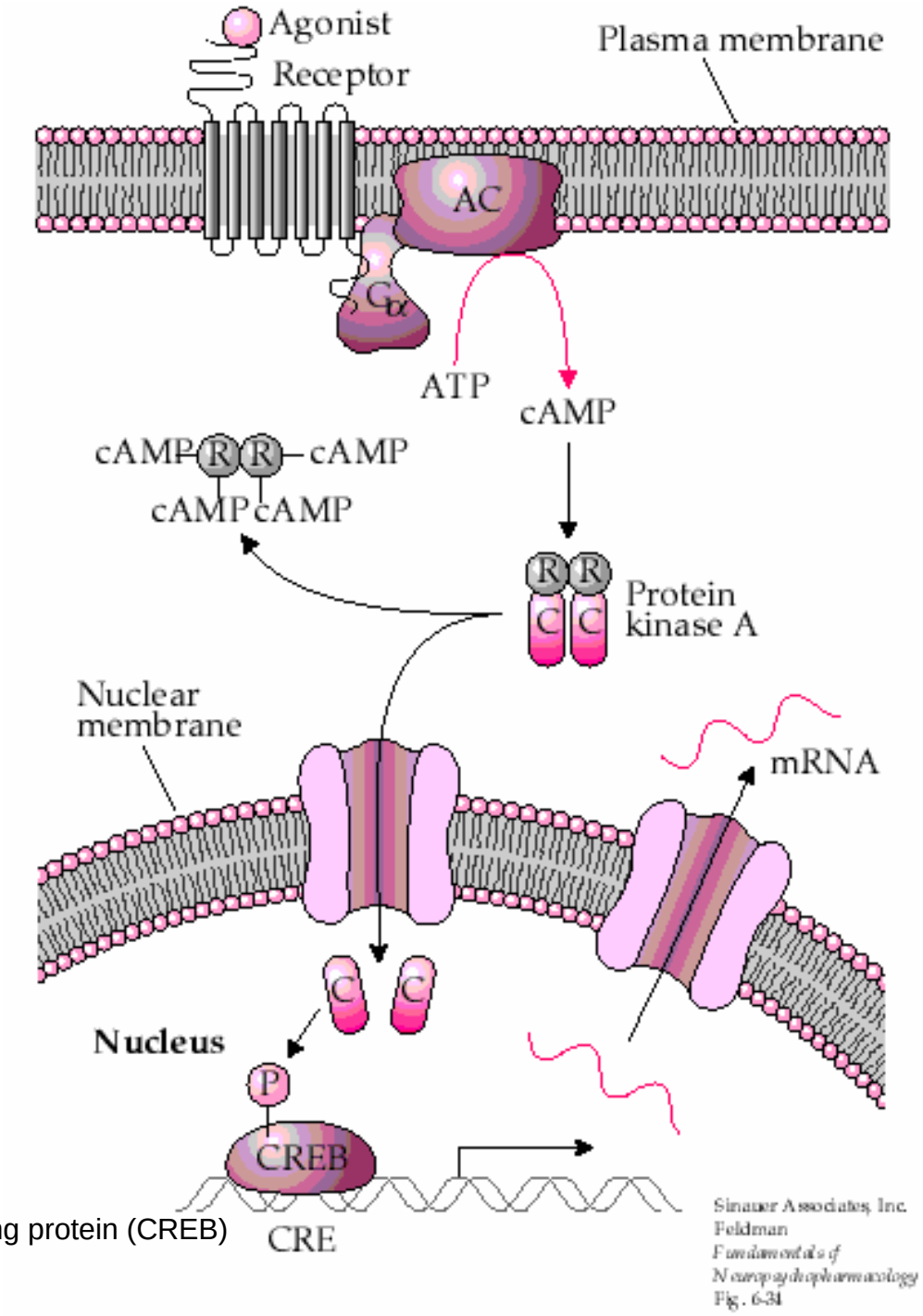
C-JUN dimerizes with C-FOS to form the AP-1 transcription factor

AP-1 activates transcription by binding to the –TGA(C/G)TCA- TPA response element (TRE) (seen in lecture 1) and interacting with the basal transcription complex



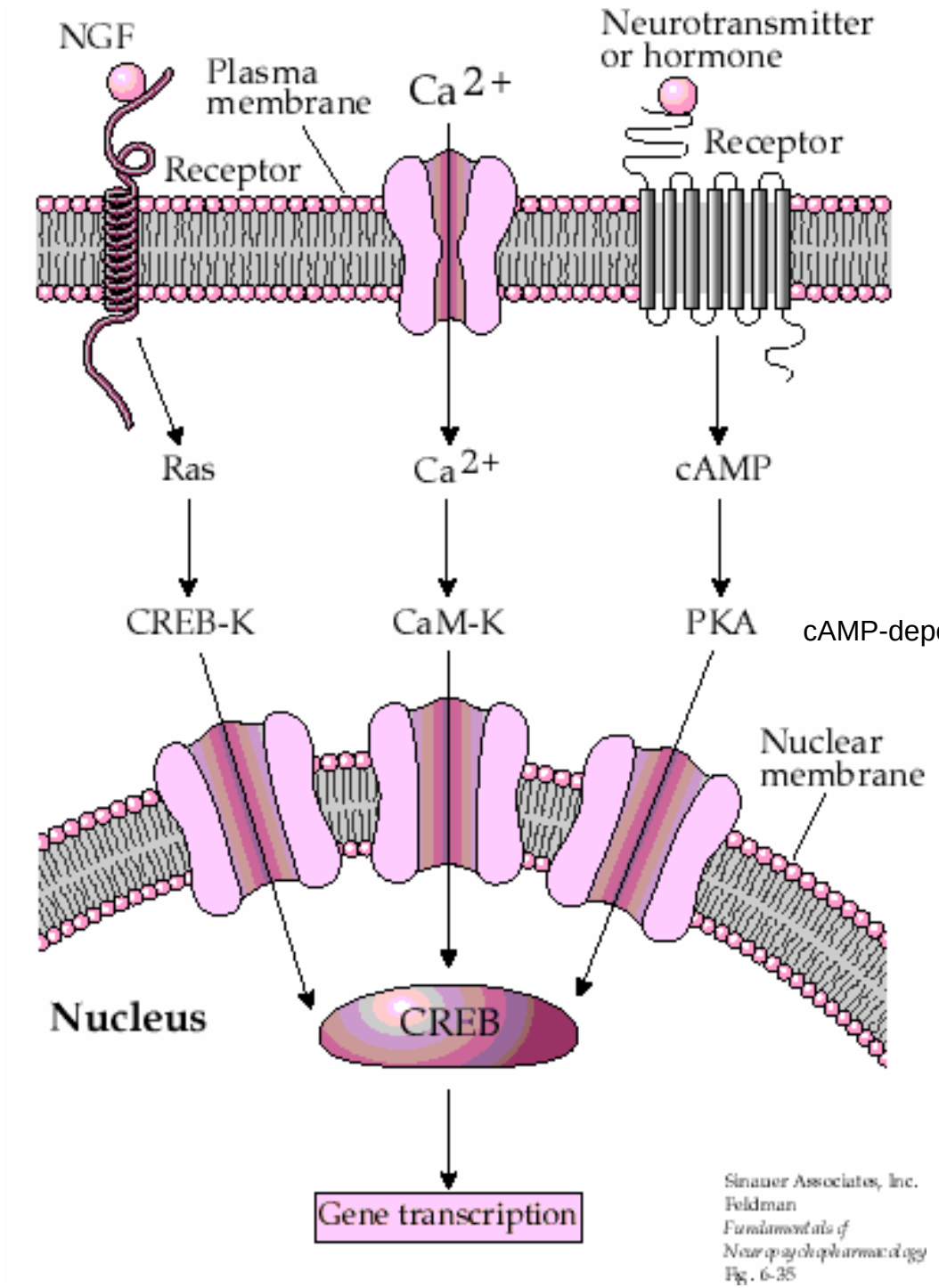
Receptor Actions in Cells



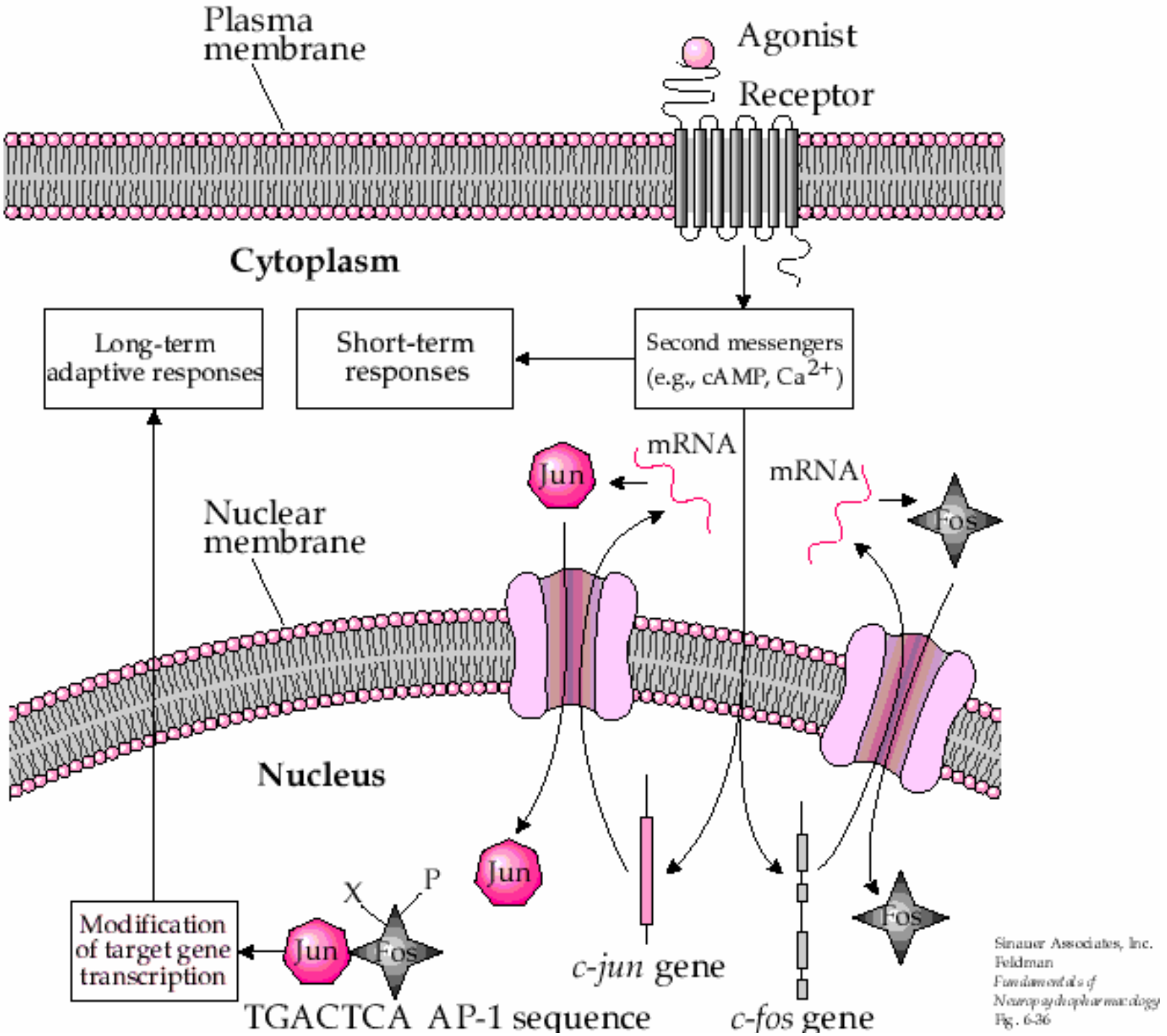


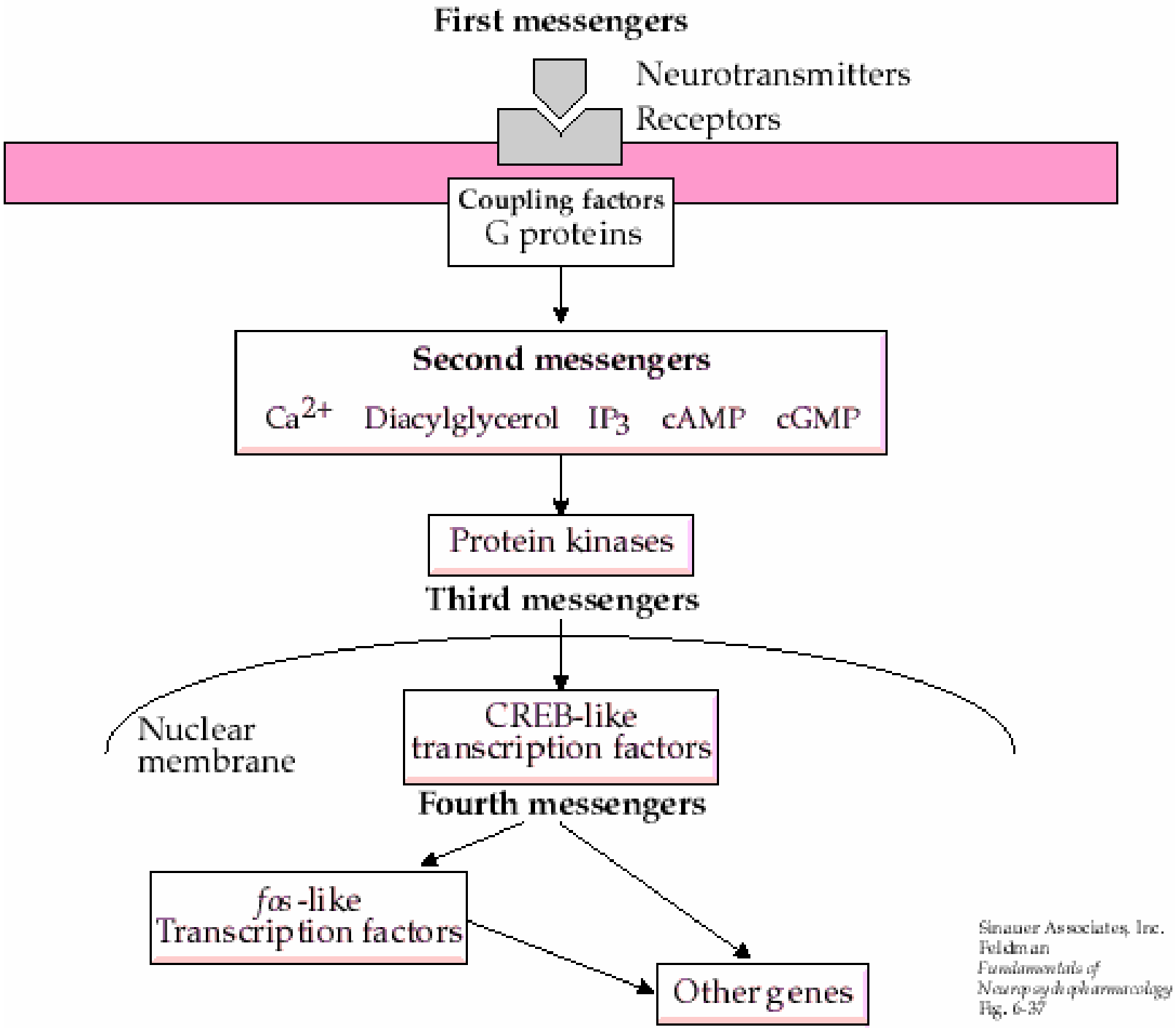
cAMP response element binding protein (CREB)

AC Adenylatcyclase



cAMP-dependant protein kinase (PKA)



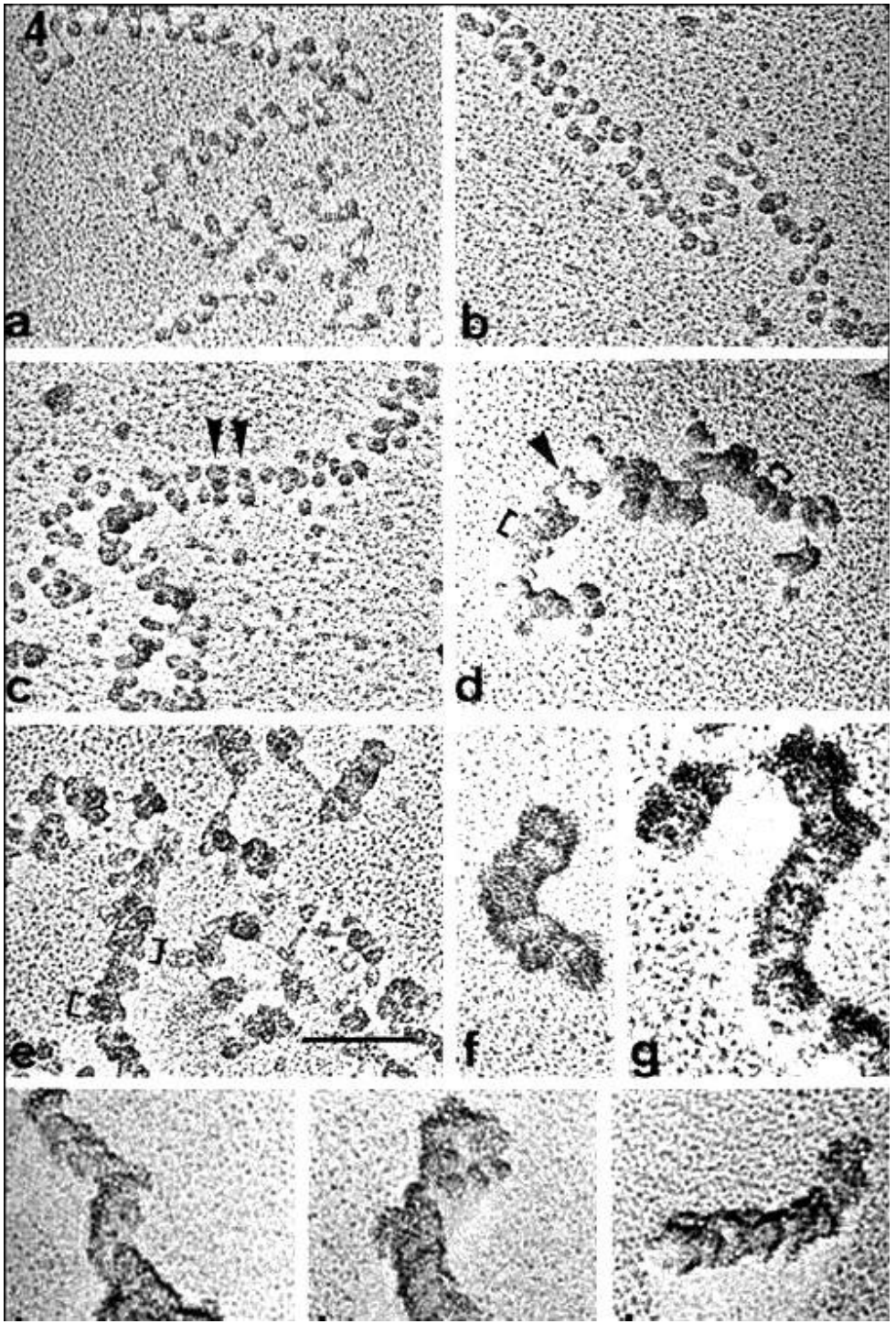


Sinauer Associates, Inc.
Feldman
Fundamentals of Neuropsychopharmacology
Fig. 6-37

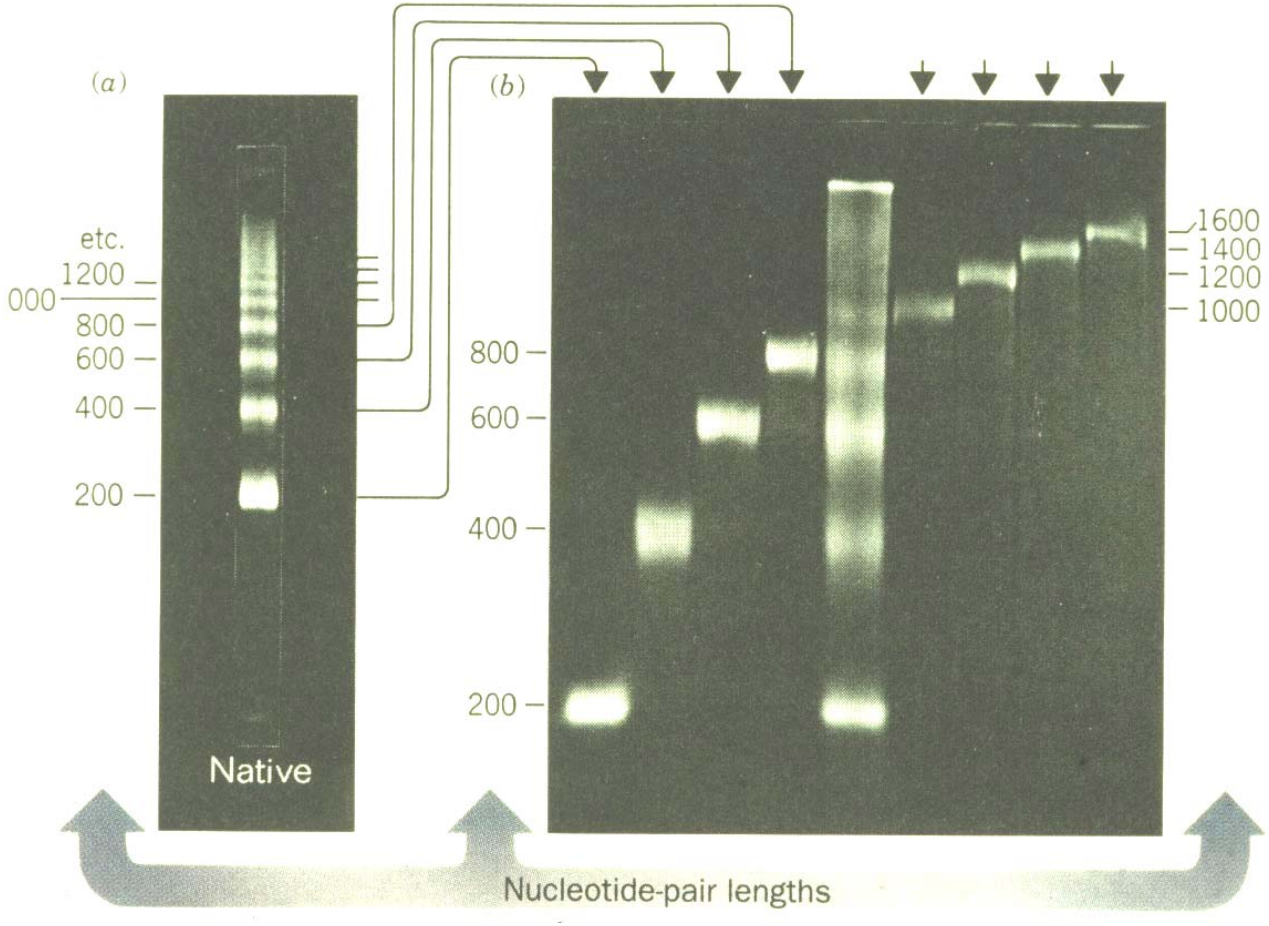
Chromatin und Genregulation

- Chromatin ist eine wesentliche Ebene der Genregulation bei Eukaryoten
- Beispiele sind Eu- und Heterochromatin
- Aktives und inaktives Chromatin entstehen durch Proteinmodifikationen und unterschiedliche Zusammensetzung des Chromatins
- Spezifische Proteine sind für die Bildung von Heterochromatin verantwortlich (z.B. HP1, SuVar-Proteine, Histonacetyltransferasen etc)

DNA in Chromatin: verschiedene Verpackungs-ebenen

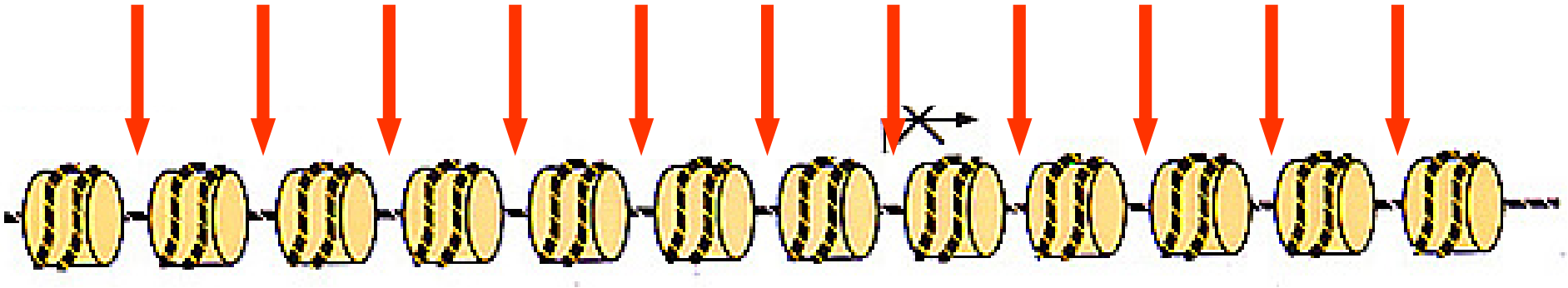


Nuclease-Verdau von Chromatin ergibt diskrete DNA-Fragmente von ca. 180 Bp Länge

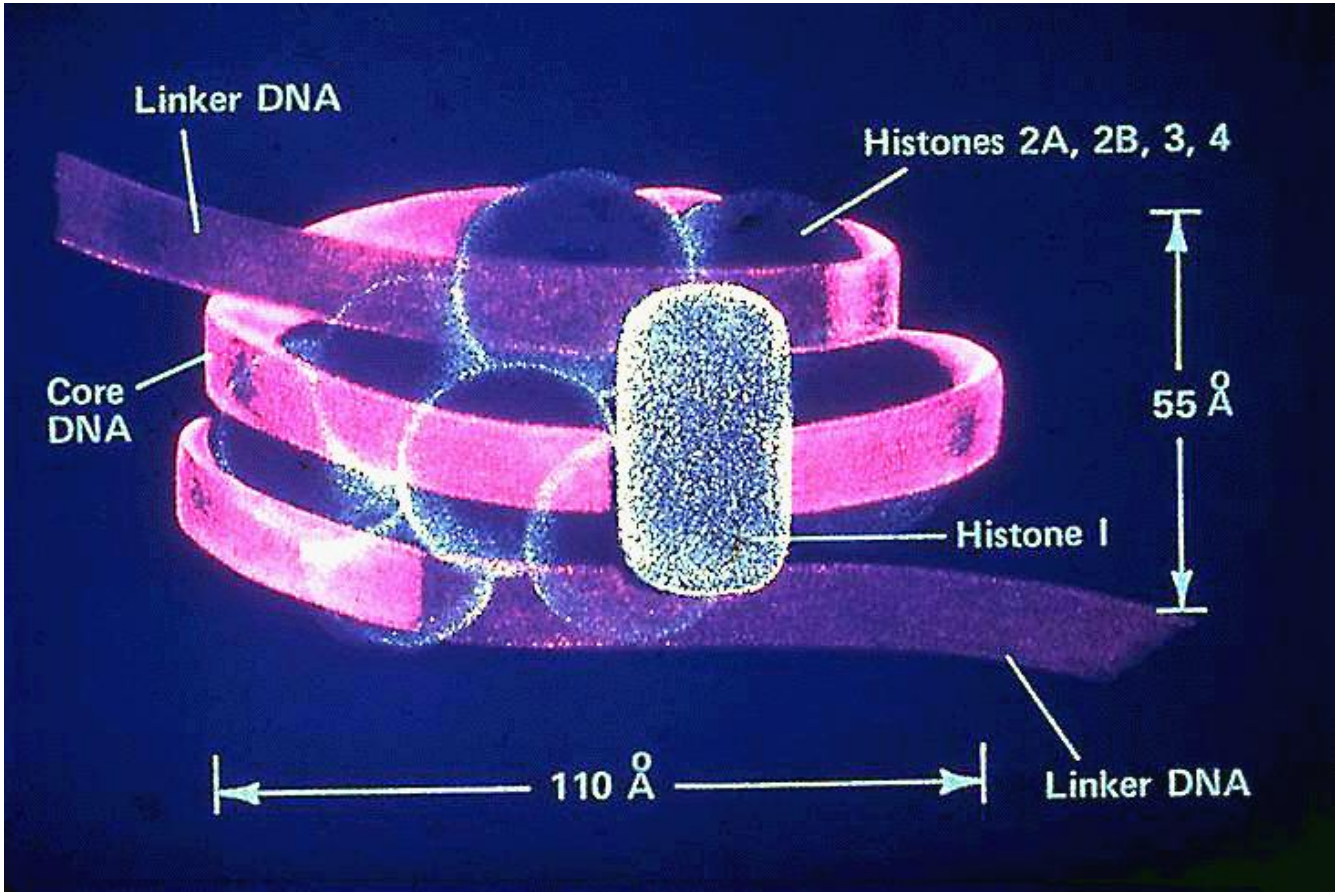


Chromatin und Genaktivität

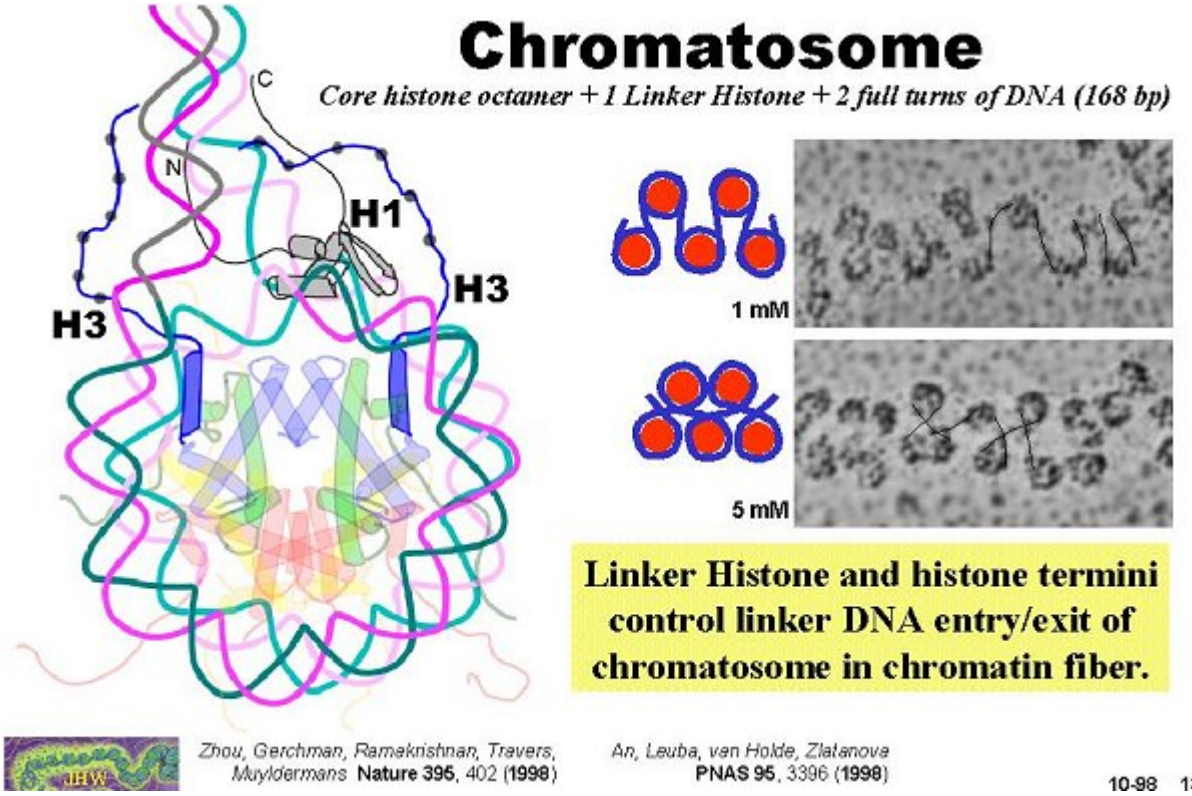
Nuklease zugängliche Chromatinbereiche



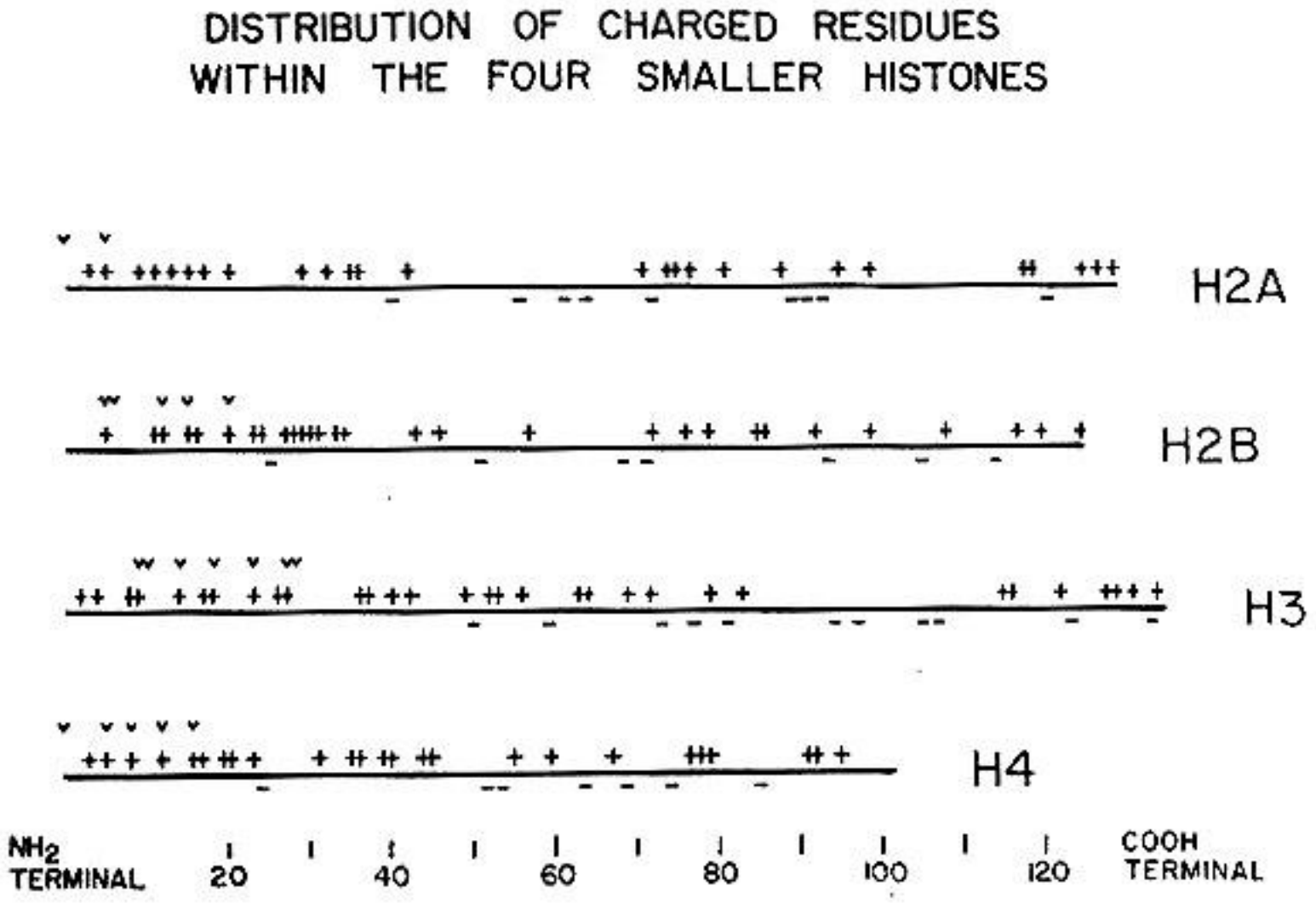
Organisation des Mononukleosoms



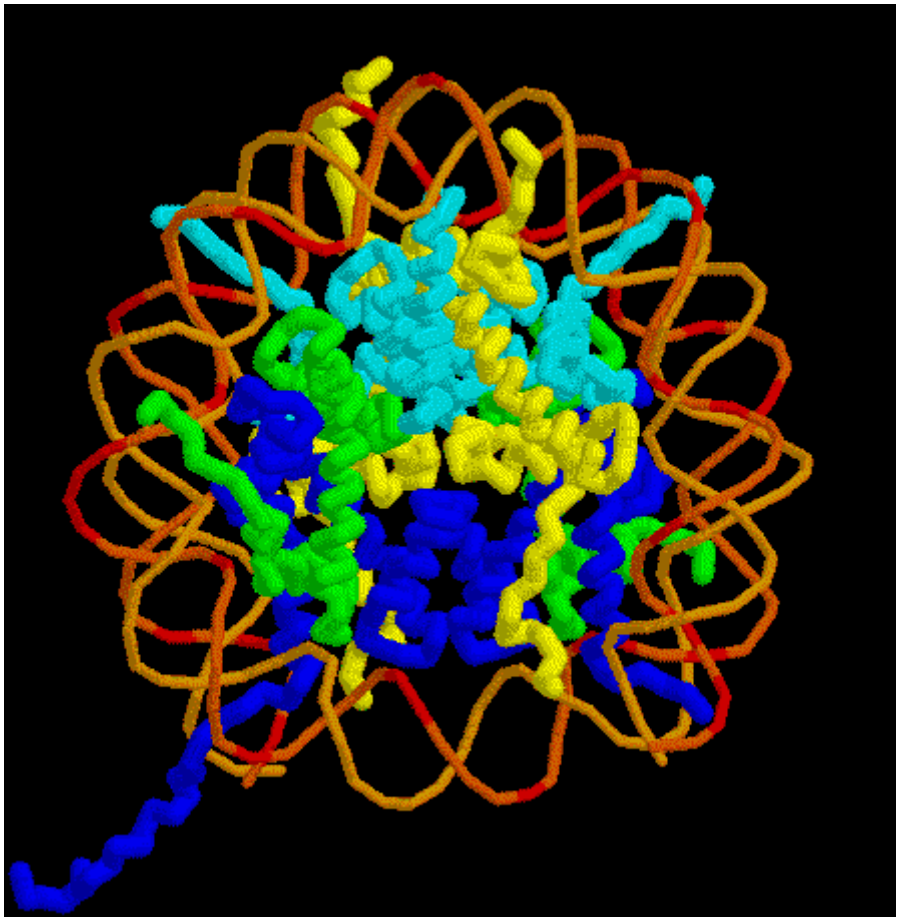
Histon H1 – „Linker Histon“

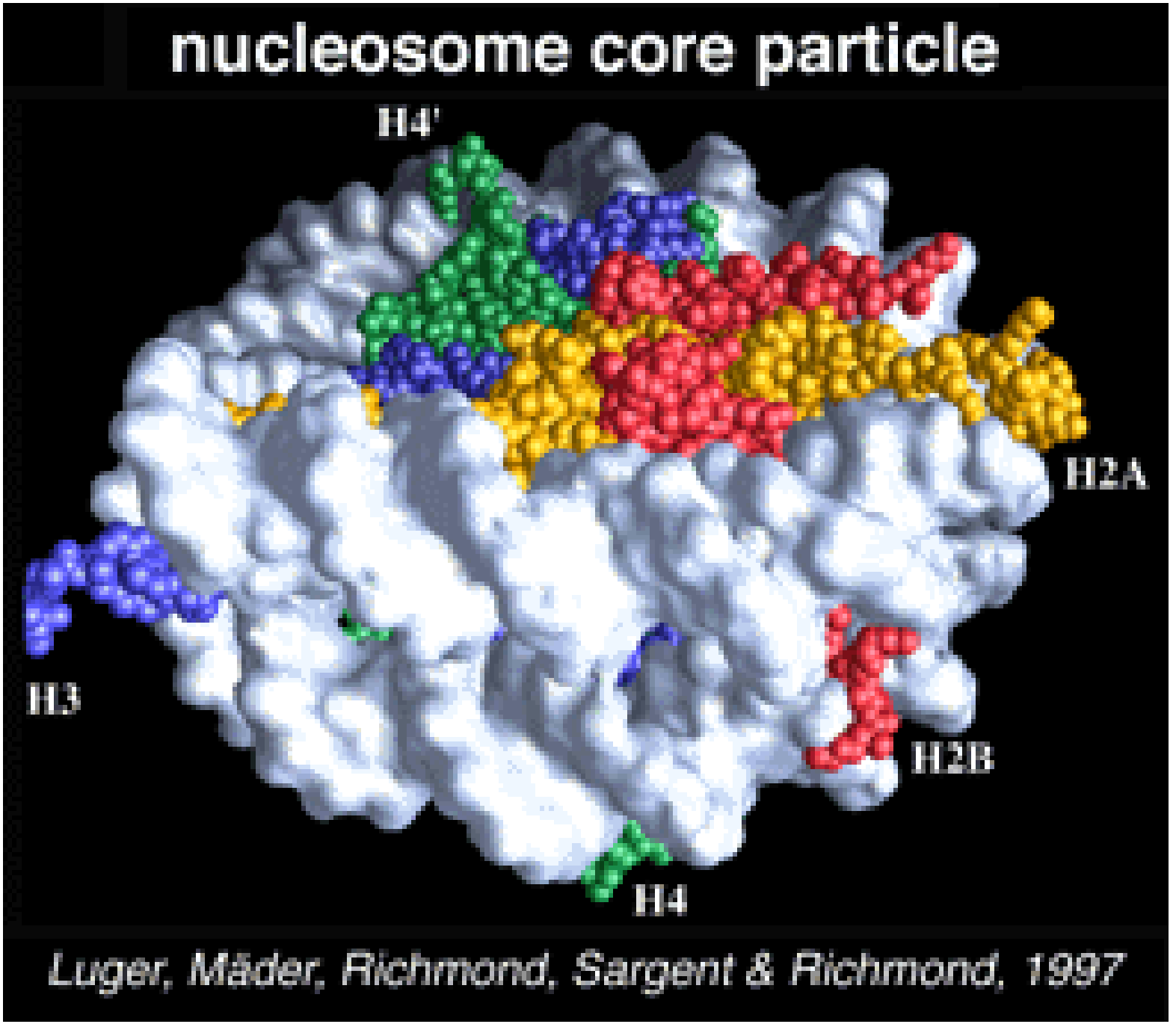


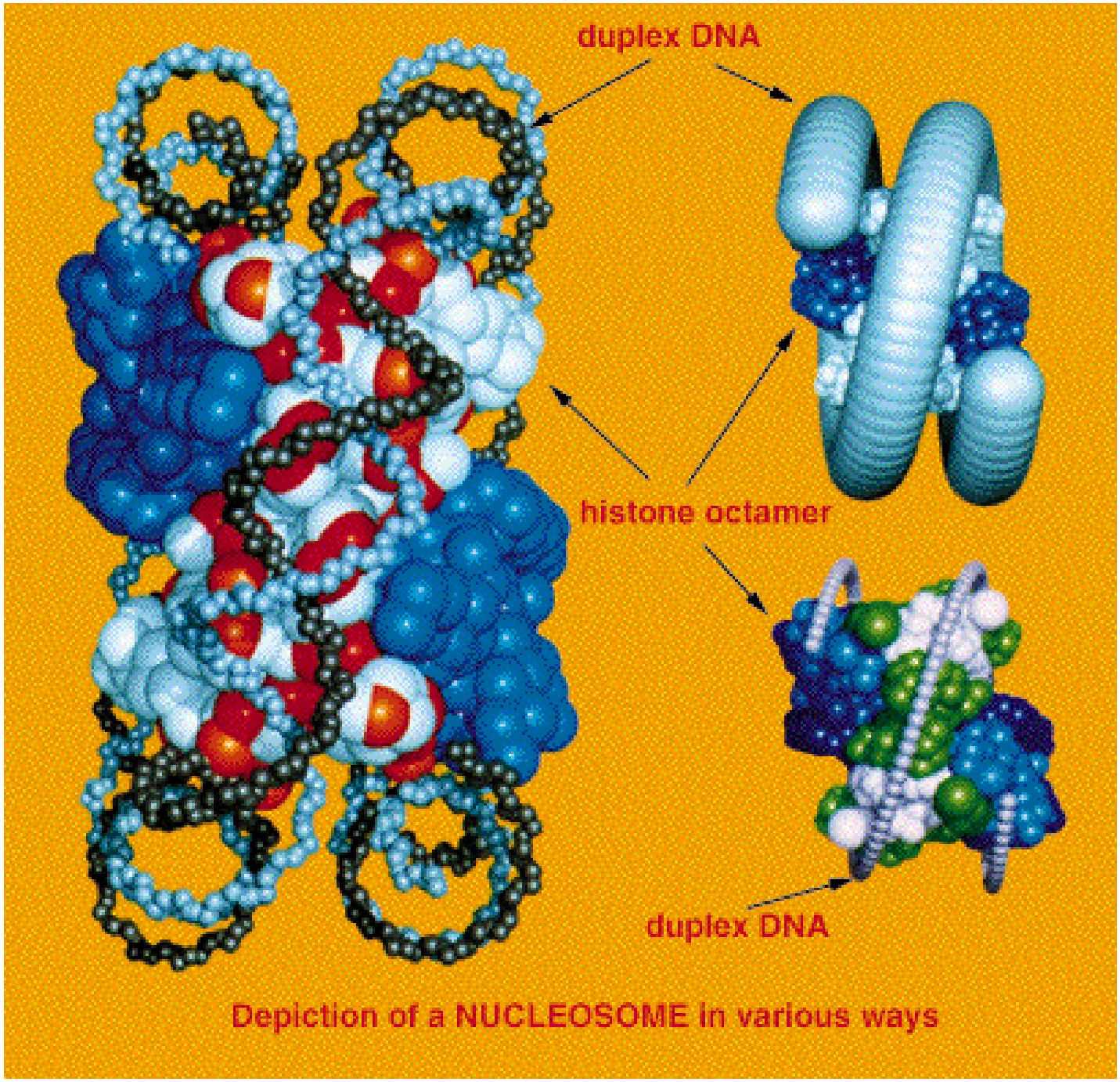
Die Core Histone:
H2A, H2B, H3, H4

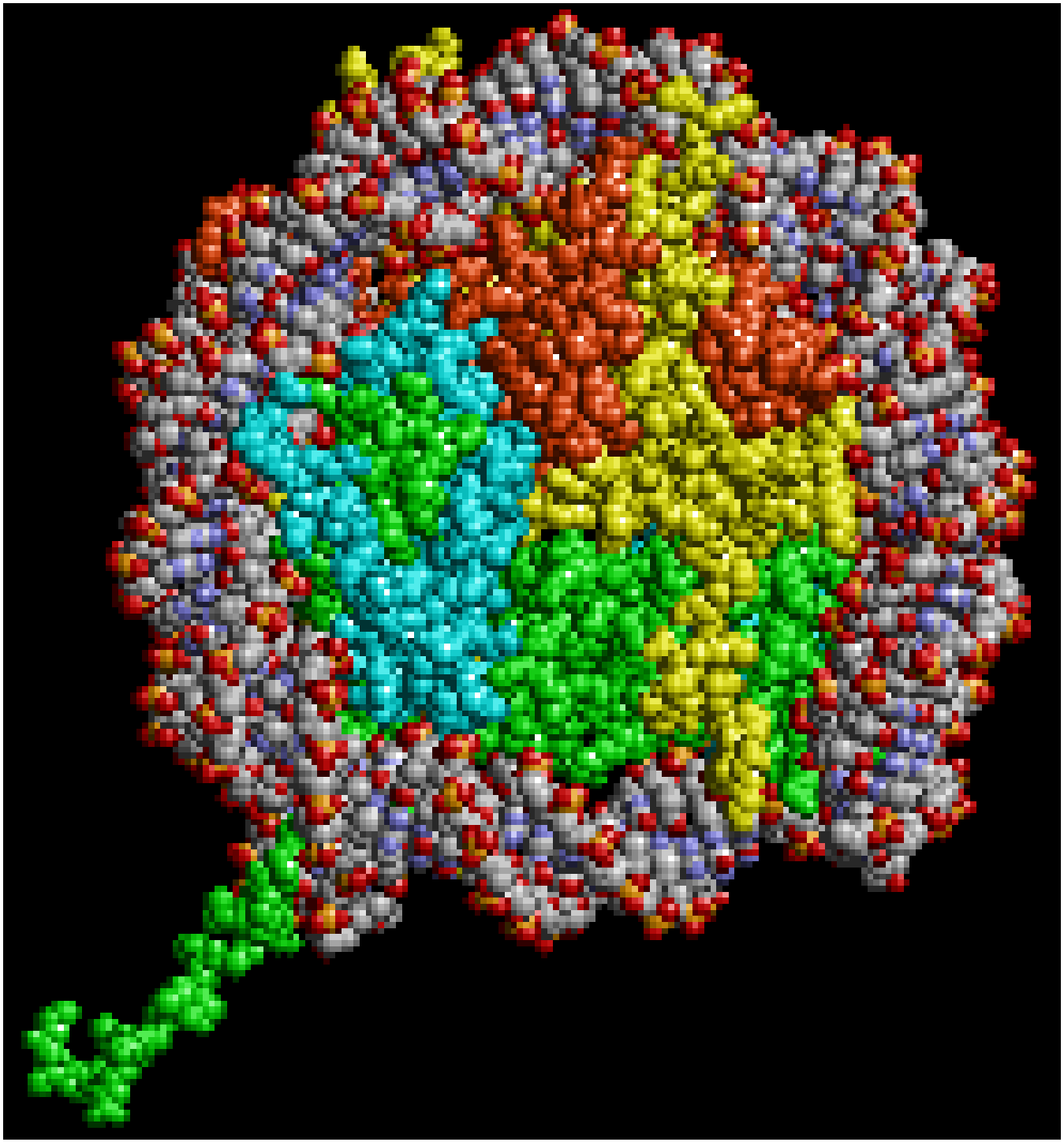


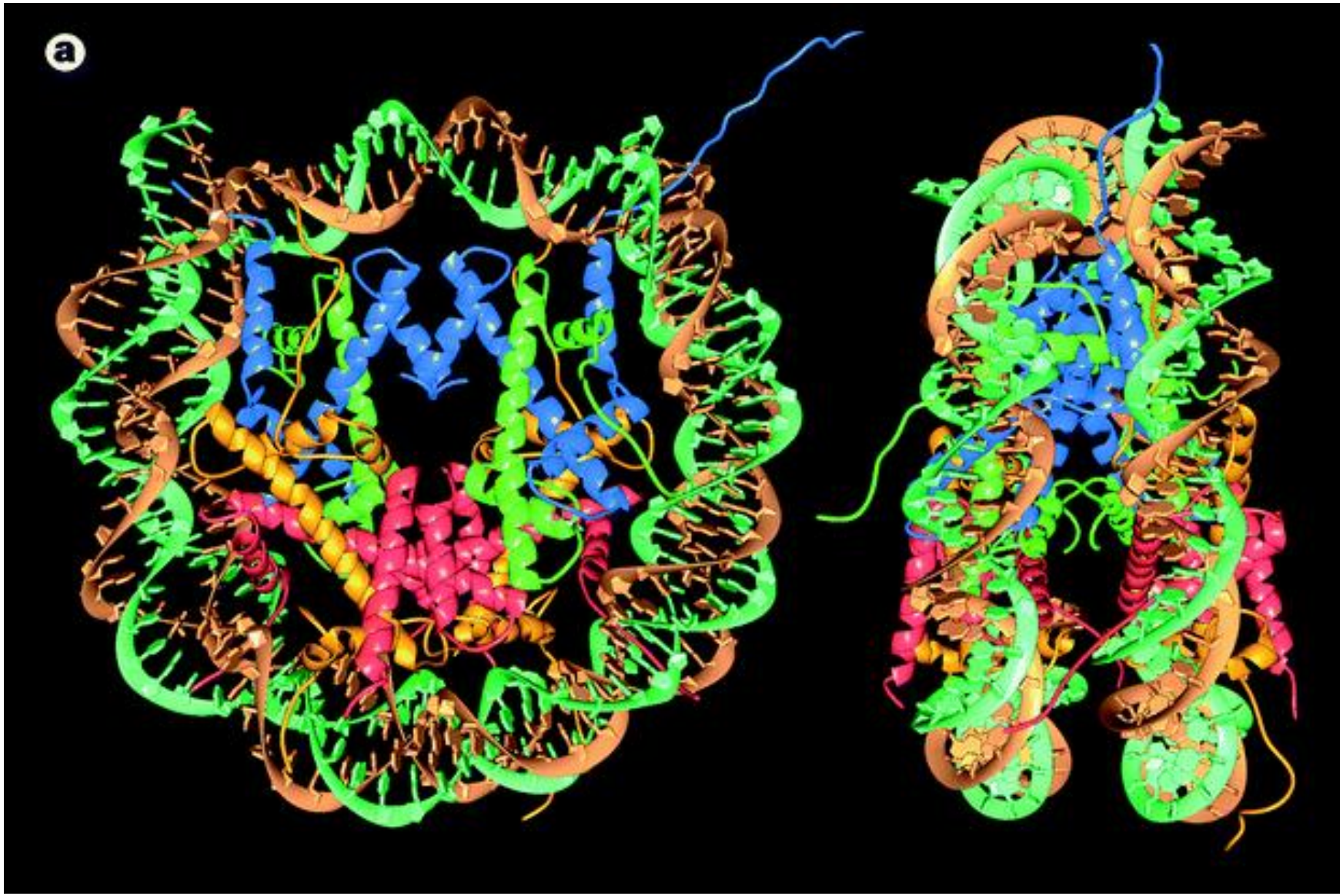
Der Aufbau des „Core“-Nukleosoms ist im Detail aufgeklärt:



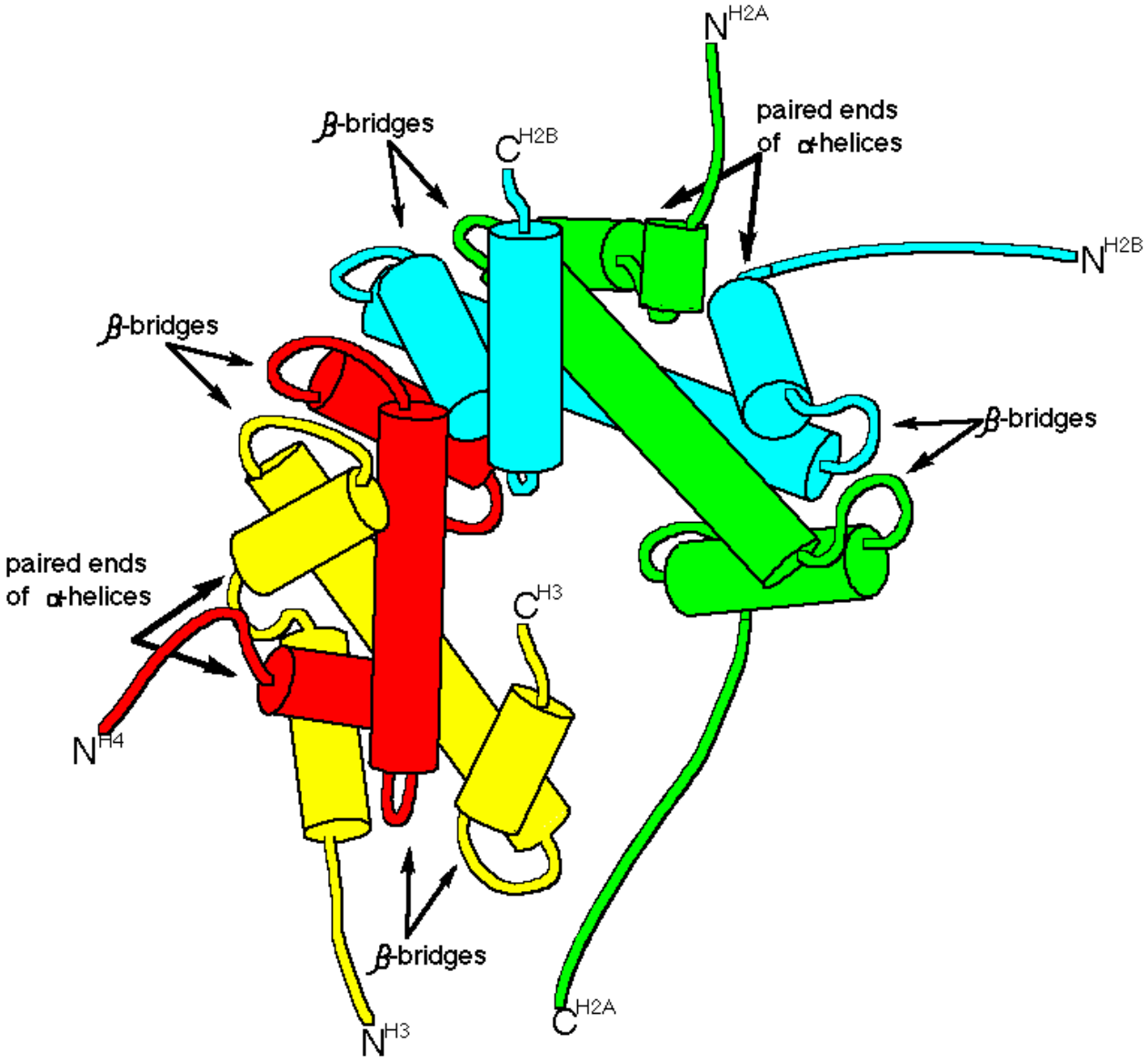

nucleosome core particle
H4'
H2A
H3
H2B
H4
Luger, Mäder, Richmond, Sargent & Richmond, 1997

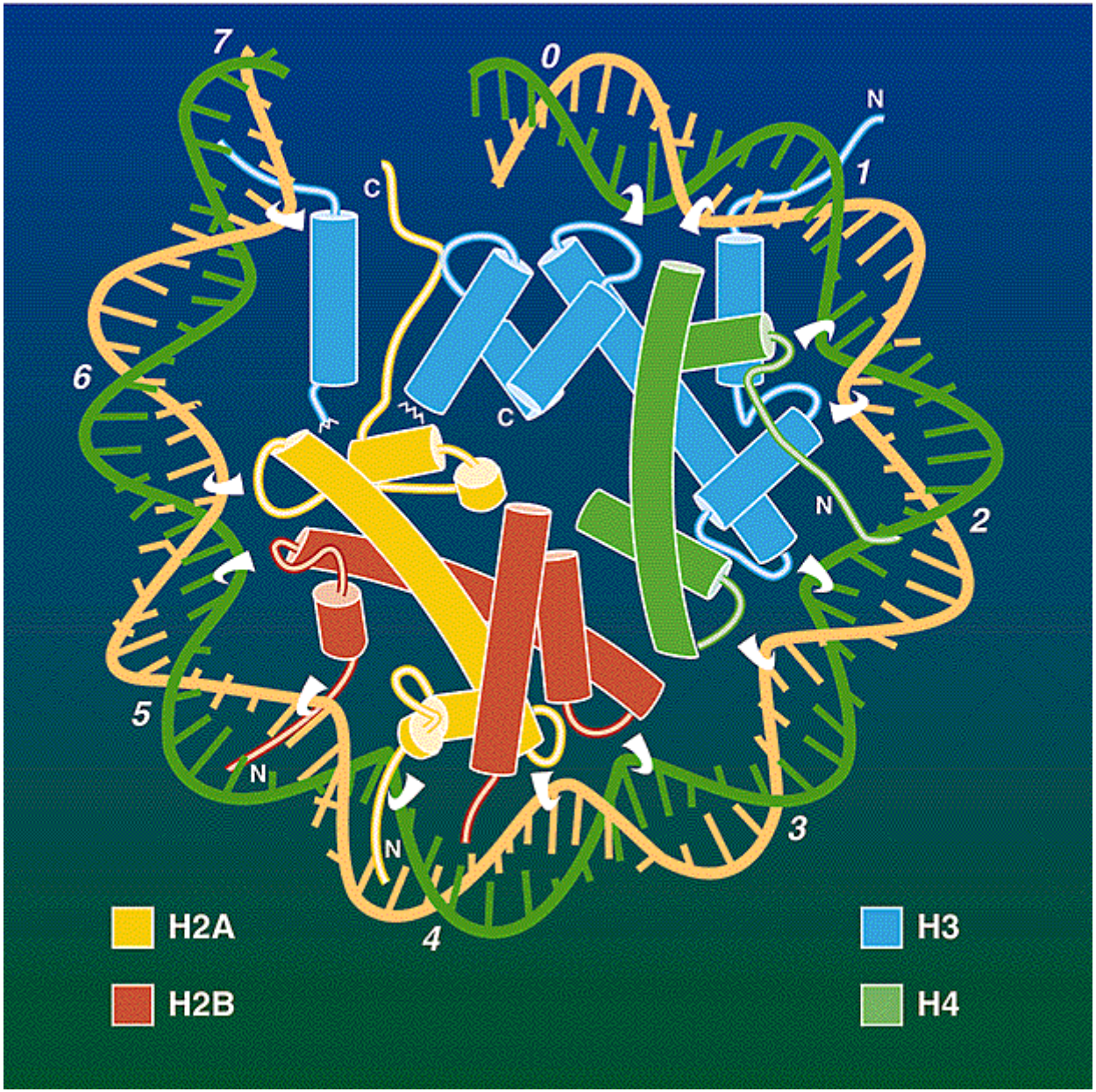

duplex DNA
histone octamer
duplex DNA
Depiction of a NUCLEOSOME in various ways

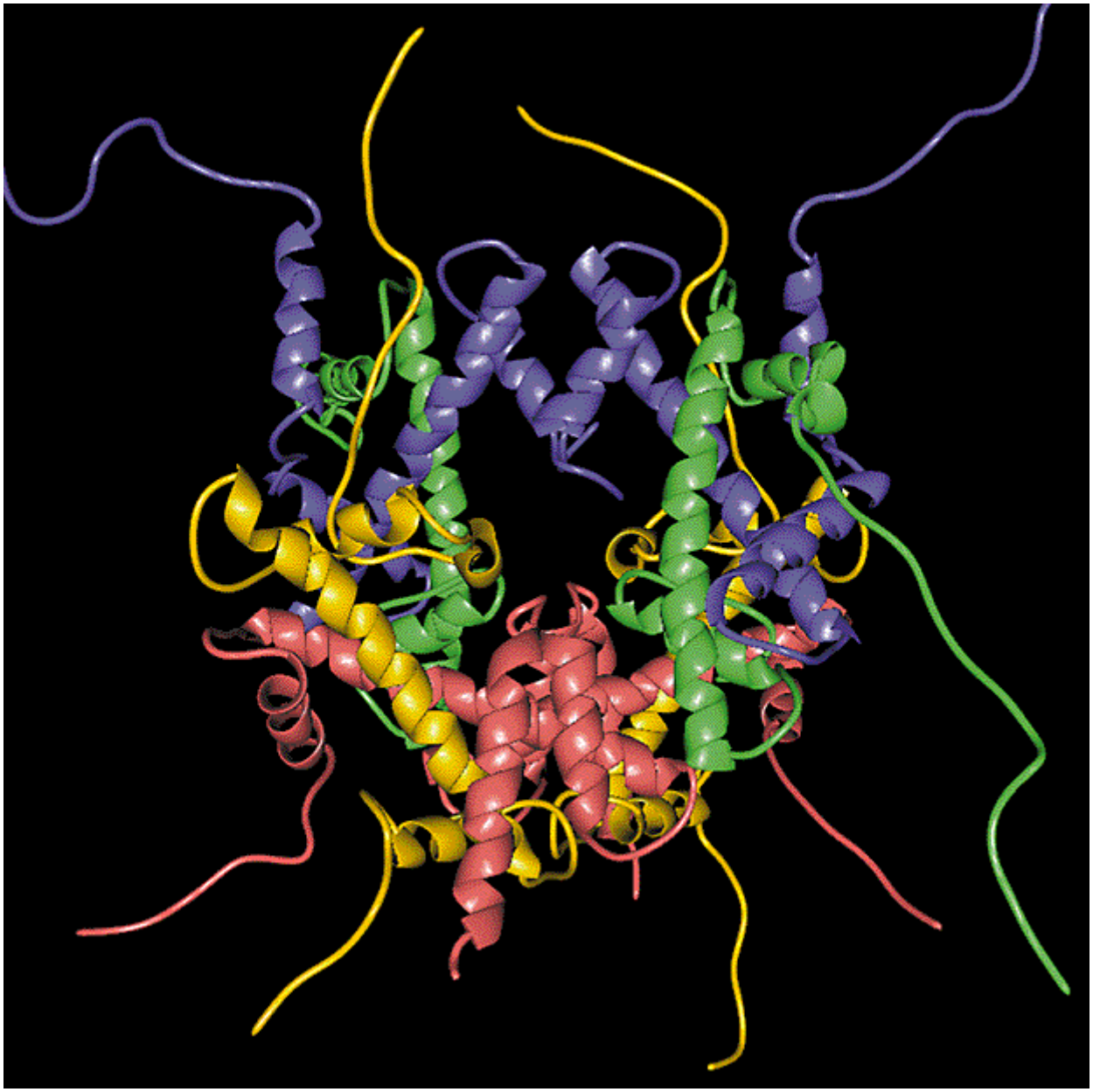


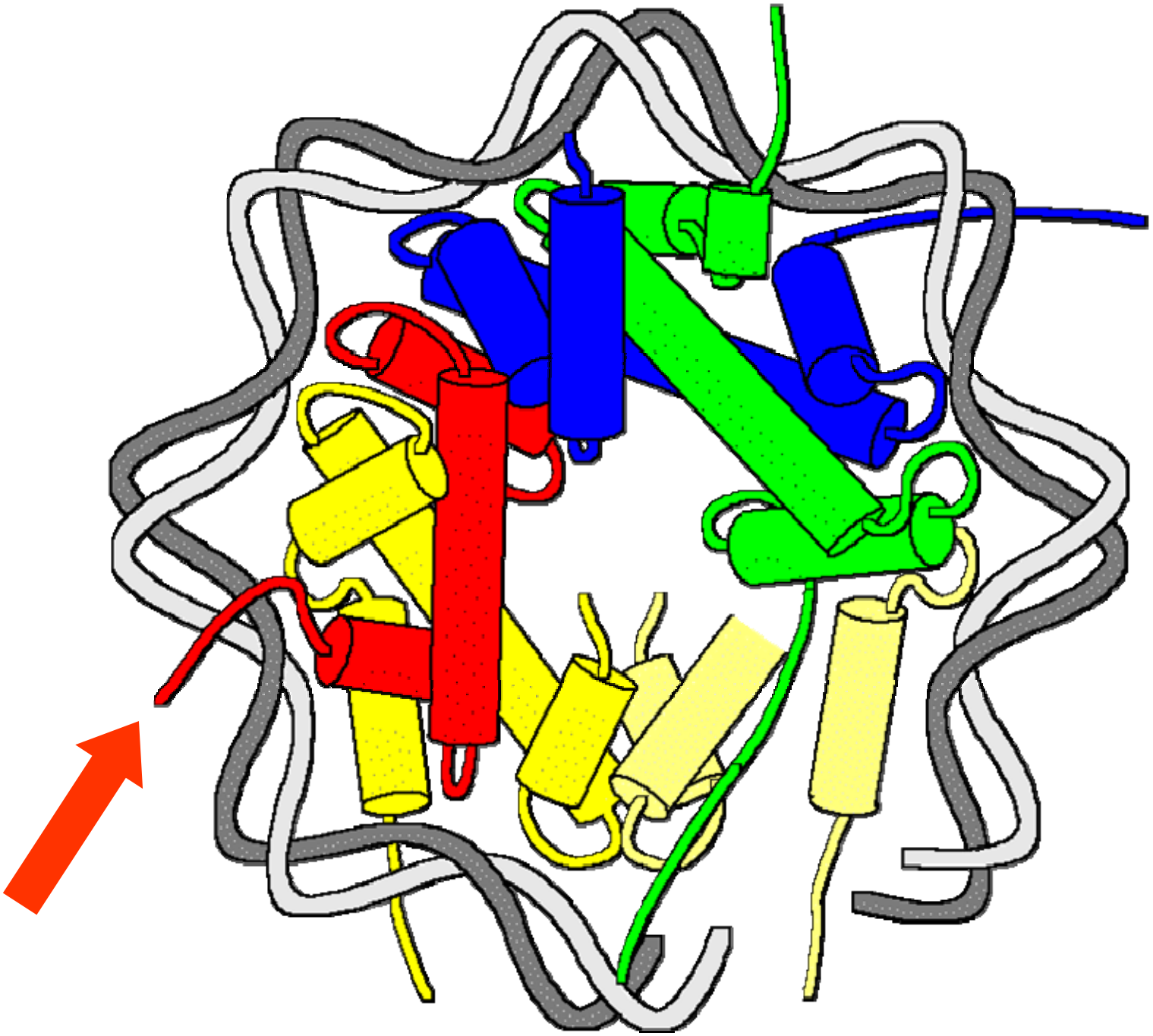

a

Histonoktamer die Abbildung zeigt nur ein „halbes“ Oktamer

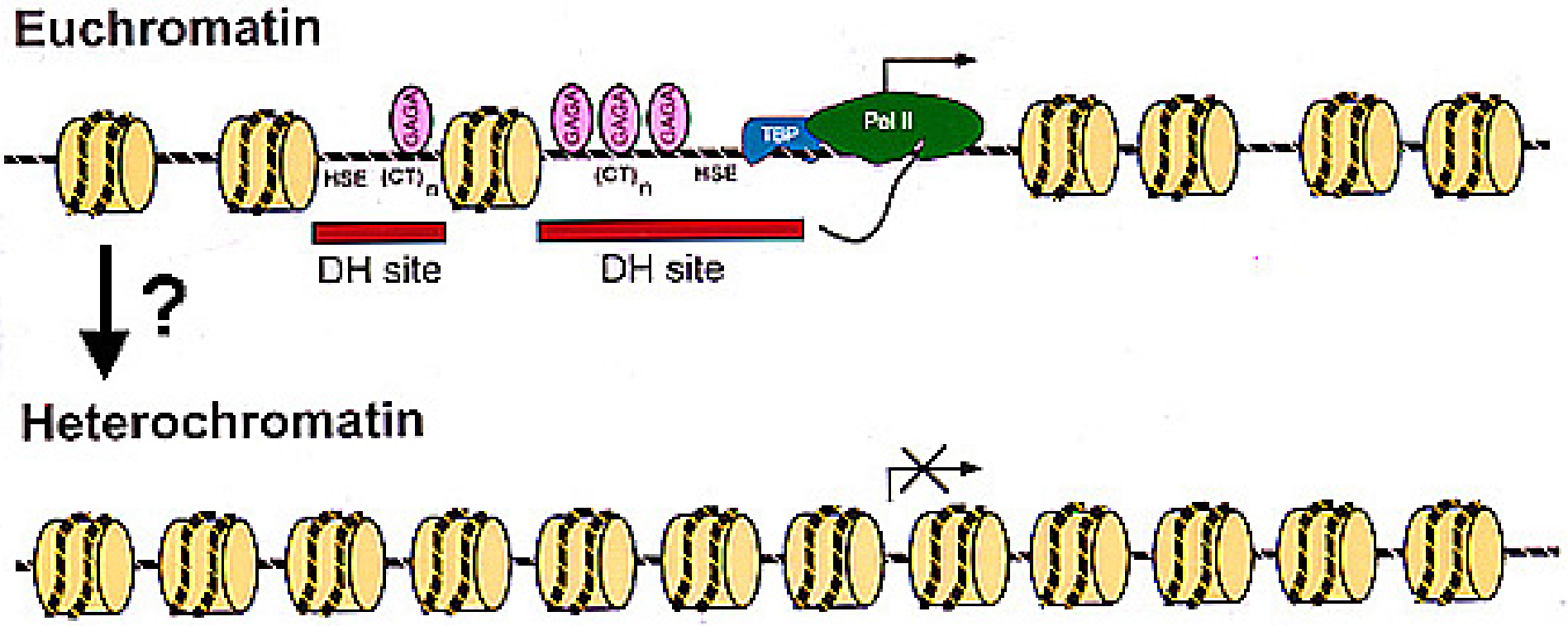



7
0
N
1
C
6
C
N
2
5
N
N
3
4
H2A
H2B
H3
H4

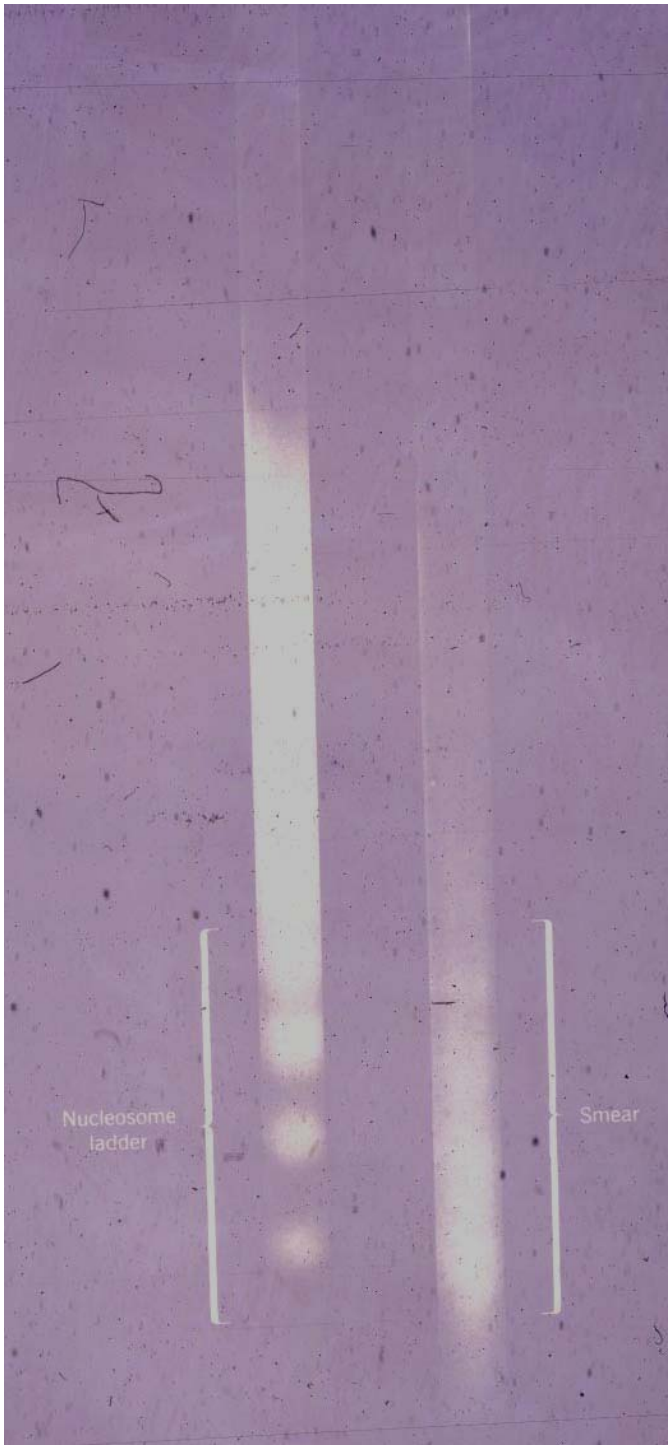




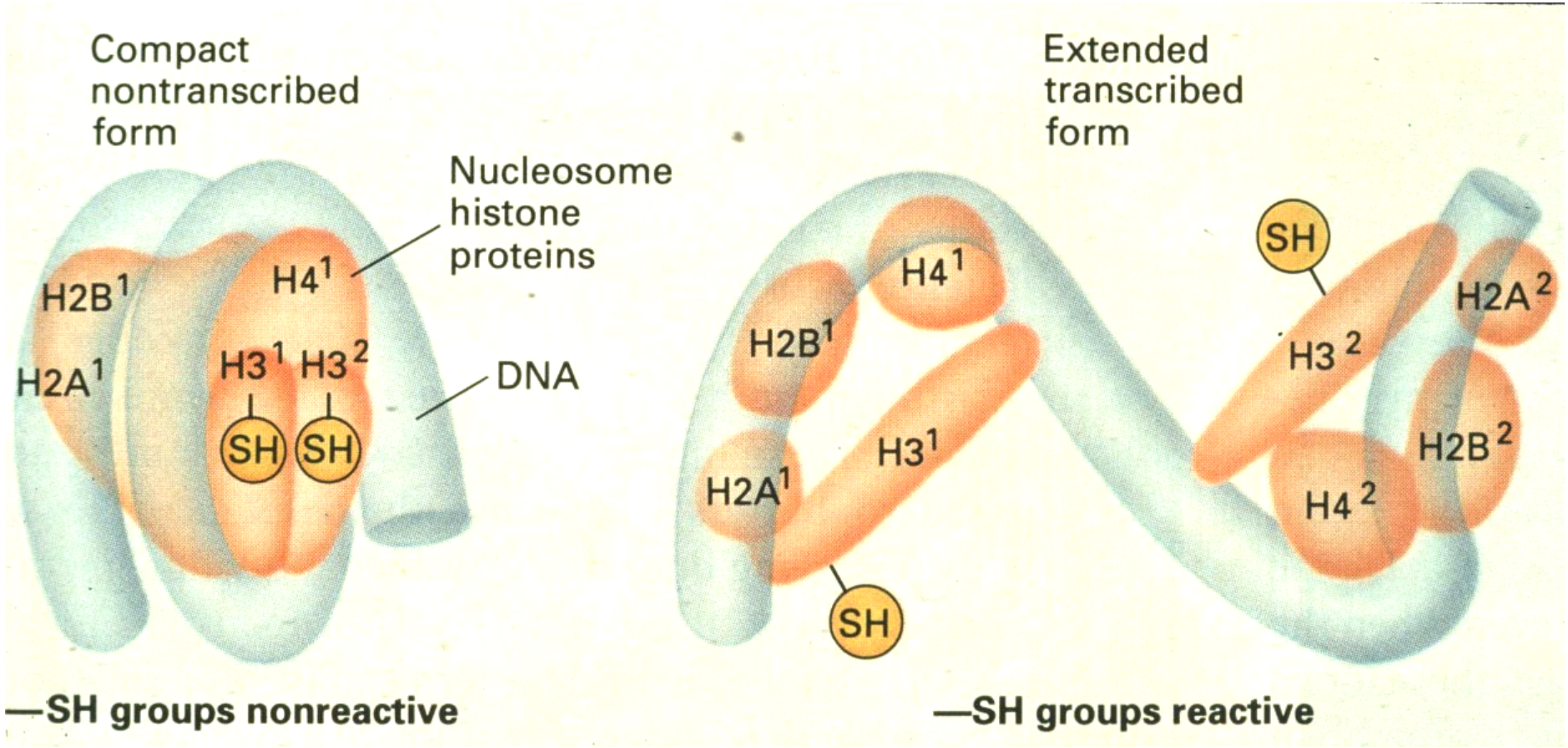
Chromatin und Genaktivität

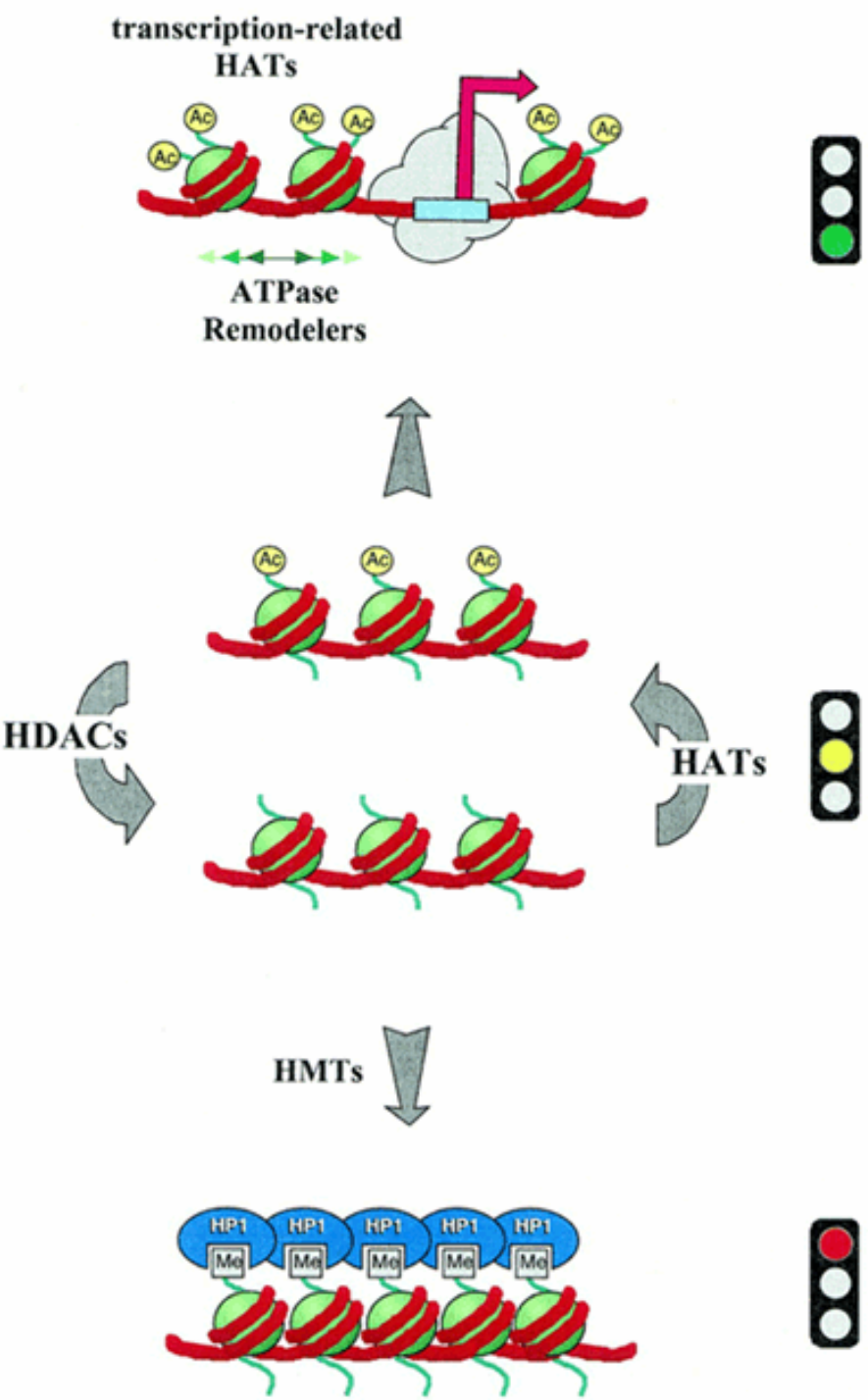


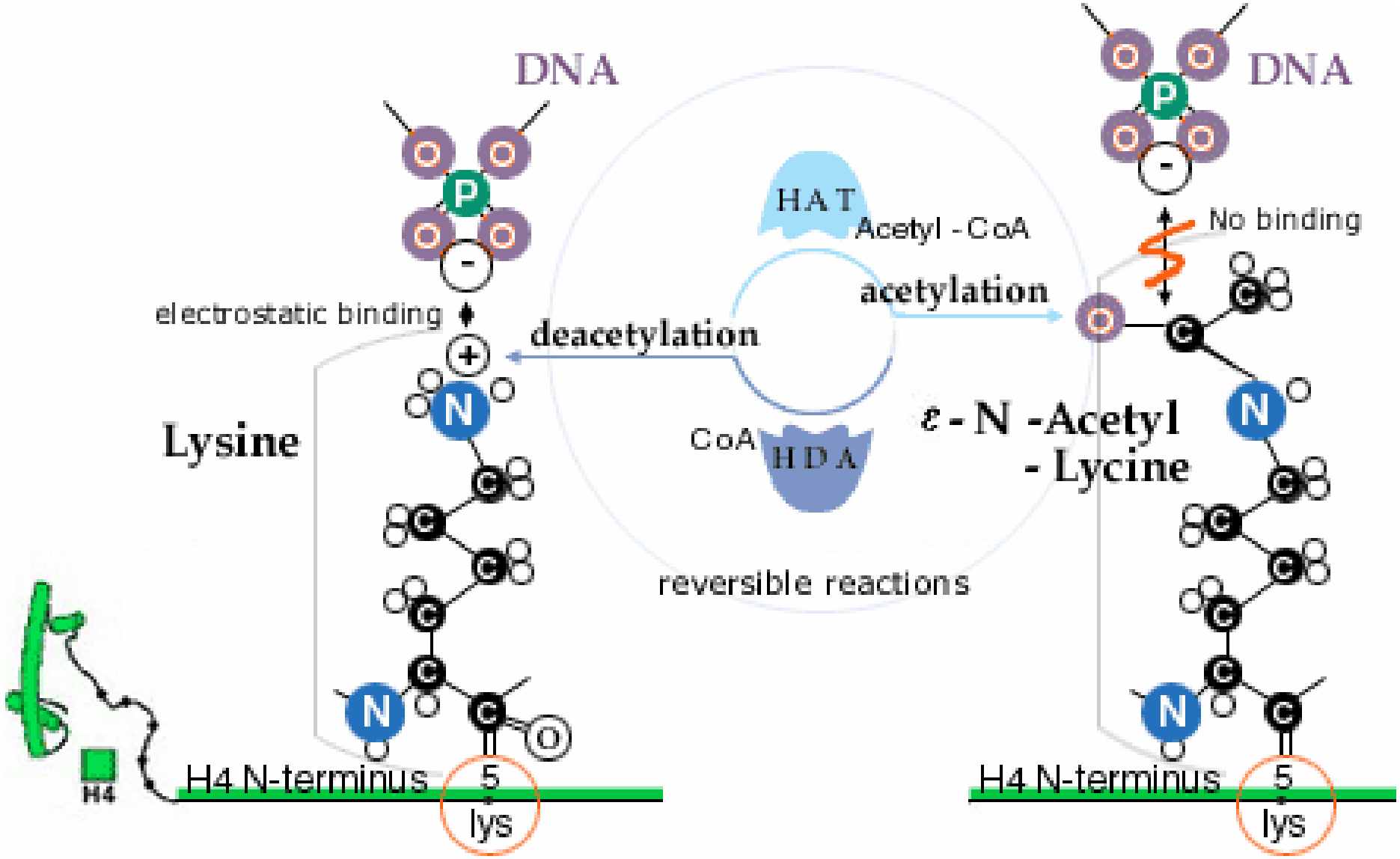
Aktives Chromatin ist nicht in Nukleosomen organsisiert



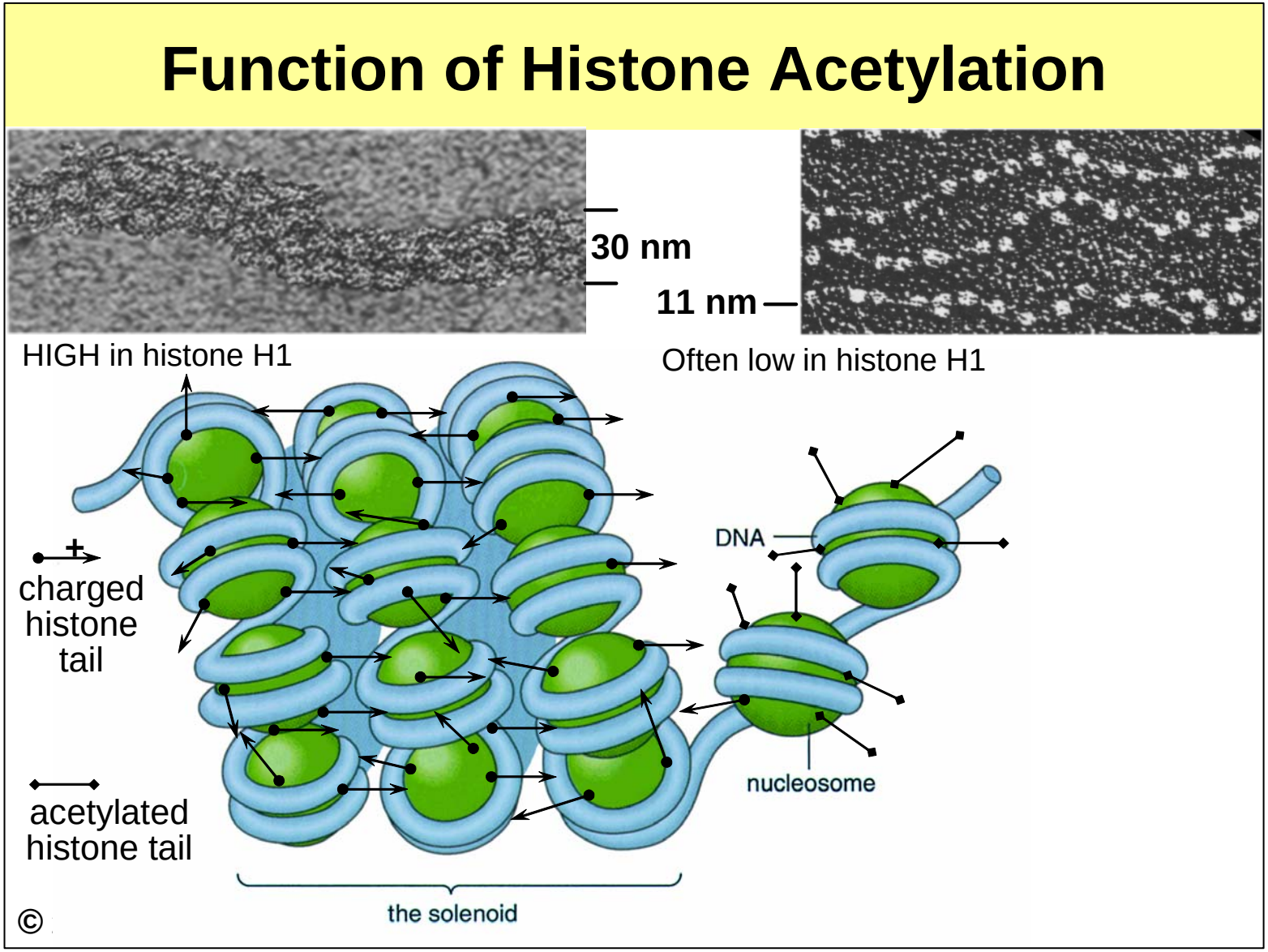
Inaktive / Aktives Chromatin




transcription-related
HATs
Ac
ATPase
Remodelers
HDACs
HATs
HMTs
HP1
Me

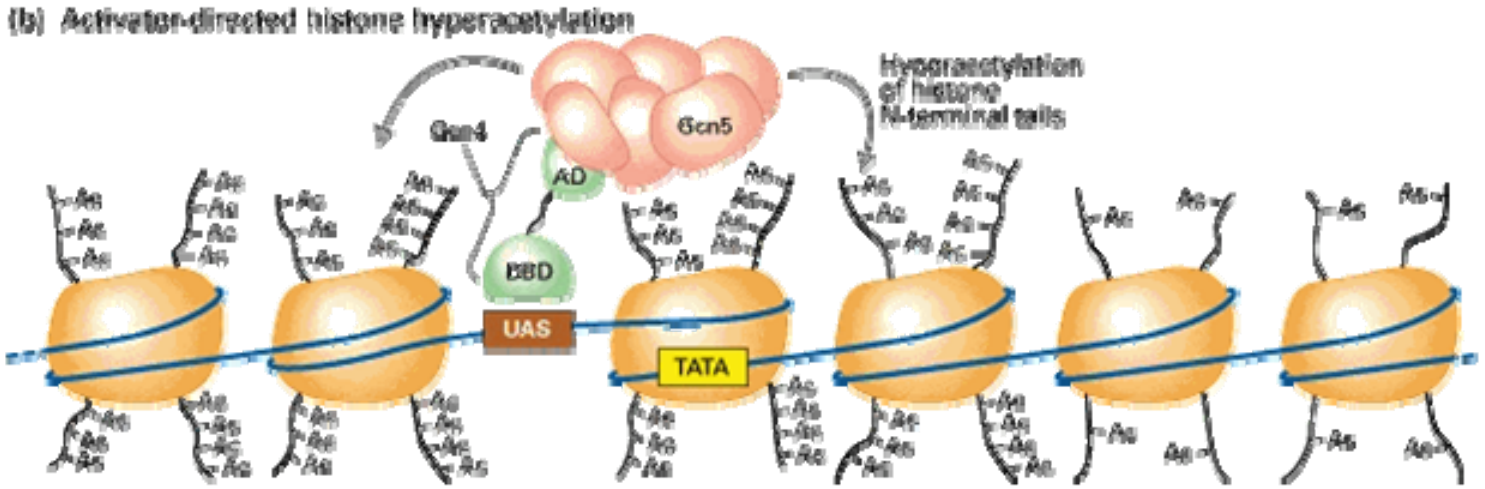

DNA
electrostatic binding
Lysine
deacetylation
HAT
Acetyl - CoA
acetylation
CoA
HDA
reversible reactions
DNA
No binding
ε - N -Acetyl
- Lycine
H4
H4 N-terminus
5
lys
H4 N-terminus
5
lys

Histon Acetylierung



Histone acetylationand gene activation

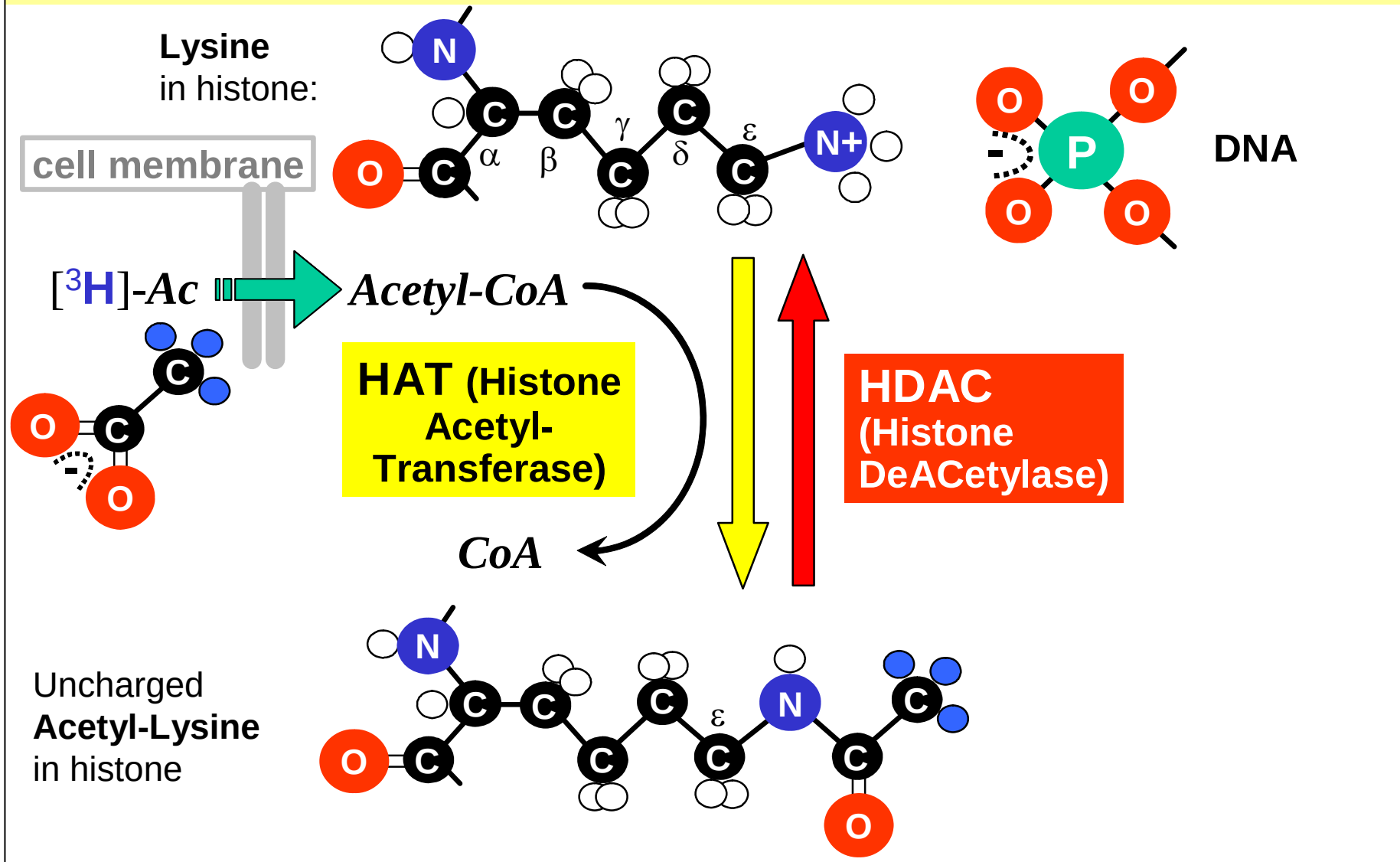
Model for how HATs might activate gene expression in chromatin



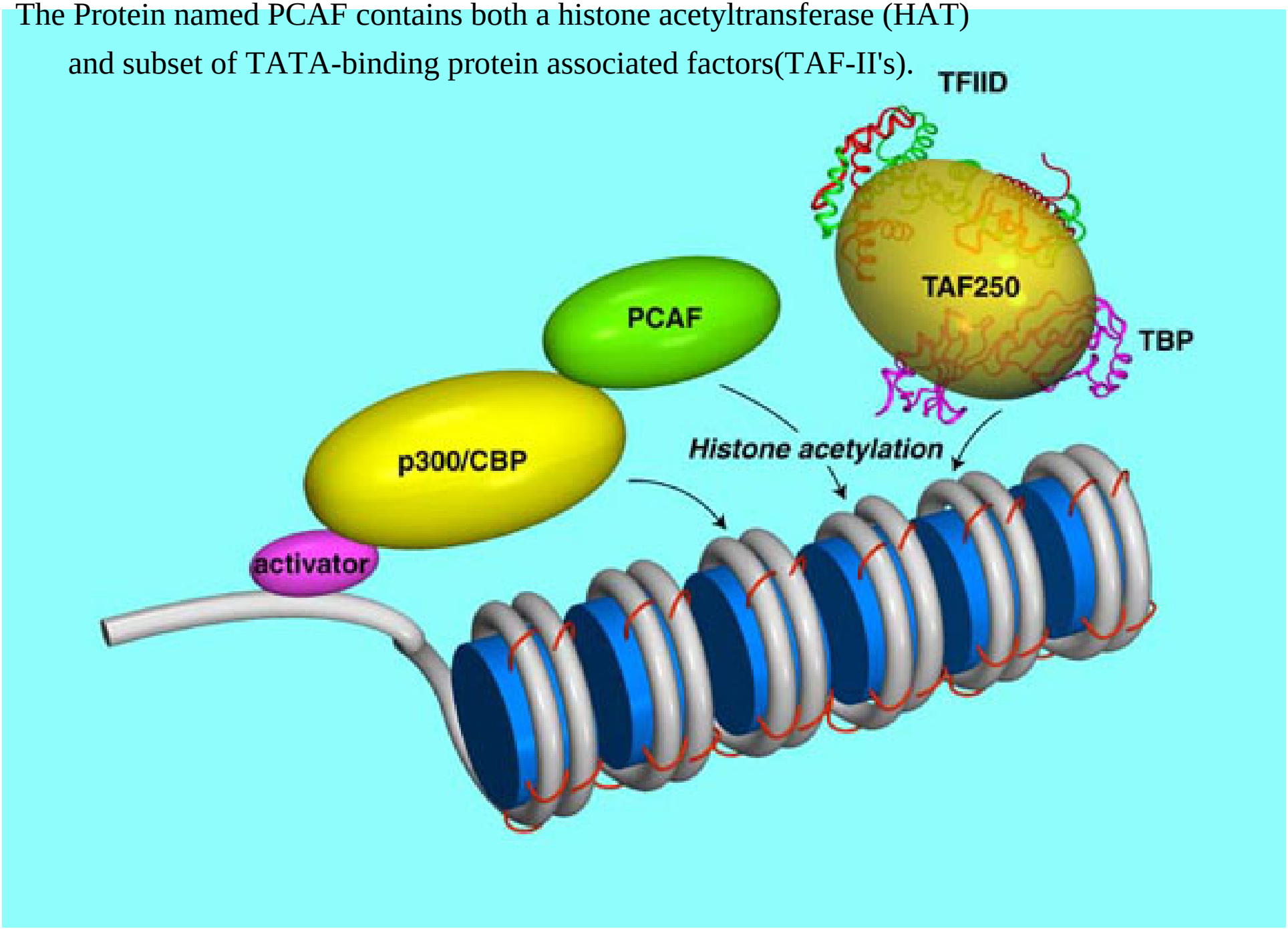
- GCN5, a known co-activator of gene expression is yeast, was shown to be closely related to the first HAT isolated biochemically from human cells
- GCN 5 was shown to be part of a large multi-protein complex
- One of the subunits of the complex was shown to interact directly with GCN4
- GCN4 is a gene-specific TF that binds to a specific DNA sequence in the promoter-proximal region of genes regulated by GCN4

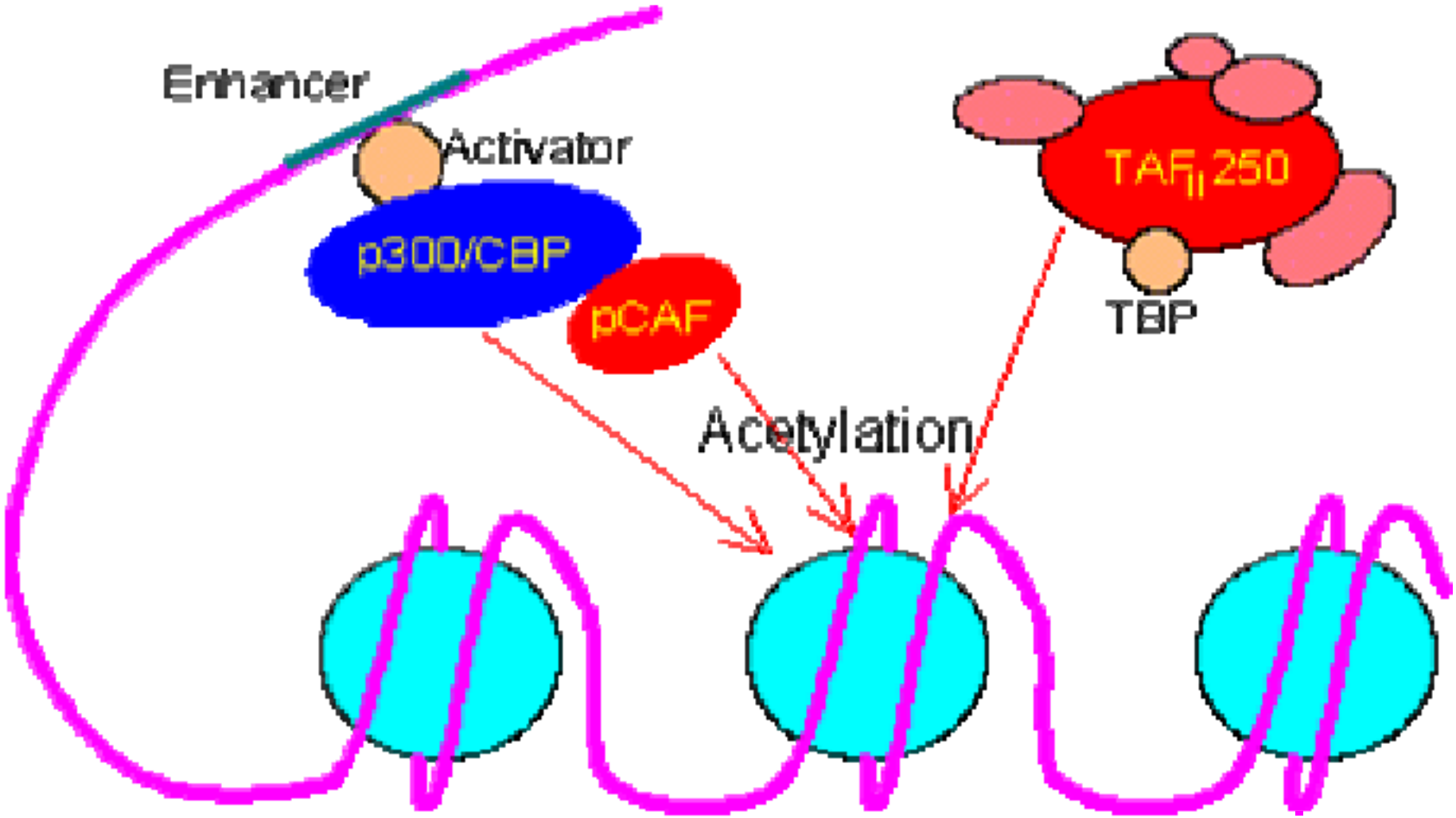
ÿ

Histone Acetylation Enzymes

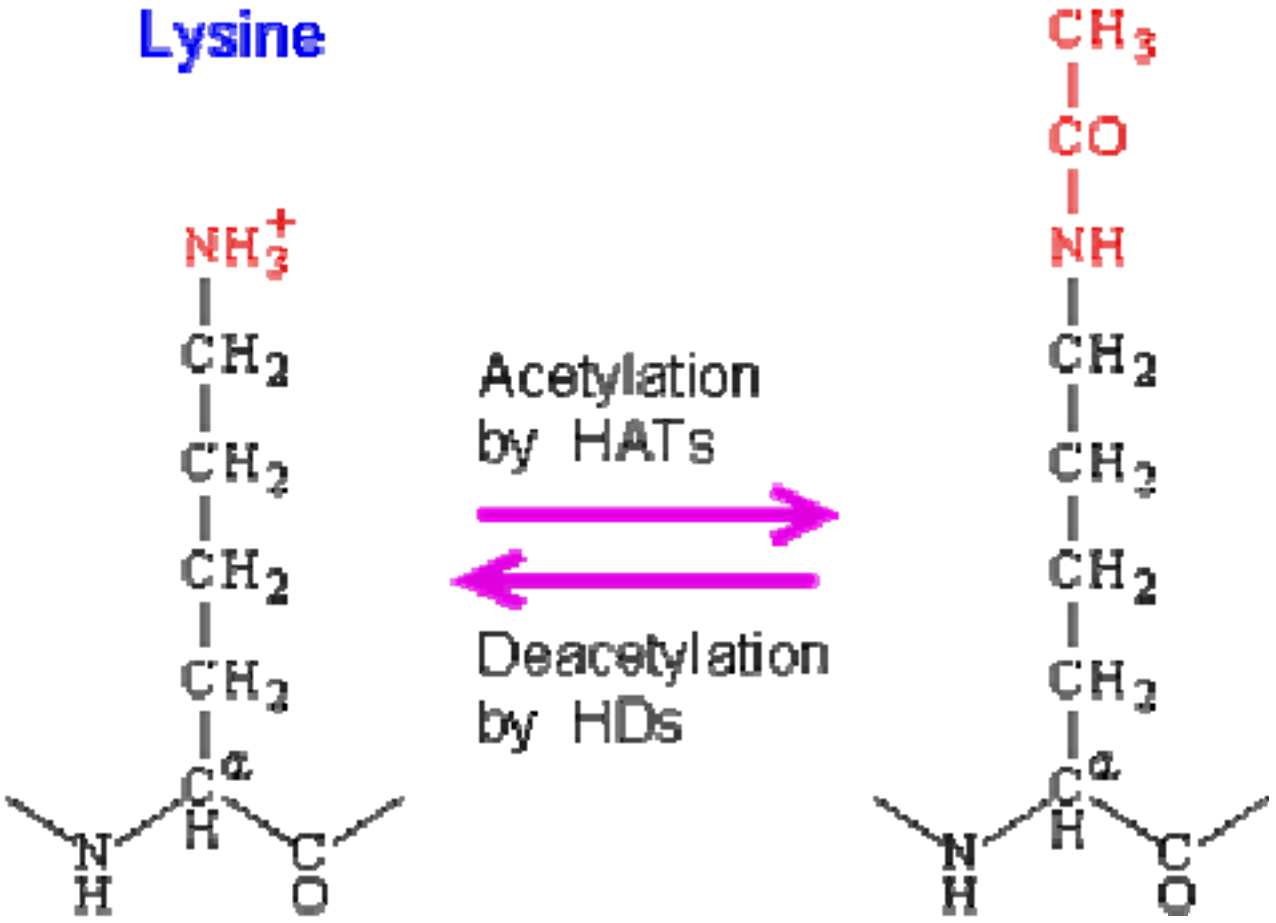


The Protein named PCAF contains both a histone acetyltransferase (HAT)
and subset of TATA-binding protein associated factors(TAF-II's).




Enhancer
Activator
p300/CBP
pCAF
TAF$_{II}$250
TBP
Acetylation

Acetylierung und Deacetylierung



Acetylated Histones – a choice

N-terminus of histone H3

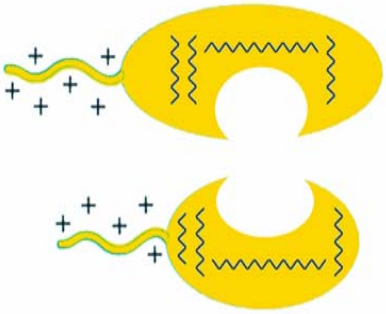
H_3N-ARTKQTARKS
5 9
m m

TGGKAPRKQL
14 18

ASKAARKSAP…
23 27
m

3-6 Ac

H3 : 3-6Ac



H2A : 1-2Ac

H2B : 3-6Ac

H4 : 4 or 5 Ac

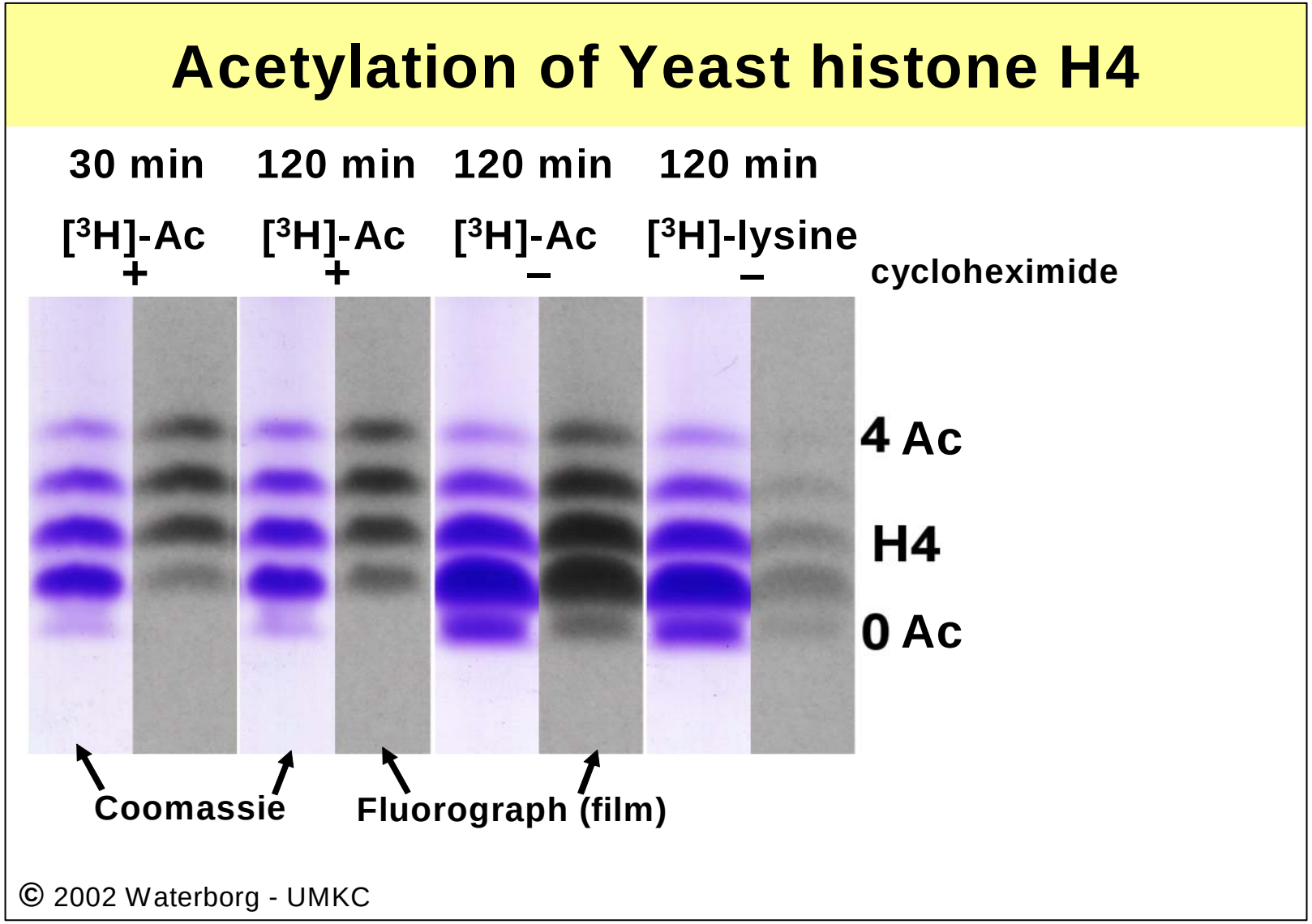
N-terminus of histone H4

Ac-HN-SGRGKGGKGL GKGGAKRHRK ILR…
5 8 12 16 20
(m)

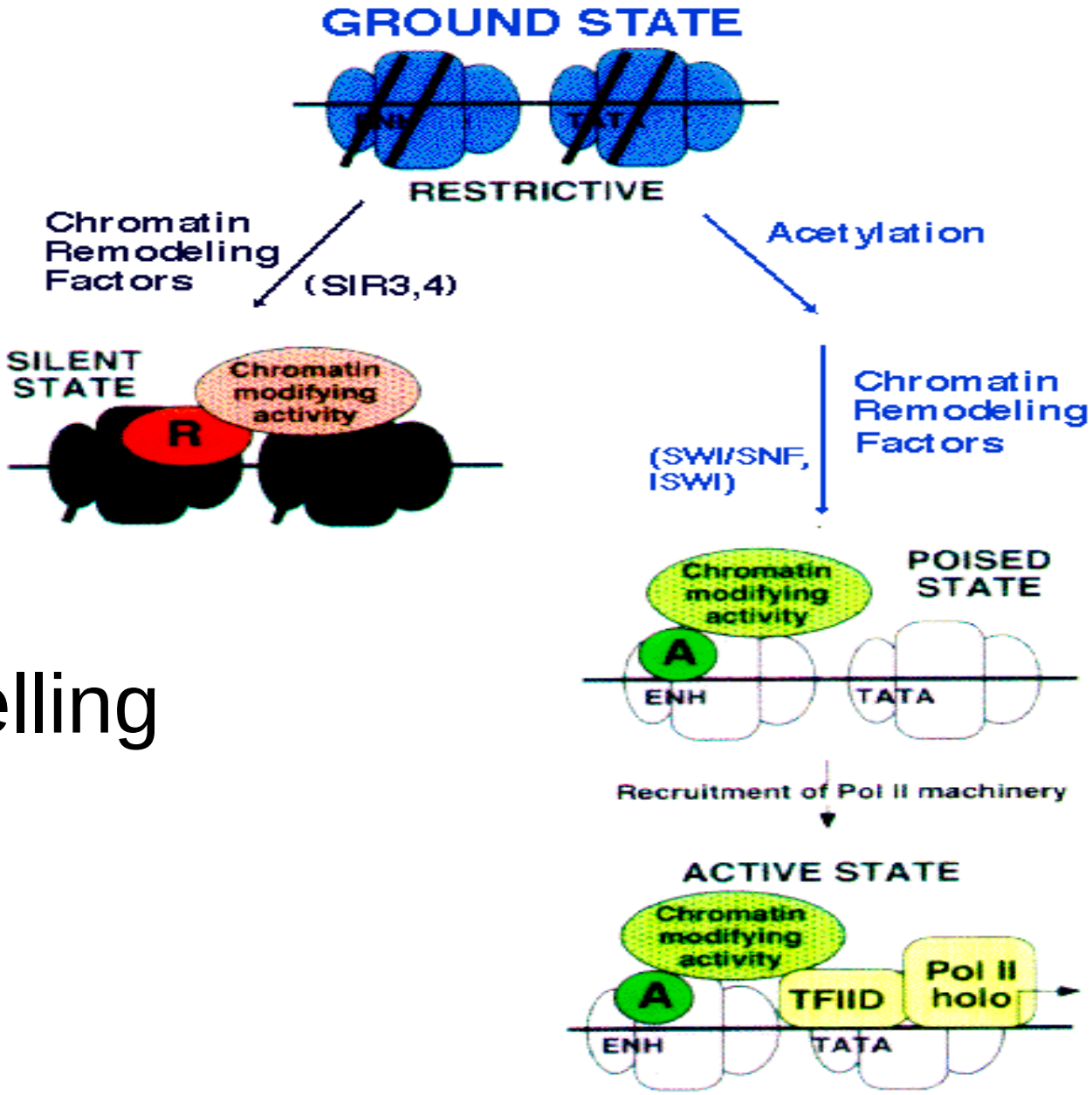
4 or 5 Ac

Note the "standard" colors for the core histones

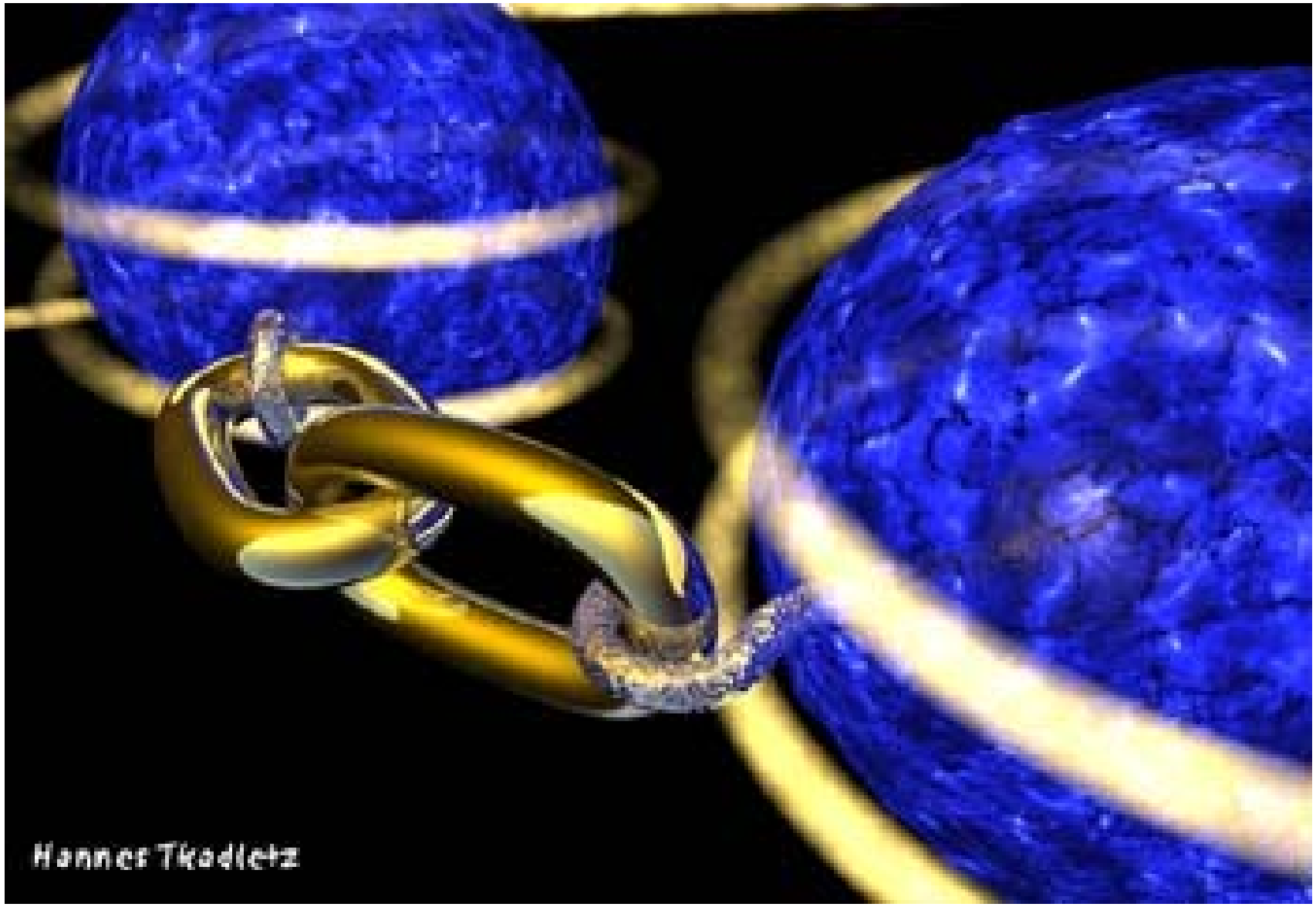
H4-Acetylierung



„Remodelling
“



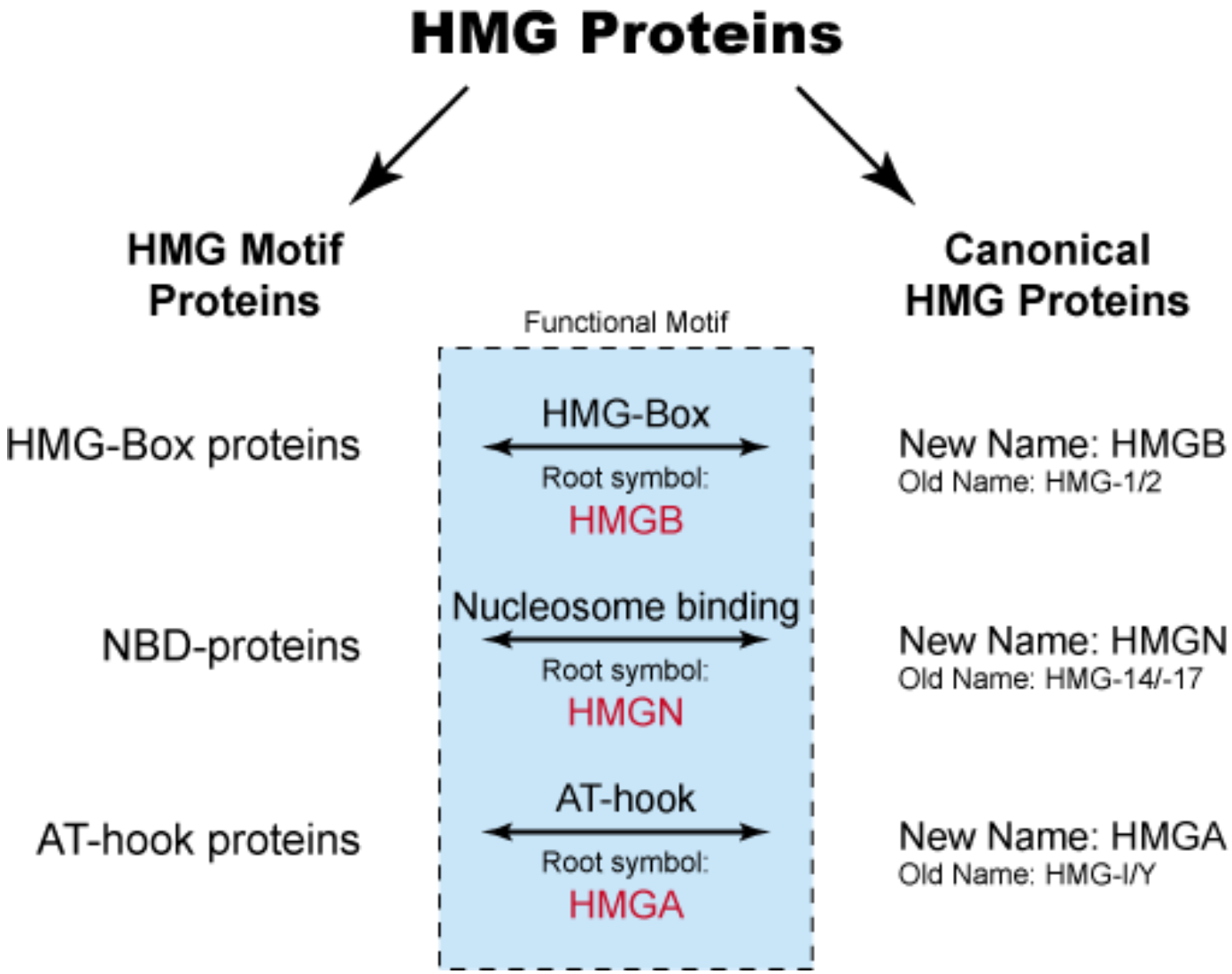
Inaktivierung durch Histonmethylierung



Weiere Modifikationen an Chromatin:

Core histones can be reversibly modified
by acetylation,
methylation, phosphorylation,
ubiquitination or ADP-ribosylation
(Strahl and Allis, 2000)

Weitere Chromatinproteine



Nuklesomenstruktur des Chromatins informative Internetadressen:

http://www.average.org/~pruss/nucleosome.html
http://bama.ua.edu/~hsmithso/class/bsc_495/chromatin/chromatin_web.html
http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v389/n6648/full/389231a0_fs.html
http://www.biochem.arizona.edu/classes/bioc462/462a/NOTES/Nucleic_Acids/nucleosome.html
http://info.bio.cmu.edu/Courses/03438/Nsome/1AOI.htm
http://arapaho.nsuok.edu/%7Ebiology/Tutorials/Nucleosome.htm
http://medweb.bham.ac.uk/research/chromatin/nucleosome.html
http://www.sciencemag.org/cgi/content/abstract/1060118v1
Chromatin structure:The nucleosome core all wrapped up Histonmethylierung:
Nature **389**, 231 - 232 (1997)

X-Inaktivierung

Barr body in epidermal spinous cell layer

Nuclear appendage in white blood cells

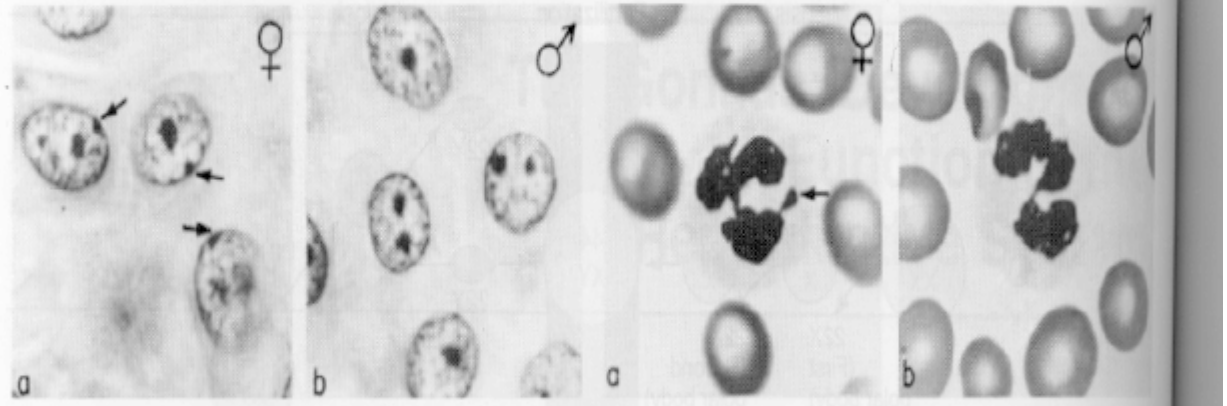


Figure 23–3. ***Left:*** Barr body (arrows) in the epidermal spinous cell layer. ***Right:*** Nuclear appendage ("drumstick") identified by arrow in white blood cells. (Reproduced, with permission, from Grumbach MM, Barr ML: Cytologic tests of chromosomal sex in relation to sex anomalies in man. *Recent Prog Horm Res* 1958;**14**:255.)

Mechanism of X-inactivation

Inactivation of an X chromosome requires a gene on that chromosome called ***XIST***.

XIST encodes a large molecule of **RNA** (of a type different from those, e.g., mRNA, used in protein synthesis).

XIST RNA accumulates along the X chromosome containing the active *XIST* gene and proceeds to inactivate all (or almost all) of the other hundreds of genes on that chromosome.

XIST RNA does not travel over to any other X chromosome in the nucleus.

Barr bodies are inactive X chromosomes "painted" with *XIST* RNA.

During the first steps of embryonic development of the female, the *XIST* locus on each of her two X chromosomes is expressed but the *XIST* RNA is quickly broken down. Then something happens to tip the balance in favor of one or the other of the X chromosomes.

transcription **continues** on one of the X chromosomes, leading to an accumulation of *XIST* RNA and converting that chromosome into an inactive Barr body.

transcription of *XIST* **ceases** on the other X chromosome allowing all of its hundreds of other genes to be expressed. The shut-down of the *XIST* locus on the active X chromosome is done by methylating *XIST* regulatory sequences. DNA methylation usually results in gene repression so methylation permanently blocks *XIST* expression and permits the continued expression of all the other X-linked genes.

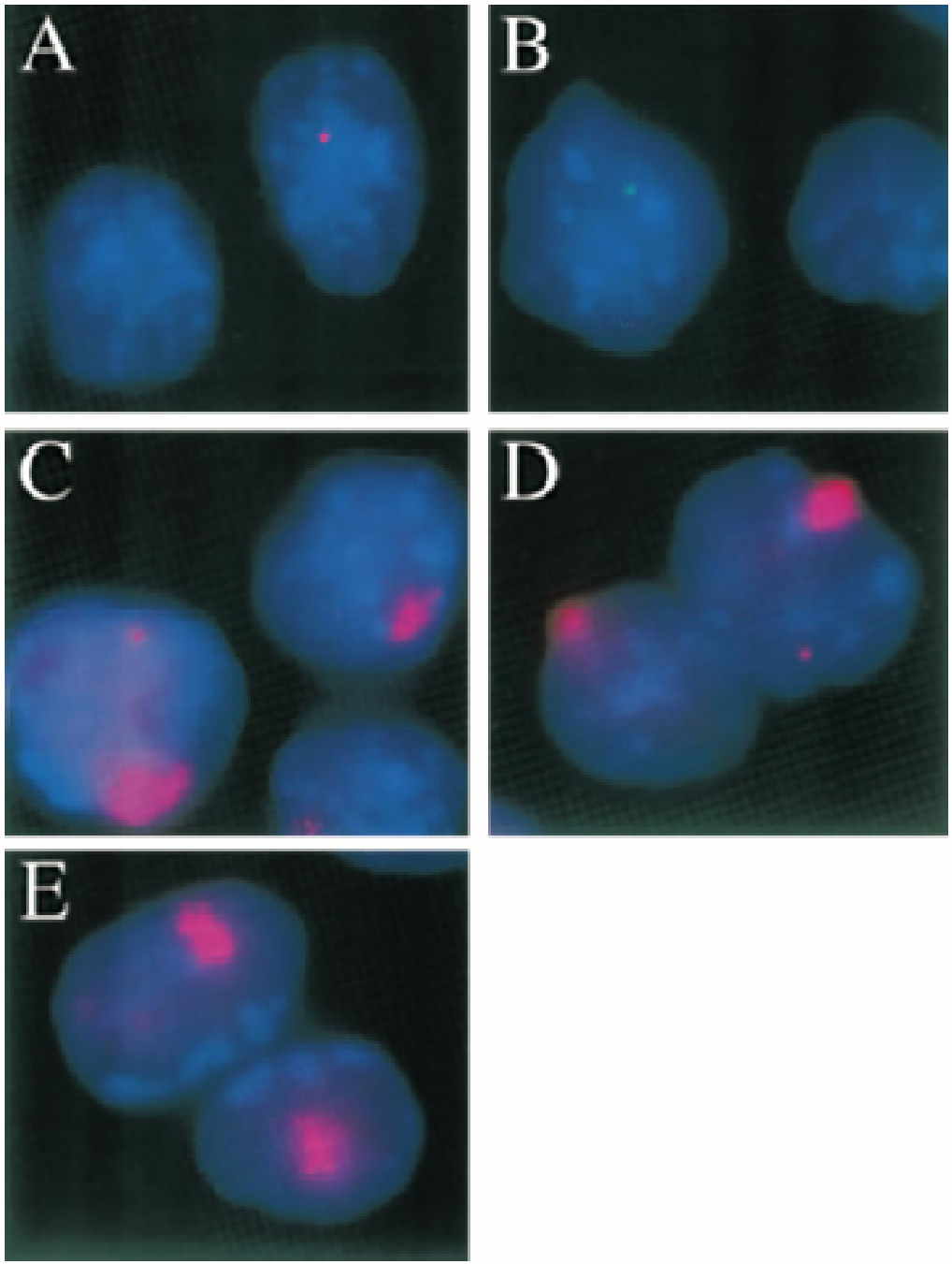
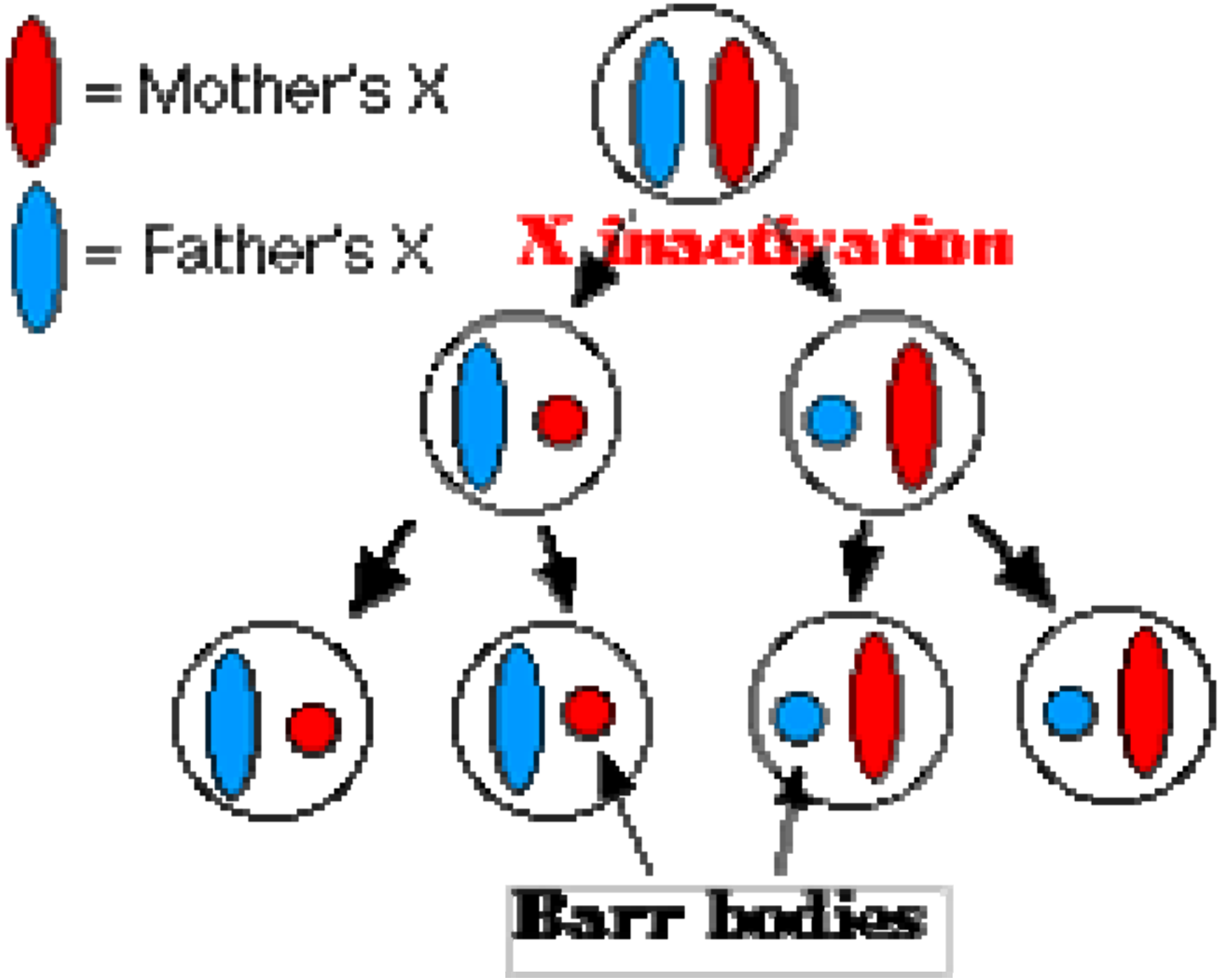


Figure 4. *Xist* Expression in Cells From Female *Xist*$^{+/-}$ Embryos at 7.5 Days of Gestation

Fluorescence in situ hybridization was performed for *Xist* (pink/red) and *Pgk-1* (green) RNA. The full probe was used in (A), (C), and (D); the deletion probe was used in (B) and (E). A probe for *Pgk-1* was also used in (B). (A) *Xist*$^{-}$ male; (B) *Xist*$^{-}$ male; (C) *Xist*$^{+/+}$ female; (D) *Xist*$^{+/-}$ female; (E) *Xist*$^{+/-}$ female.

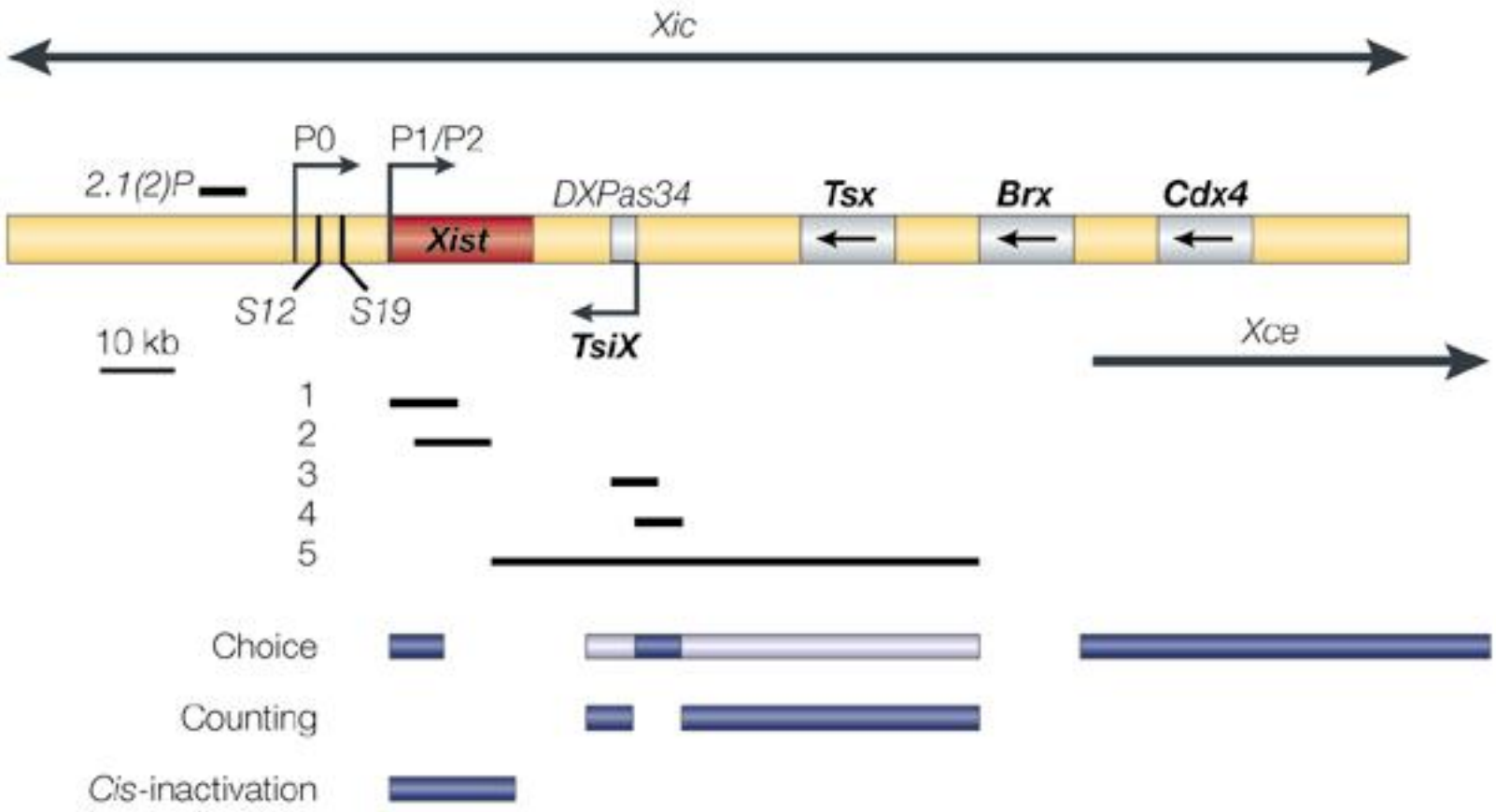

= Mother's X
= Father's X
X inactivation
Barr bodies

Because of X inactivation, female mammals are mosaic

XIC

- signal from X inactivation center, spread along chromosome, promoted by booster elements (Fig 3)
  - signal spreading is less effective in autosomal material than in the X chromosome itself
  - LINEs may represent booster sequences
    - » X chromosome is strikingly LINE-rich
    - » Efficiency of spread into autosomalregions correlates with their LINE content
- inactive X replicates late, the late replication may help maintain the inactive signal, in which late replication prevents transcription and transcription is required for early replication

The X inactivation center



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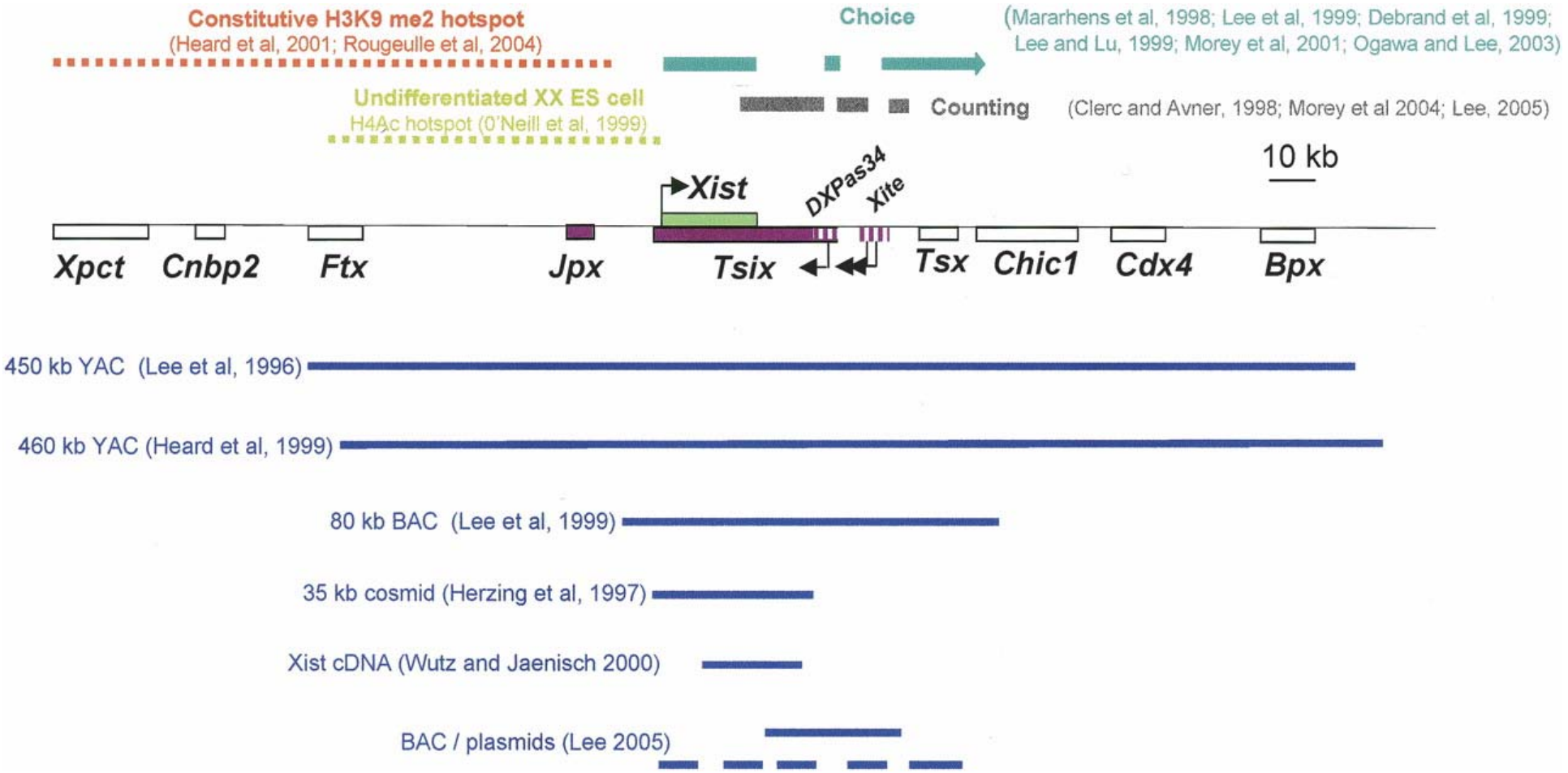
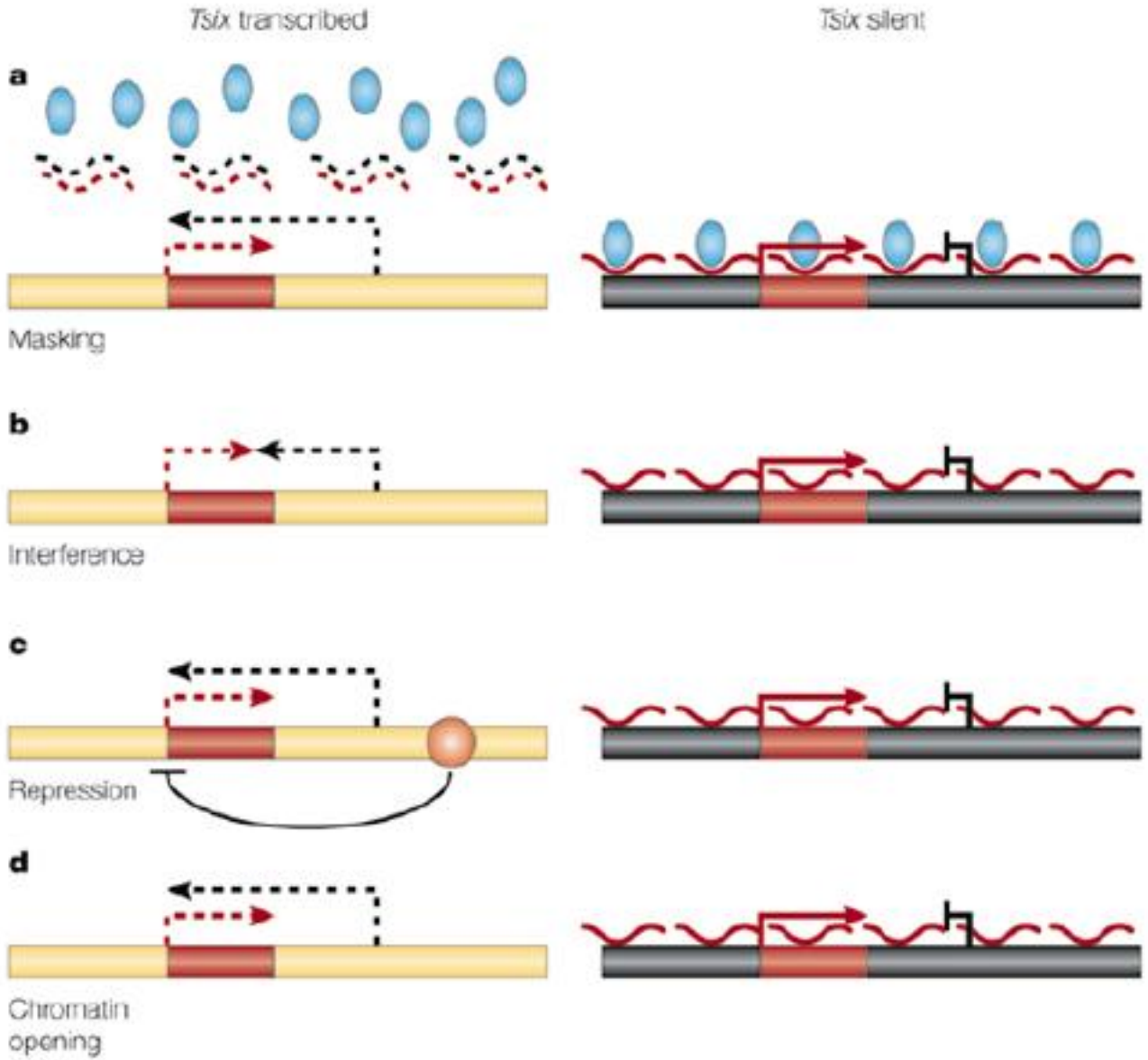

Constitutive H3K9 me2 hotspot
(Heard et al, 2001; Rougeulle et al, 2004)
Choice
(Mararhens et al, 1998; Lee et al, 1999; Debrand et al, 1999;
Lee and Lu, 1999; Morey et al, 2001; Ogawa and Lee, 2003)
Undifferentiated XX ES cell
H4Ac hotspot (O'Neill et al, 1999)
Counting
(Clerc and Avner, 1998; Morey et al 2004; Lee, 2005)
10 kb
Xist
DXPas34
Xite
Xpct
Cnbp2
Ftx
Jpx
Tsix
Tsx
Chic1
Cdx4
Bpx
450 kb YAC (Lee et al, 1996)
460 kb YAC (Heard et al, 1999)
80 kb BAC (Lee et al, 1999)
35 kb cosmid (Herzing et al, 1997)
Xist cDNA (Wutz and Jaenisch 2000)
BAC / plasmids (Lee 2005)



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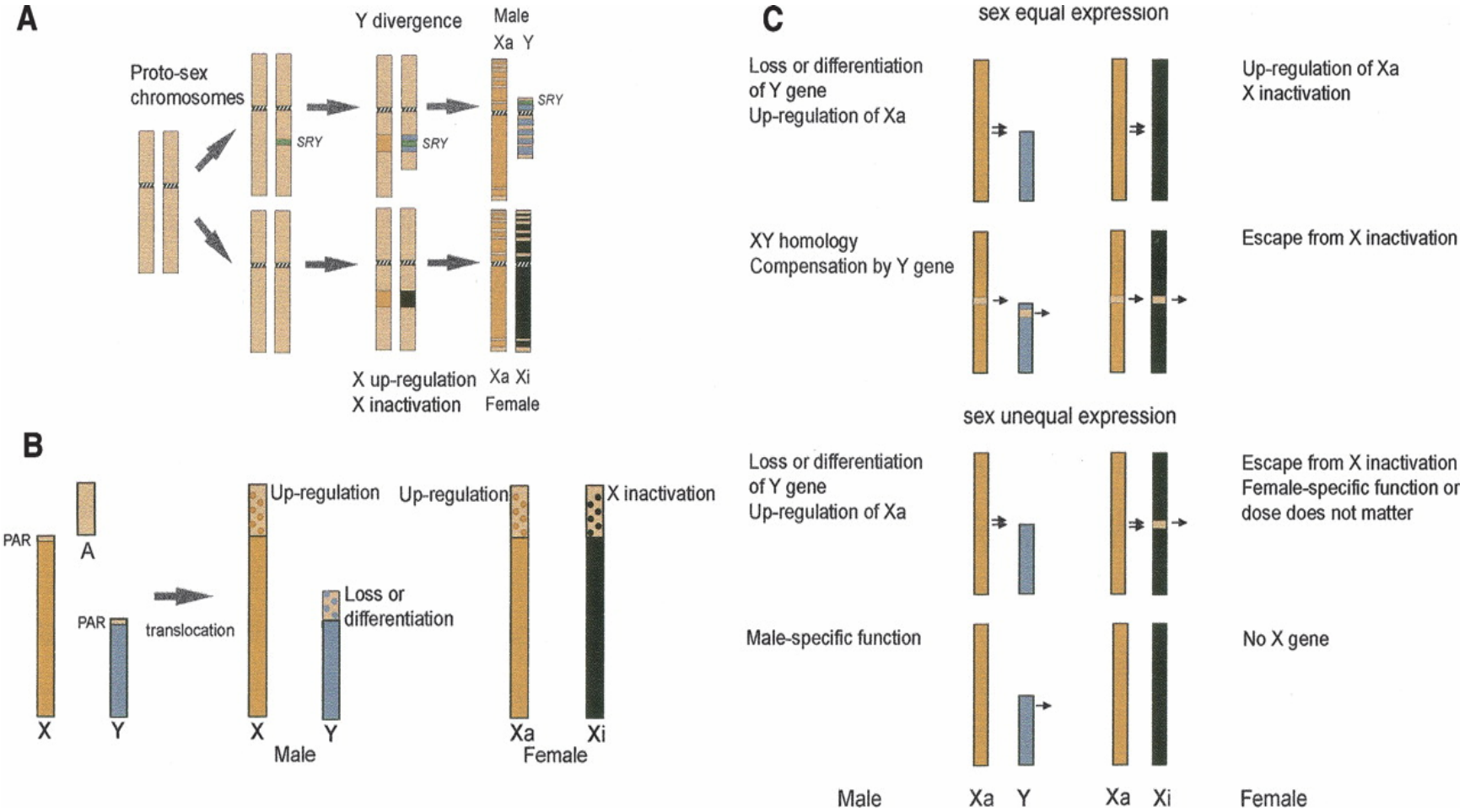
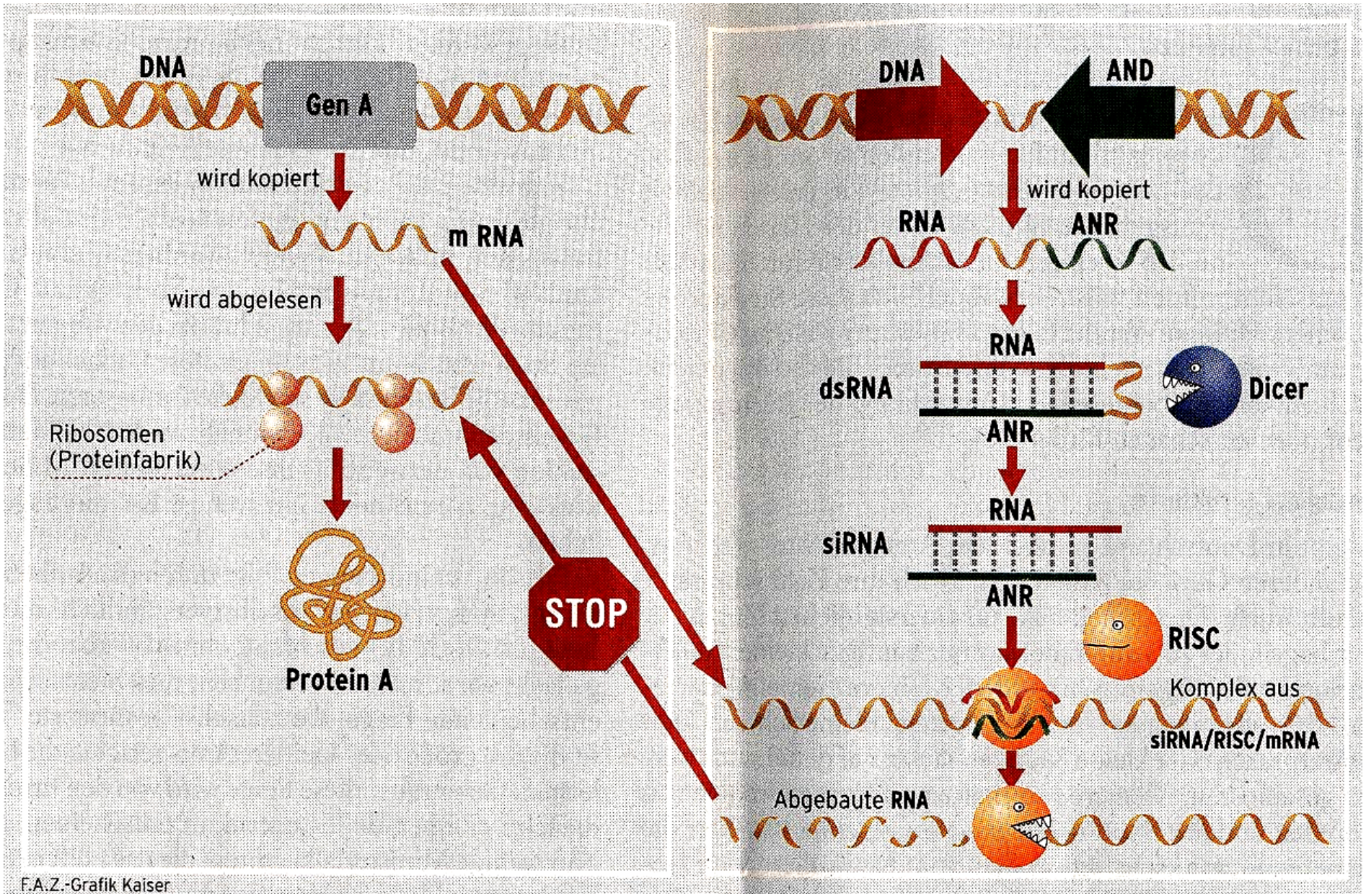
Sex chromosome evolution



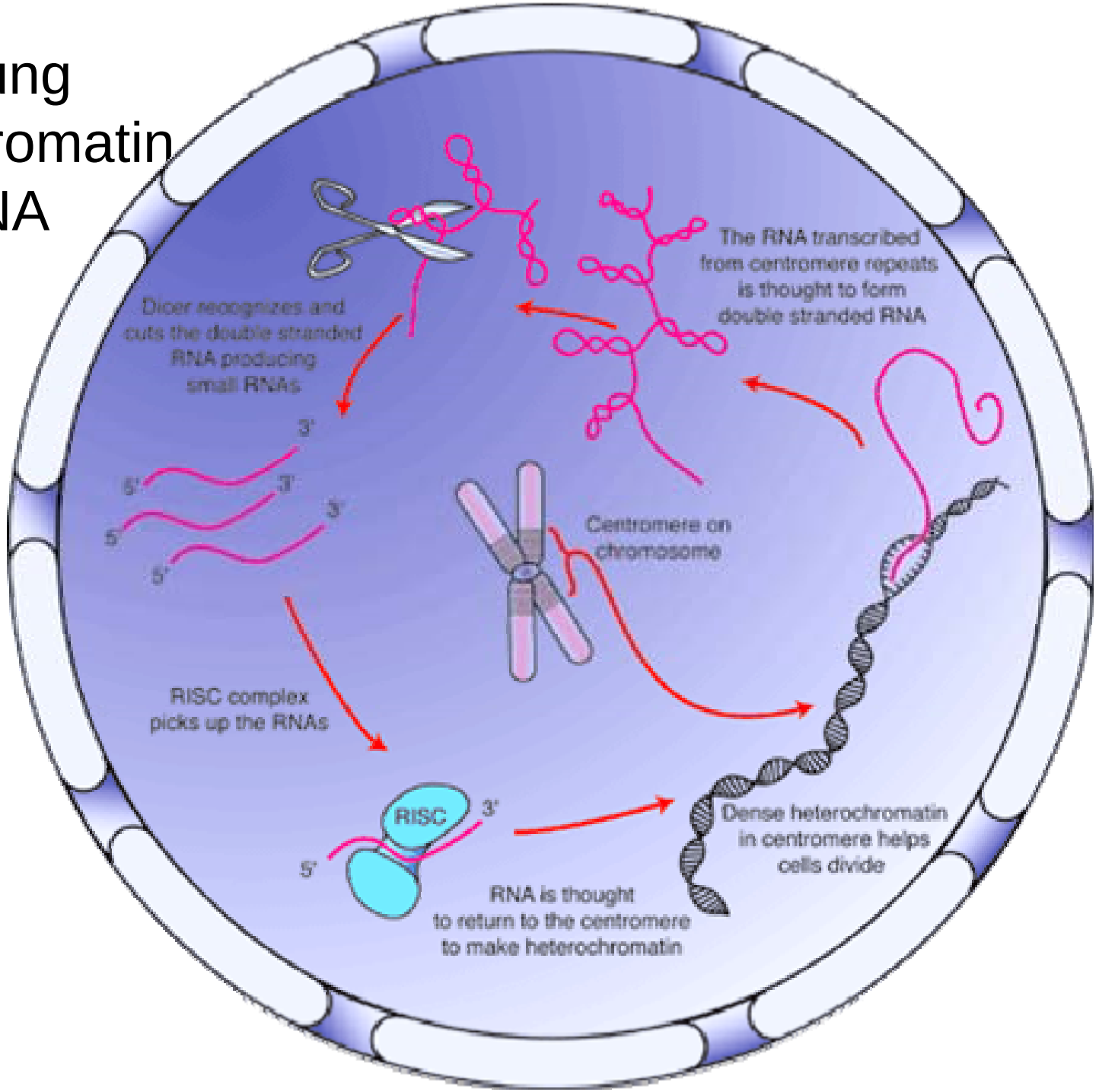
Xist continued…

- RNA is expressed, but does not code for protein
  - longest ORF (open reading frame) is 400 bp
- mouse 15 kb RNA, human 17 kb RNA
- RNA remains trapped in nucleus
- expressed only from the inactive X chromosome
- expressed in all mature female cells, and briefly in testes when an inactive X chromosome is present
- stabilization of Xist RNA mediates initiation of X inactivation

Genregulation durch RNAi



Inaktivierung von Heterochromatin durch RNA



Therapie von Leukämie durch dsRNA

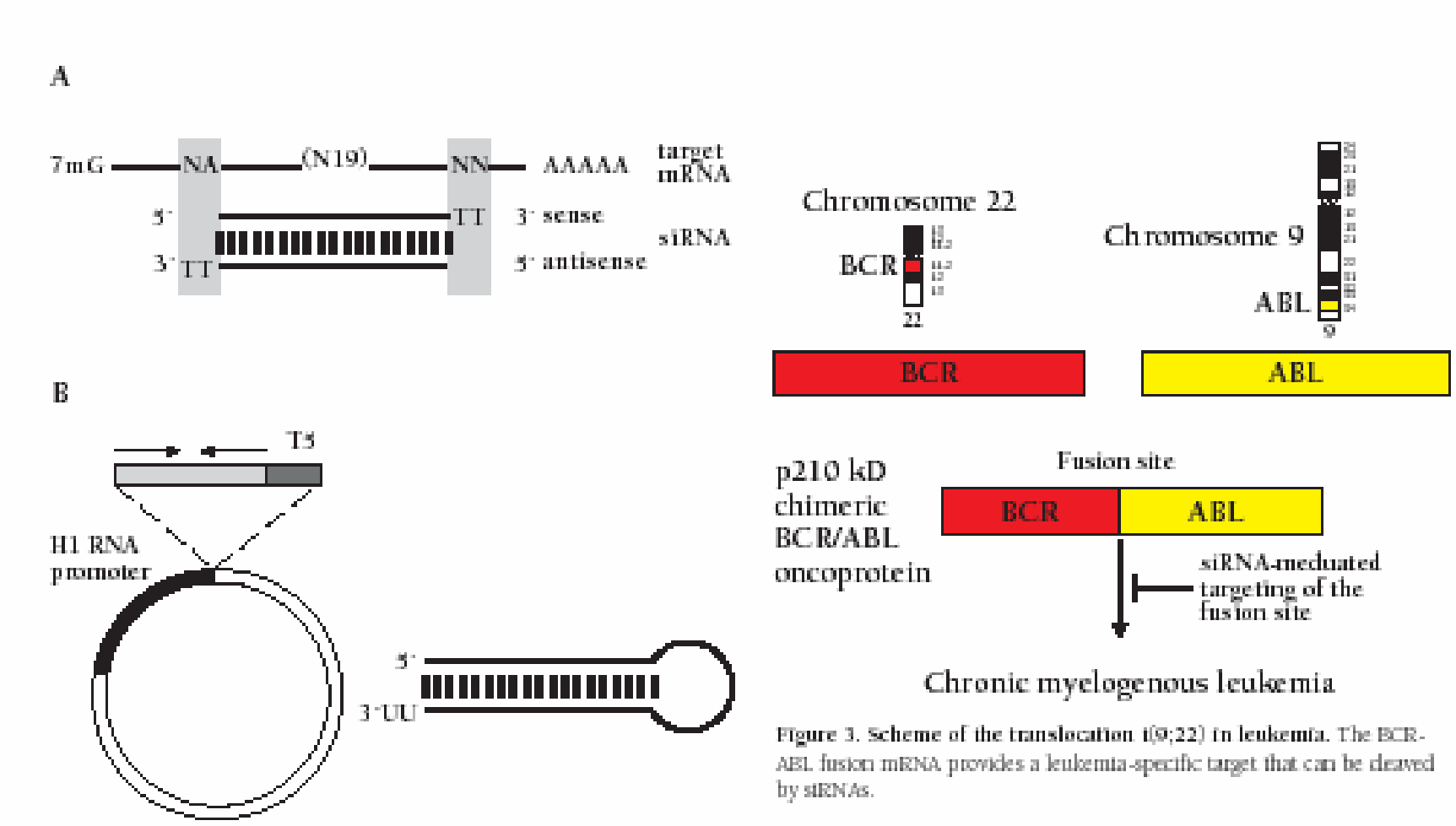


Figure 3. Scheme of the translocation t(9;22) in leukemia. The BCR-ABL fusion mRNA provides a leukemia-specific target that can be cleaved by siRNAs.

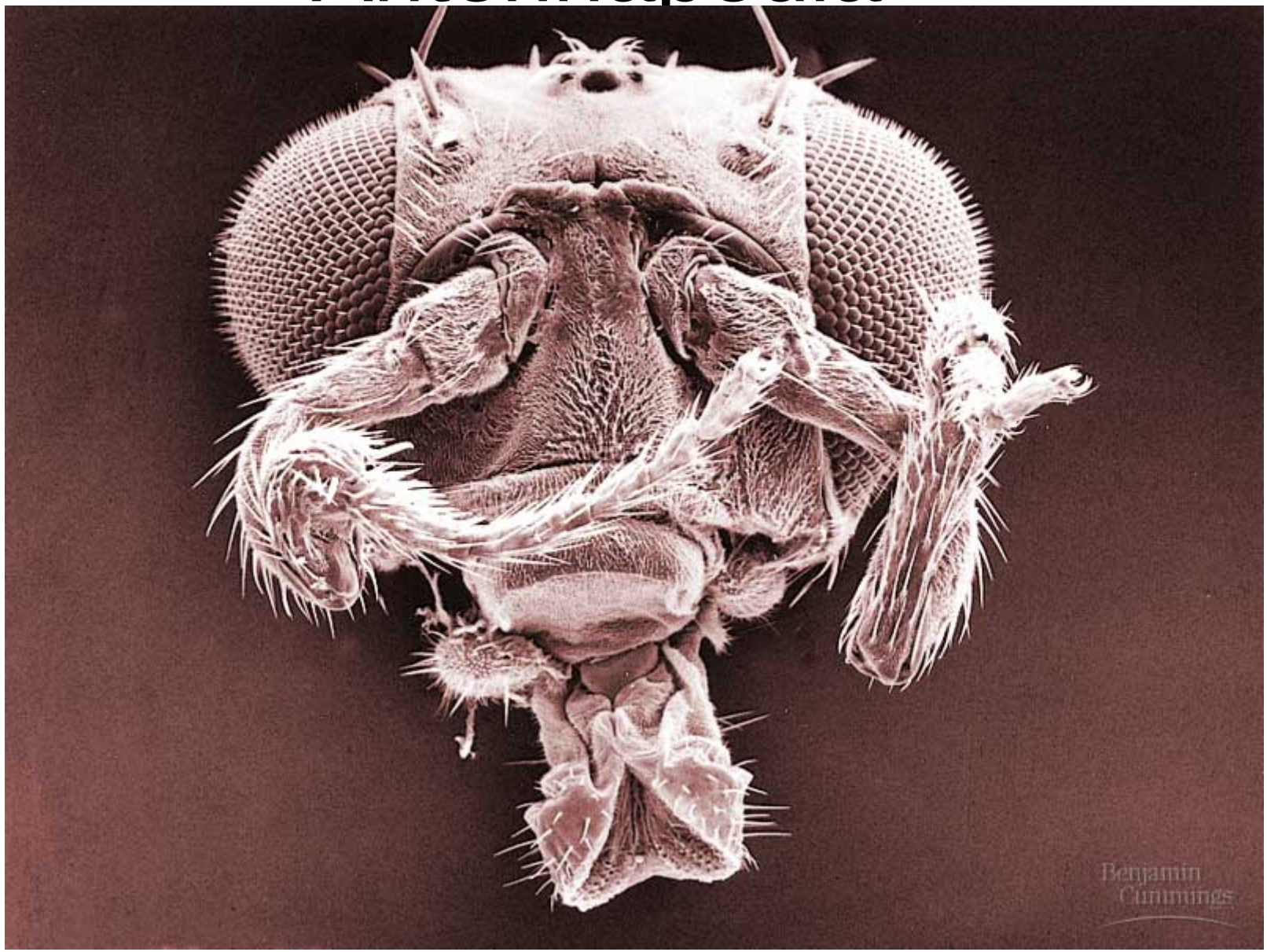
RNA interference (RNAi) represents an evolutionarily conserved cellular defense for controlling the expression of foreign genes in most eukaryotes including humans. RNAi is triggered by double-stranded RNA (dsRNA) and causes sequence-specific mRNA degradation of single-stranded target RNAs homologous in response to dsRNA. The mediators of mRNA degradation are small interfering RNA duplexes (siRNAs), which are produced from long dsRNA by enzymatic cleavage in the cell. siRNAs are approximately twenty-one nucleotides in length, and have a base-paired structure characterized by two-nucleotide 3'-overhangs. Chemically synthesized siRNAs have become powerful reagents for genome-wide analysis of mammalian gene function in cultured somatic cells. Beyond their value for validation of gene function, siRNAs also hold great potential as gene-specific therapeutic agents.

Thomas Tuschl[1] and Arndt Borkhardt[2]

[1]Department of Cellular Biochemistry, Max-Planck-Institute for Biophysical Chemistry, D-37077 Goettingen, Germany

[2]Children's University Hospital, Pediatric Hematology and Oncology, 35392 Giessen, Germany

Homöotische Mutante: Antennapedia



Bitte beachten:

- Praktikum beginnt 20. 02. 07 9 Uhr s. t.
- Praktikumsraum Molekulargenetik (Gebäude SB1, J.-J.-Becherweg 34-36)
- Laborkittel mit bringen
- Praktikumsskript kann ab Montag12. 2. im Sekretariat des Institut für Molekulargenetik abgeholt werden (kostenpflichtig)