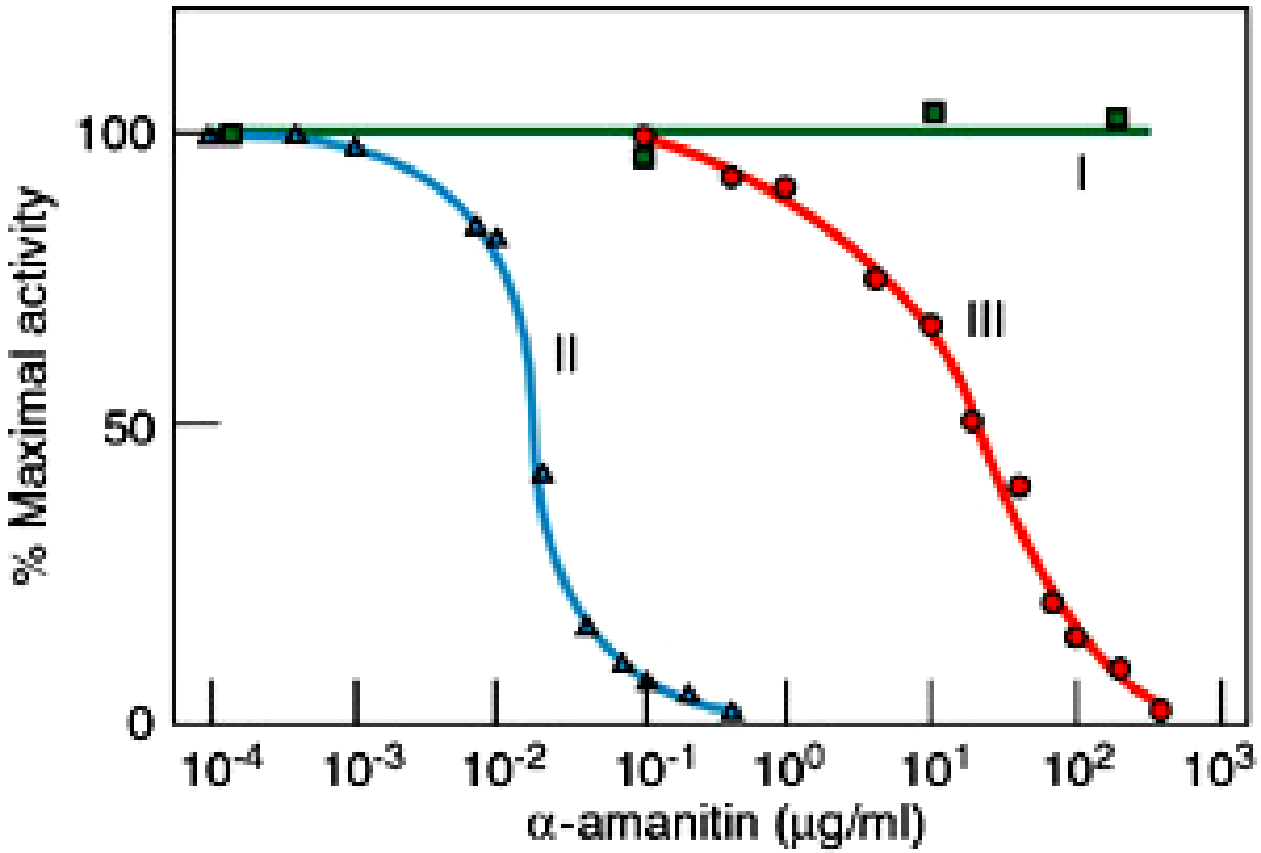
Genstruktur der Eukaryoten

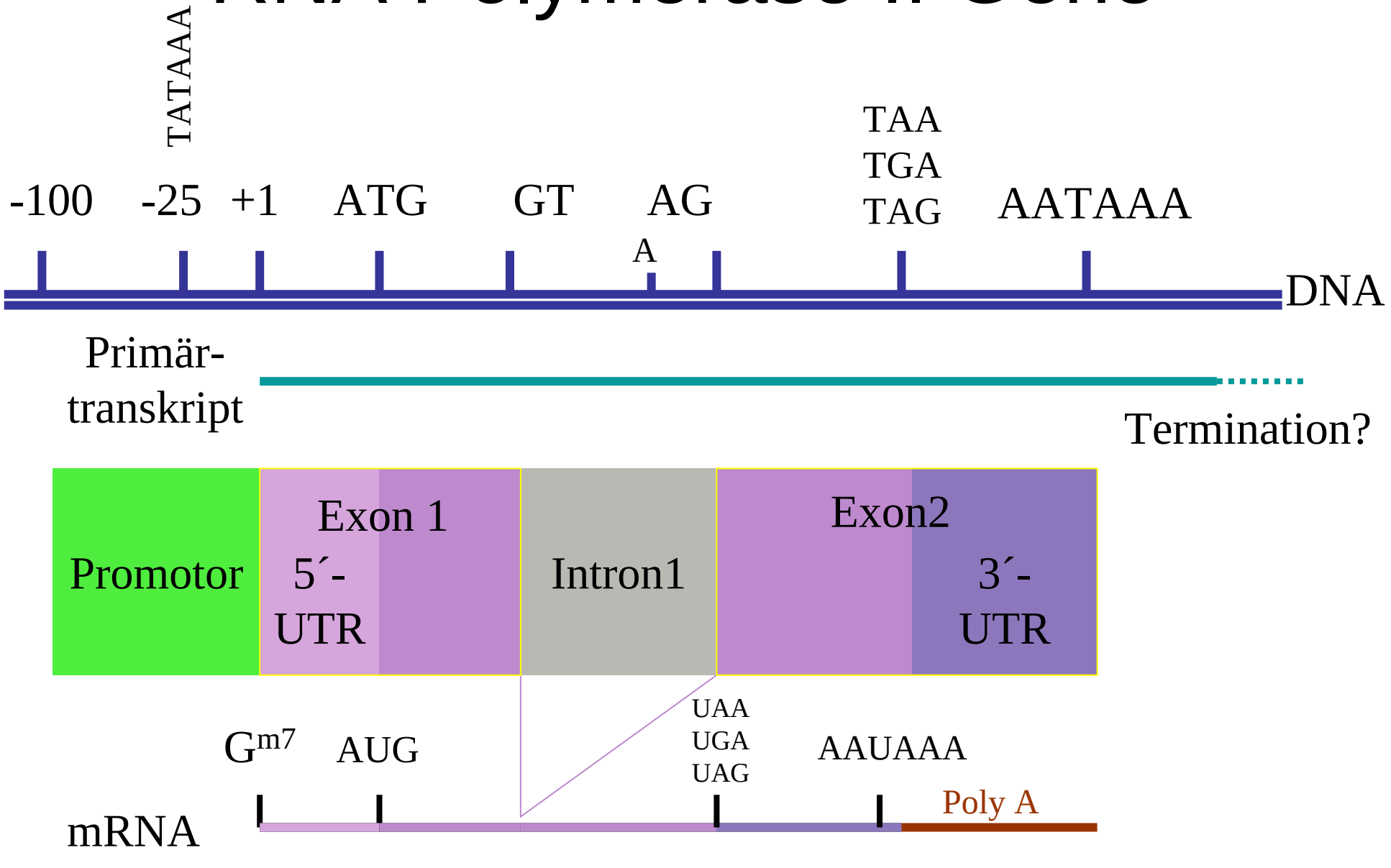
Abhängig von der Genklasse:

1. RNA Pol I – Gene: 18S, 5,8S, 28S rRNA
2. RNA Pol II – Gene: alle mRNAs
3. RNA Pol III – Gene: tRNAs, 5S rRNA, einige snRNAs

Hemmung der Polymerasen durch alpha-Amanitin



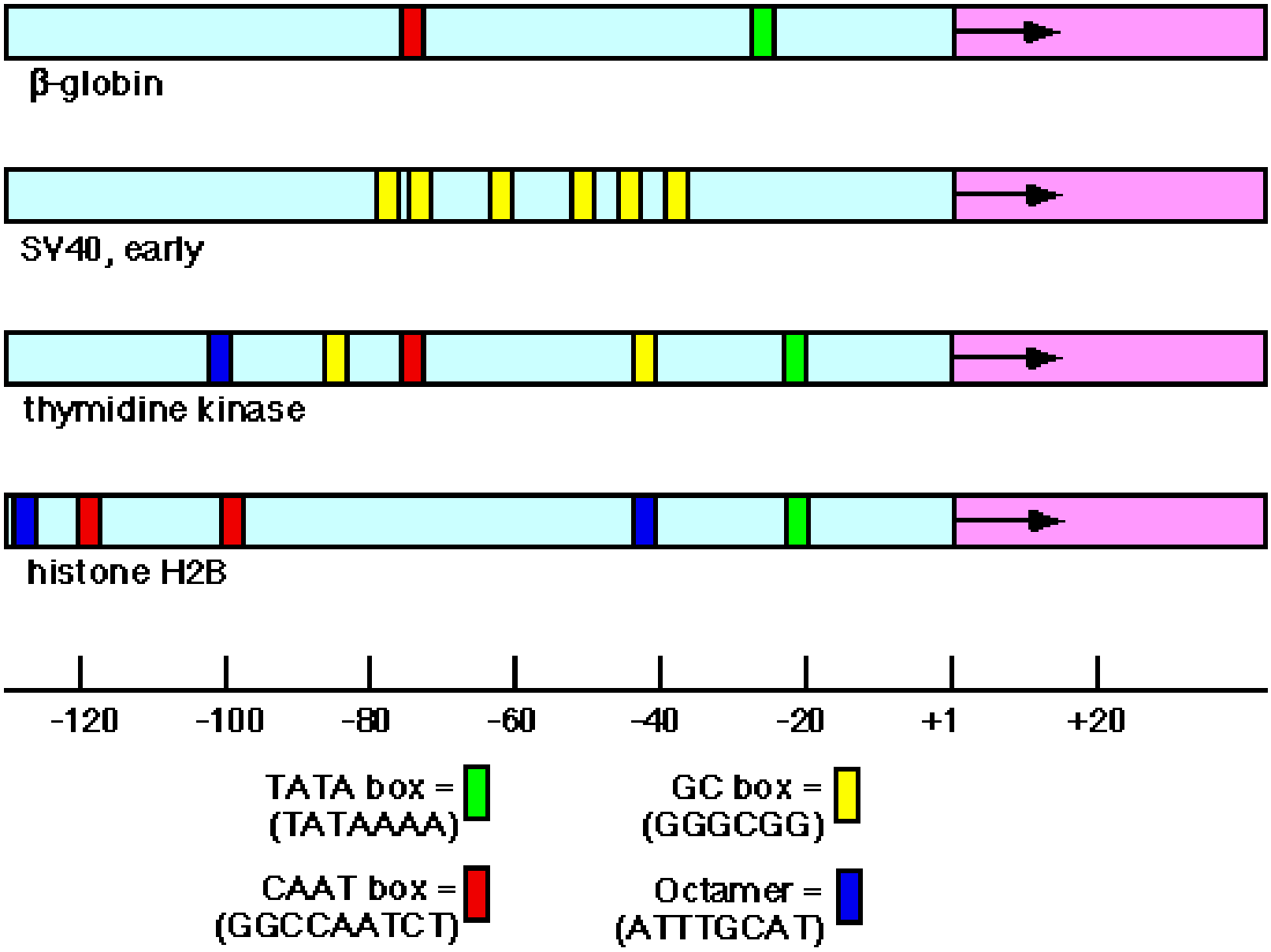
RNA Polymerase II Gene

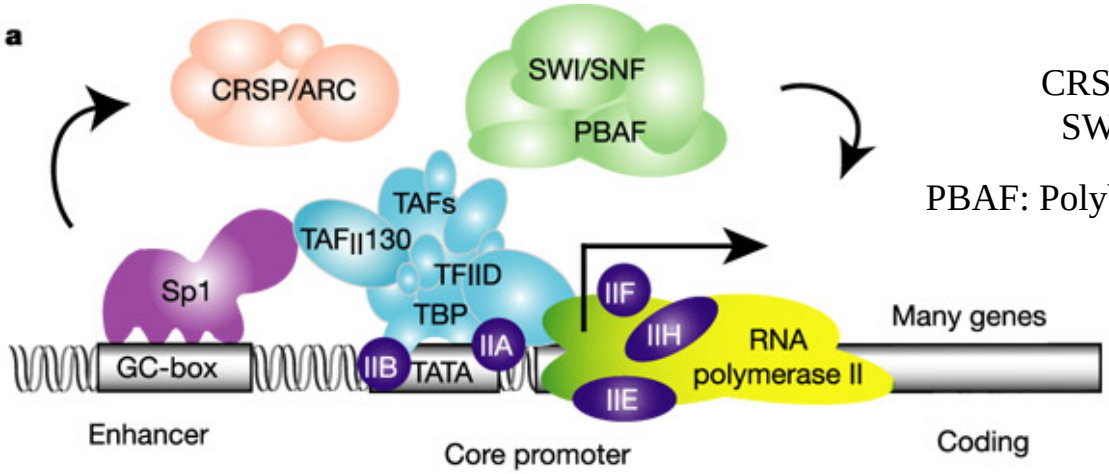


RNA Pol II Promotorelemente Sequenz:Position:Funktion

Name	Sequenz	Position	Funktion
TATA-Box Hogness-Box	TATAAA	-25 bis -30	Definiert Transkript-startpunkt
CAT-Box	GGCCAATC	-60 bis -80	Polymerase-Bindung via CBP
GC-Box	GGGCG	Variabel und mehrfach 1 bis 107	Definiert RNA-Pol Bindungstelle

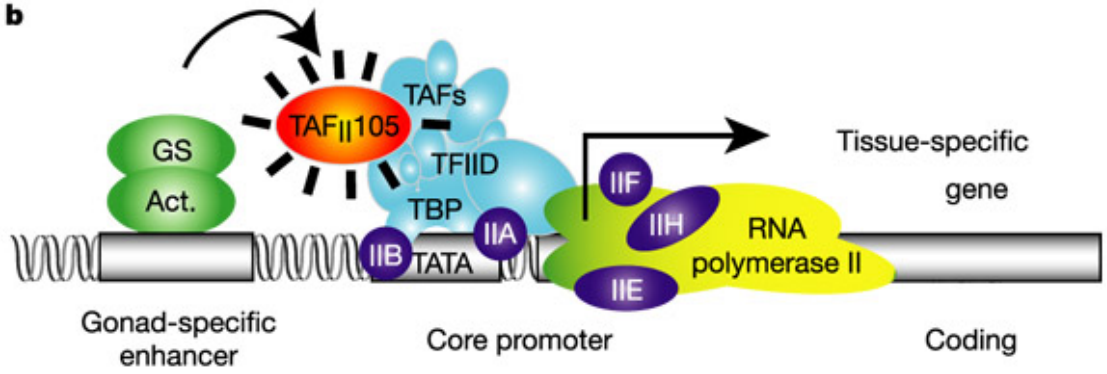
Beispiele für eukaryotische Promotoren



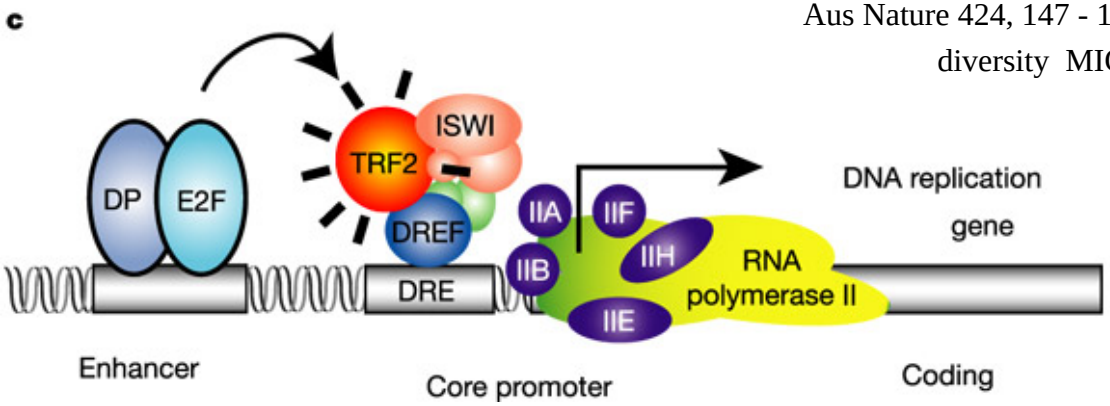


ARC: activator recruited cofactor
CRSP: Cofactor required for SP1 activation
SWI/SNF: sucrose non fermenting factor

PBAF: Polybromo- and BRG1 (603254)-associated factor



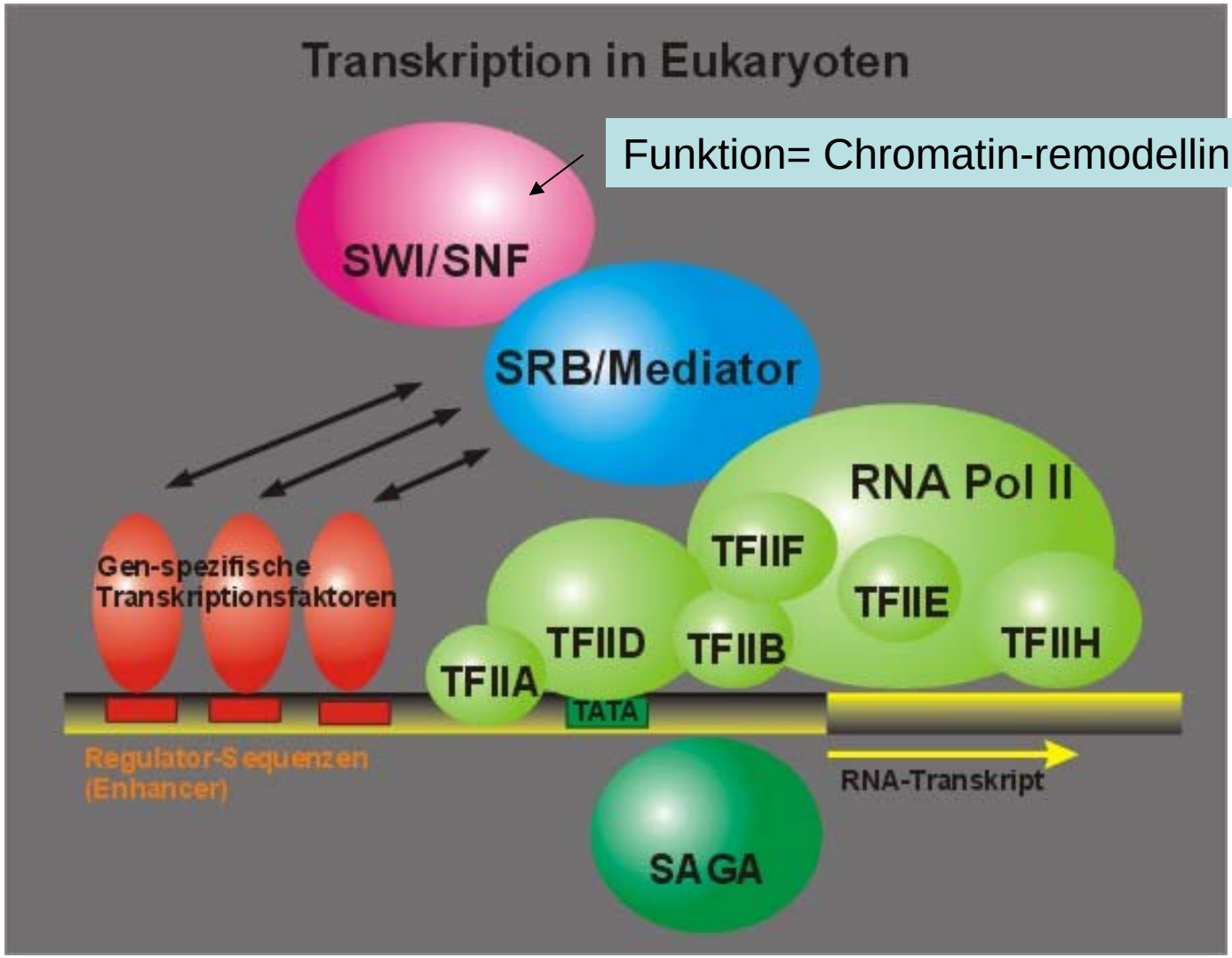
TAFs: TBP-associated factors



Aus Nature 424, 147 - 151 (10 July 2003); Transcription regulation and animal diversity MICHAEL LEVINE* AND ROBERT TJIAN*†

DRE: DNA replication related element
WSTF: Williams Syndrome Transcription Factor
TRF: TBP-related factor

An der Aktivierung von Pol II-Genen beteiligte Komplexe

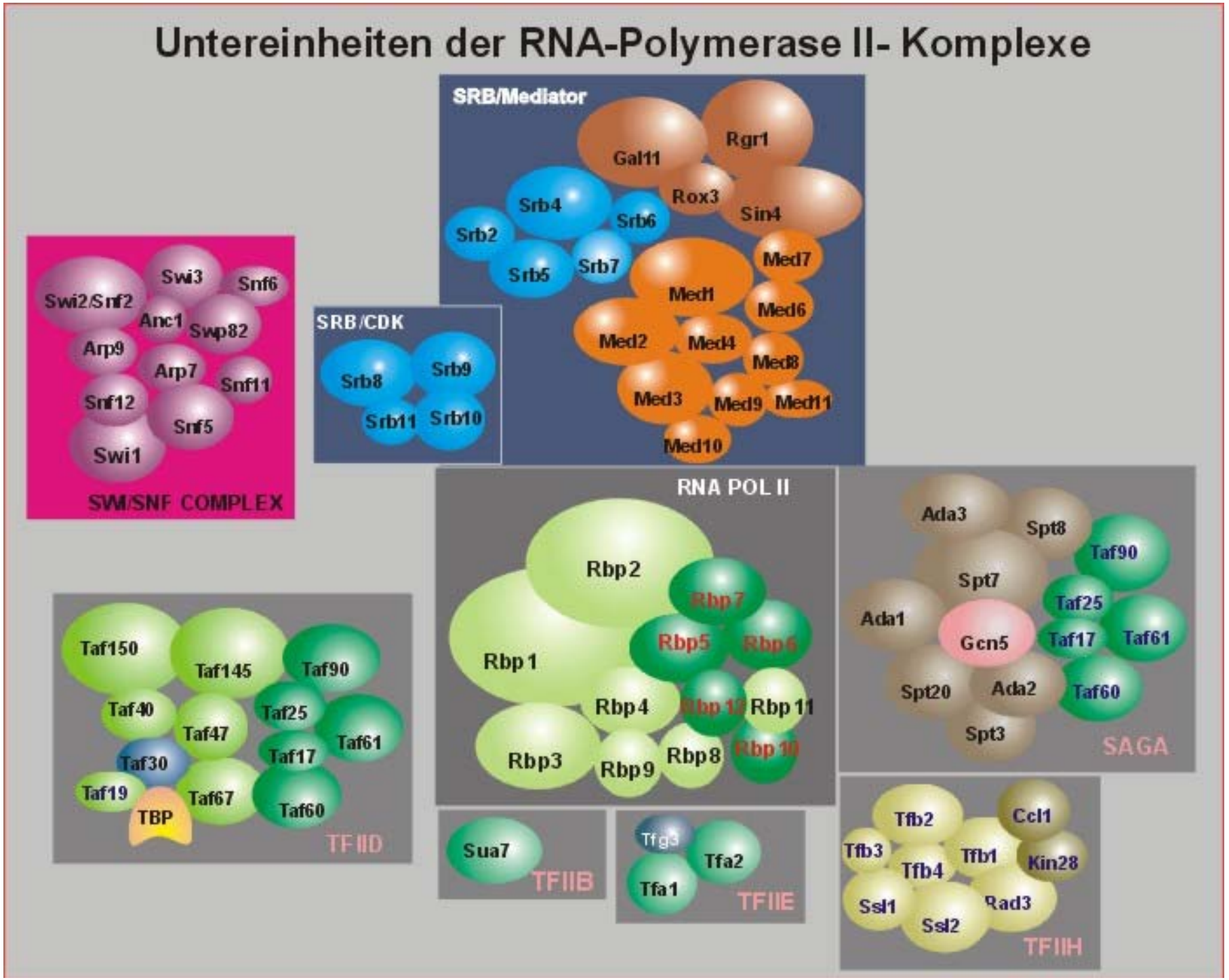


The multiprotein **Mediator complex** is a coactivator required for transcriptional activation of **RNA polymerase II** transcribed genes by DNA binding **transcription factors**.

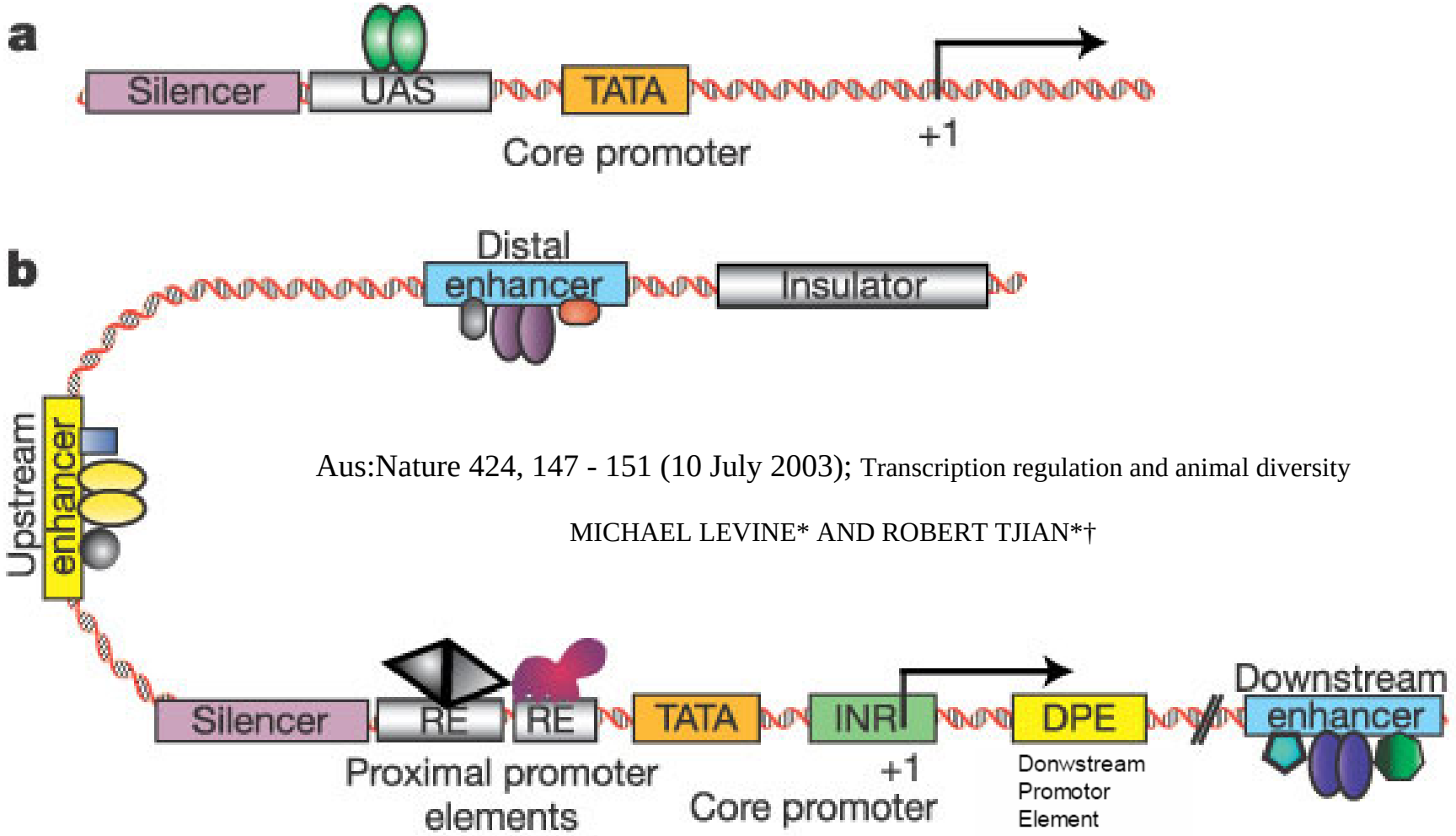
SAGA:Spt-Ada-Gcn5-Acetyltransferase

SRB: Suppressor of RNA-PolymeraseII

Untereinheiten der RNA-Polymerase II- Komplexe



Die Kontrollregion bei Eukaryoten kann sehr komplex sein

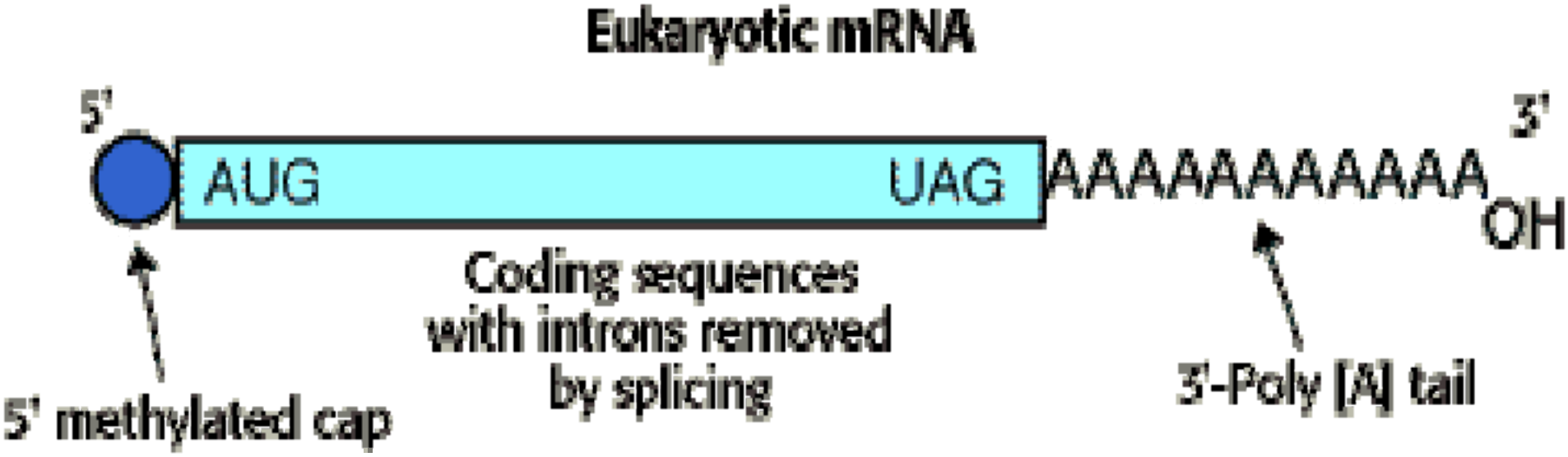


Aus:Nature 424, 147 - 151 (10 July 2003); Transcription regulation and animal diversity

MICHAEL LEVINE* AND ROBERT TJIAN*†

„posttranskriptionelle“ Modifikationen

typisch für eukaryotische mRNA:

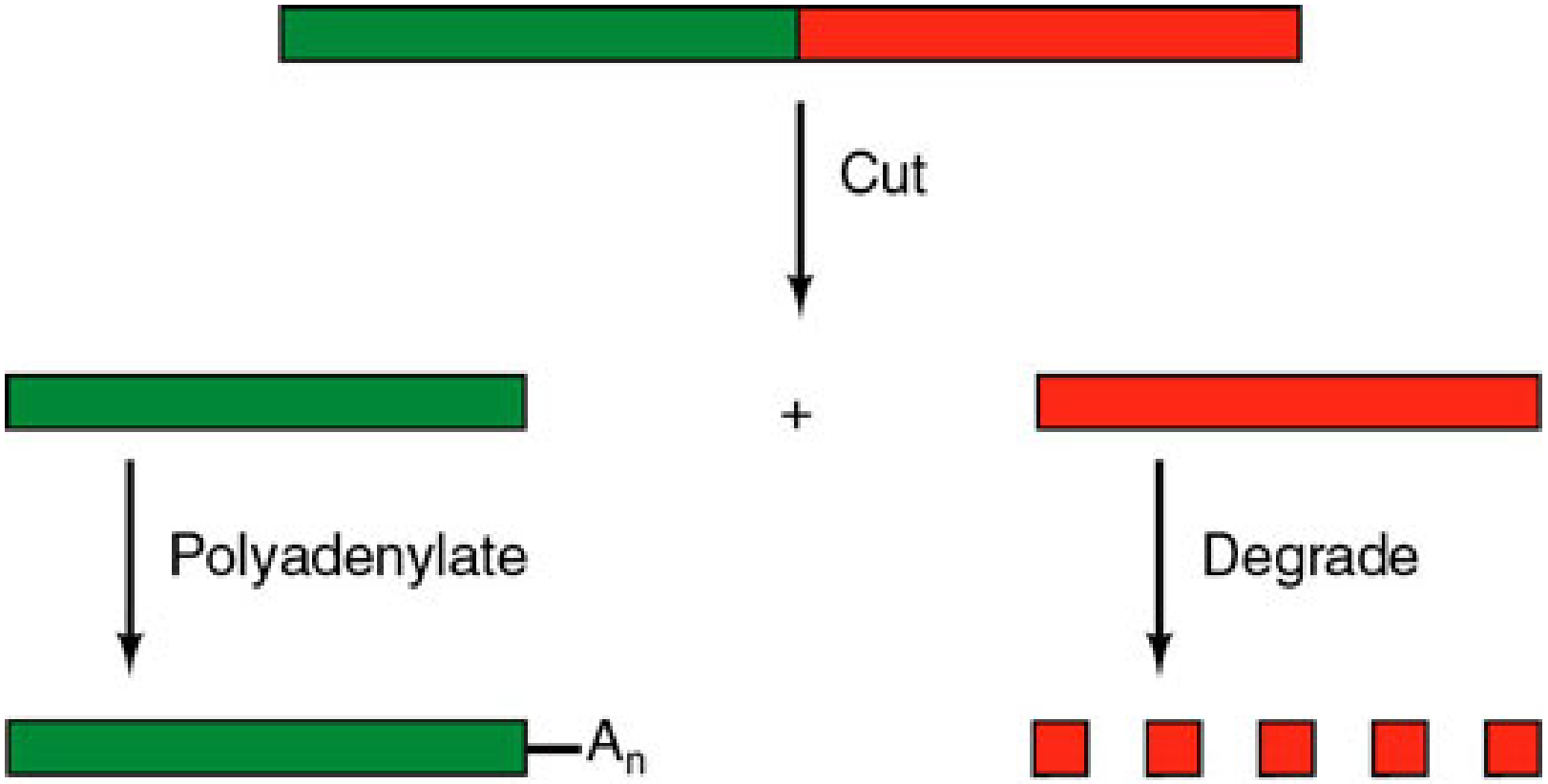


Poly-Adenylierung am 3´Ende der mRNA

1. Trimmen des Primärtranskripts an definierter Stelle (23-24 Basen stromabwärts des Poly A-Signals AAUAA)

2. Anfügen von Adenin-Nukleotiden

„Polyadenylierung“: Trimmen der mRNA und Anhängen von mehreren Adeninnukleotiden



Die Polyadenylierung ist ähnlich komplex wie die Initiation!

Aus: Strange bedfellows: polyadenylation factors at the promoter
Olga Calvo1 and James L. Manley, GENES & DEVELOPMENT 17:1321–1327 © 2003

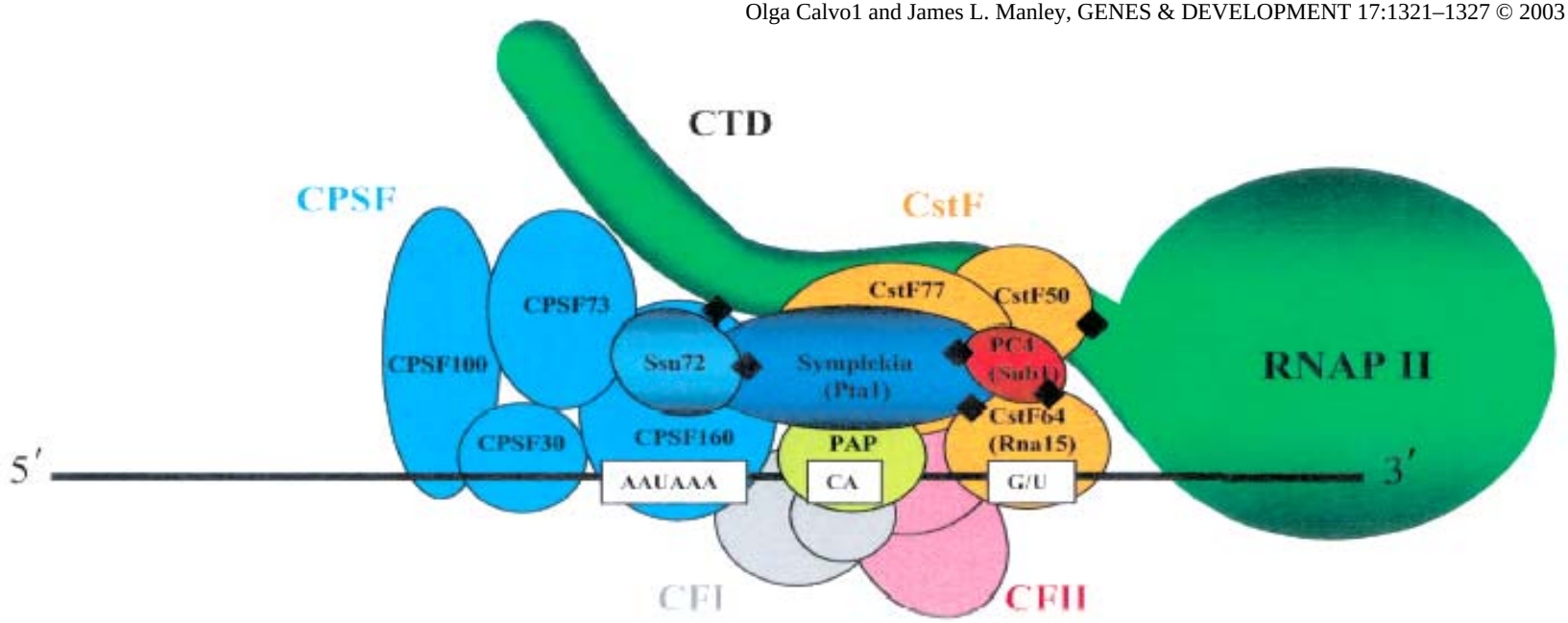


Figure 2. Schematic representation of the polyadenylation machinery. The majority of the components of the mammalian and yeast polyadenylation complexes are conserved, including all currently known factors that function in the transcription connection. For simplicity, only the mammalian nomenclature is depicted; the yeast names of factors that have important roles in the events described here are also indicated. (Note that although an apparent human homolog of Ssu72 exists, it has not yet been characterized functionally). ◆, documented protein–protein interactions that help link transcription and 3′ processing (see text). Polyadenylation signal sequences (upstream AAUAAA, CA cleavage site consensus, and downstream G/U-rich region) are boxed. CPSF, cleavage-polyadenylation specificity factor; CstF, cleavage stimulation factor; CFI and CFII, cleavage factors I and II, respectively; PAP, poly(A) polymerase.

Poly-Adenylierung am 3´Ende der mRNA

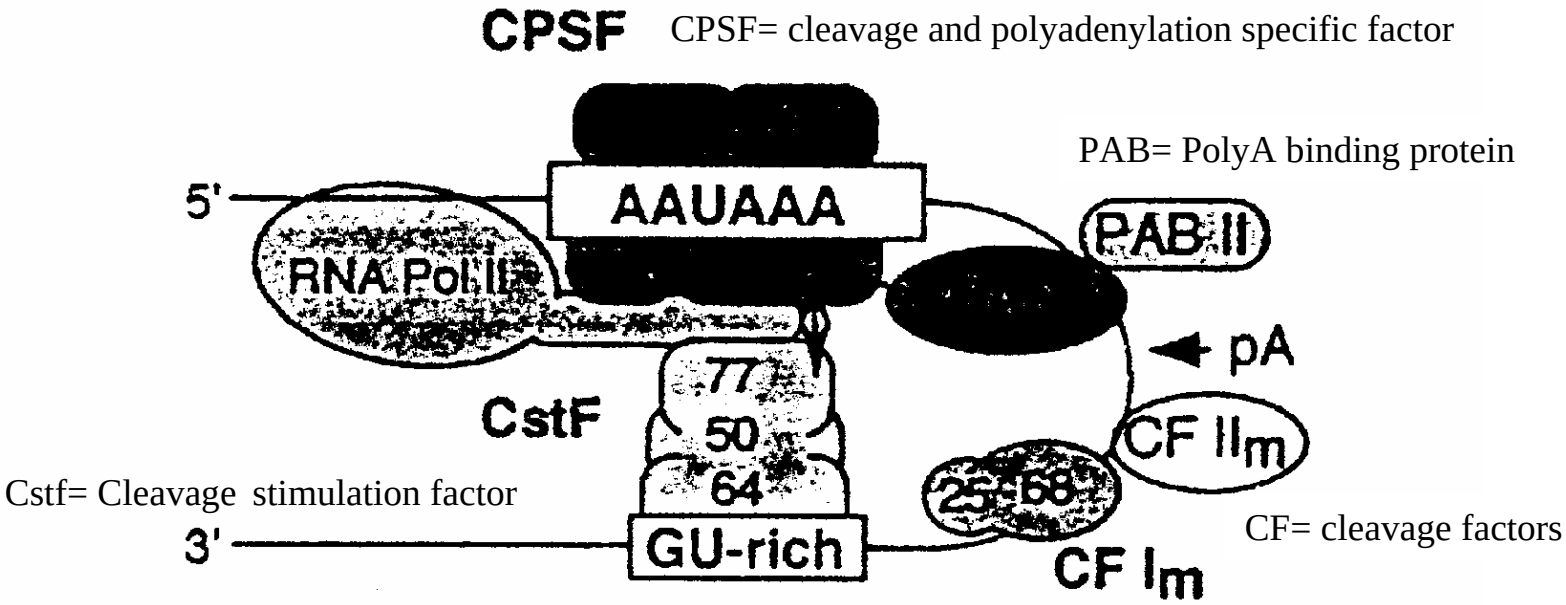


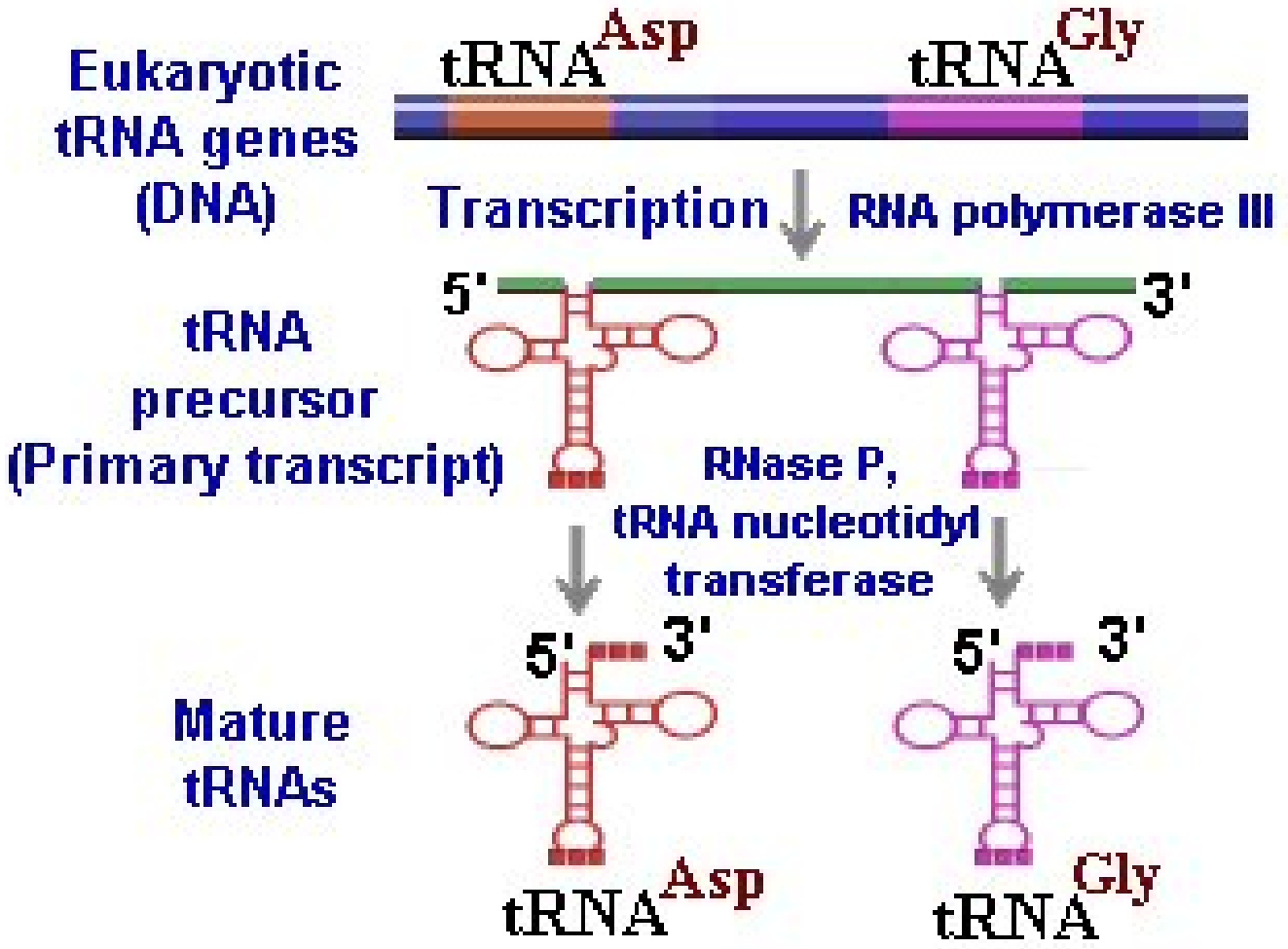
Figure 1. The Mammalian pre-mRNA 3′ End Processing Complex

Experimentally demonstrated protein:protein interactions are indicated by double-headed arrows. pA indicates the poly(A) addition site.

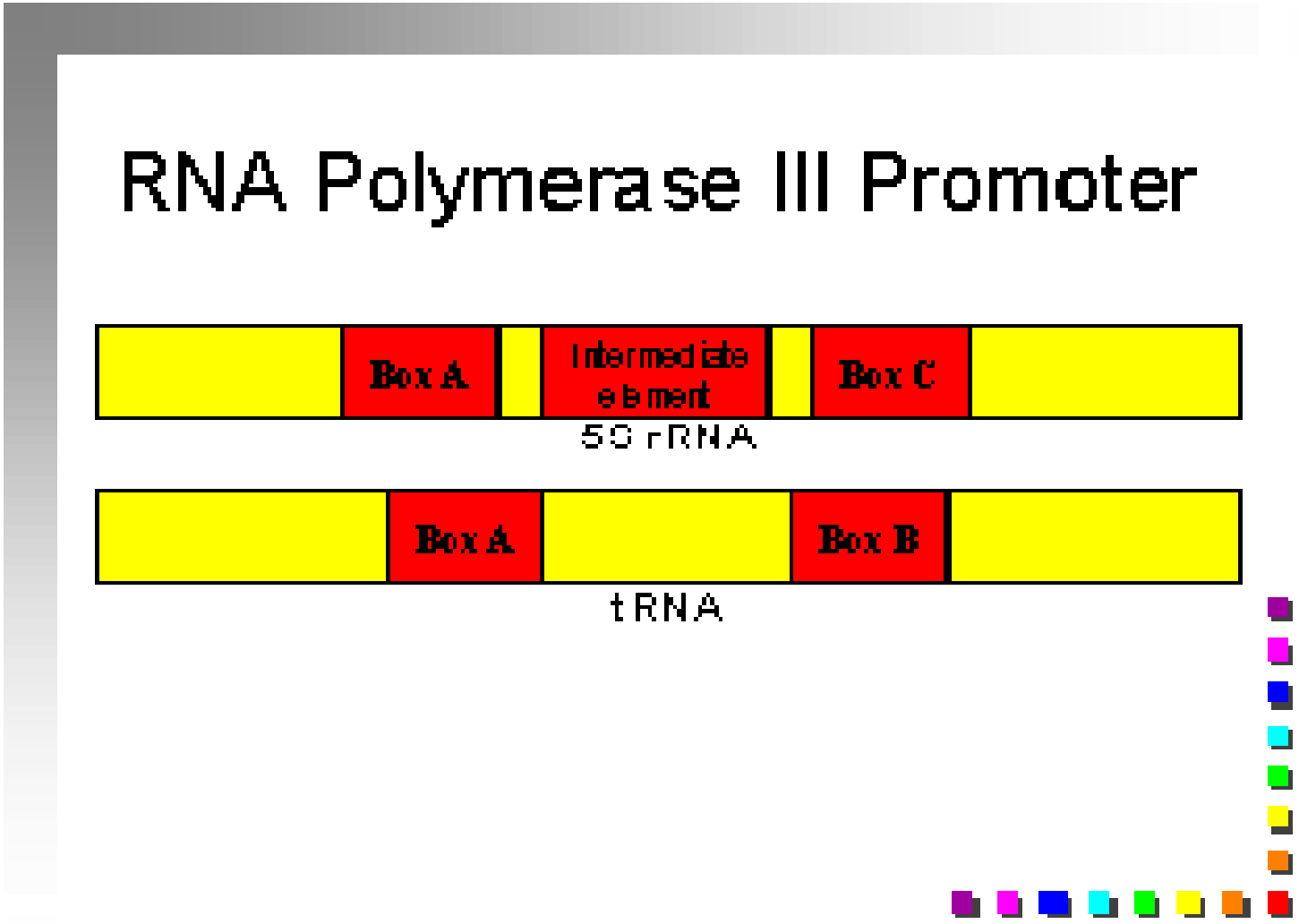
Funktion der Poly-Adenylierung?

- Stabilität der mRNA
- Translatierbarkeit

Polymerase III – Gene: tRNAs, 5S rRNA, snRNAs

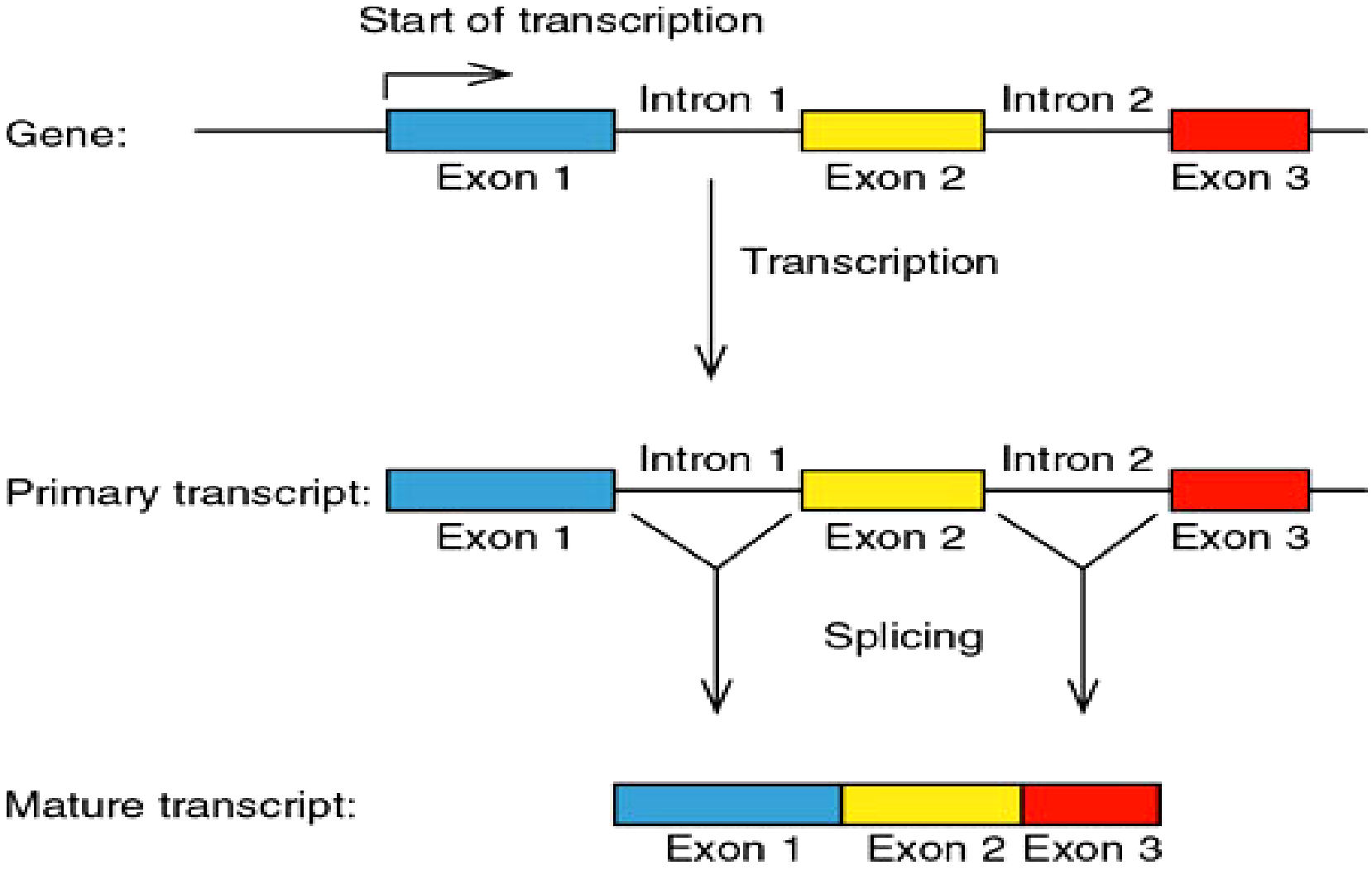


RNA Polymerase III -Gene
haben **interne!** Promotoren
d. h. die Promotoren liegen im transkribierten Bereich



Splicing (Spleißen)

wichtiger Teil der RNA-Prozessierung; die Intronabschschnitte werden aus dem Primärtranskript entfernt



Je nach Spleißmechanismus werden vier verschiedene Gruppen von Introns unterschieden:

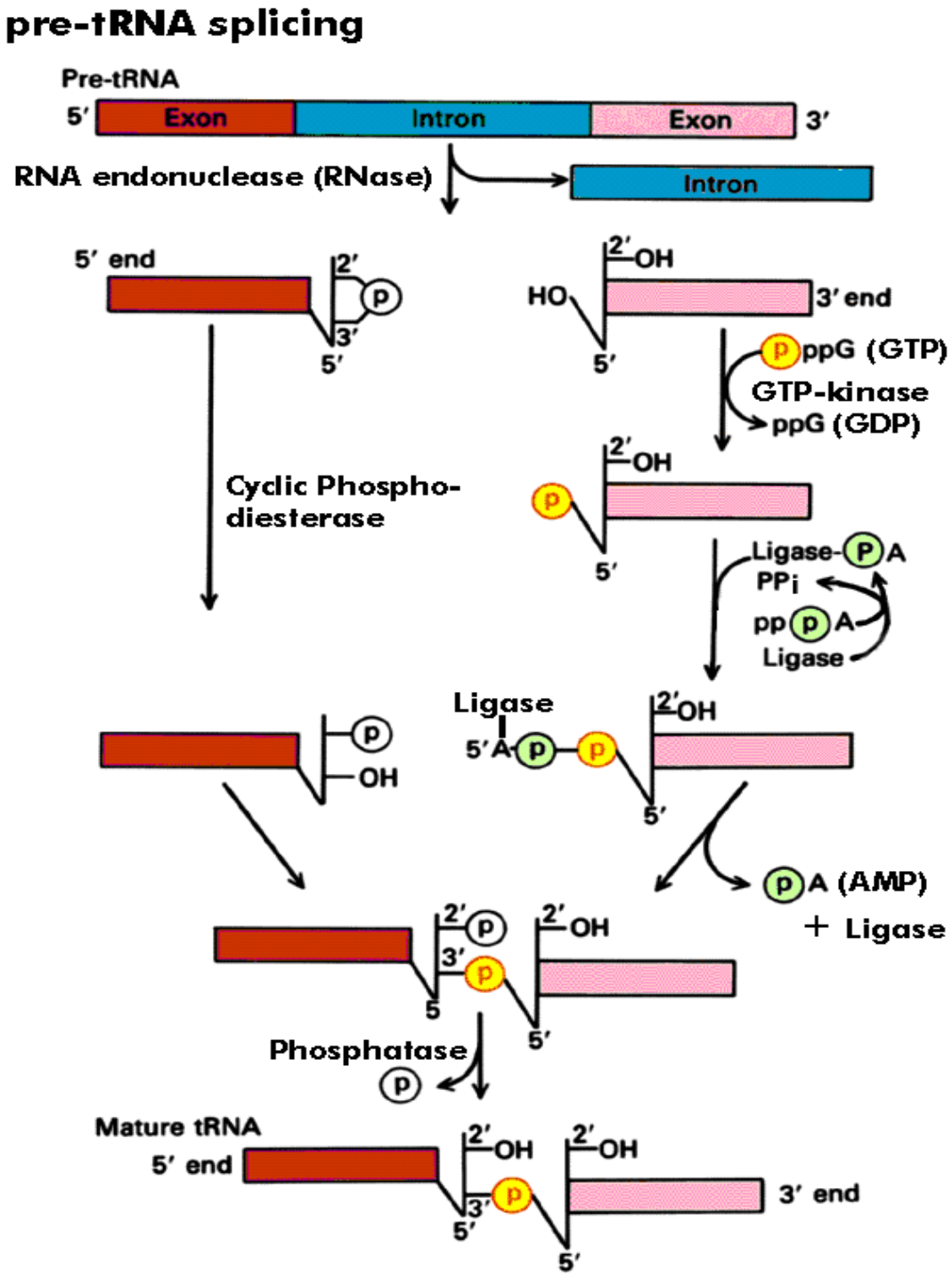
- tRNA Introns
- Autokatalytische Introns Gruppe I
- Autokatalytische Introns Gruppe II
- hn-/mRNA Introns

tRNA Splicing:

= zwei Stufenprozess

1. Herausschneiden des Introns durch Endonuklease

2. Verknüpfen der Exons durch RNA-Ligase

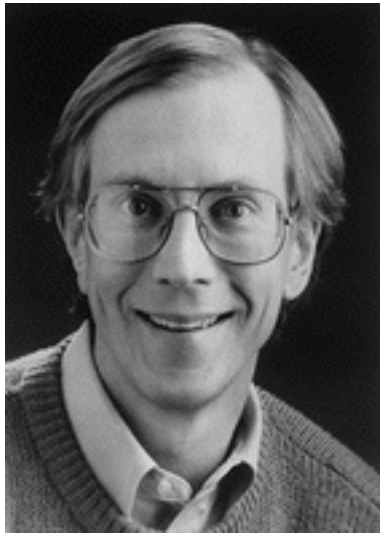


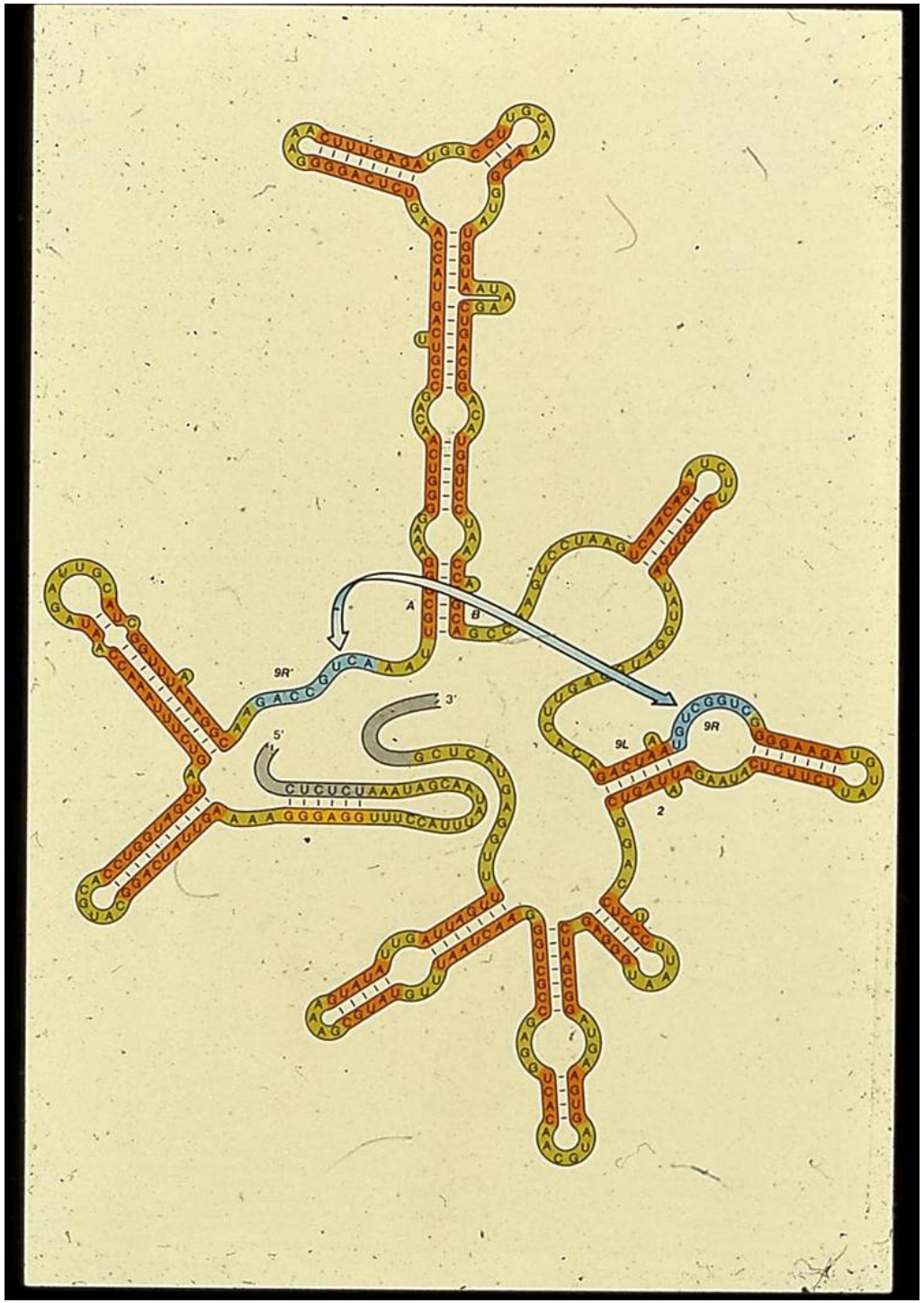
Autokatalytisches Splicing
Ribozyme

Beim **autokatalytischen Spleißen** sorgt die Intron-RNA selbst (autokatalytisch) dafür, dass die RNA an den Intron-Exon-Grenzen geschnitten und die beiden Exon-Enden (3´-Ende von Exon n mit dem 5´-Ende von Exon m) über eine Phosphodiesterbindung verknüpft werden. Weil die RNA bei diesem Prozess wie ein Enzym katalytisch aktiv ist, werden diese RNAs auch als **Ribozyme** bezeichnet

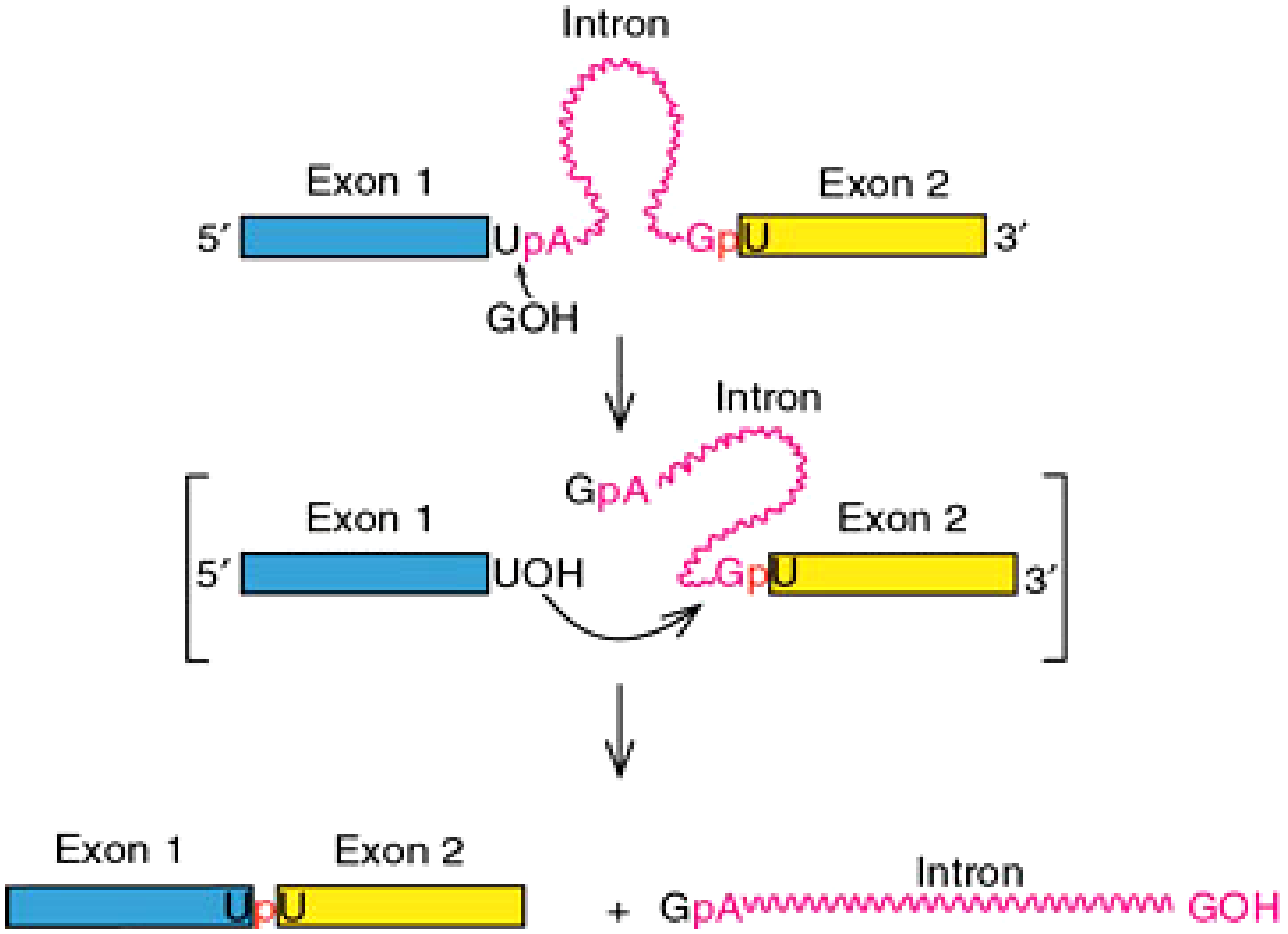
Ribozym-Struktur

Entdecker der Ribozyme
Th. R. Cech; Sydney Altman
Nobelpreis 1989

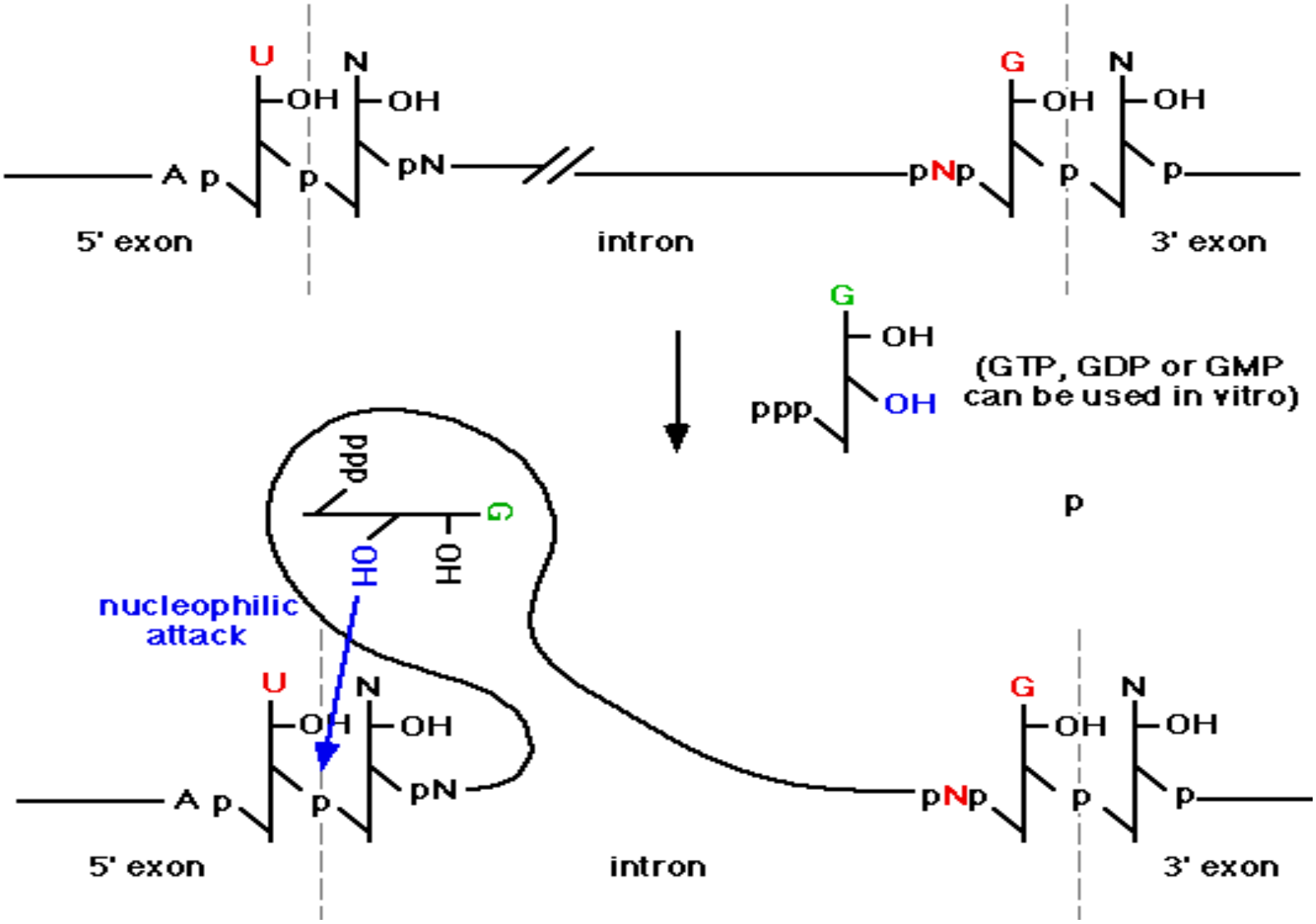




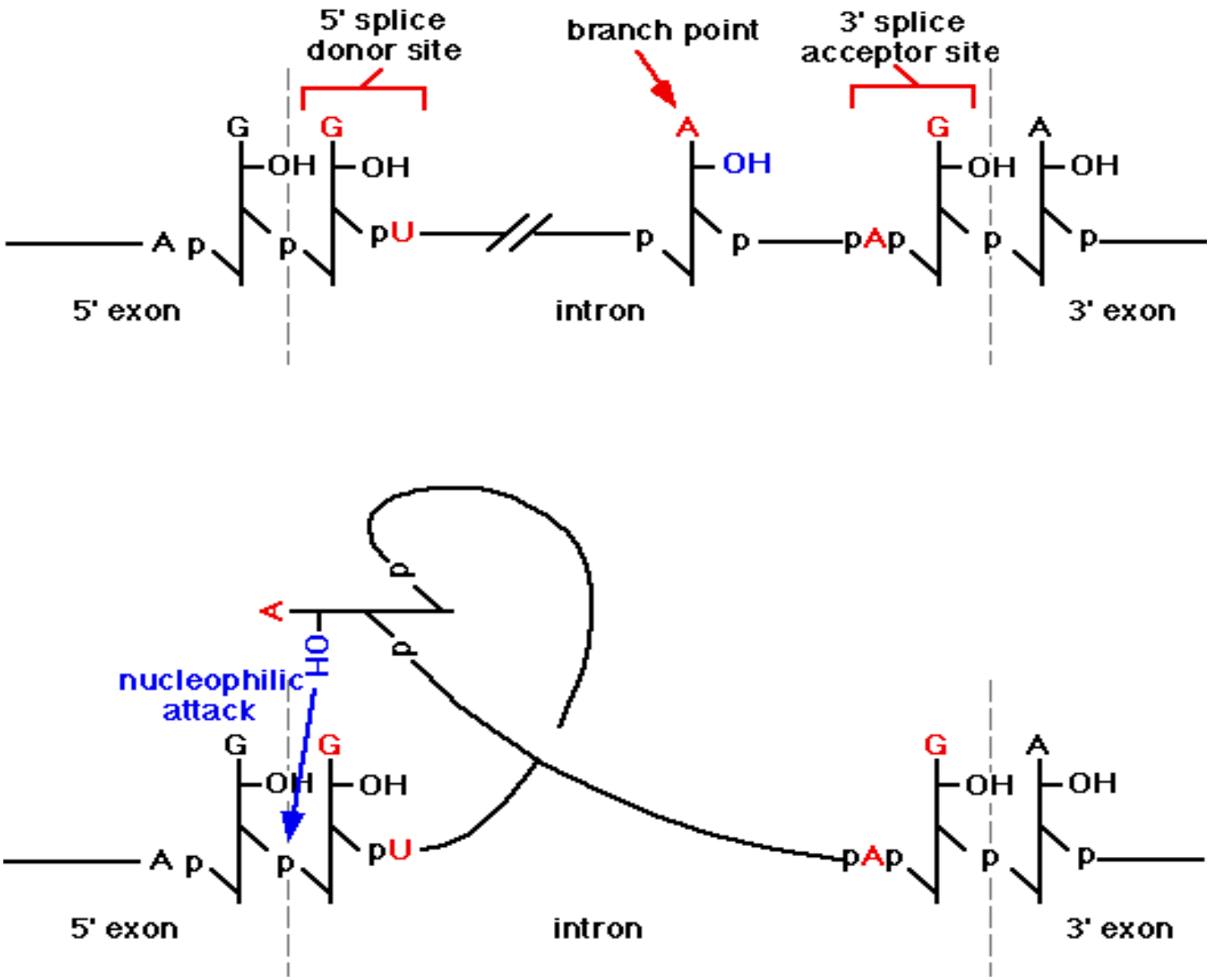
Autokatalytisches Spleißen der Gruppe I Introns bei Prä-rRNA von Tetrahymena



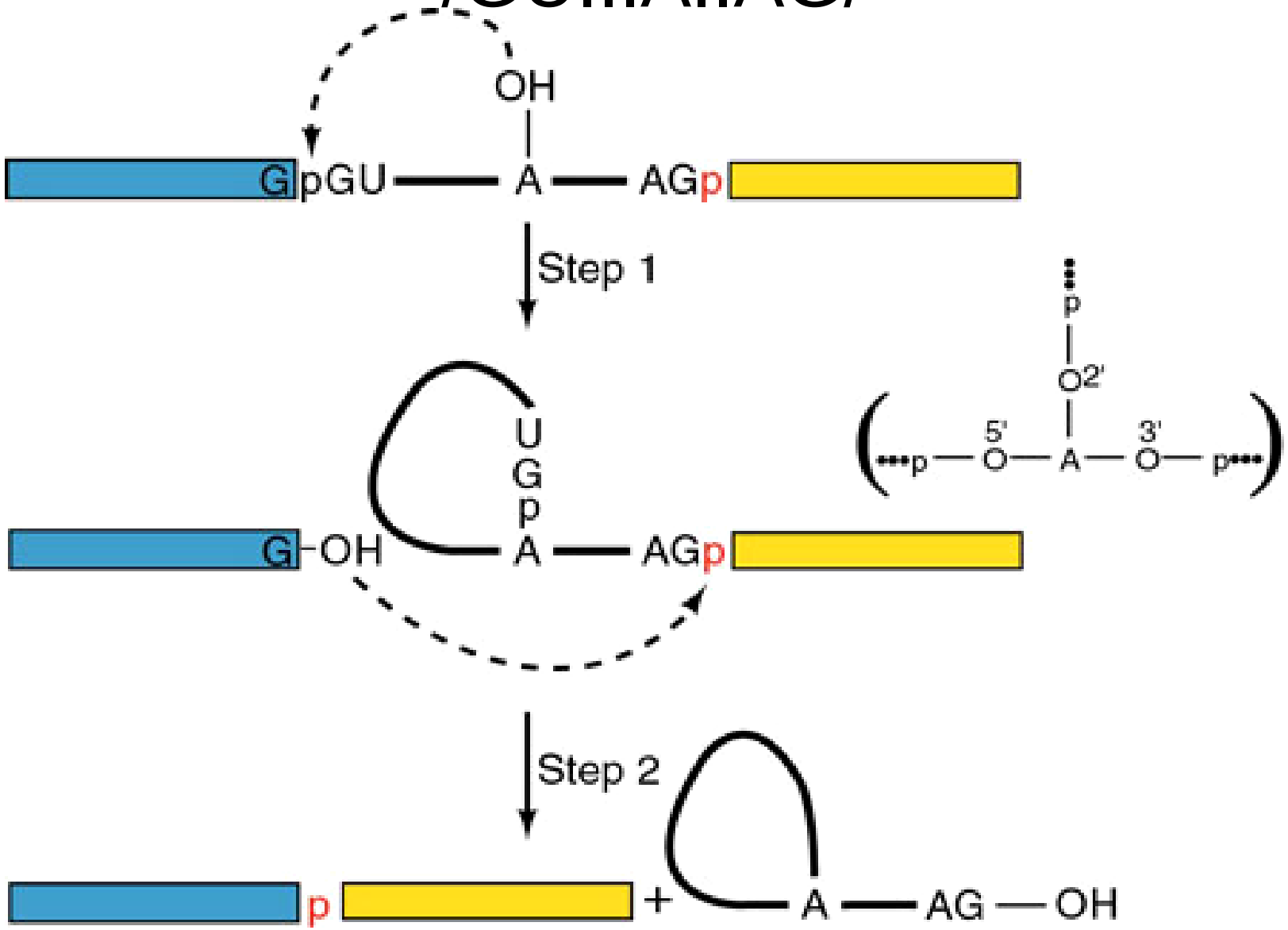
Mechanismus der autokatalytisch spleißenden Gruppe I Introns



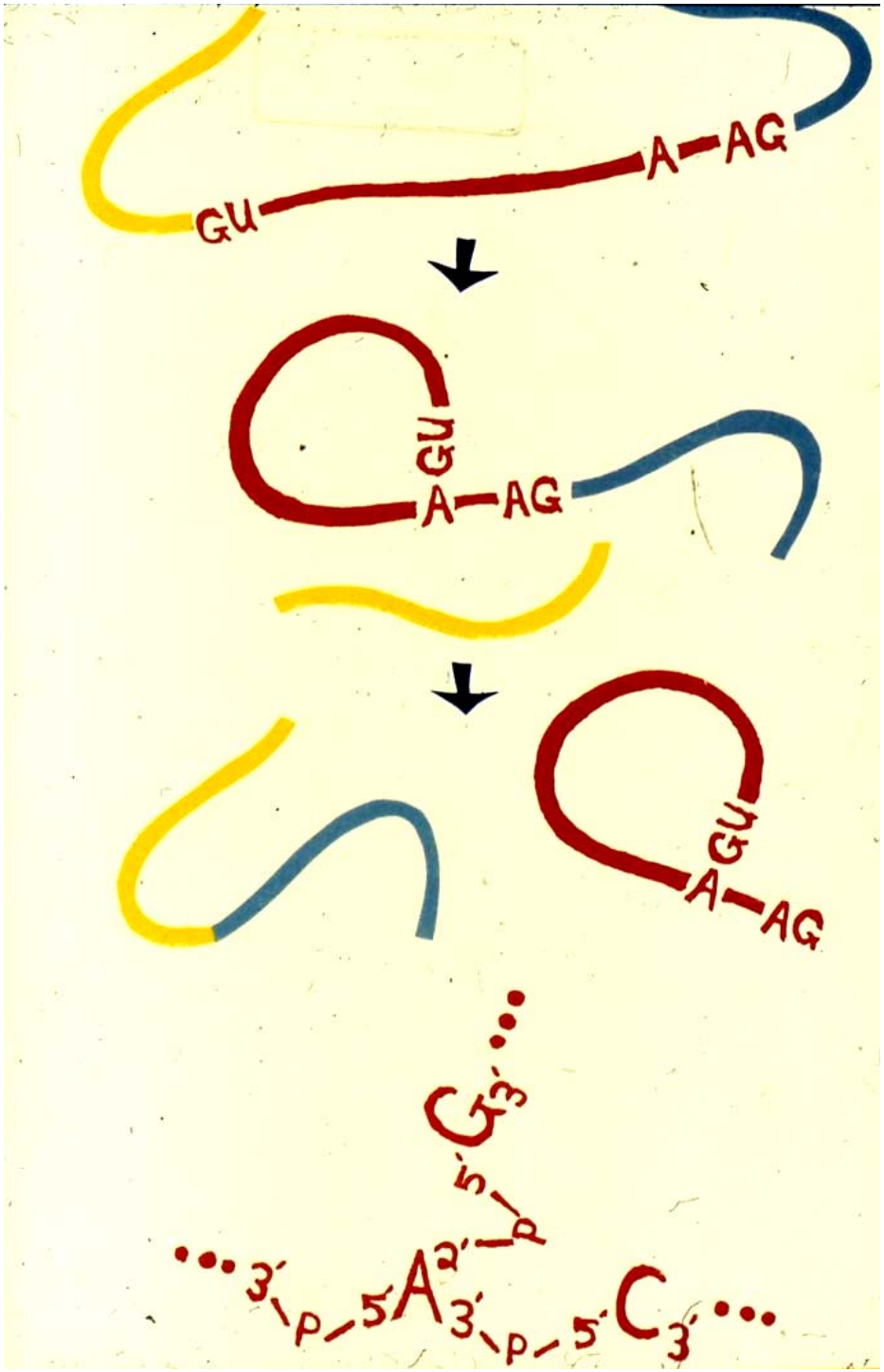
Autokatalytisches Splicing Gruppe II Introns



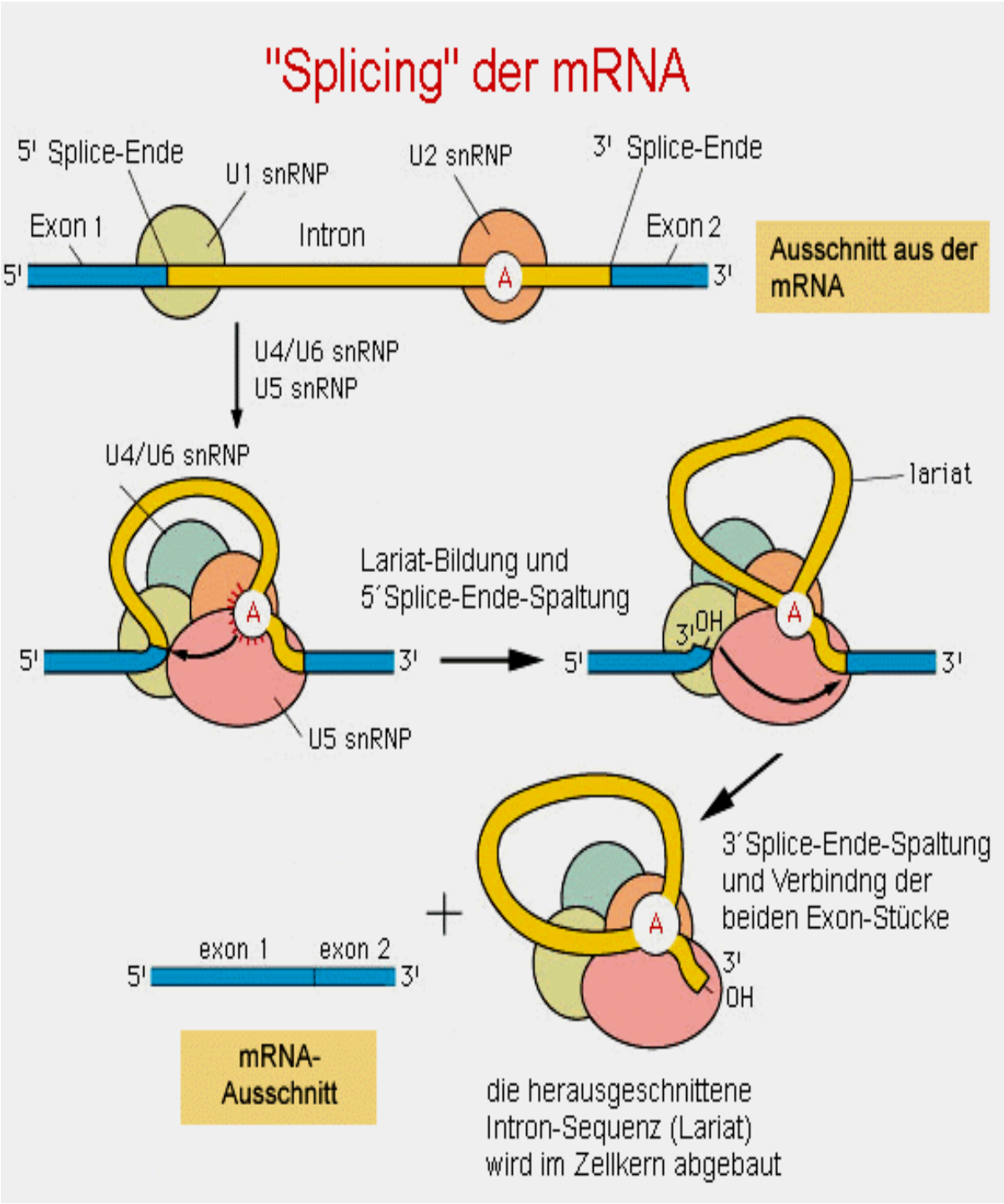
mRNA-Spleißen an der „Consensus Splice Site“ /GU...A..AG/



Beim Spleißen bildet sich ein „Lariat“ im heraus gelösten Intron über eine 2´-5´ Phospho-diesterbindung

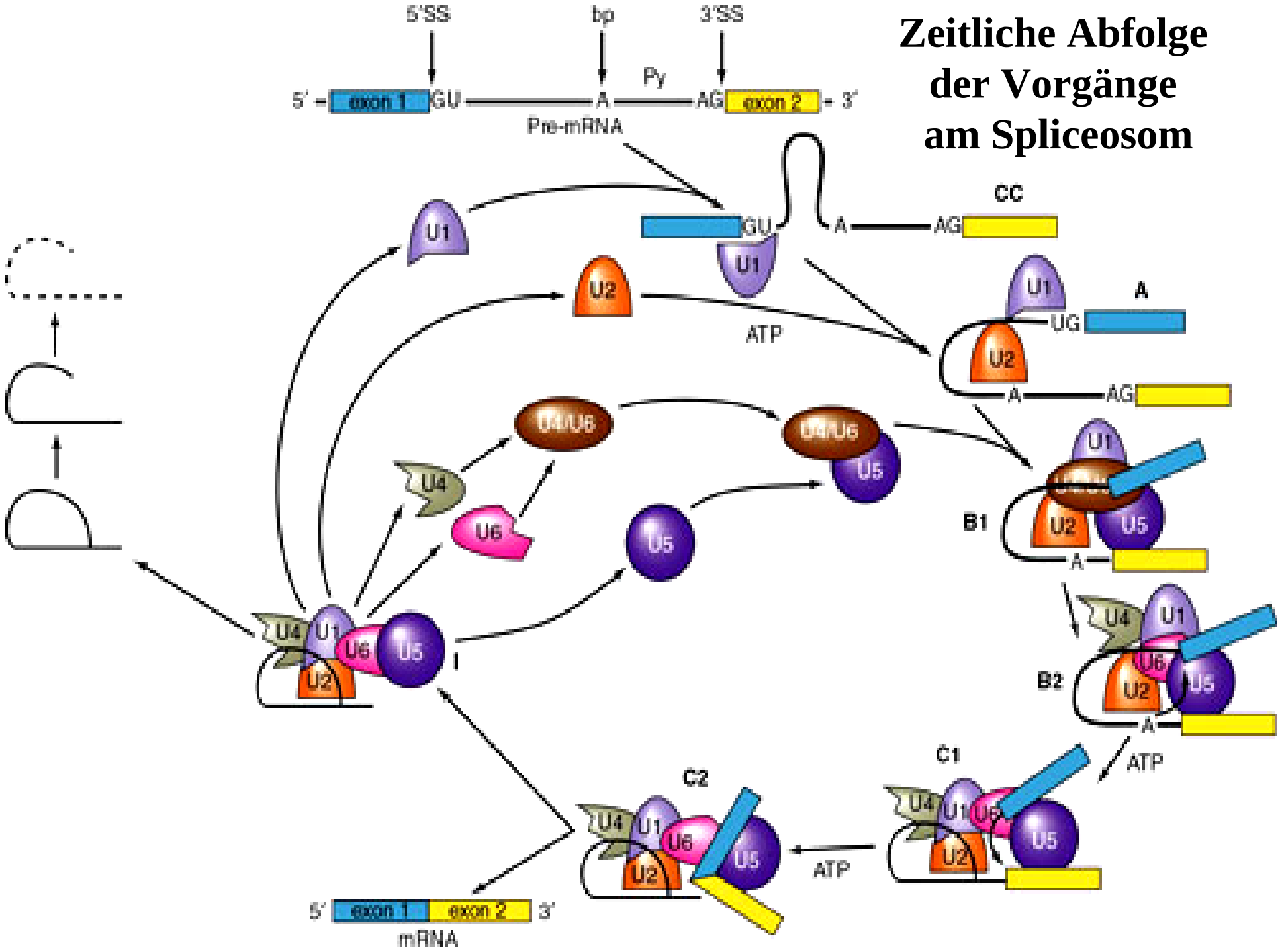


Am Spleißen von mRNAs sind Spliceosomen mit „SN(U)RPS“ beteiligt



snRNPs (SNURPS) enthalten die
snRNAs (sn= „small nuclear“)
U1, U2, U4/6 und U5
(snRNPs= small nuclear ribonucleoprotein)

Zeitliche Abfolge der Vorgänge am Spliceosom



Zusammenfassung

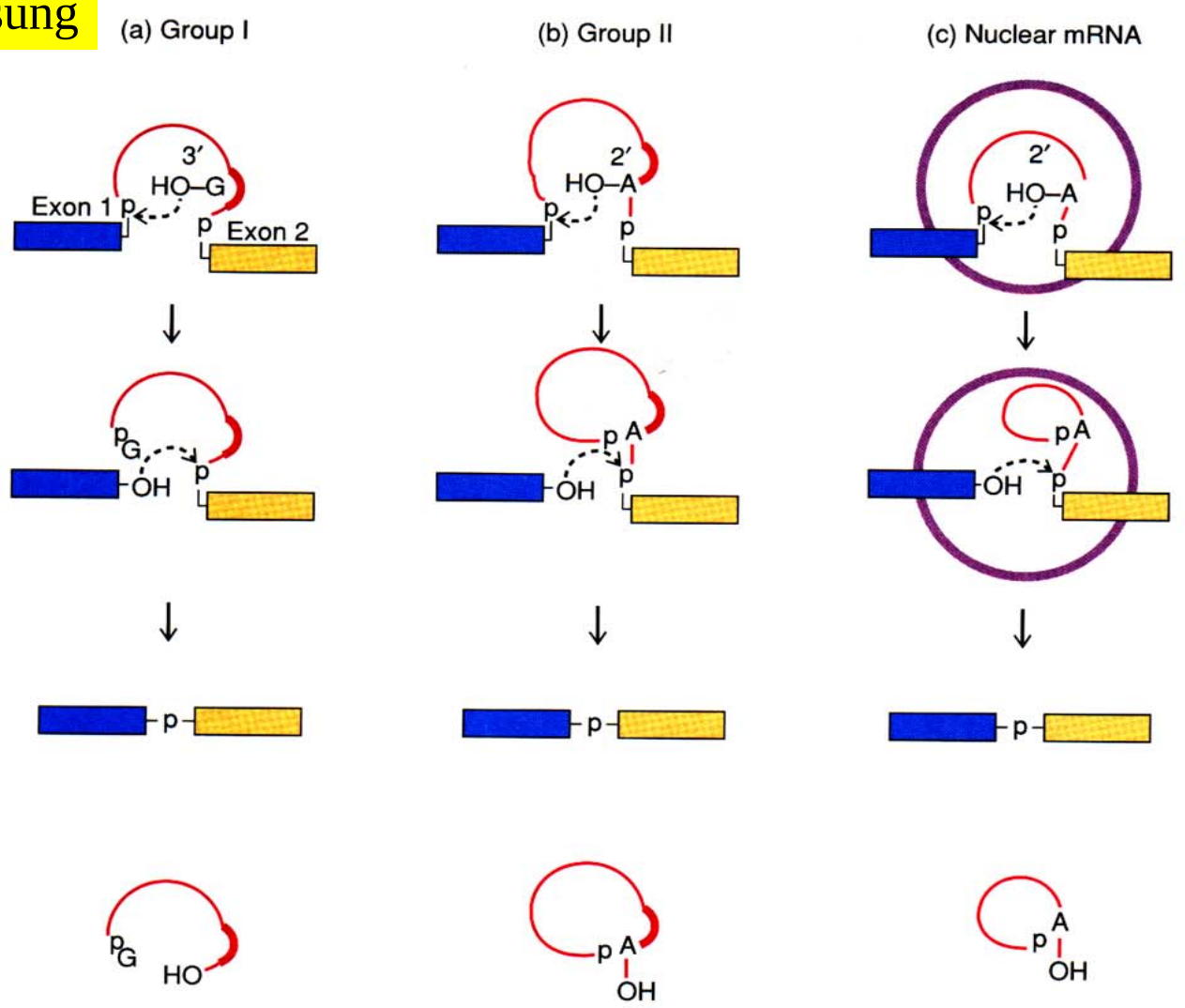
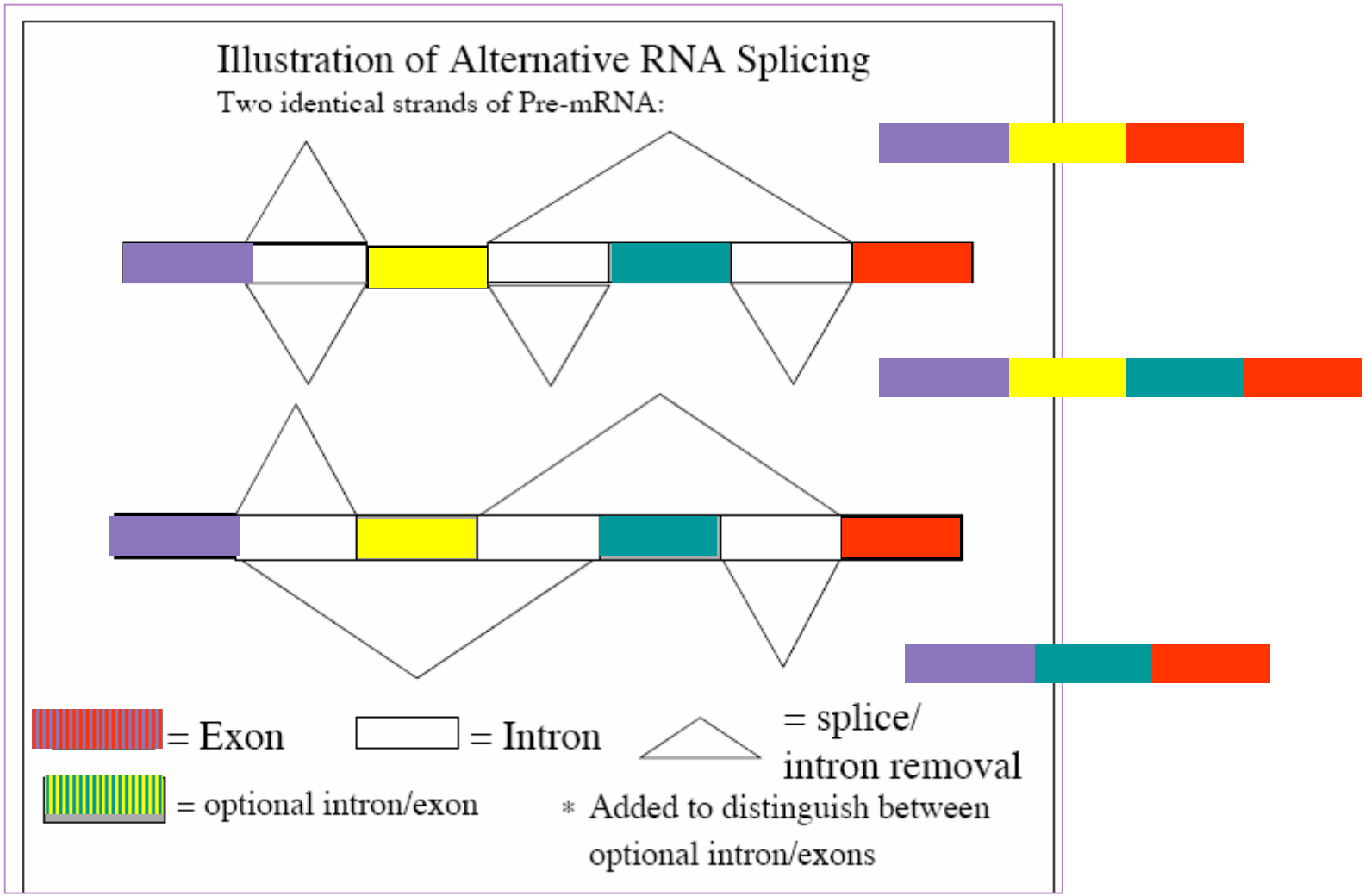


FIGURE 9.42 Summary of three splicing schemes. The major differences in these mechanisms lie in the first step. The self-splicing of group I introns (*a*) is initiated by a guanosine nucleotide that presumably resides in a pocket in the intron (represented by a thickened semicircle). This guanosine attacks the phosphate linking exon 1 (blue) and the intron (red). In group II (*b*), an adenosine nucleotide that is part of the intron itself plays this initiation role, resulting in a lariat-shaped intermediate. This adenosine is represented as adjacent to a pocket similar to the one in group I introns that harbors the initiating guanosine. Nuclear mRNA precursors (*c*) follow a splicing scheme remarkably similar to that used by group II introns. The major difference is that nuclear mRNA splicing requires a spliceosome (purple).

Warum überhaupt Introns?

- Erleichtern die Enstehung komplexer Gene!
- (mehrere Minigene = Exons werden zu Makrogene zusammengepackt)
- „Exon shuffling“
- (einzelne Exons kodieren für Proteindomämen)“Module“, verschiedene Module ergeben zusammen immer wieder neue Proteine)
- Alternatives Spleißen
- (durch Kombination verschiedener Exons auf Ebene der RNA kann ein Gen für viele Proteine kodieren)
- Transspleißen

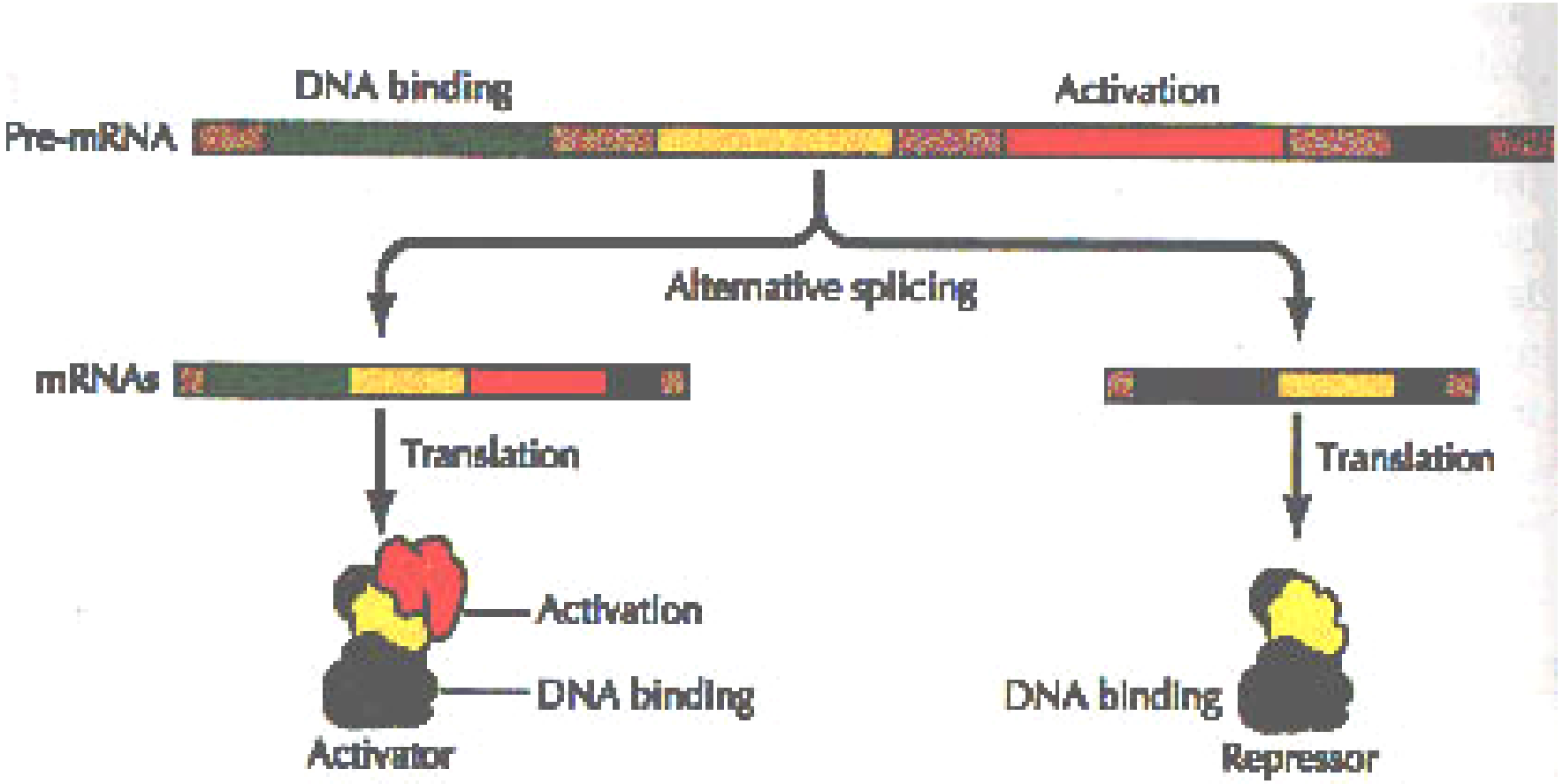
Prinzip des alternativen Spleißens



Alternatives oder differenzielles Spleißen erhöht die Zahl der Proteine

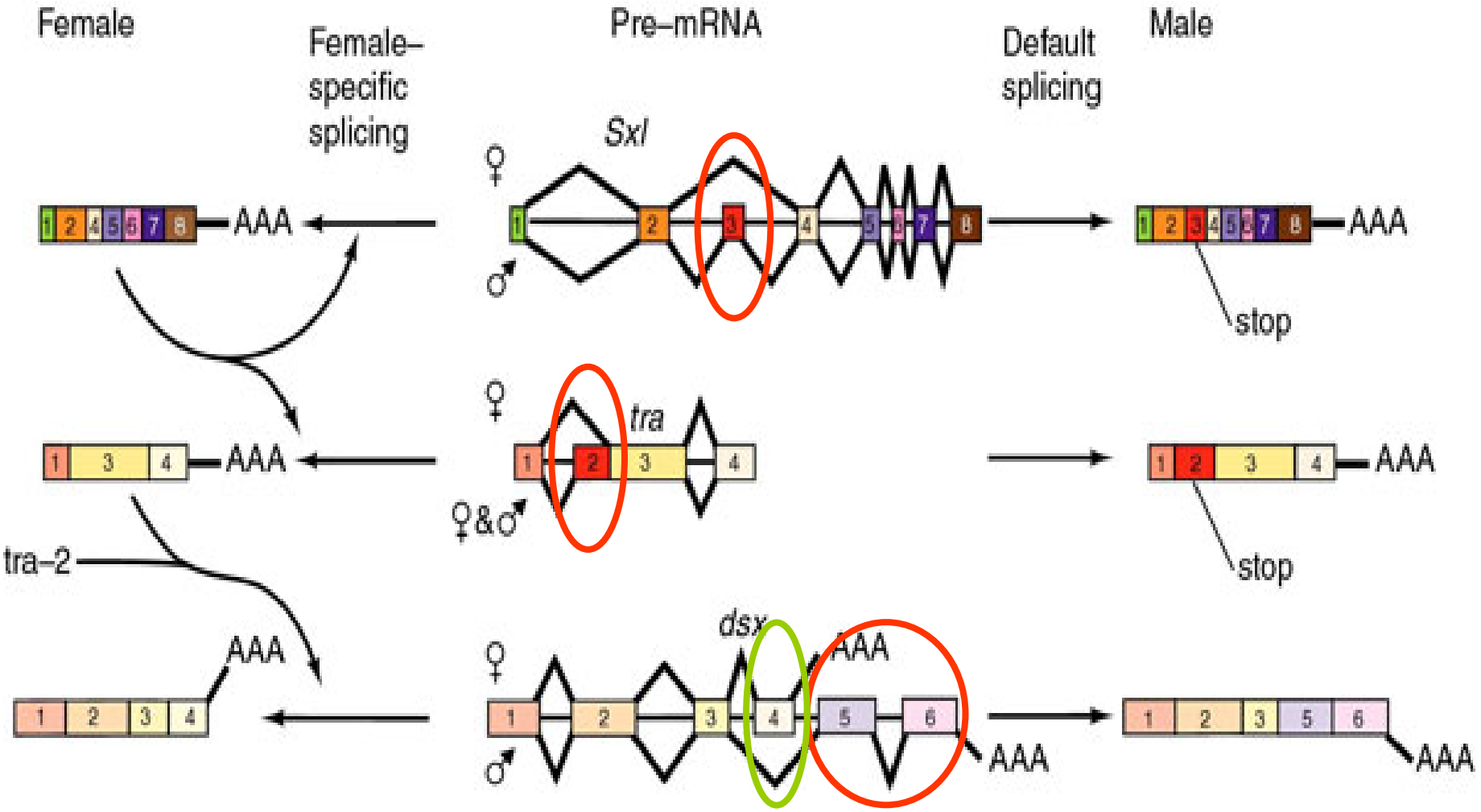
22 – 59% unserer Gene sollen alternative Spleißprodukte bilden!

Modrek, B. and C. Lee, 2002, 'A genomic view of alternative splicing', *Nature Genetics,* 30: 13-19.

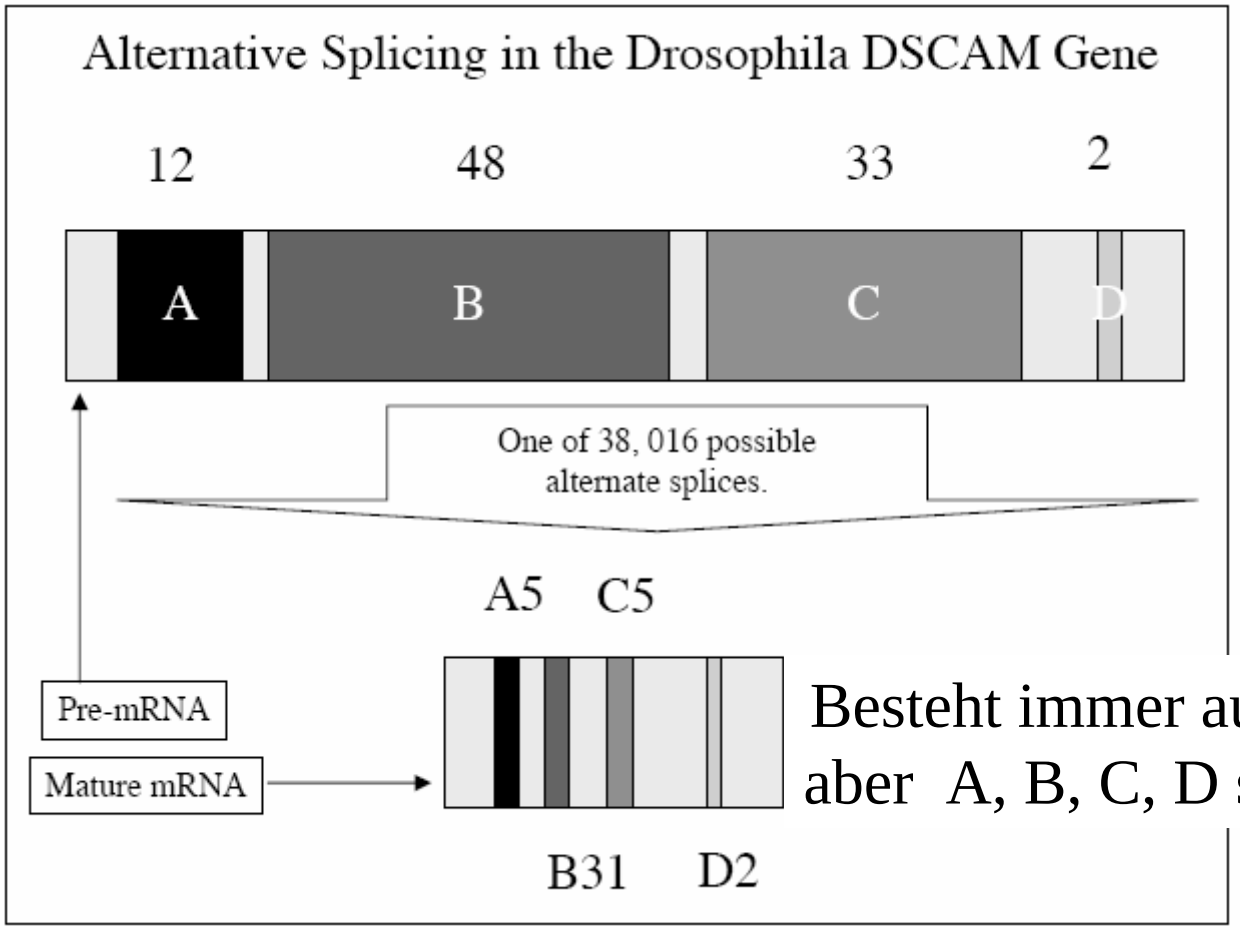


Nur für Interessierte!

Alternatives Spleißen bestimmt bei *Drosophila melanogaster* das Geschlecht



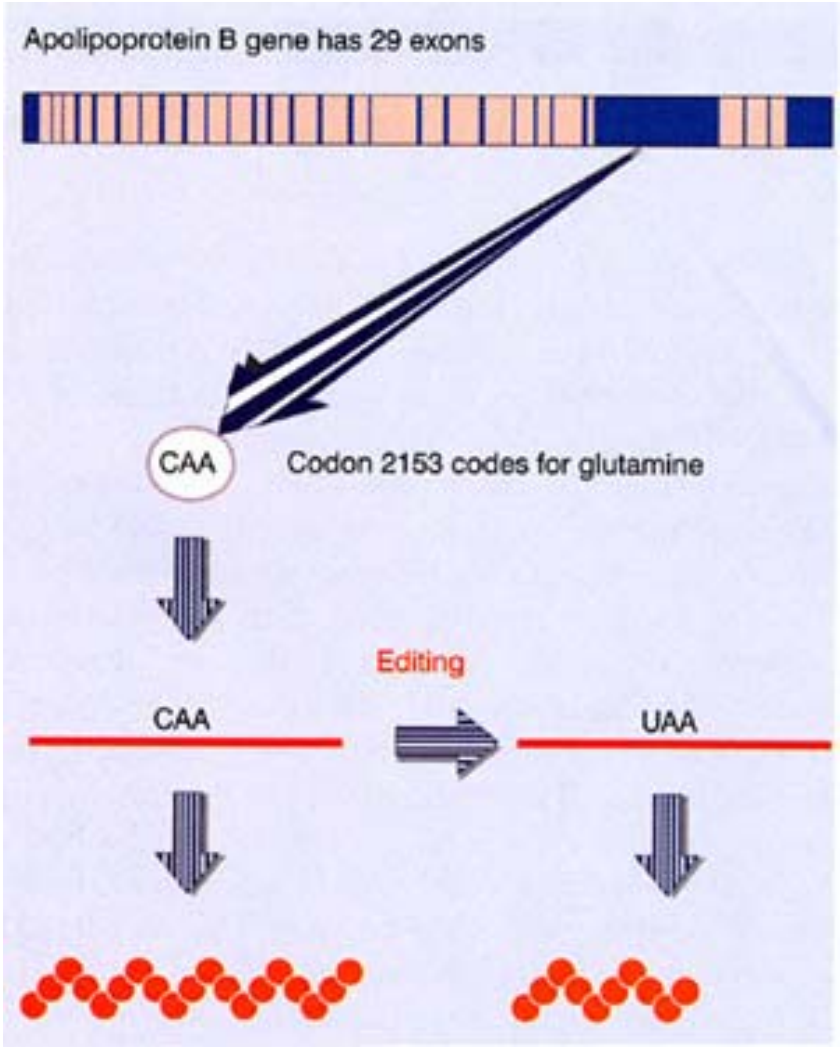
Beim DSCAM-Gen (115 Exons) gibt es bis zu **38.016** verschiedene Spleißvarianten

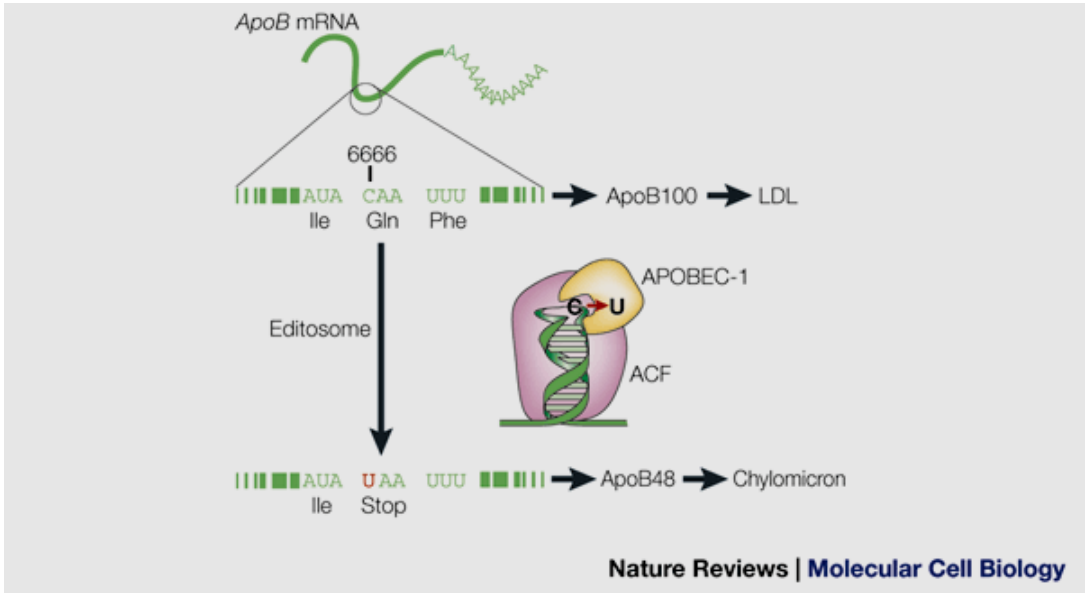


Besteht immer aus 24 Exons, aber A, B, C, D sind variabel

RNA-Editierung

http://dna.kdna.ucla.edu/rna/index.aspx





RNA-Editierung

bei ca. 5- 6 % der menschlichen Gene der Fall

"Nature Biotechnology" (Bd. 22, S. 1001, August 2004)

Neues Thema:
Steuerung der Transkription

- Die Aktivierung von Genen erfolgt durch **Transkriptionsfaktoren** (TFs)
- Es gibt **basale TFs** (immer vorhanden und für jede Transkription notwendig) und **spezifische TFs** (gewebs-/zellspezifisch; hormoninduziert; entwicklungsspezifisch etc.)
- Jede Genklasse hat eigene TFs

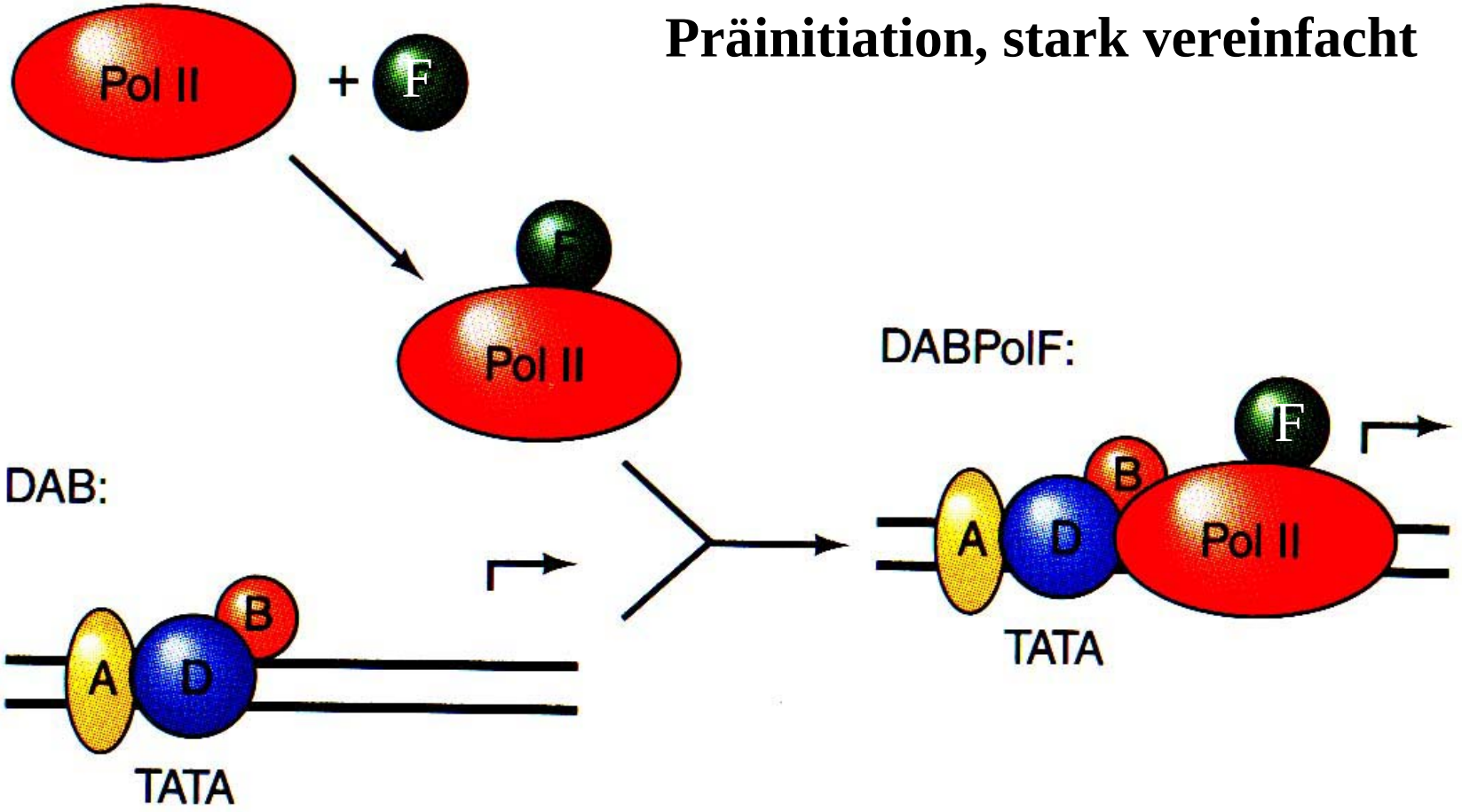
Die basalen Transkriptionsfaktoren:

- Je nach Genklasse werden die Transkriptionsfaktoren TF IA, TF IB ...;

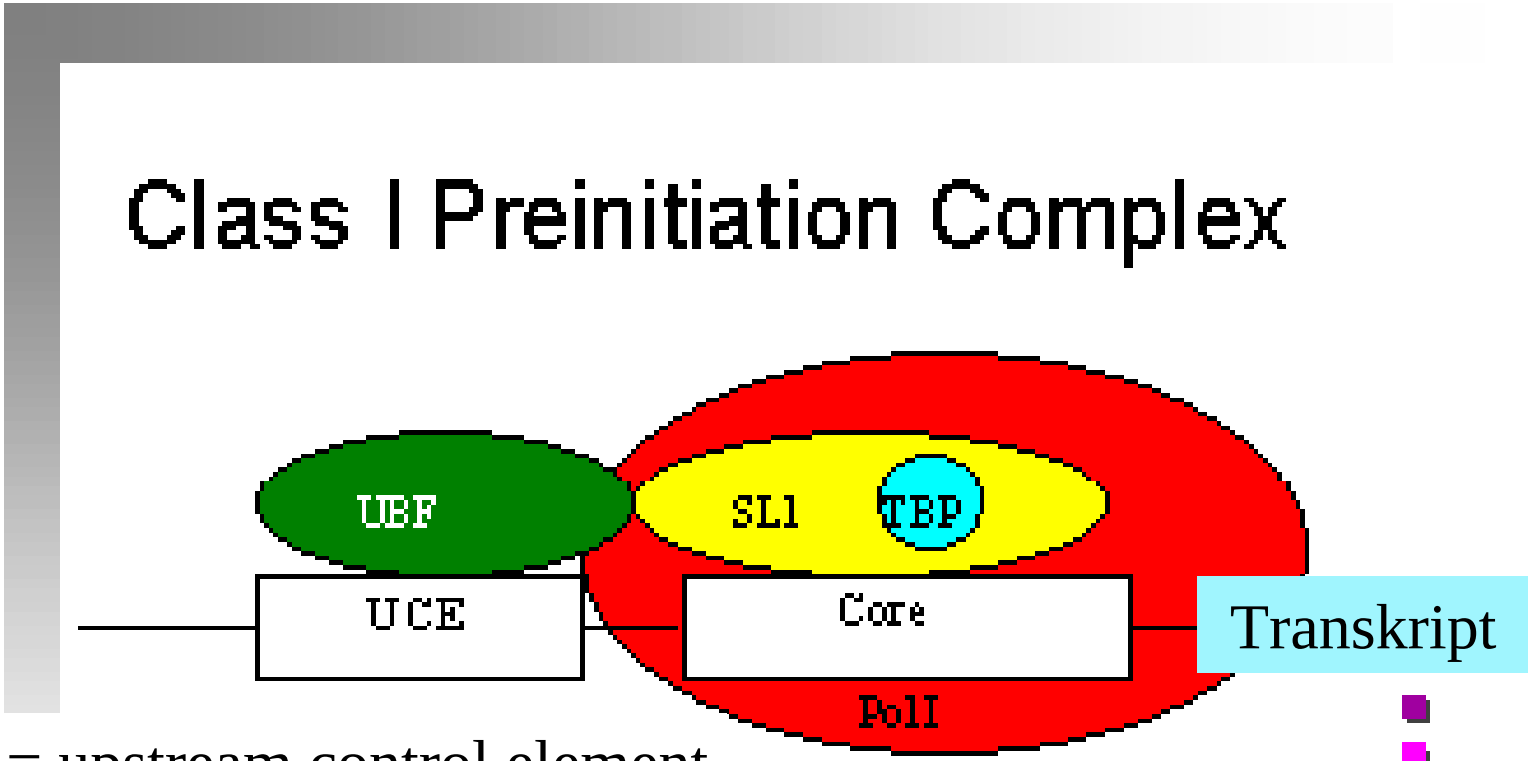
  TF IIA, TF IIB ..; TF IIIA etc. bezeichnet

Daneben gibt es eine Reihe anders benannter Proteine, die die Genaktivität steuern und nicht immer Teil des basalen Transkriptionskomplexes sind (z. B. SP1).

Der Basale Transkriptionskomplex (Präinitiationskomplex) der Pol II – Gene:
TF IID. TF IIA. TF IIB + Pol II-TFIIF



RNA Polymerase I Promotor und Initiationskomplex



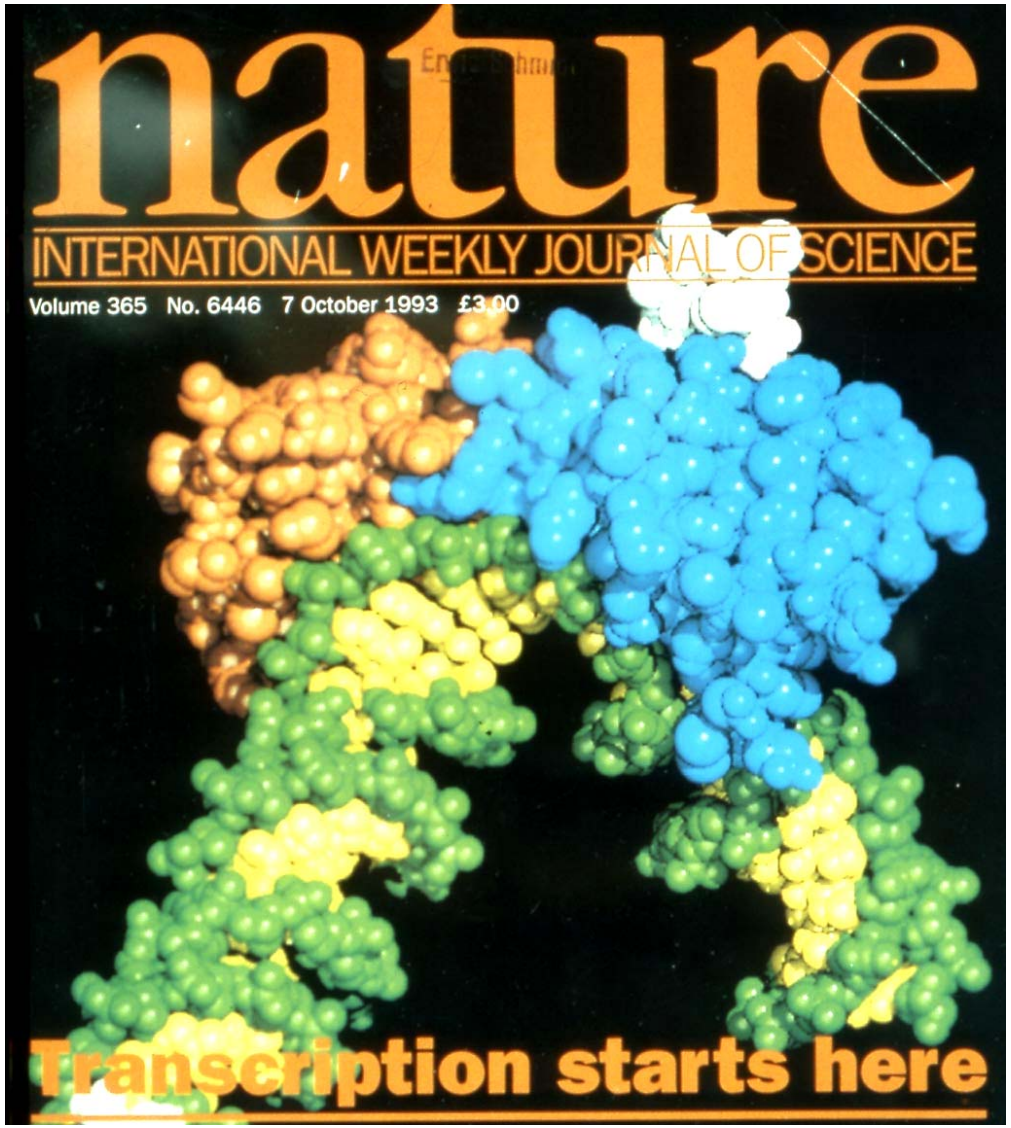
= upstream control element

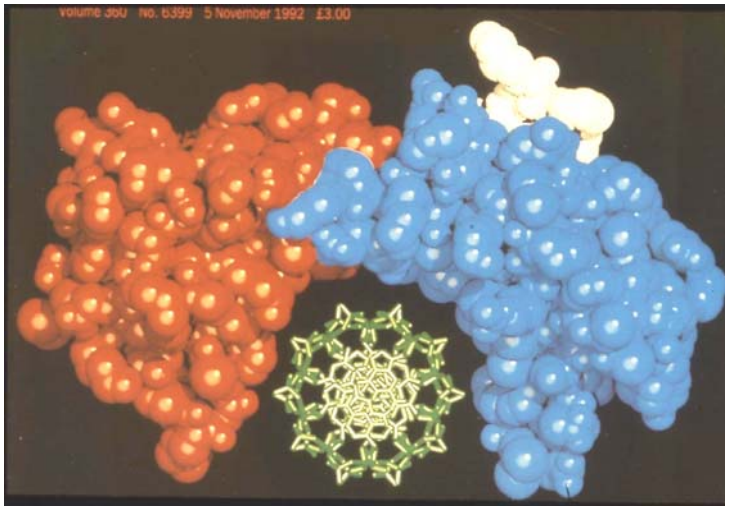
RNA Polymerase I Gene
haben einen 5´ aufwärts Promotor

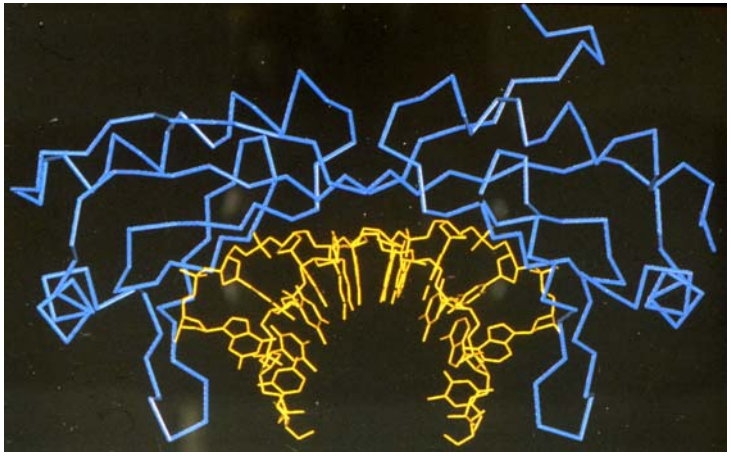
Besondere Rolle von TF IID:

- **TF IID** enthält als Untereinheit das **TBP** („TATA-Box binding Protein). Das TBP erkennt die TATA-Box und bindet als erstes Protein an den Gen-Promotor. Erst danach erfolgt die Bindung der anderen TFs und schließlich zuletzt die der RNA-Polymerase II in Verbindung mit TF IIF.

TATA-Box binding protein (TBP)



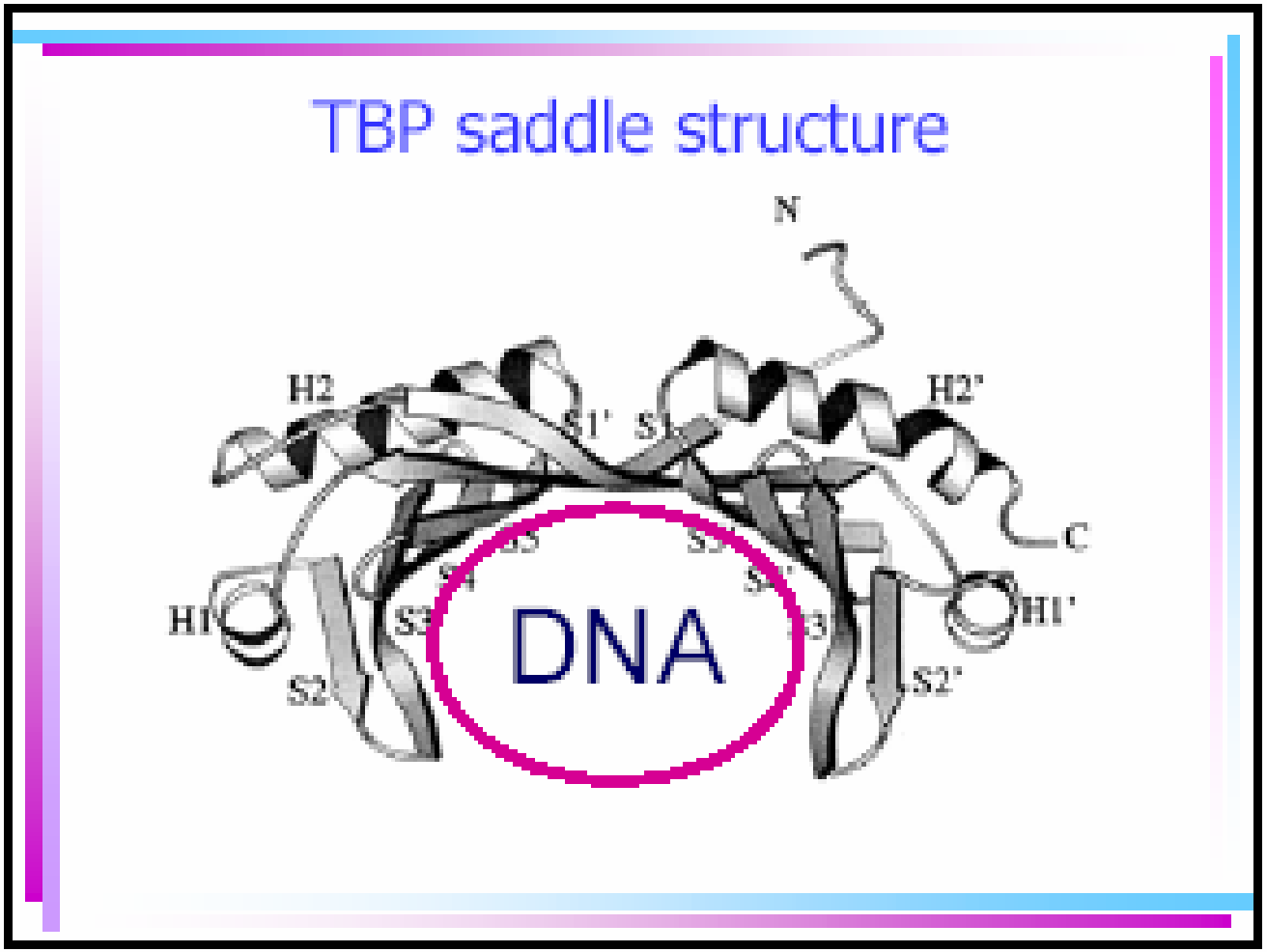




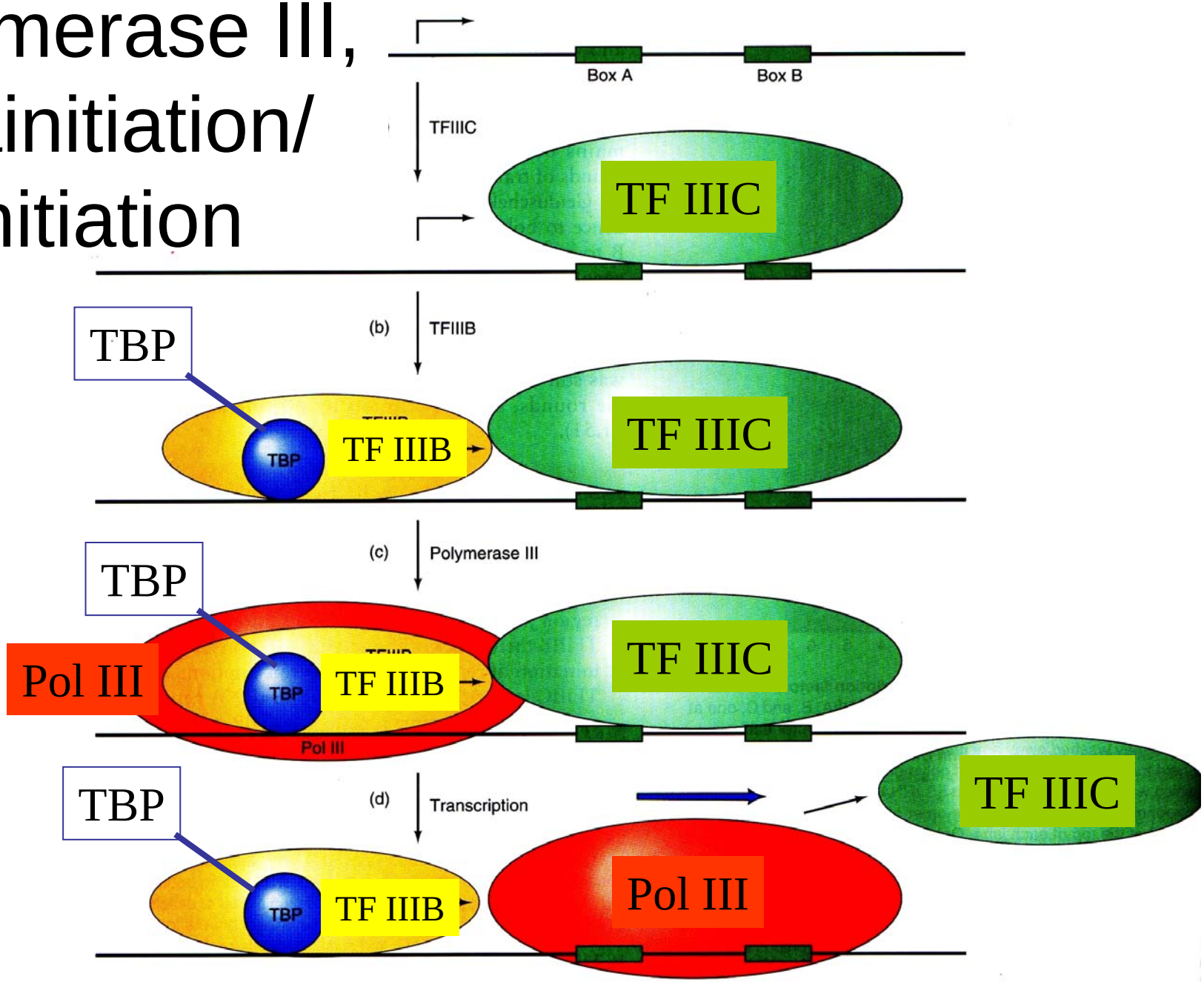
TBP (TATA-box binding protein)

- TBP bindet im Gegensatz zu den meisten DNA-bindenden Proteinen in der „kleinen Grube“ der DNA
- TBP krümmt die DNA durch die Bindung und verursacht so einen scharfen „Knick“
- TBP vermittelt die Bindung weiterer TFs an den Promotor
- TBP ist auch bei Genen ohne TATA-Box am Präinitiationskomplex beteiligt, und zwar auch bei Pol I- und Pol III-Genen

Sattel-Struktur des TBP auf der DNA



Polymerase III, Präinitiation/ Initiation

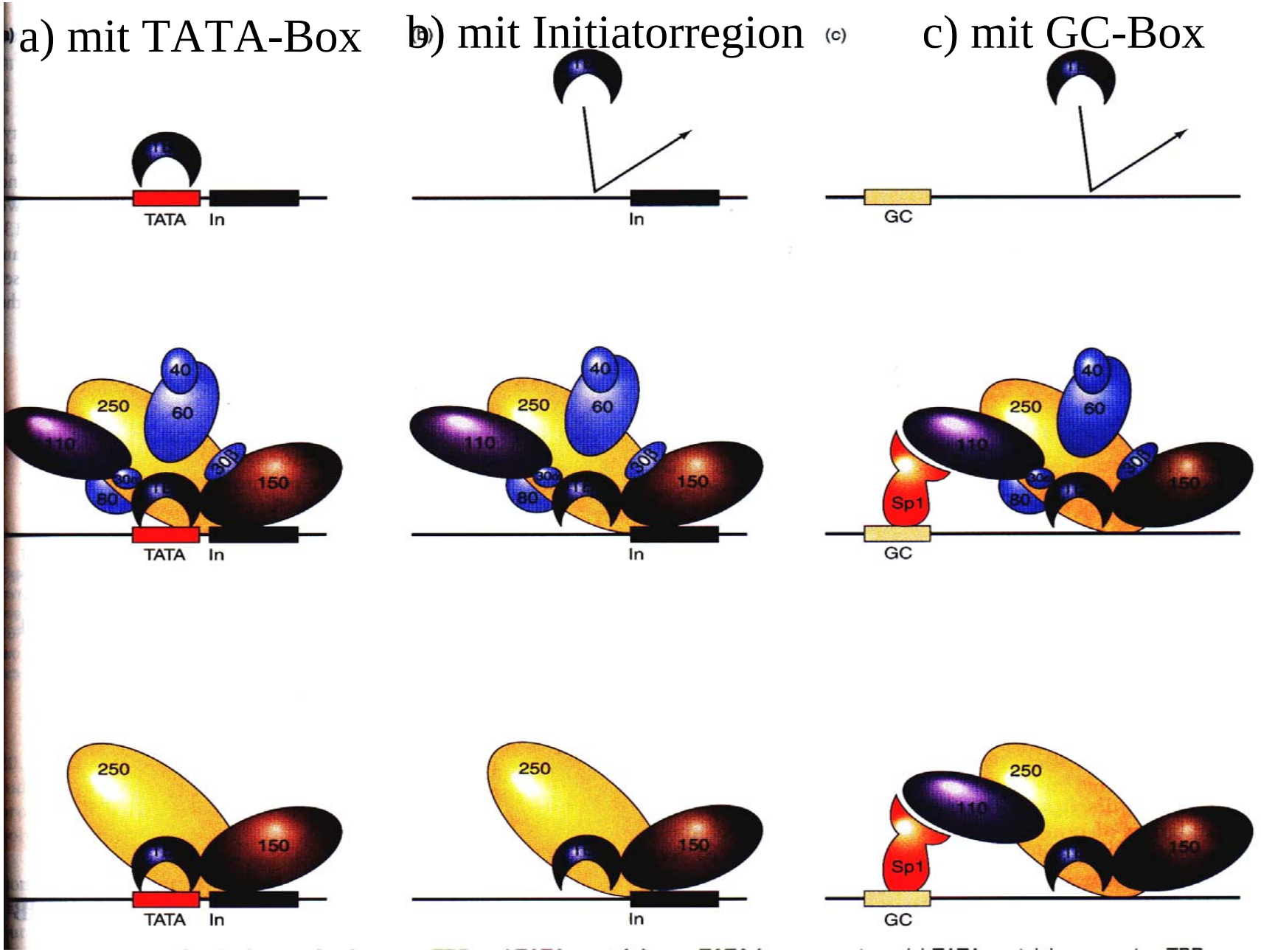


Präinitiation bei Genen

a) mit TATA-Box

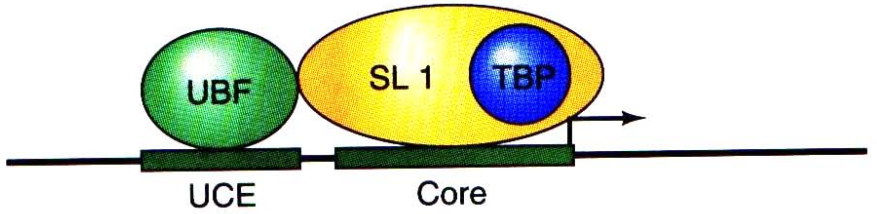
b) mit Initiatorregion

c) mit GC-Box

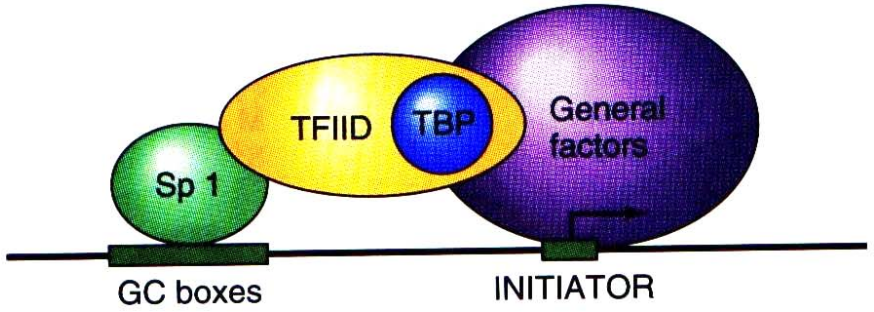


Zusammen-fassung: Präinitiations-komplexe der verschiedenen Genklassen

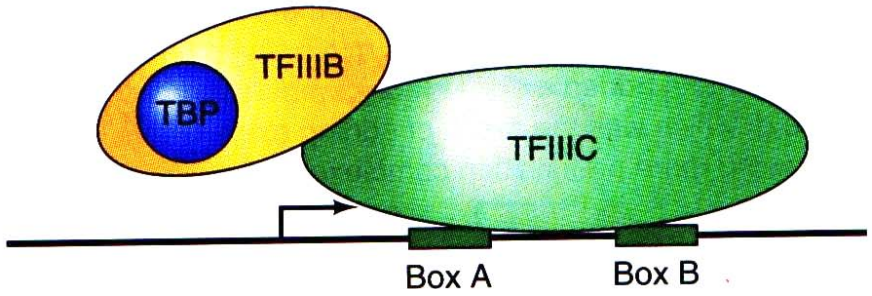
CLASS I
rRNA

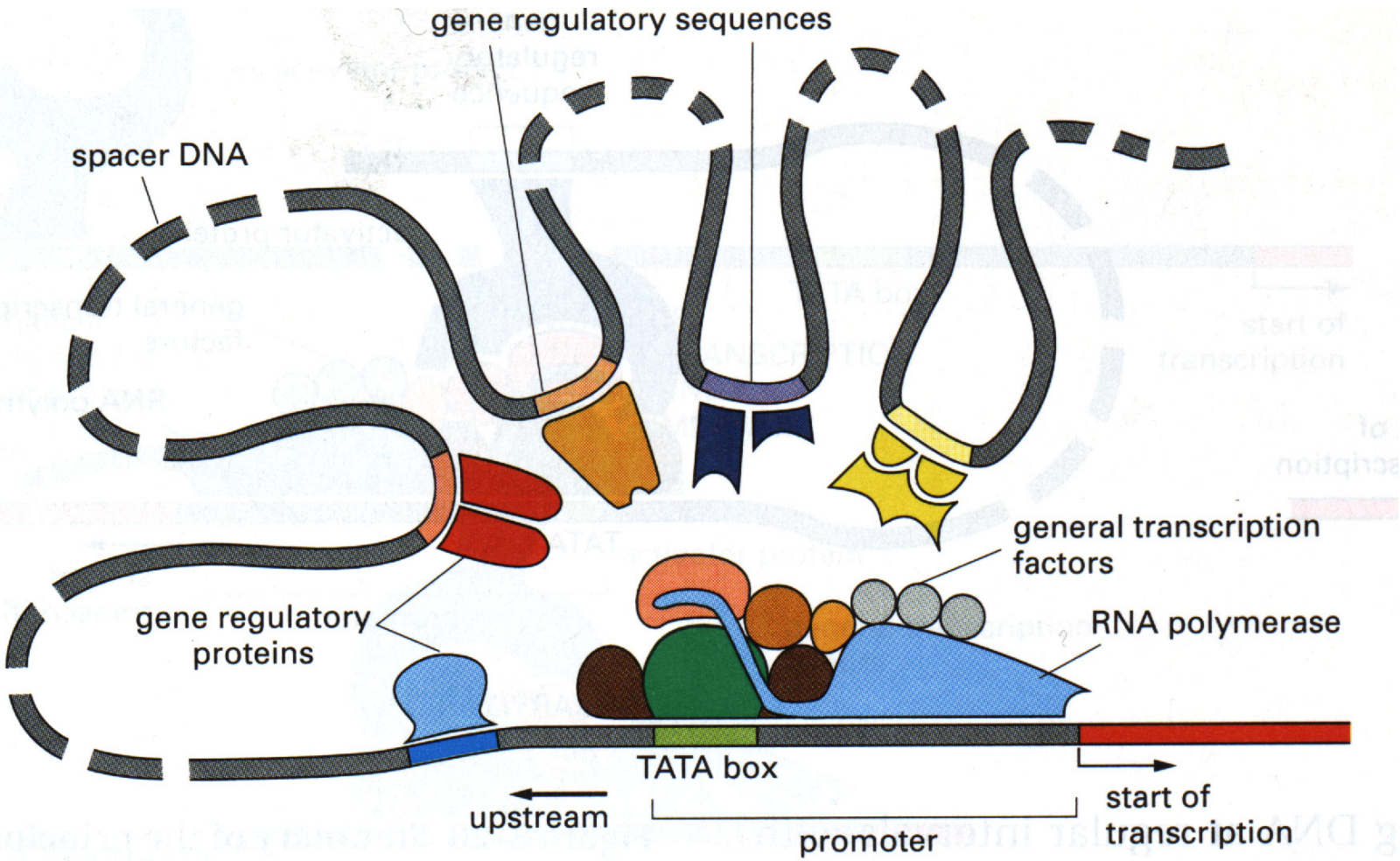


CLASS II
G_6I

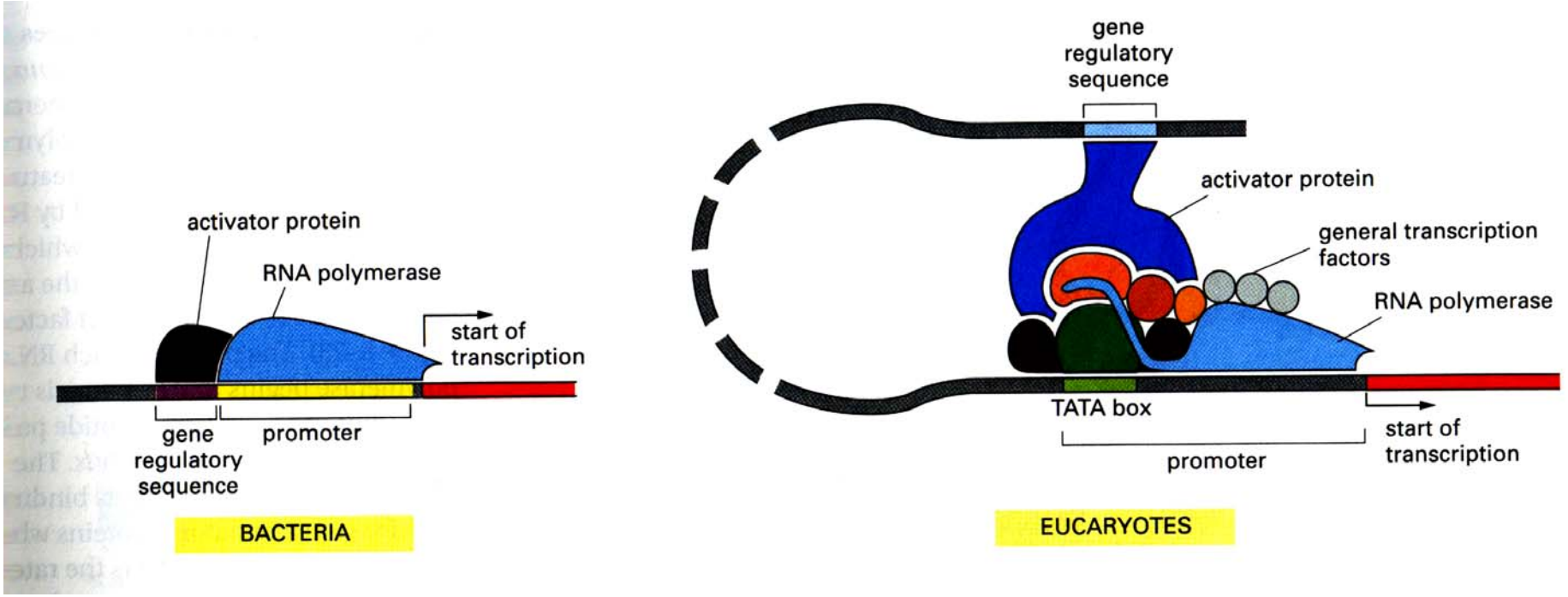


CLASS III
VA_I




gene regulatory sequences
spacer DNA
general transcription factors
RNA polymerase
gene regulatory proteins
TATA box
upstream
start of transcription
promoter

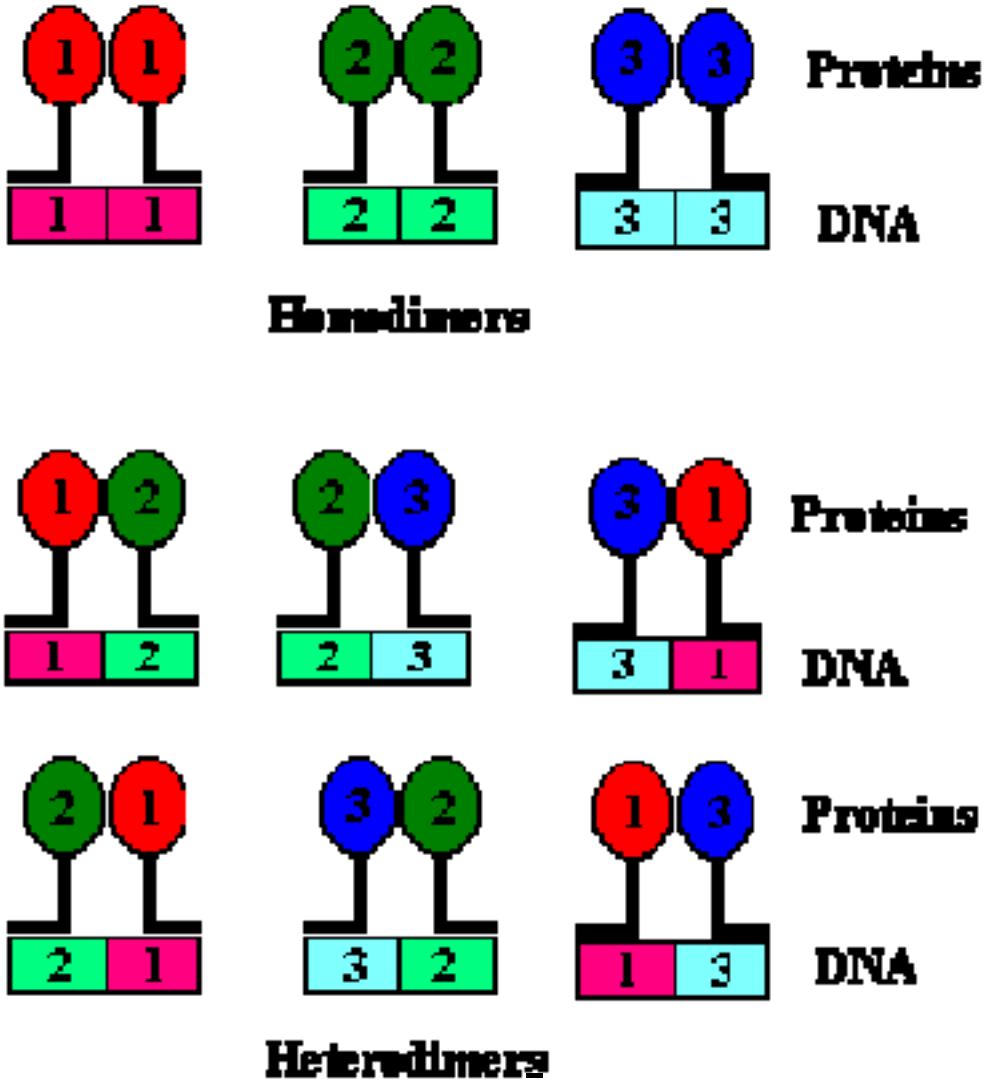
Vergleich Prokaryoten - Eukaryoten



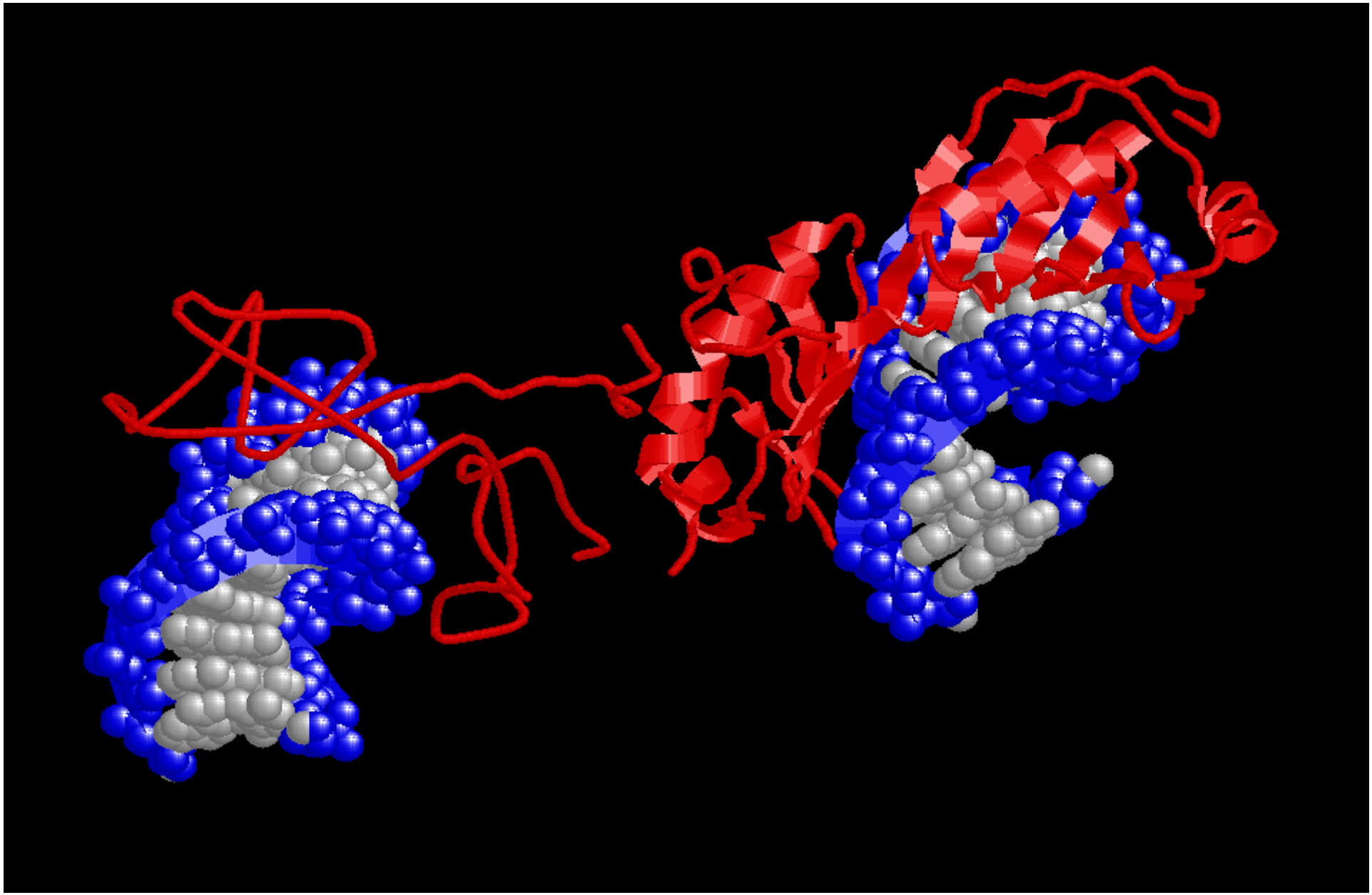
DNA-bindende Proteine:

- Die wichtigsten „Interpreter“ des DNA-Kommando-Codes
- Die Vermittler zwischen ankommenden Signalen und Umsetzung durch die Gene
- „transaktive“ Steuerungselemente von Genen oder ganzen Gengruppen
- Globale oder lokale Modifikatoren der Chromatinstruktur und damit der Genaktivität

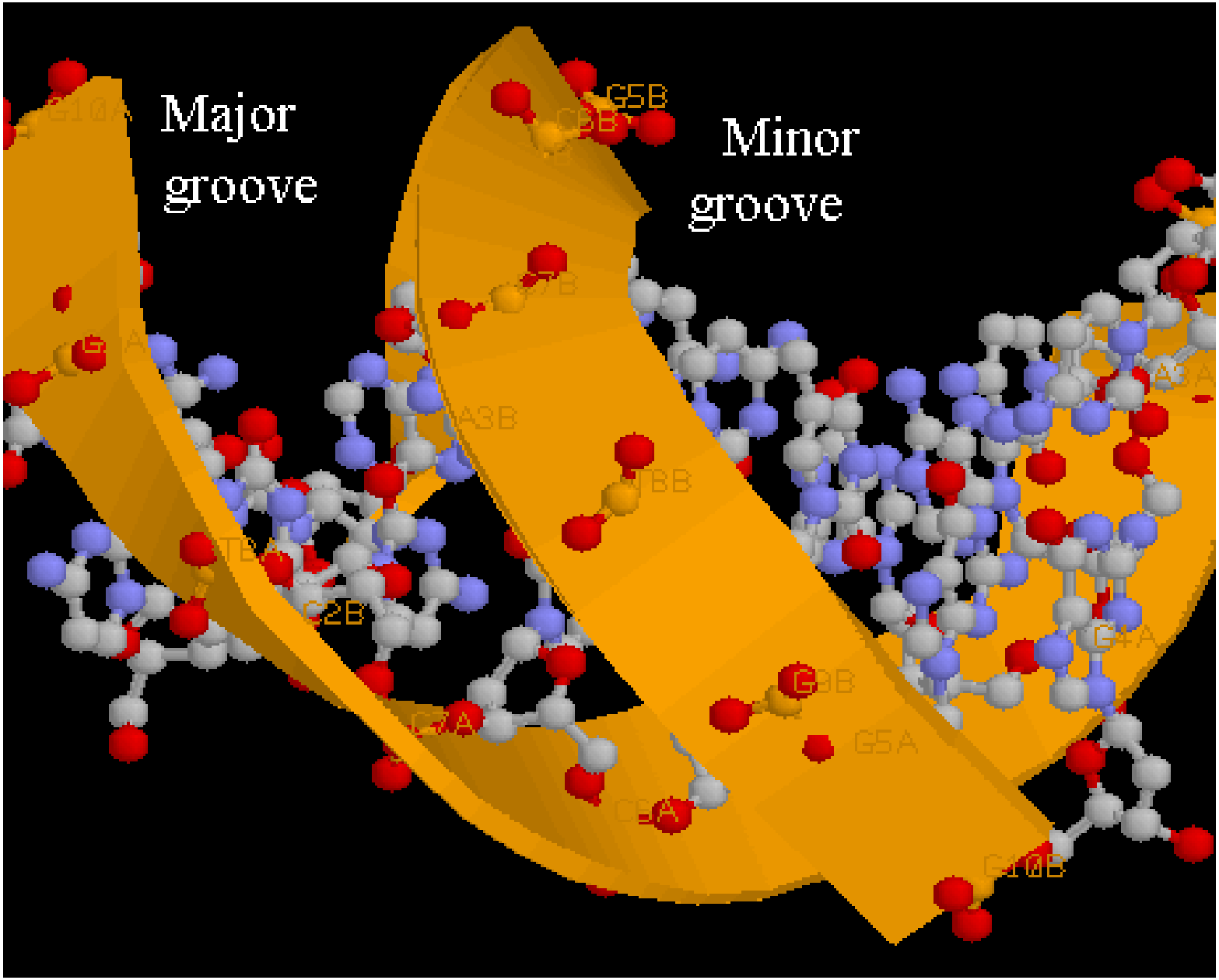
DNA bindende Proteine haben eine DNA-Bindedomäne und binden oft als Dimere



TATA-Box binding Protein

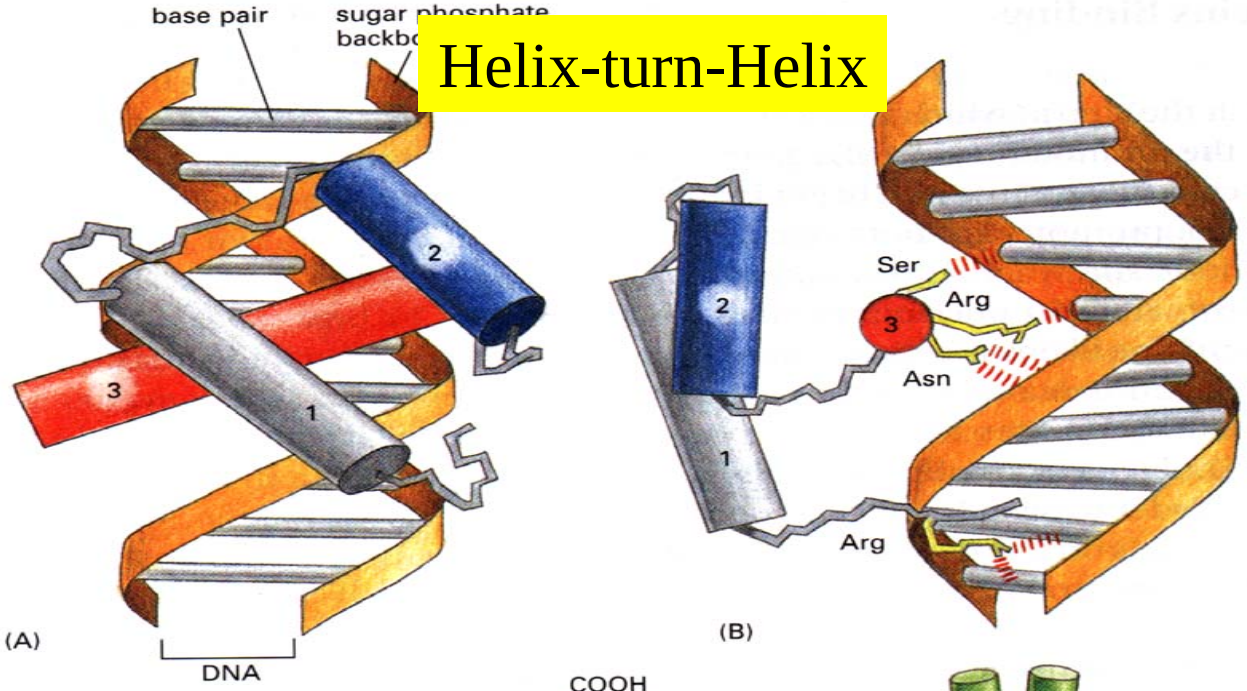


Bindung „große Grube“/kleine Grube

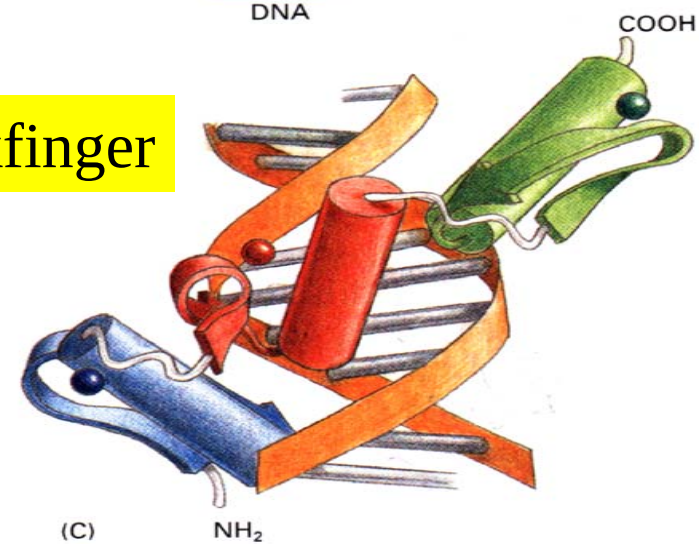


Die wichtigsten DNA-binde-Proteine

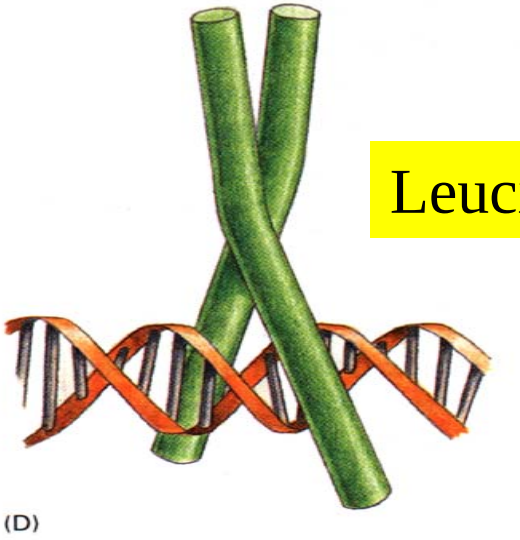
Helix-turn-Helix



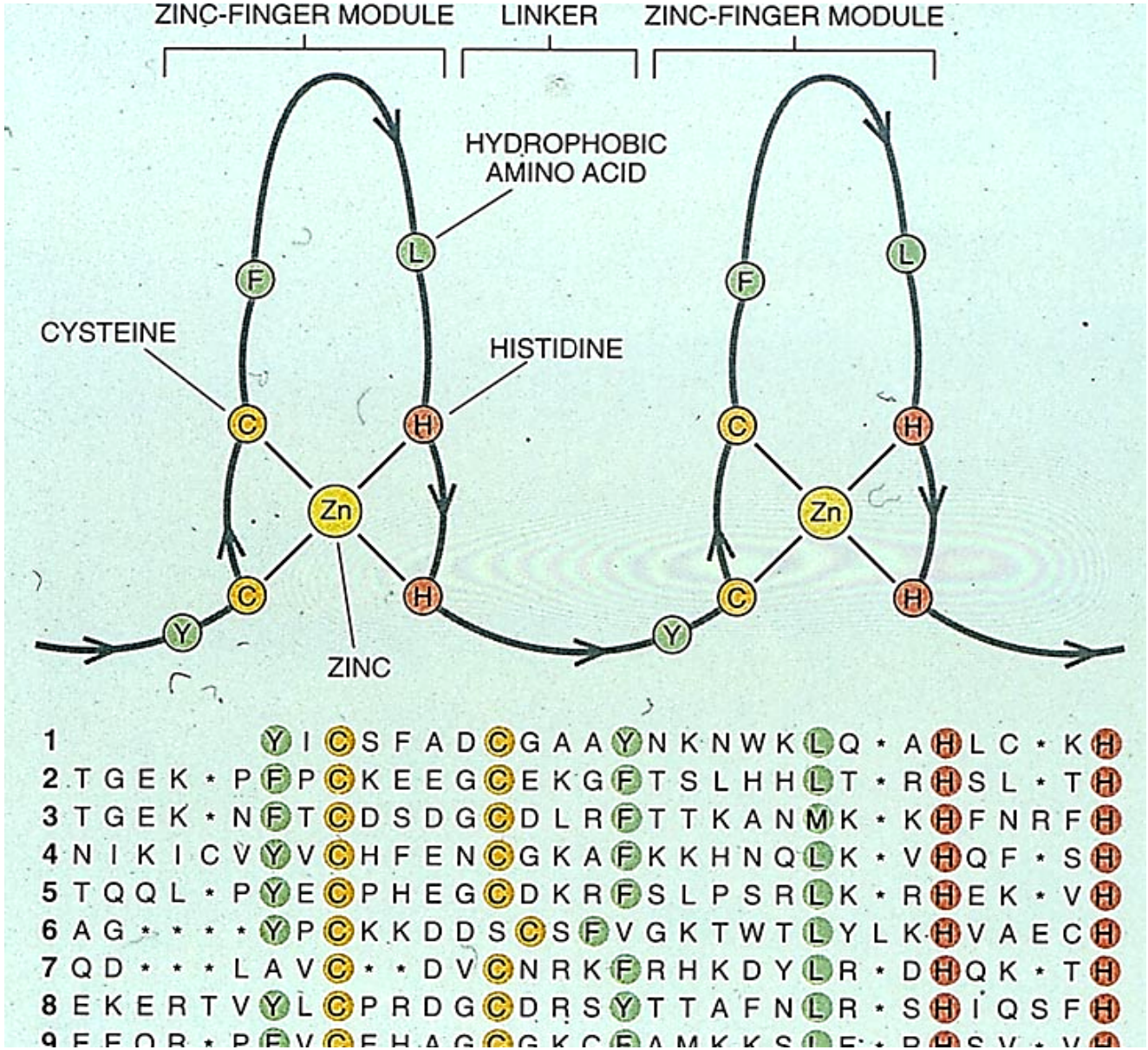
Zinkfinger



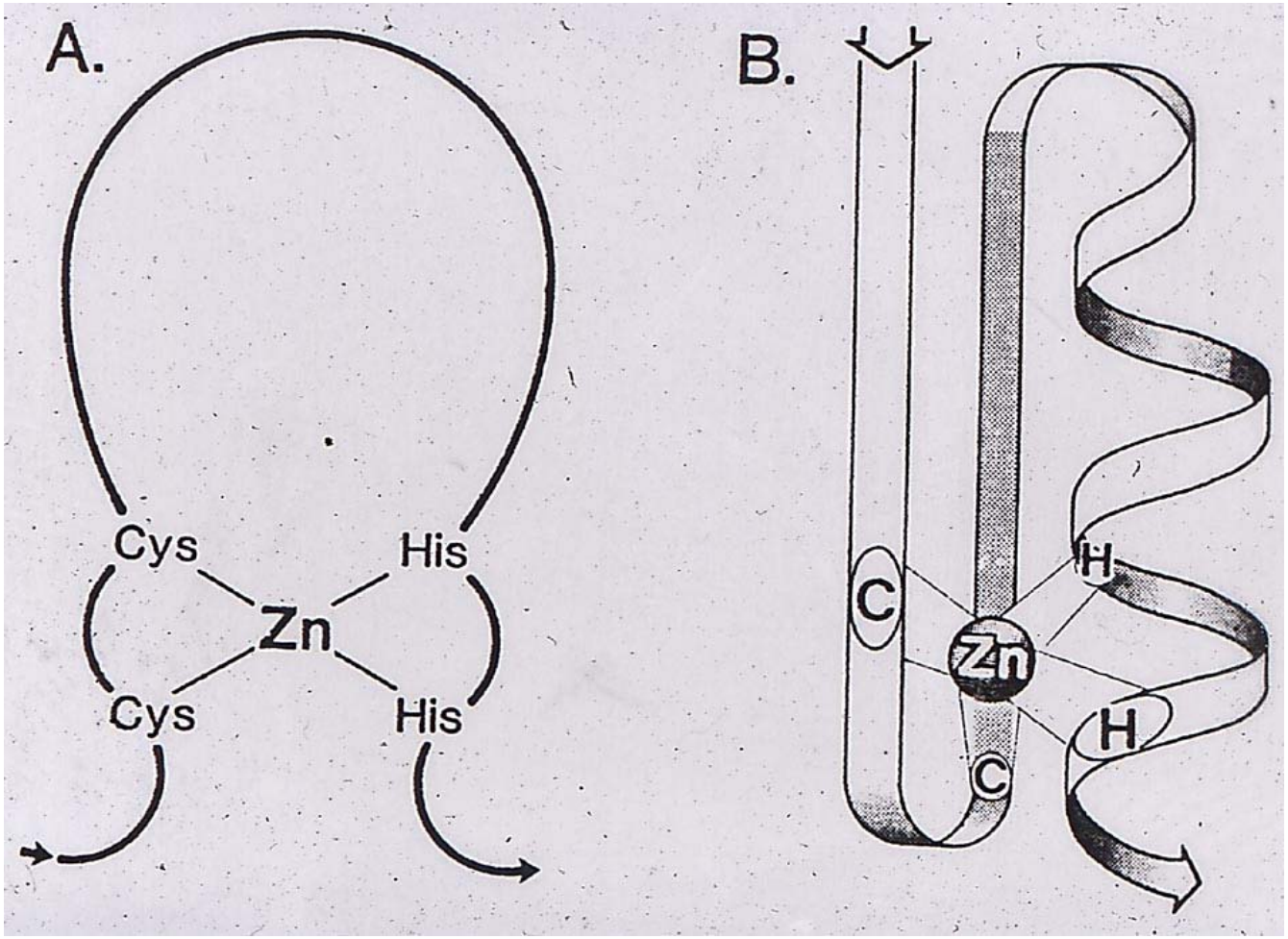
Leucin Zipper



ZinkfingerProteine (ZF)



Die wichtigsten DNA-binde-Proteine: Zinkfinger-Proteine



Die wichtigsten DNA-bindeProteine: Zinkfinger-Proteine: **Cys-His-Typ**

C_2H_2: F/Y-X- C $-X_{2-4}-$ C $-X_3$-F-X_5-L-X_2- H $-X_{3-4}-$ H $-X_{\beta}$

	Repeats	Binds DNA In vitro	Trans-Acting	Organism
TFIIIA[a]	9	+	+	Xenopus
ADR1[b]	2		+	yeast
SP1[c]	3	+	+	human
NGFI-A[d]	3			rat
Krüppel h[e]	2(+)			Drosophila
Krüppel[f]	4	+		Drosophila
Hunchback[g]	4+2			Drosophila
Serendipity β[h]	5			Drosophila
Serendipity δ[h]	6+1			Drosophila
Snail[i]	4			Drosophila
MKR1[j]	7(+)			mouse
MKR2[j]	9(+)			mouse
TDF[k]	13(+)			human
Xfin[l]	6+6+8+ 7+3+5			Xenopus

The two classes of finger proteins are listed with the general primary structure of each shown. Amino acids in bold are invariant and potentially coordinate metal, where "X" indicates intervening amino acid residues. A "+" between finger repeat units represents a linker of greater than 8 amino acids separating two groups of repeat units, "(+)" indicates data from a partial coding sequence, and "Trans-Acting" denotes demonstrated ability to transcriptionally regulate a gene(s).

Die wichtigsten DNA-binde-Proteine: Zinkfinger-Proteine **Cys-Cys-Typ**

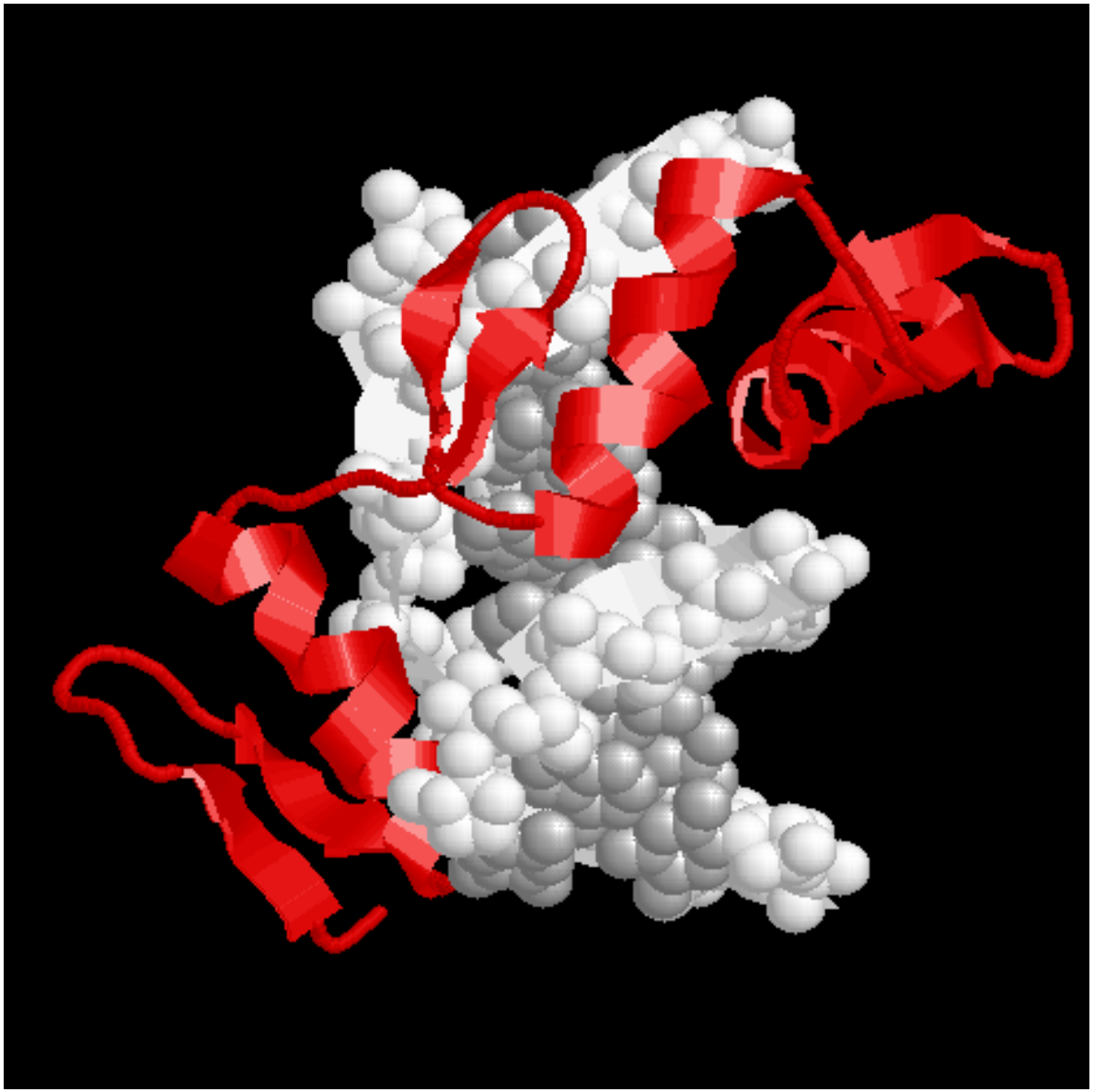
C_x C_4: C $-X_2-$ C $-X_{13}-$ C $-X_2-$ C

C_5: C $-X_5-$ C $-X_9-$ C $-X_2-$ C $-X_4-$ C

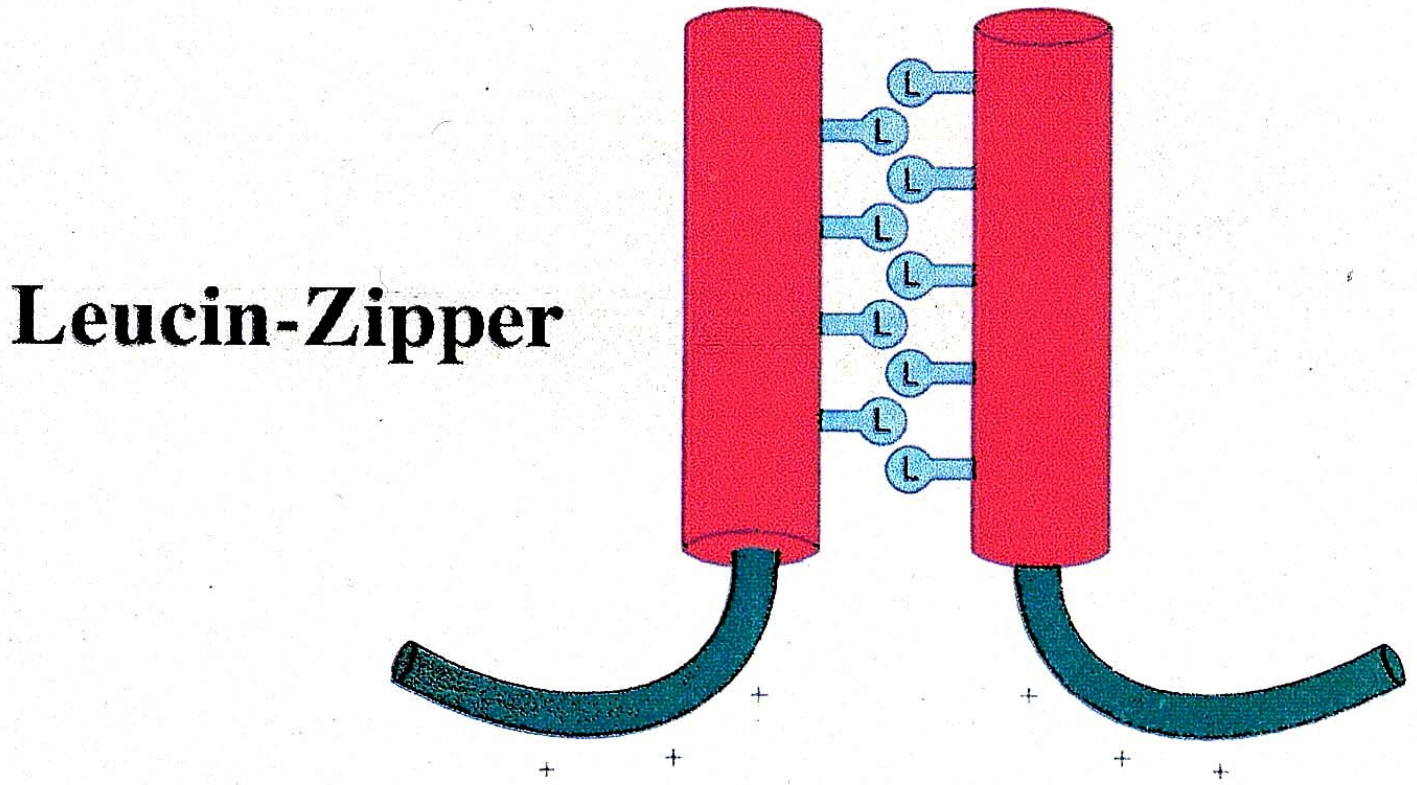
C_6: C $-X_2-$ C $-X_6-$ C $-X_6-$ C $-X_2-$ C $-X_6-$ C

	Finger Type	Binds DNA In vitro	Trans-Acting	Organism
GAL4[m] (PPR1/ARGR11/ LAC9/qa-1F)	C_6	+	+	yeast
E1A[n]	C_4	-	+	adenovirus
Steroid Hormone Receptor Superfamily[o]	C_4+C_5	+	+	human/rat/ mouse/ chicken

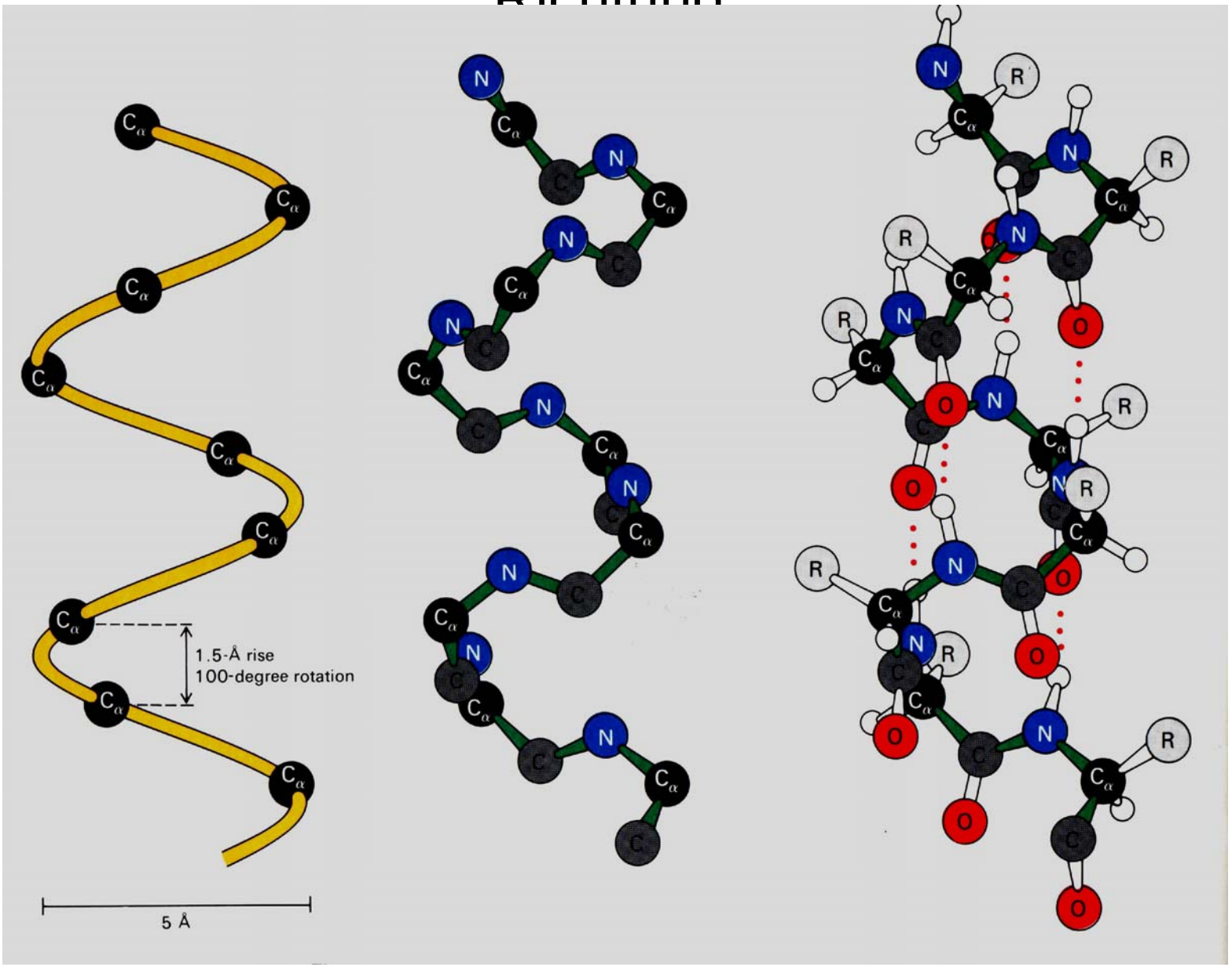
Zinkfinger-Motiv



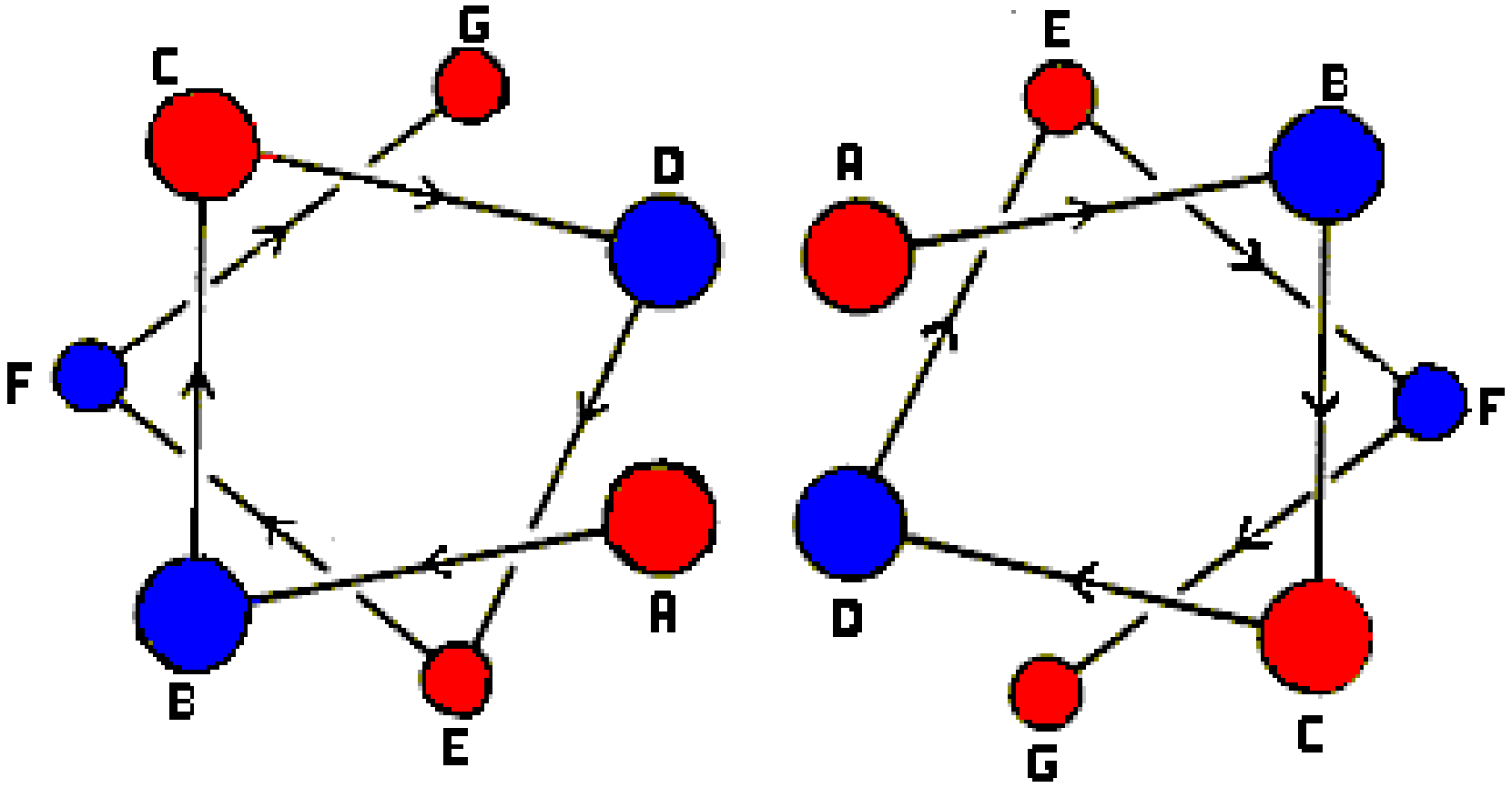
Leucin „Zipper“



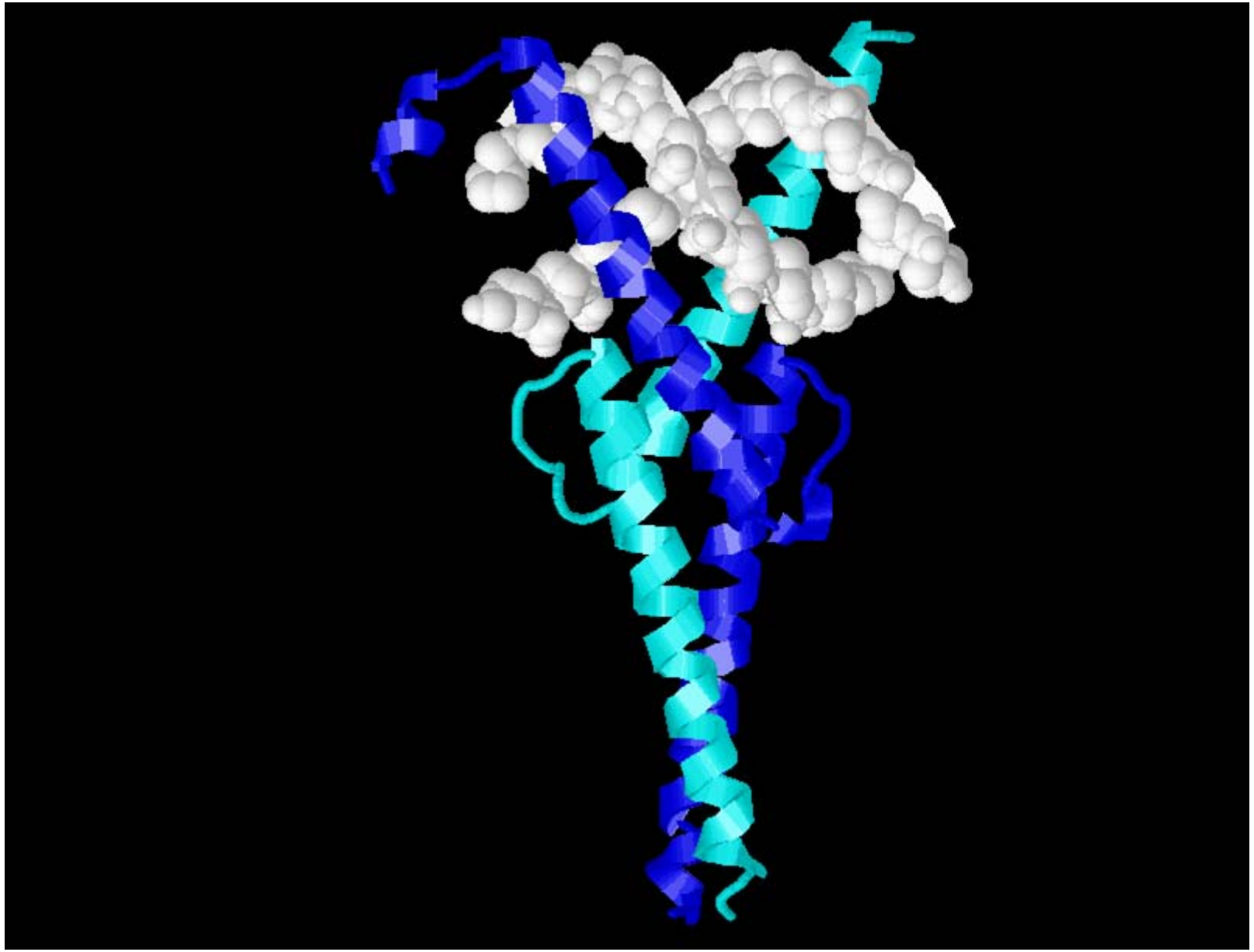
alpha-Helix der Proteine:
alle 7 Aminosäuren weist die Helix in die gleiche Richtung



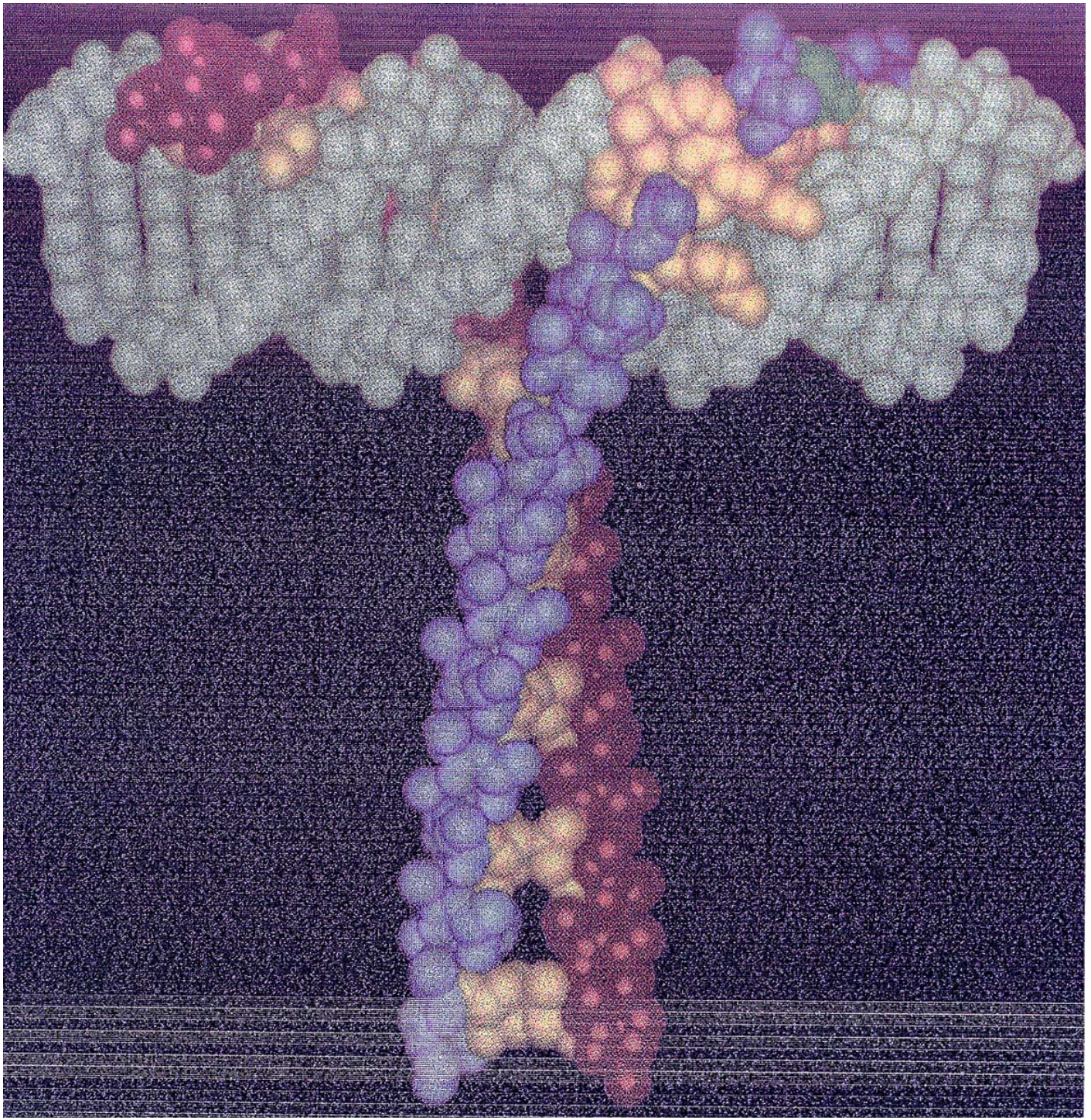
Alpha-Helix



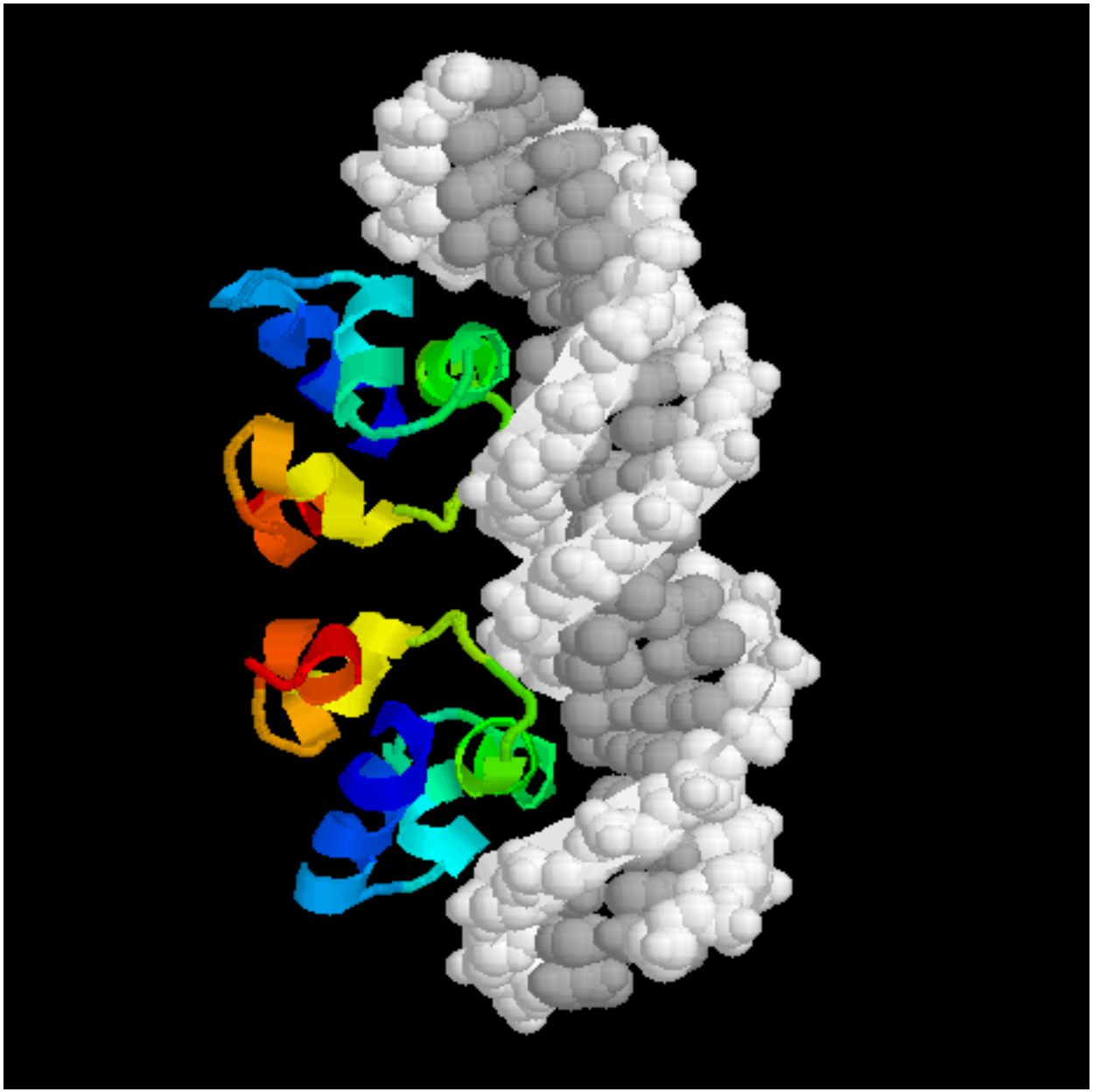
Leucin-Zipper



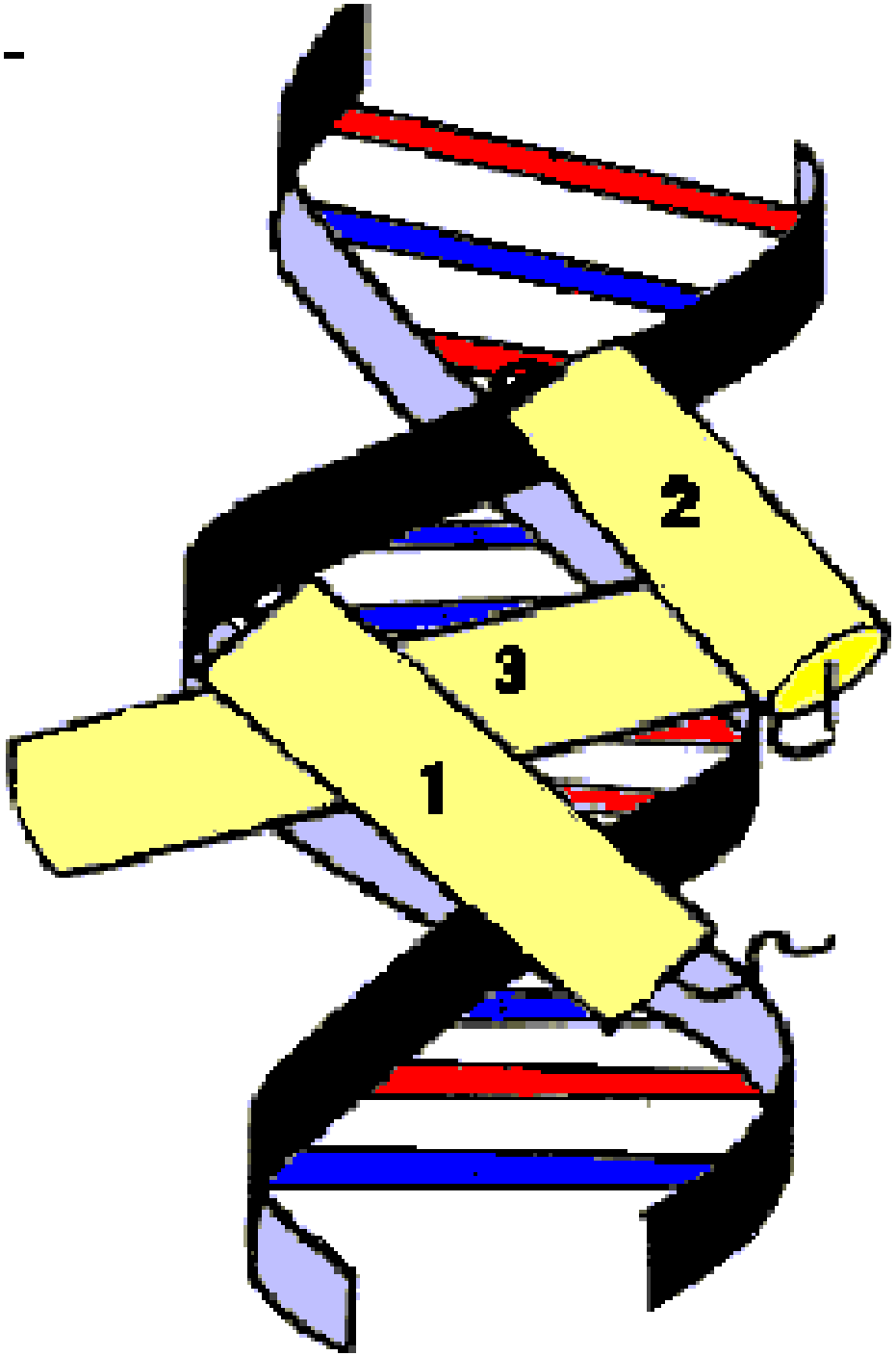
Leucin „Zipper“



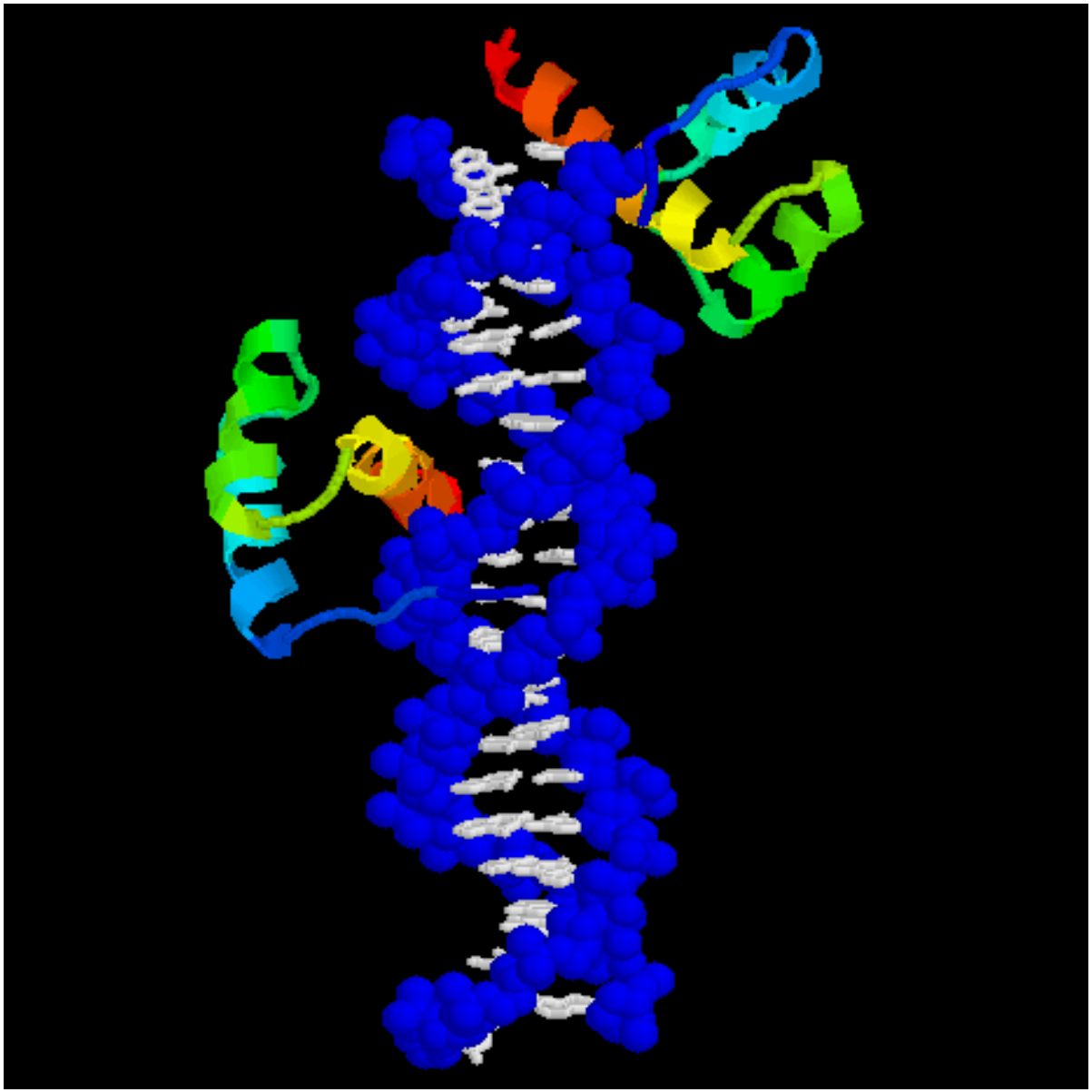
Helix-turn-Helix-Protein



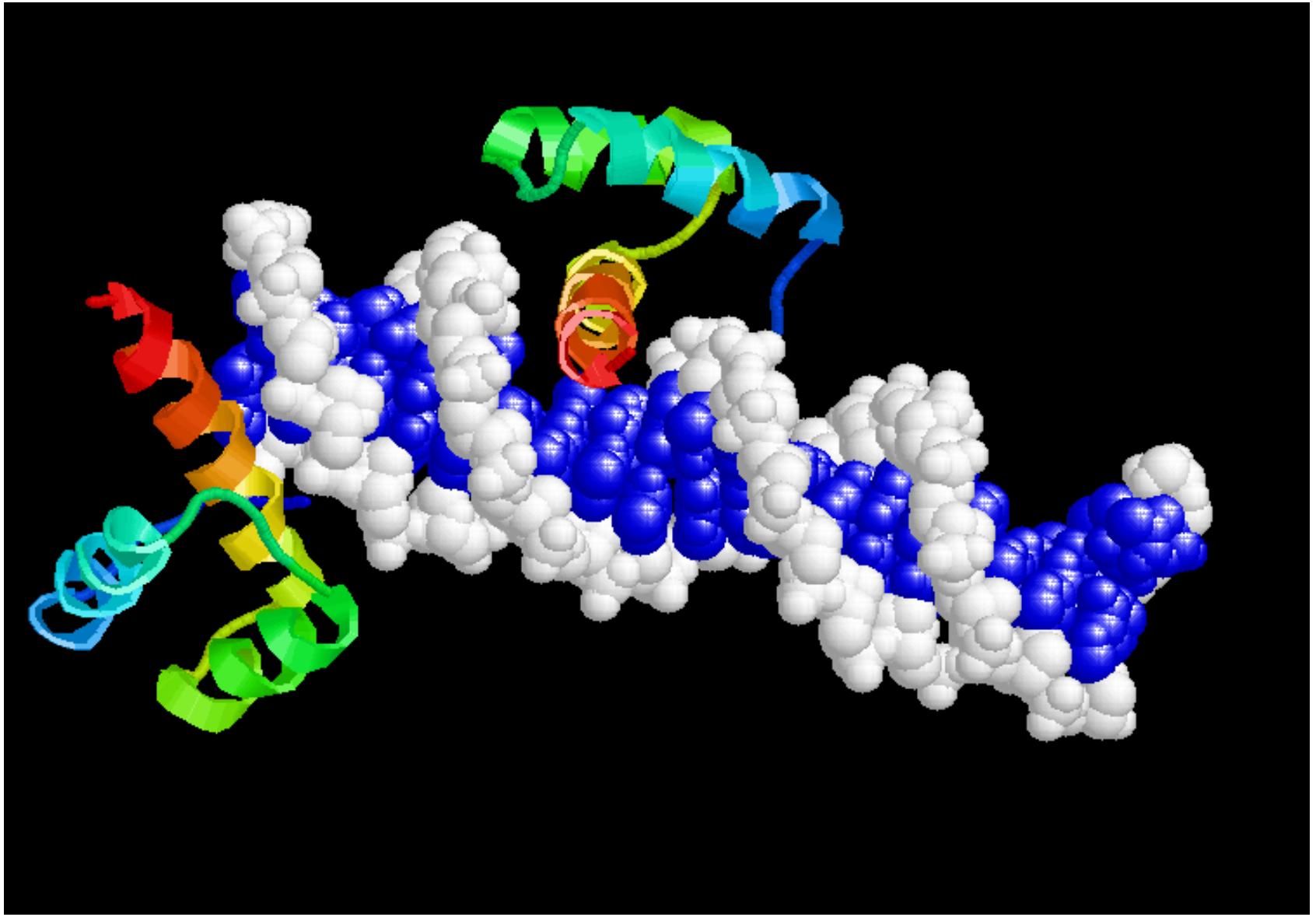
Helix-turn-Helix-Proteine



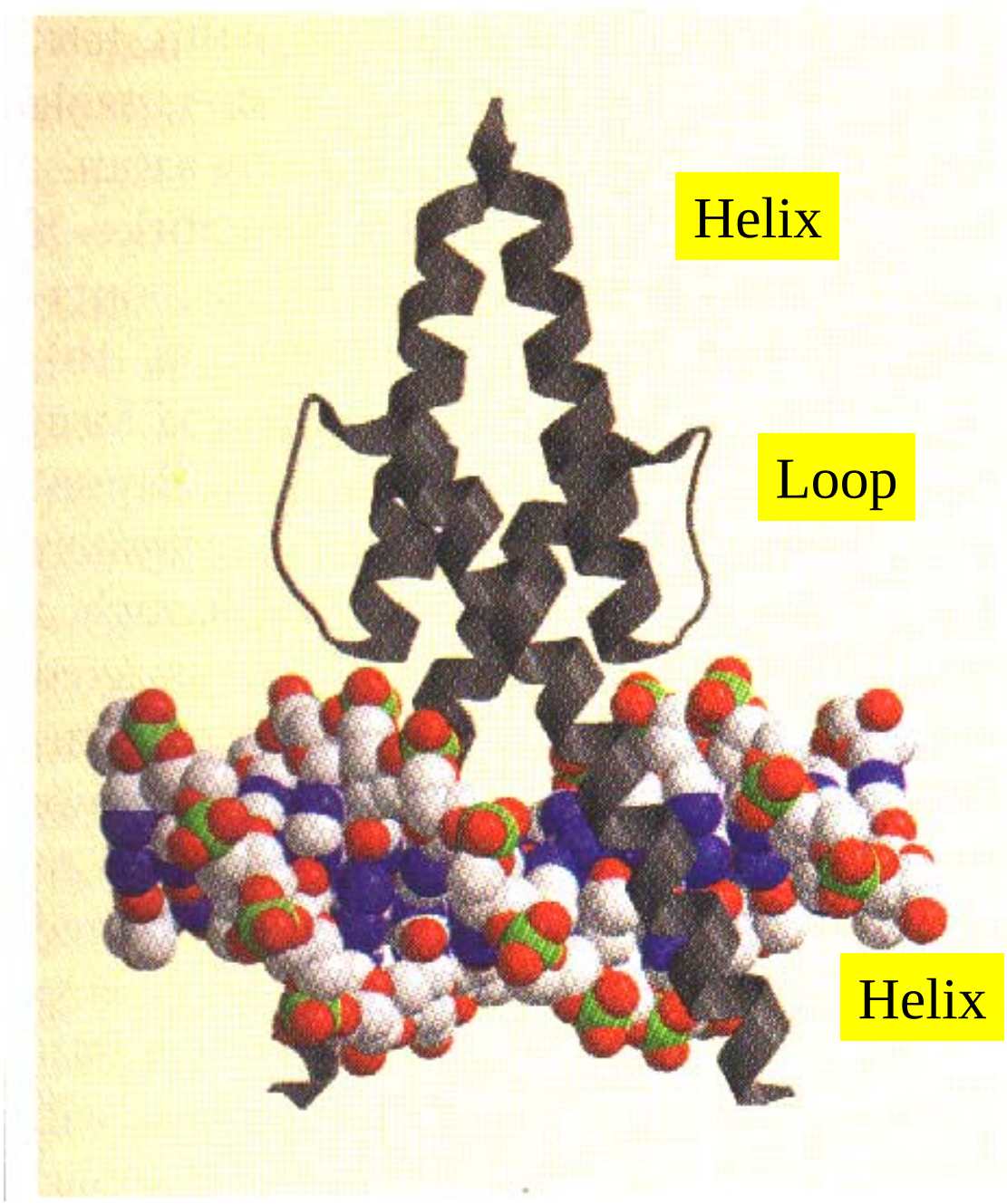
Homeodomän-Protein



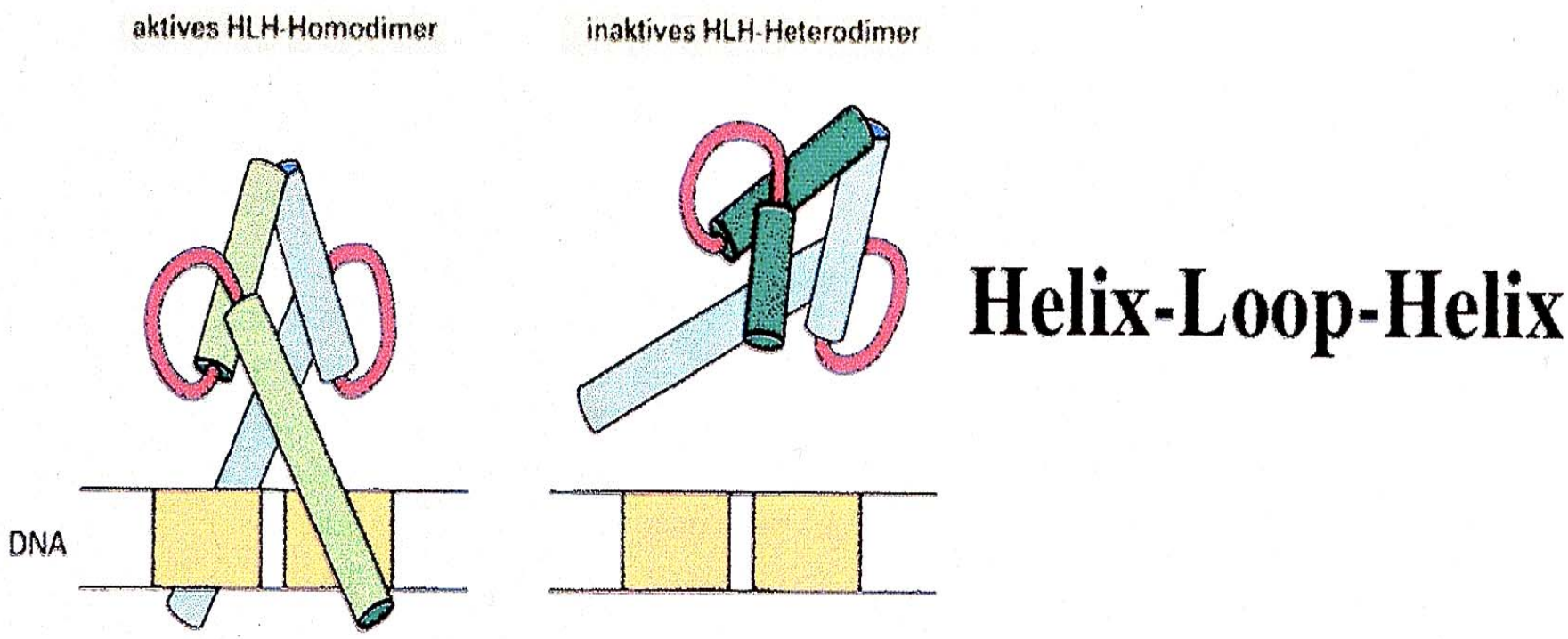
Drosophila TF „engrailed“



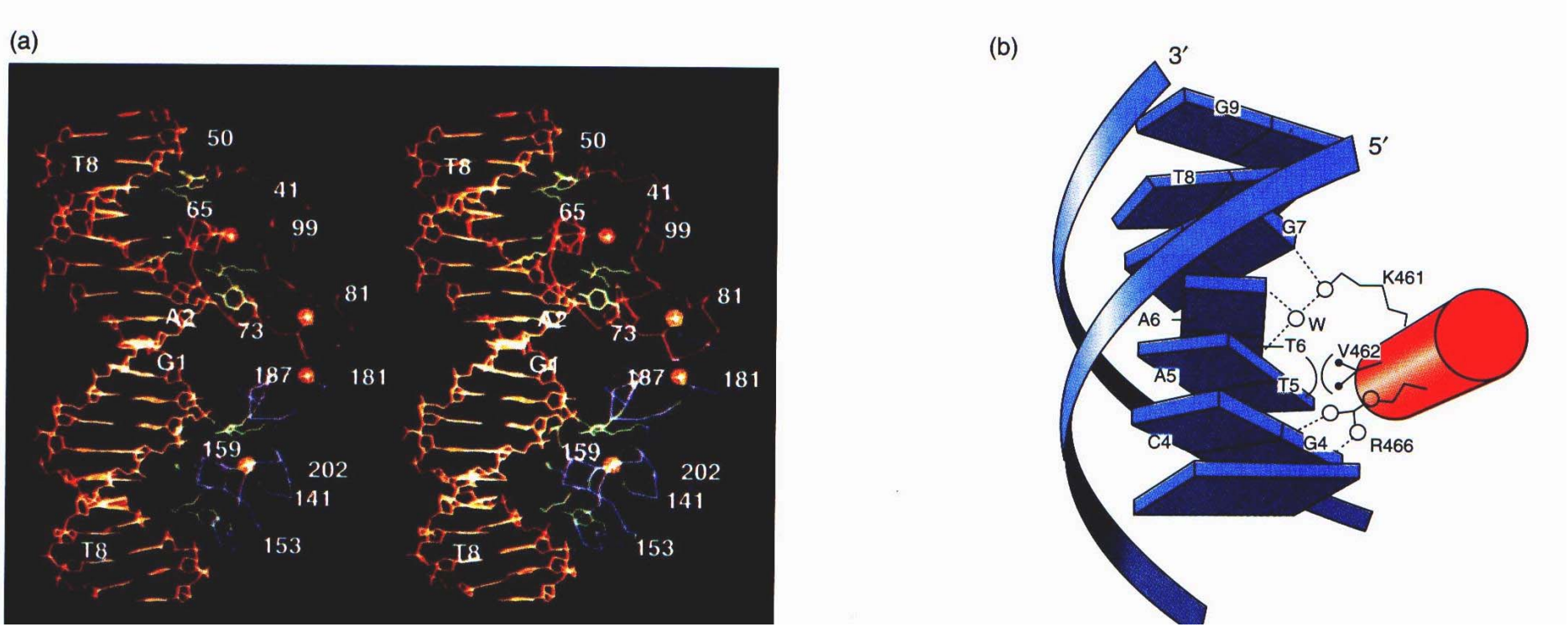
DNA-binde-Proteine: Basisches Helix-loop-Helix-Protein (bHLH)



Basisches Helix-Loop-Helix

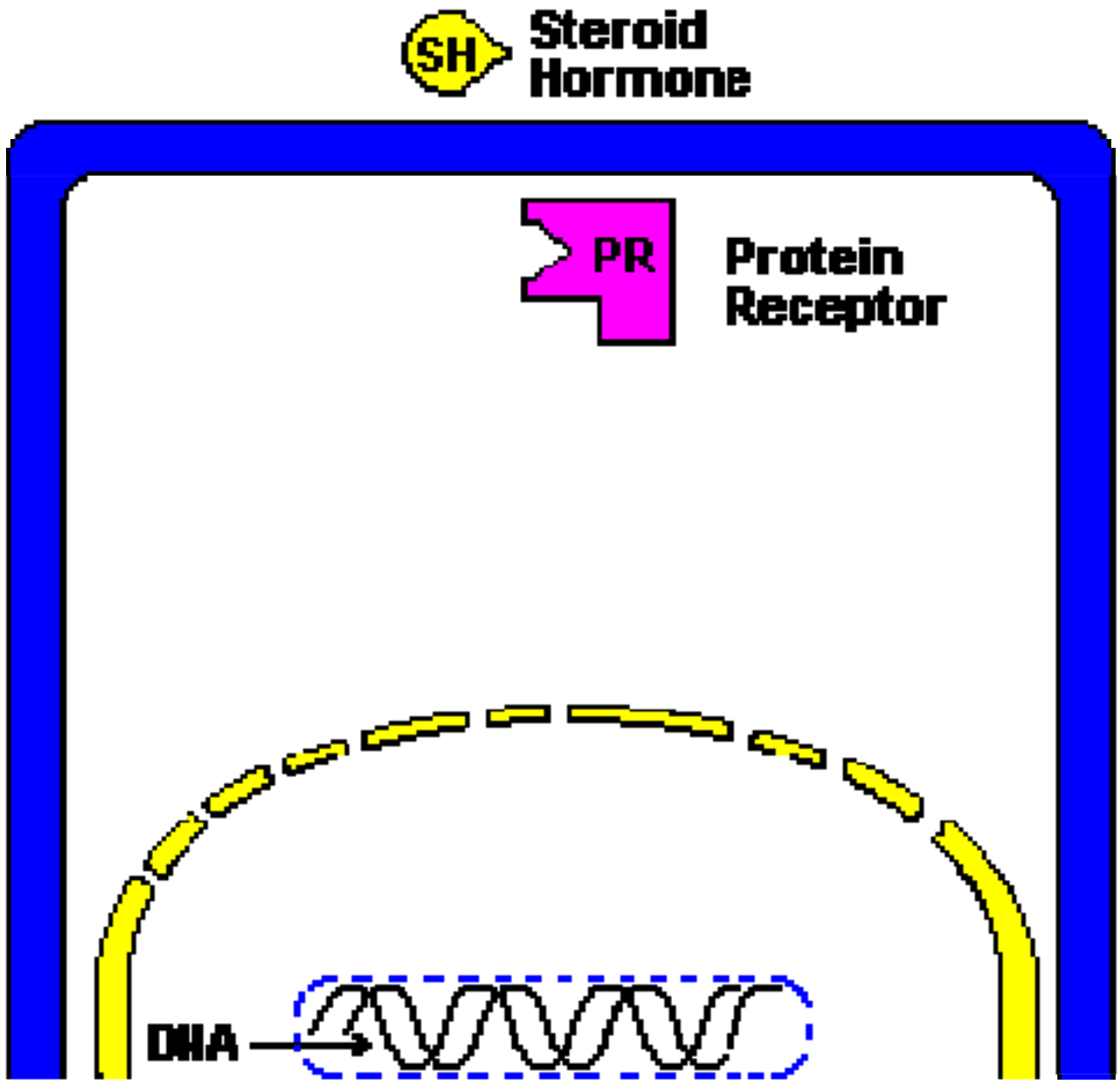


H-Brückenbindungen zwischen Aminosäuren des Proteins und Basen der DNA über die große Grube stellen die sequenzspezifische Bindung sicher



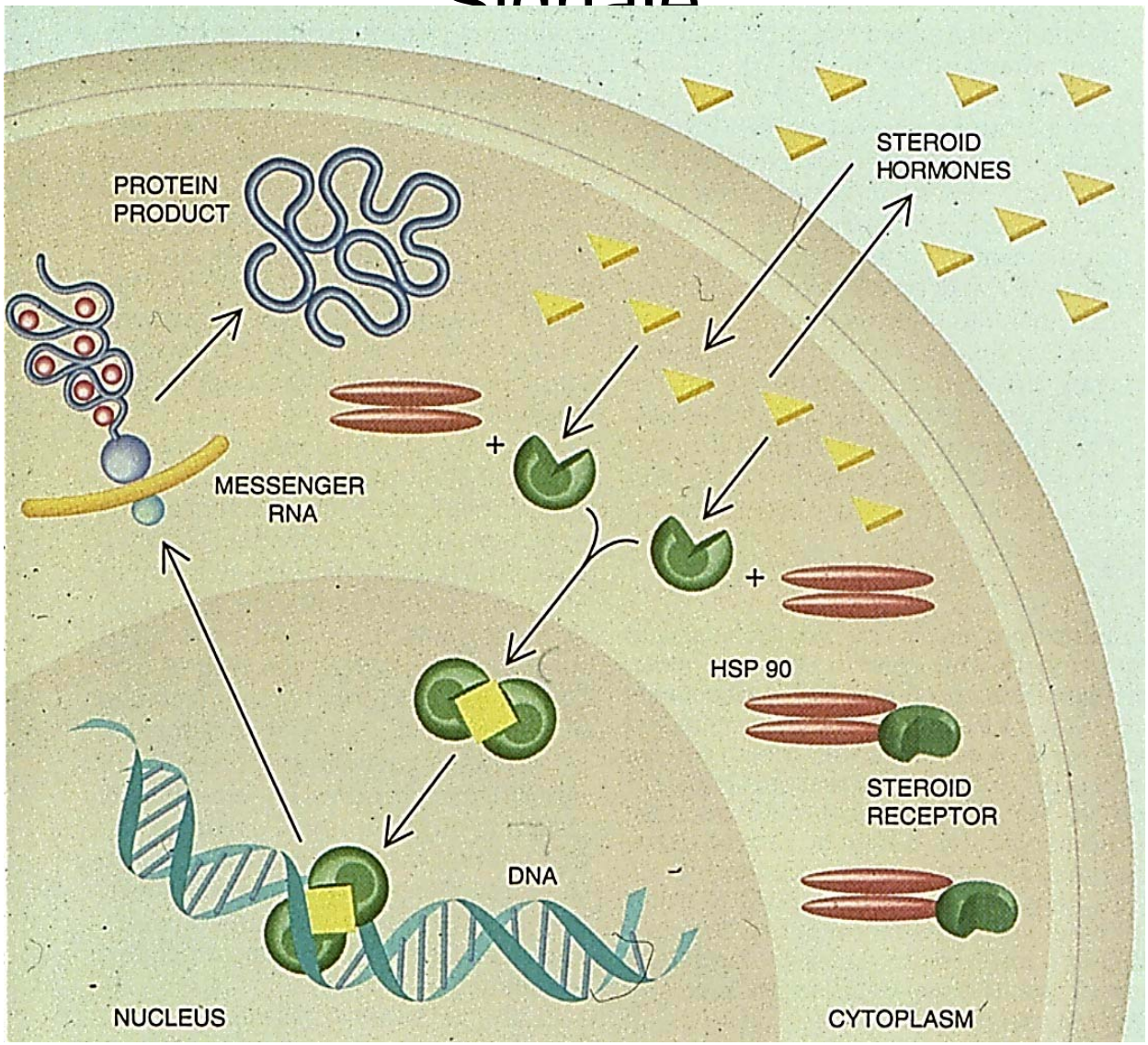
Die Aminosäuren der DNA-bindenProteine interagieren über die große Grube direkt mit den Basen der DNA über H-Brückenbindungen

Genregulation durch Steroidhormon



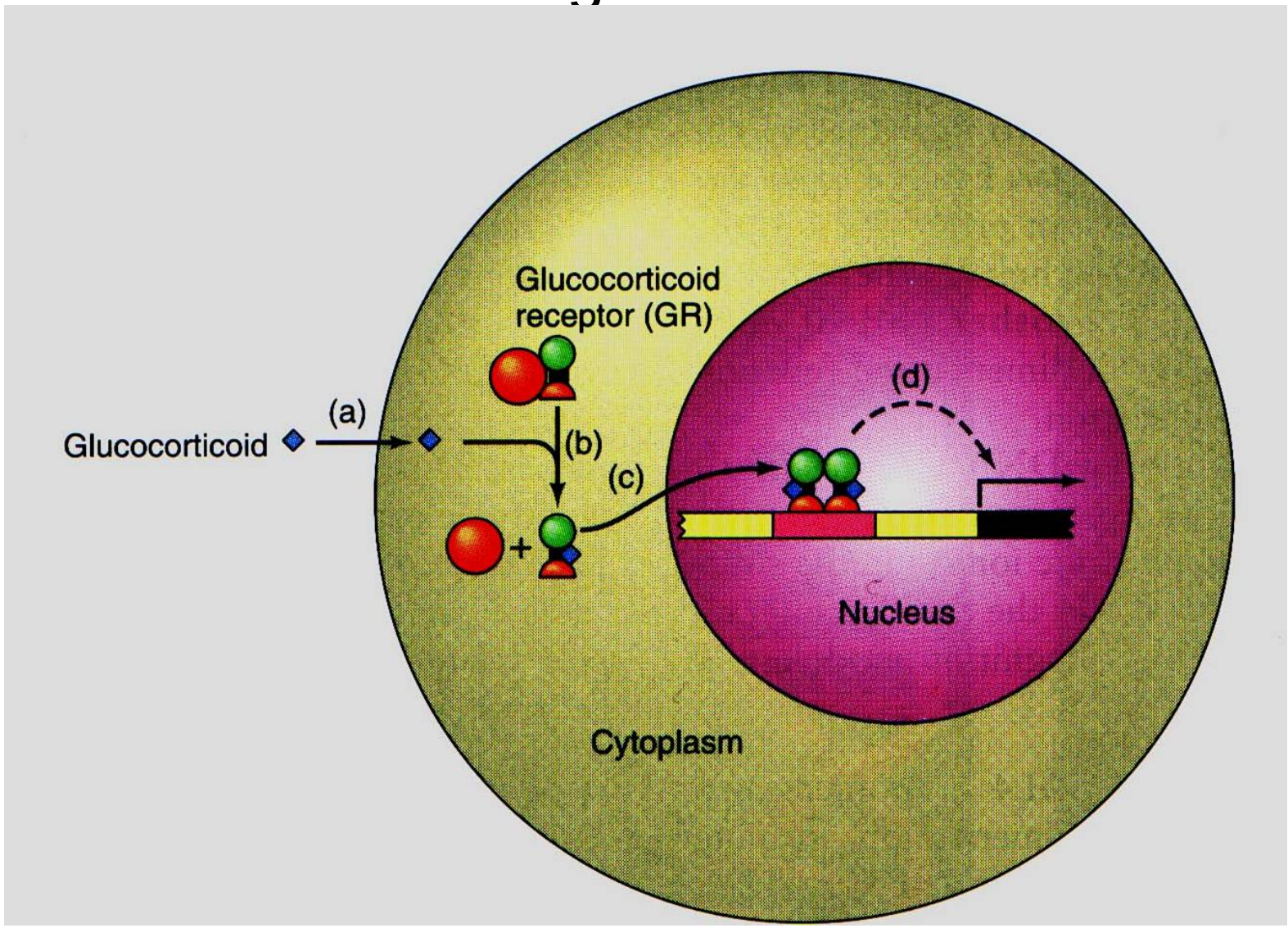
Hormon induzierte Genaktivität

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

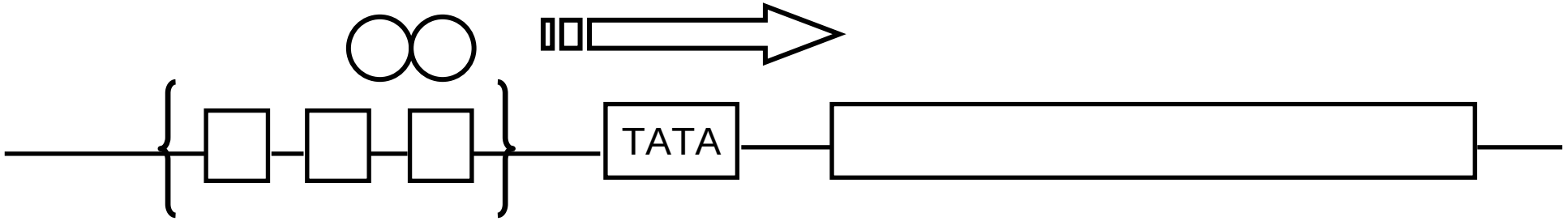
Binding of hormone

Dissociation from hsp90

Dimerisation

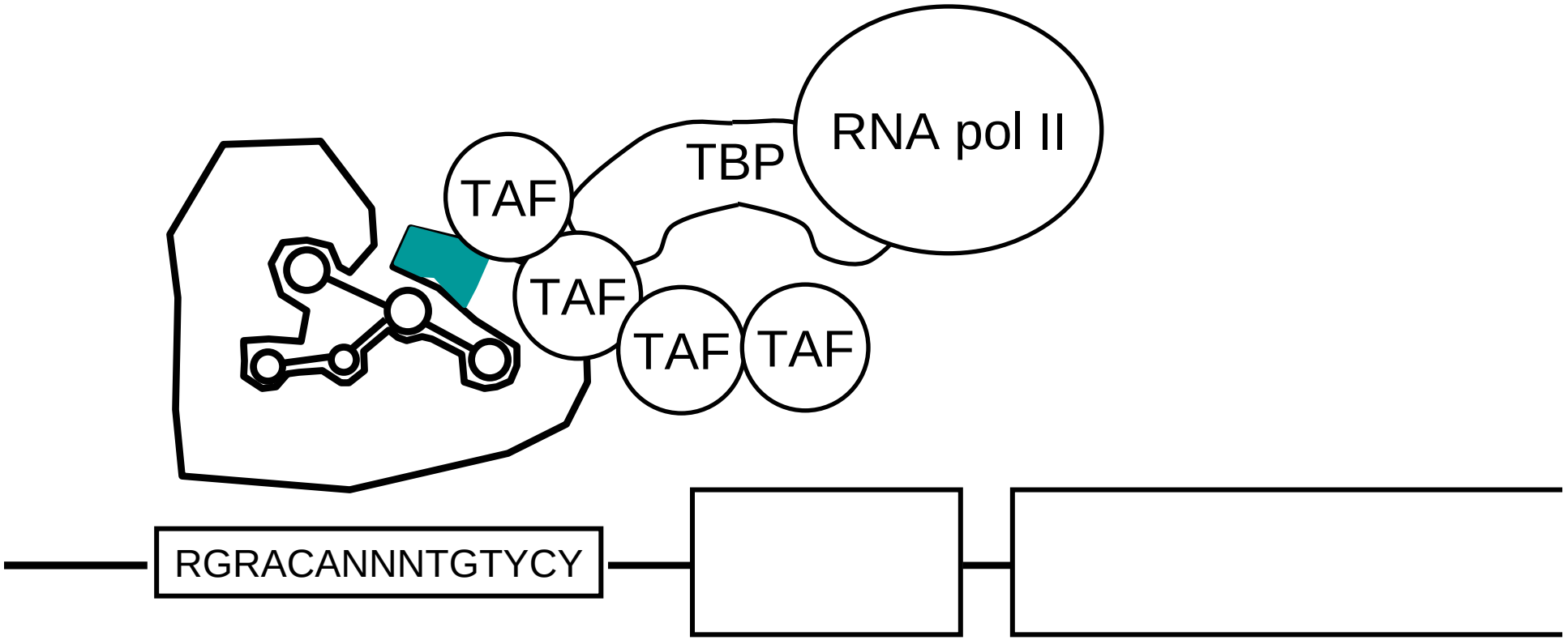
Migration to nucleus

Binding to URE

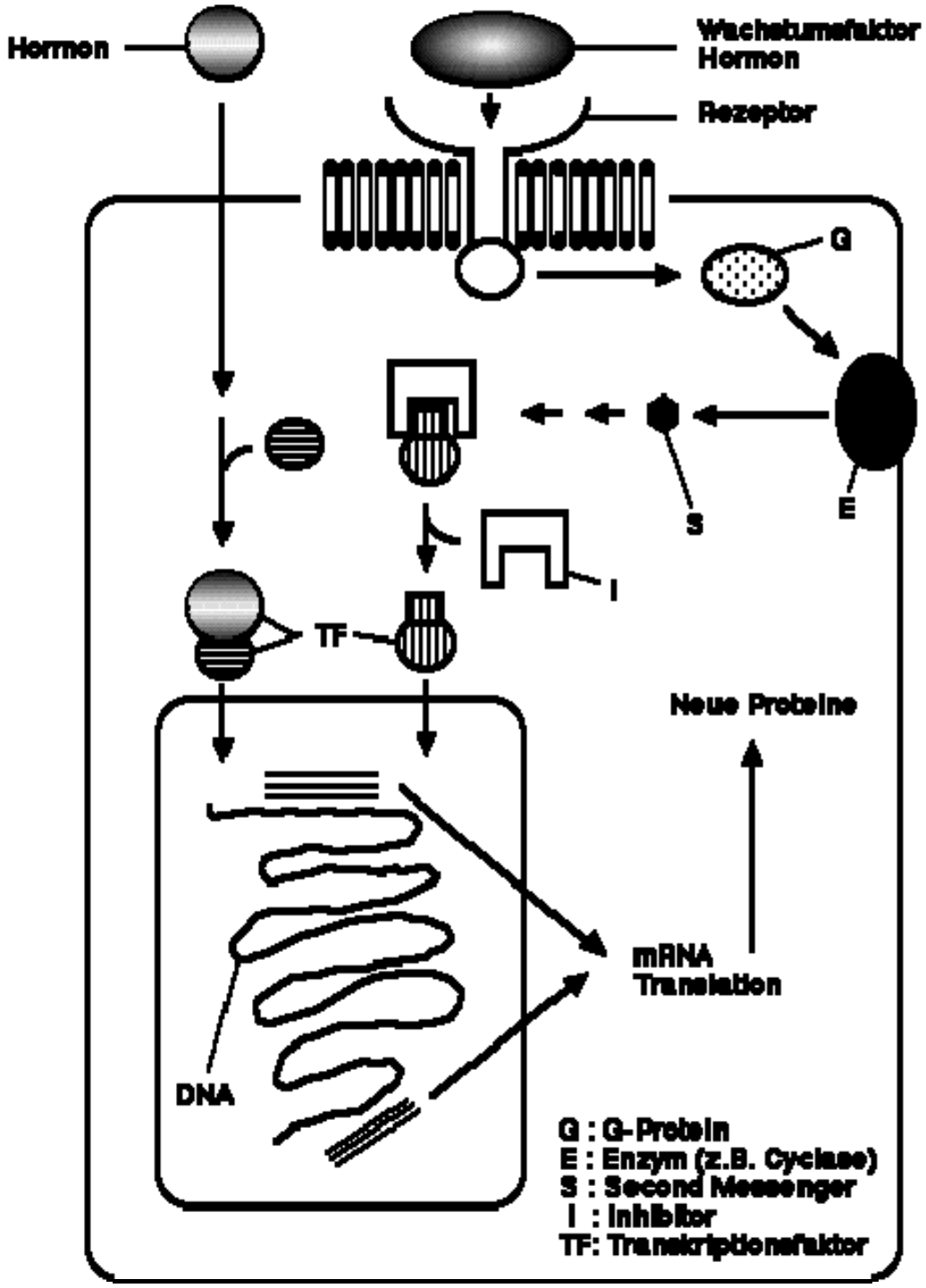


Nuclear Receptors

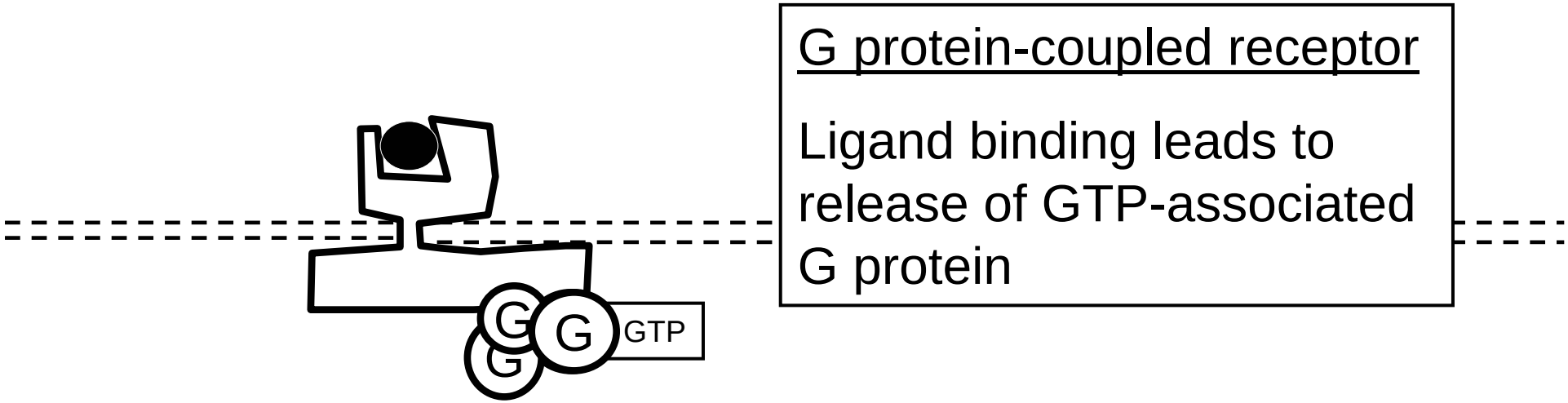
Binding of ligand causes conformational change allowing transactivation domain to interact with transcriptional machinery



Hormon-regulation von Genen



cAMP signalling

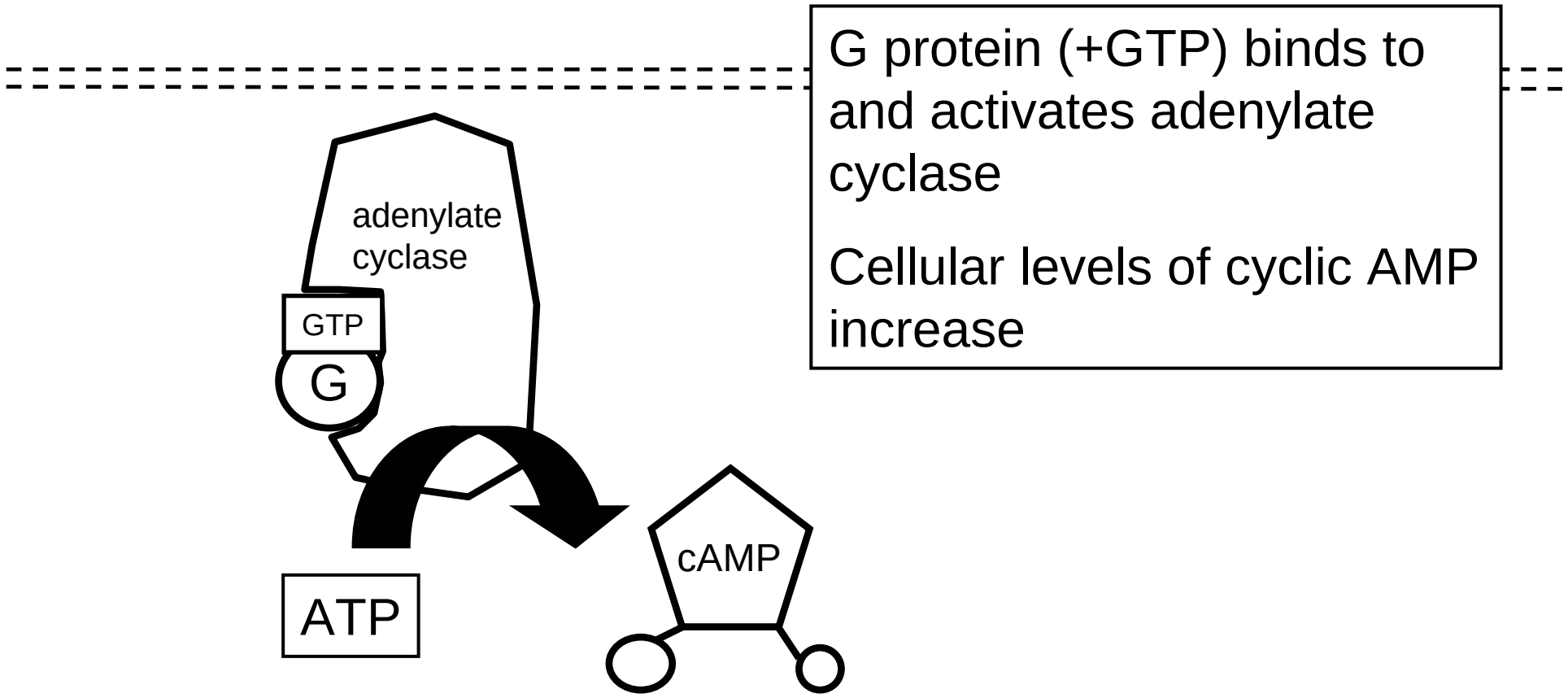


Small GTP-binding proteins

Example: ras – covered in earlier lecture

Alternate between inactive GDP and active GTP bound forms

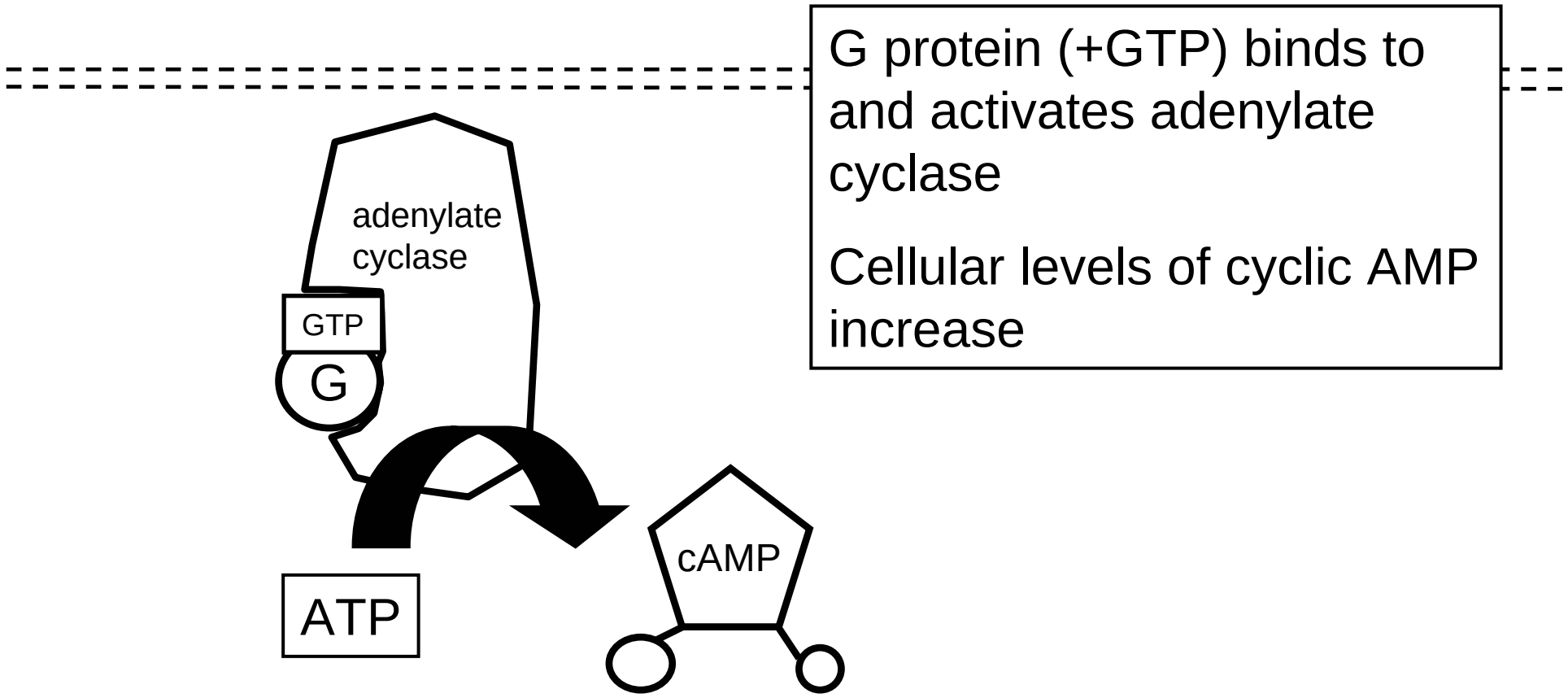
cAMP signalling



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

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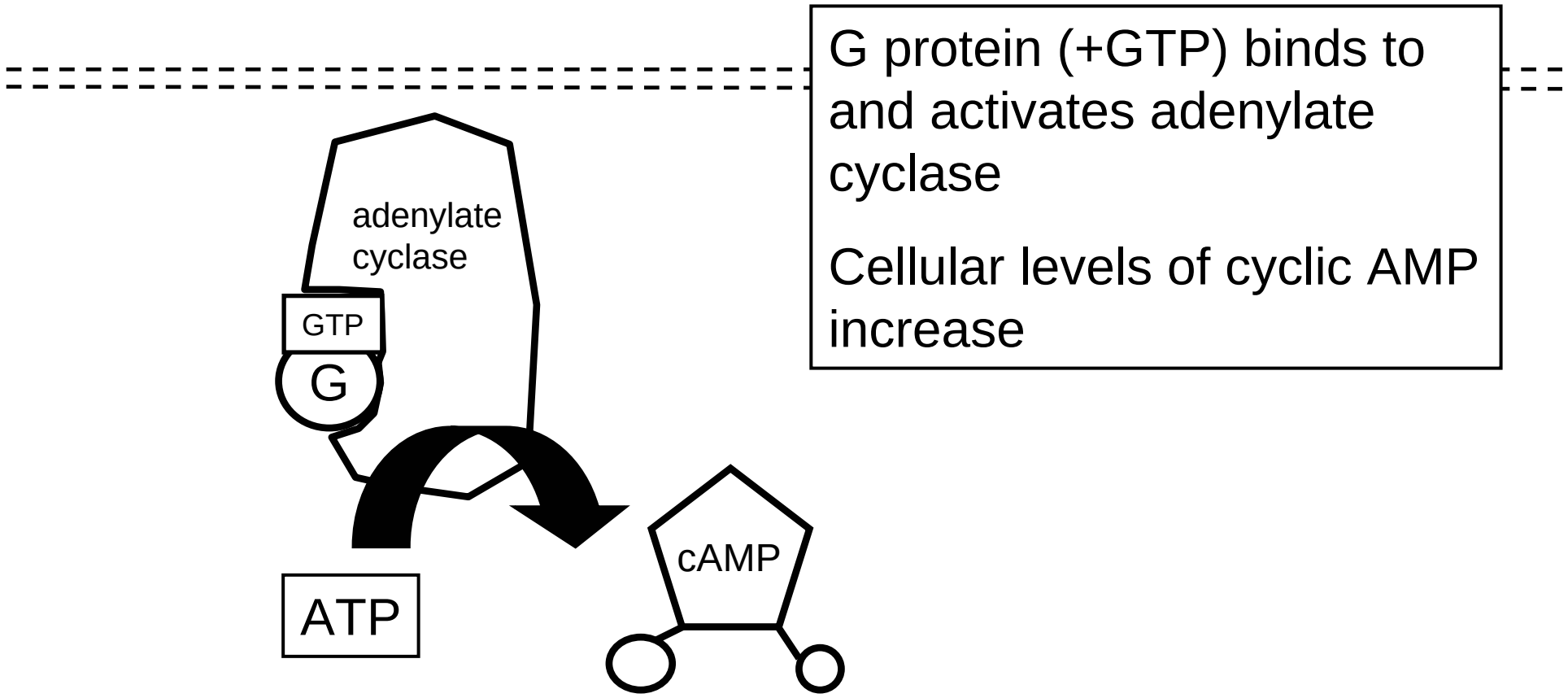
cAMP signalling



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

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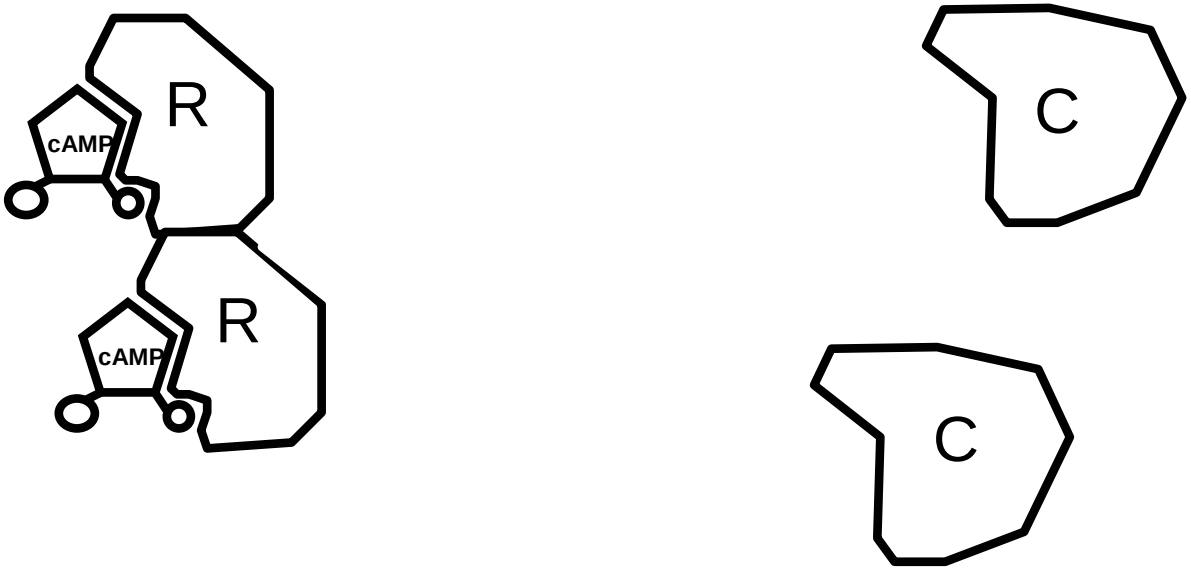
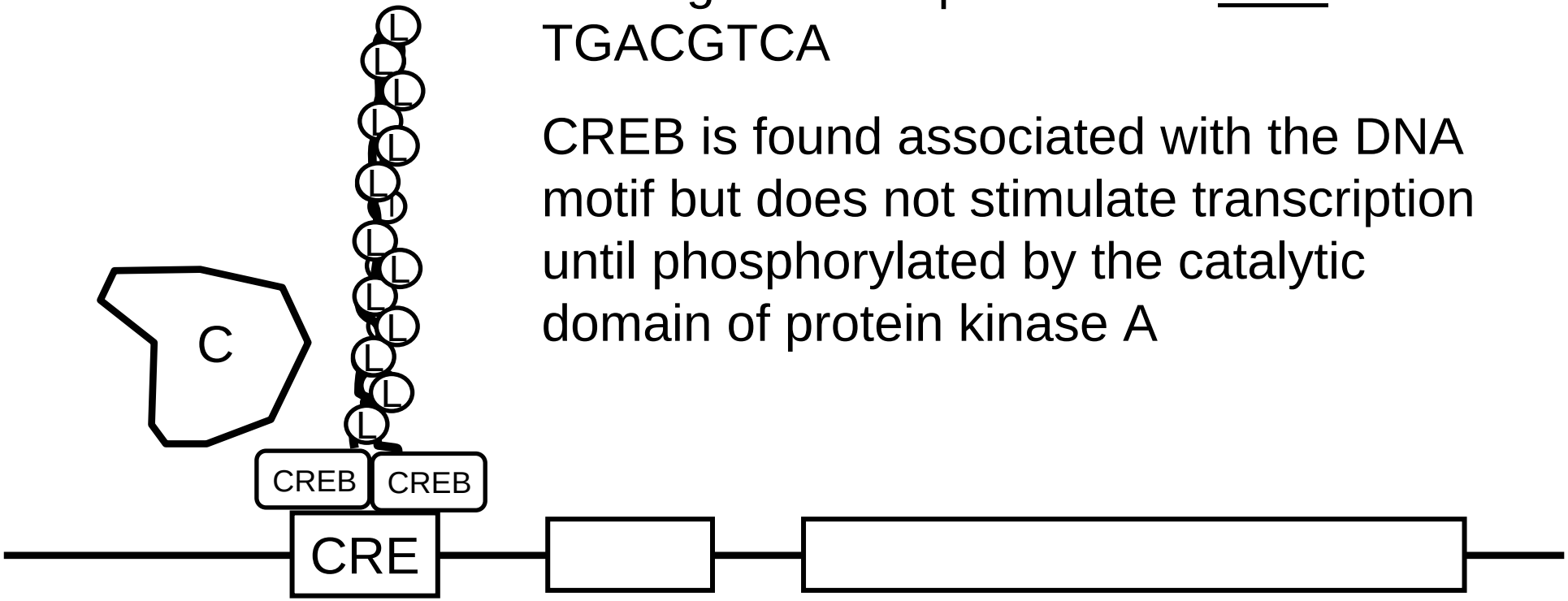


Cyclic AMP Response Element Binding protein

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

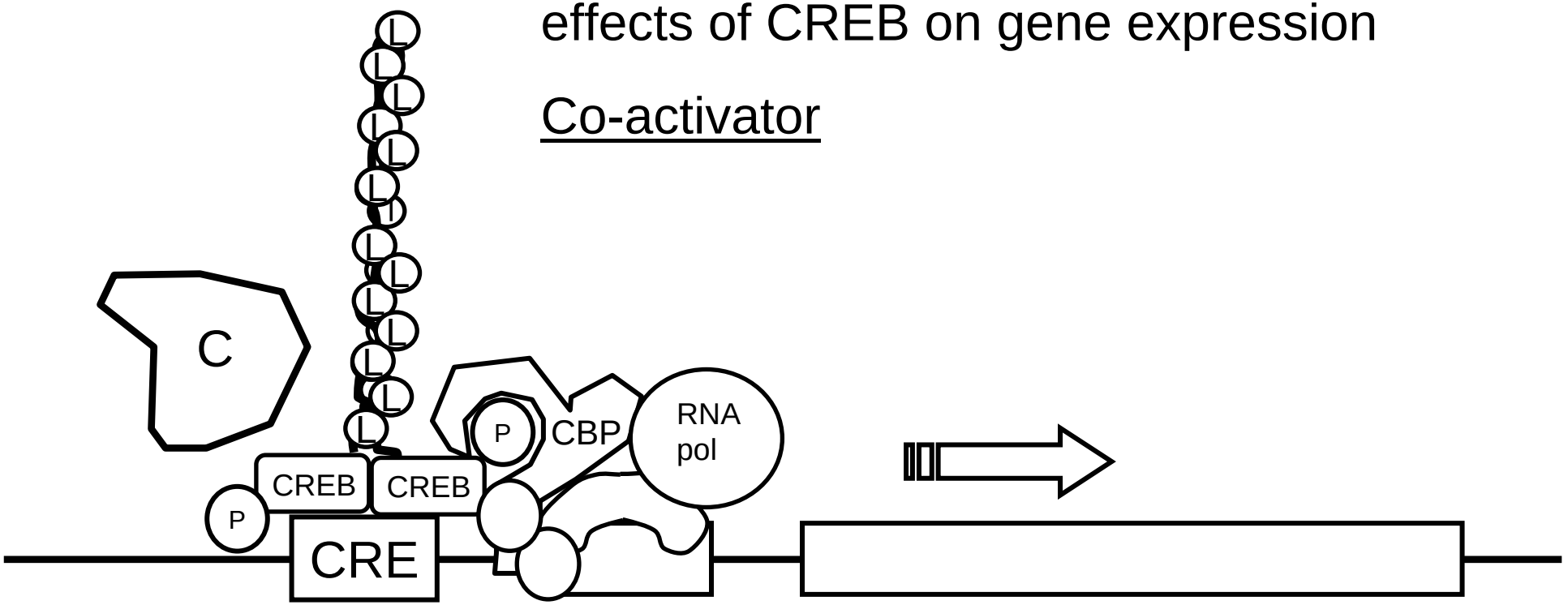


CREB binding protein

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

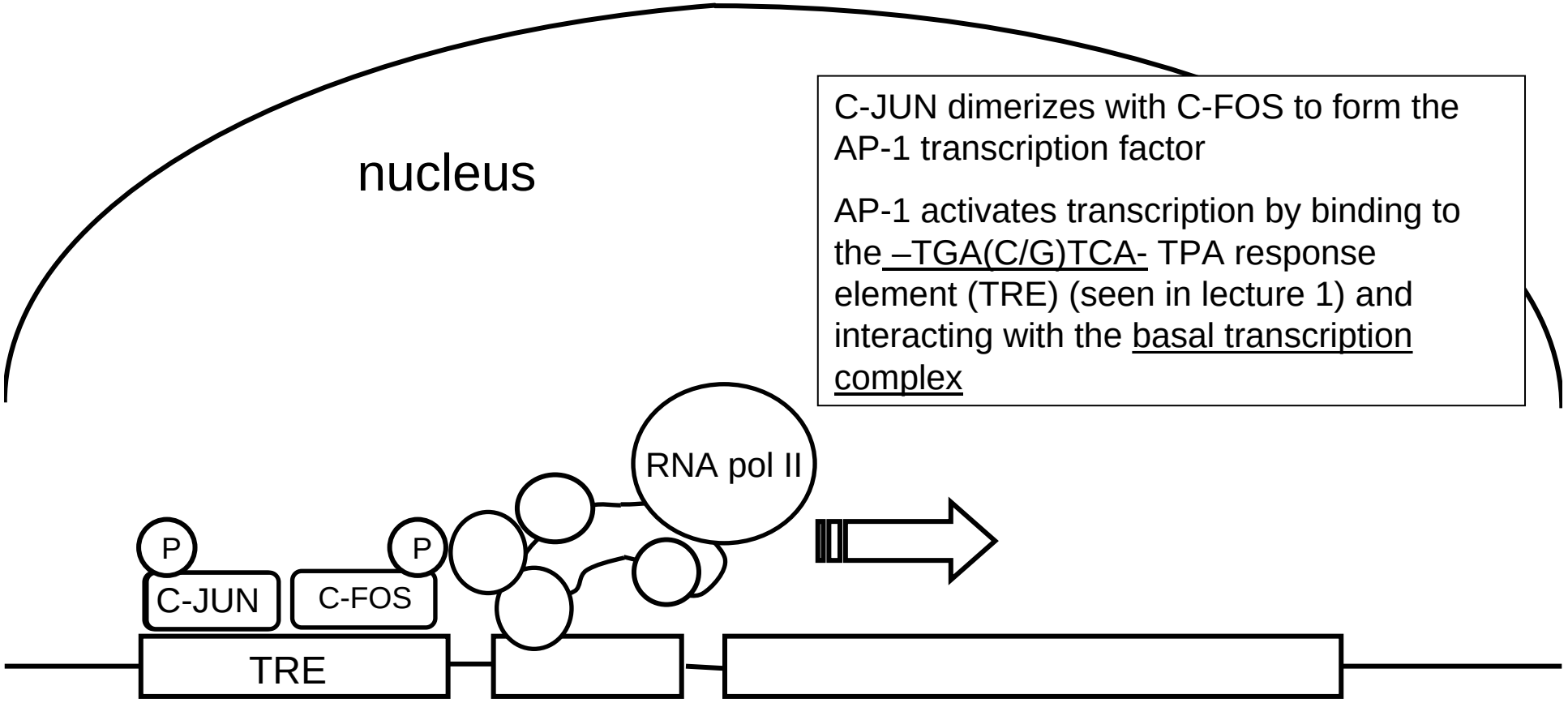


Kinase Cascades

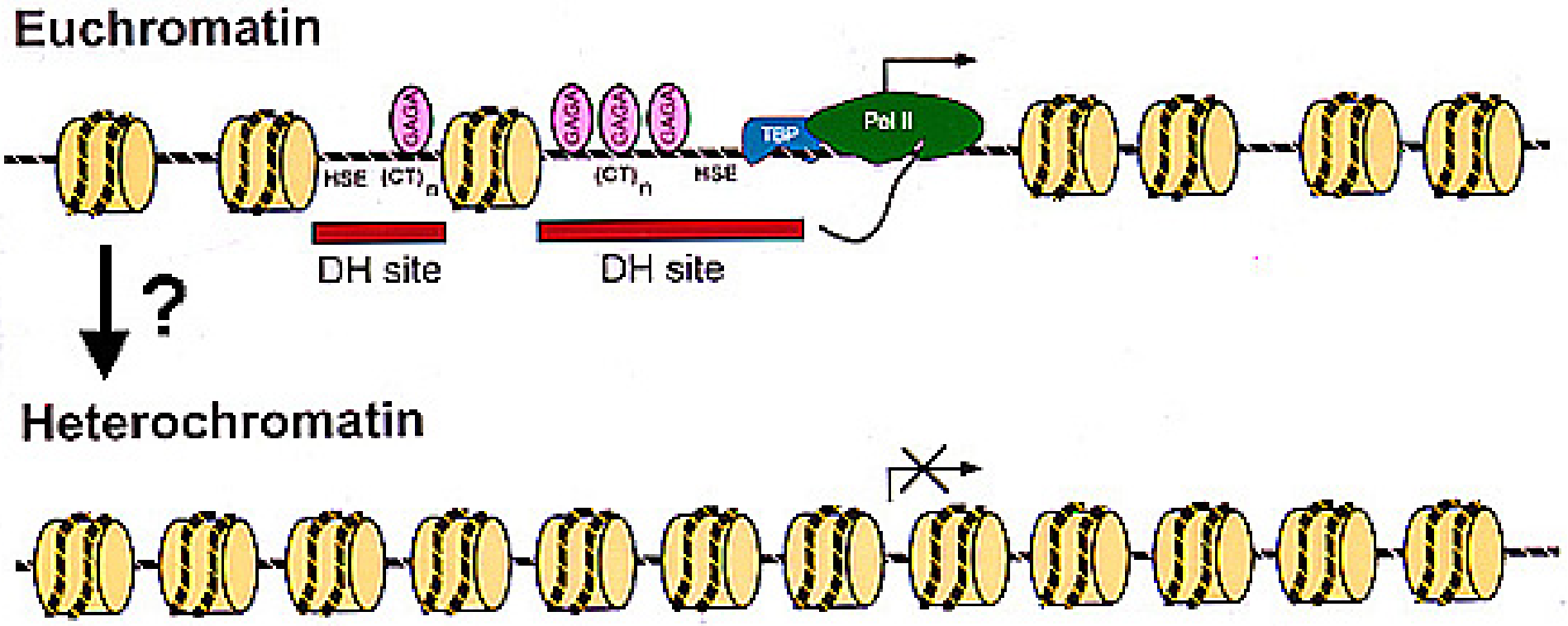
Active JNK travels to the nucleus and phosphorylates the bZIP transcription factor C-JUN

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

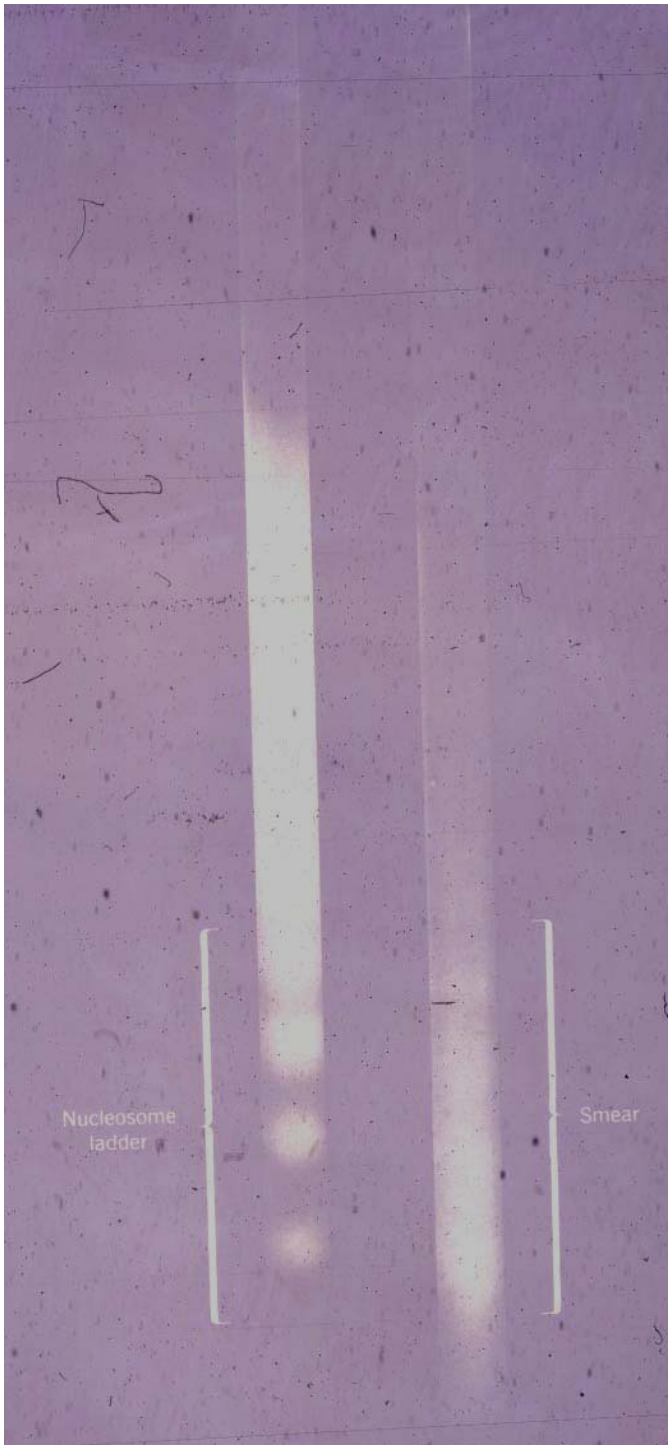
AP-1 activates transcription by binding to the –TGA(C/G)TCA- TPA response element (TRE) (seen in lecture 1) and interacting with the basal transcription complex



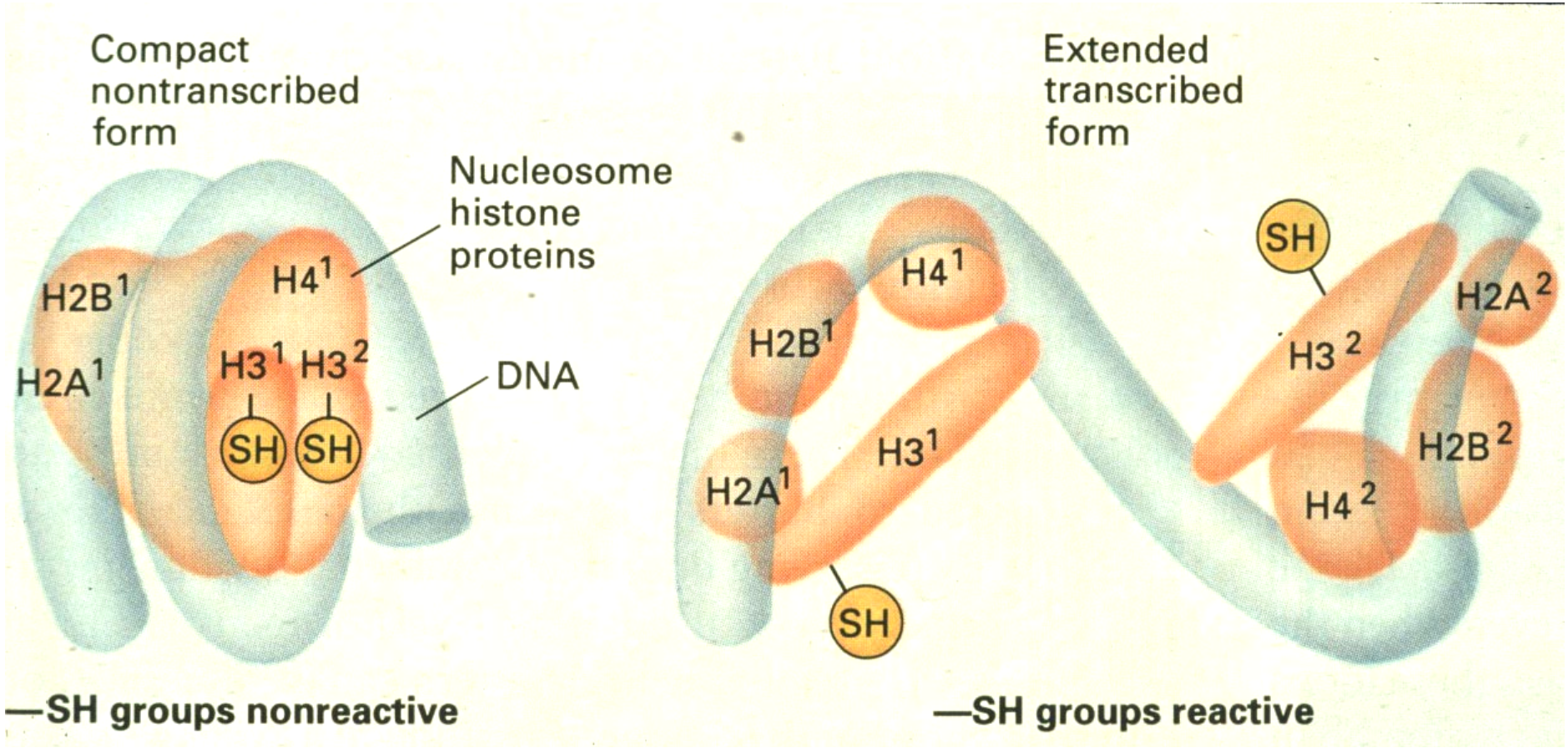
Chromatin und Genaktivität

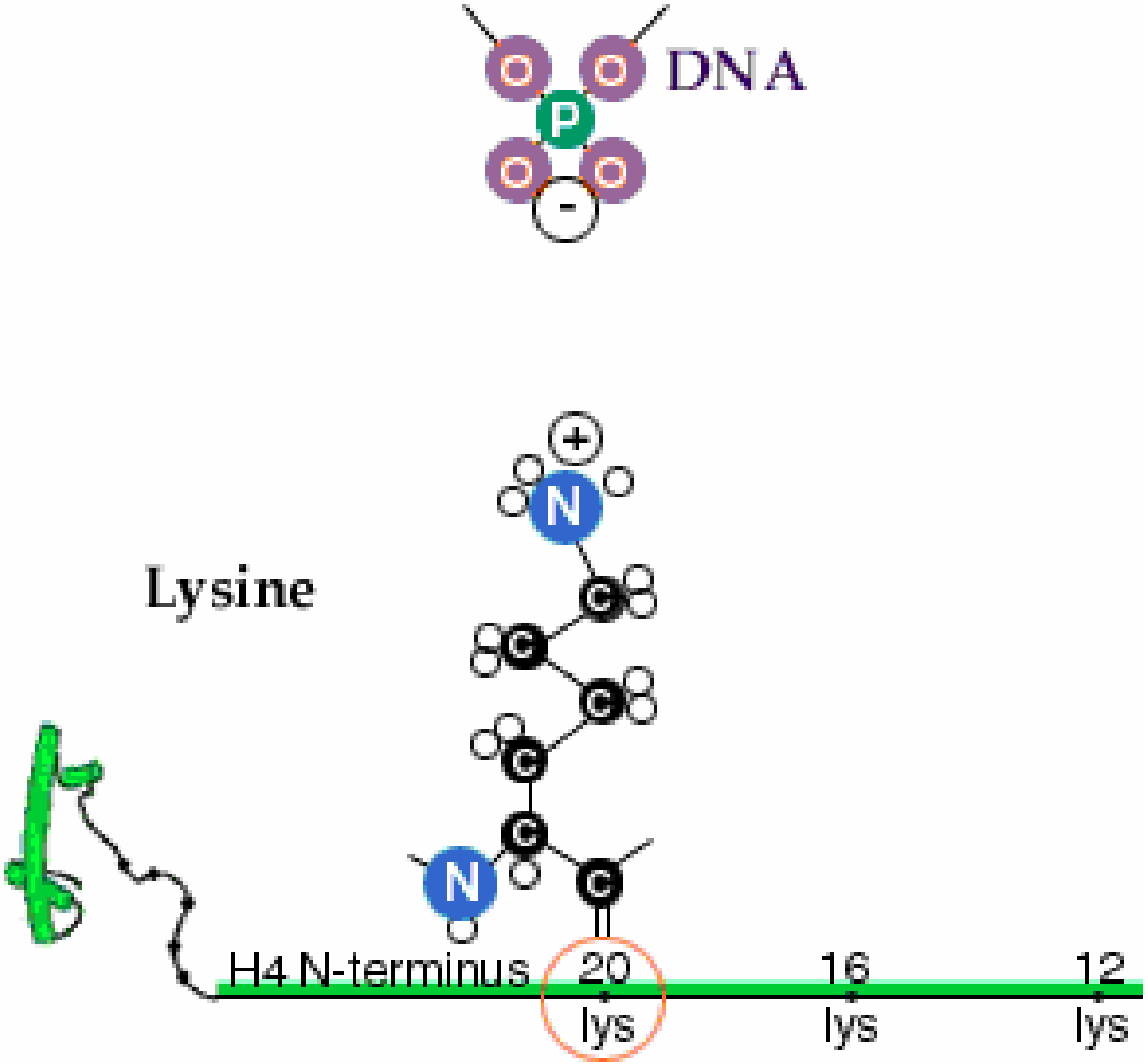


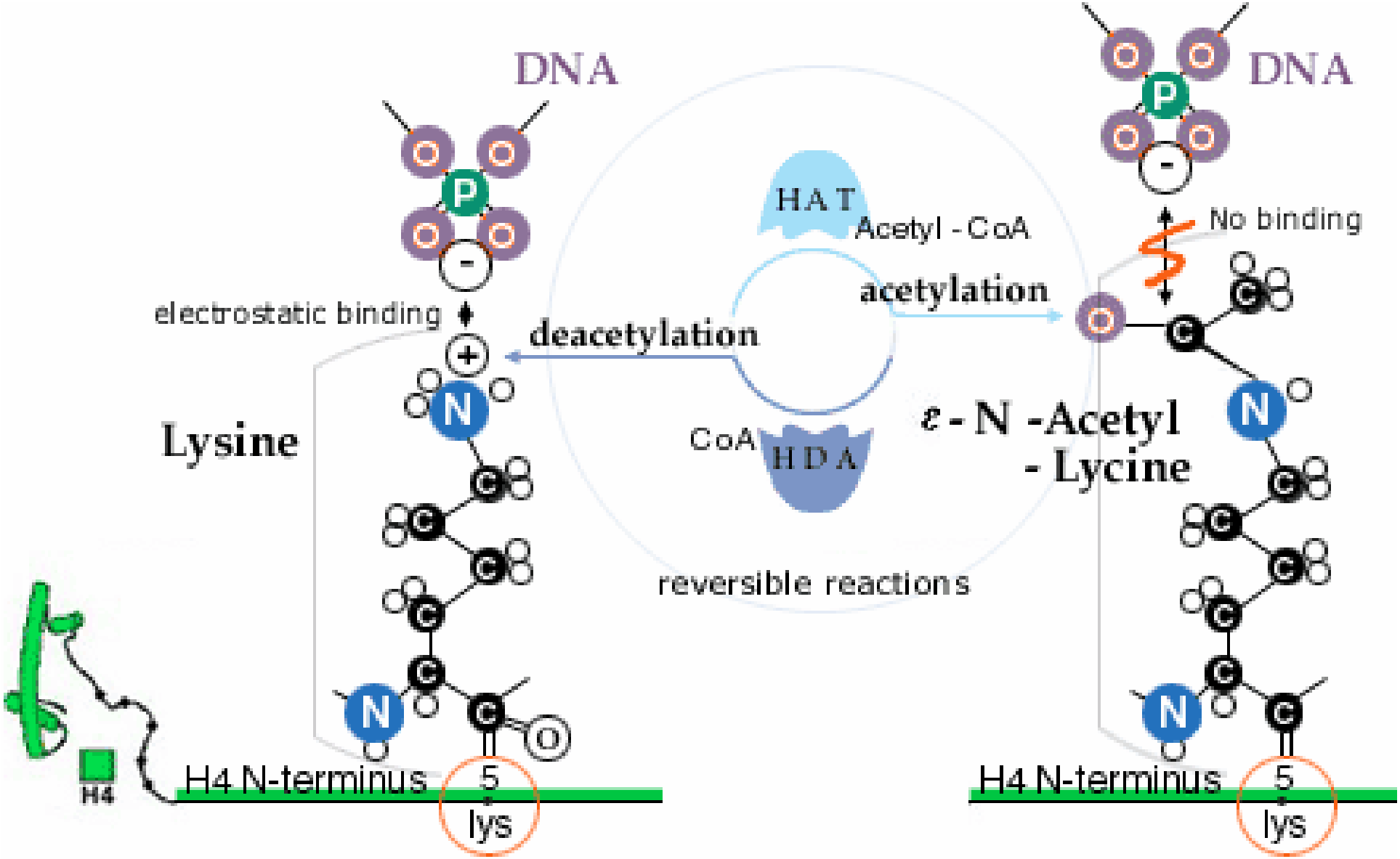
Aktives Chromatin ist nicht in Nukleosomen organsisiert

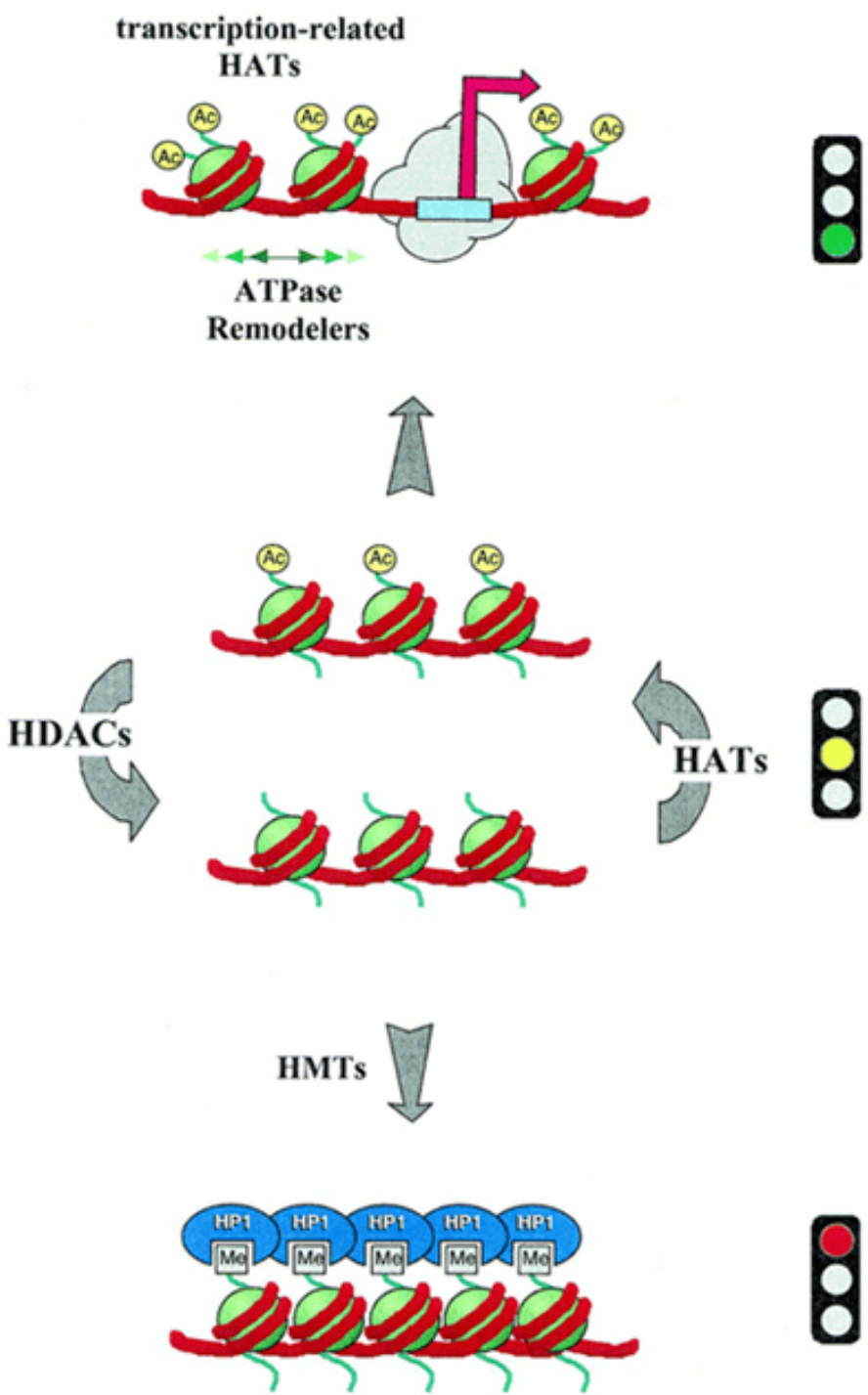


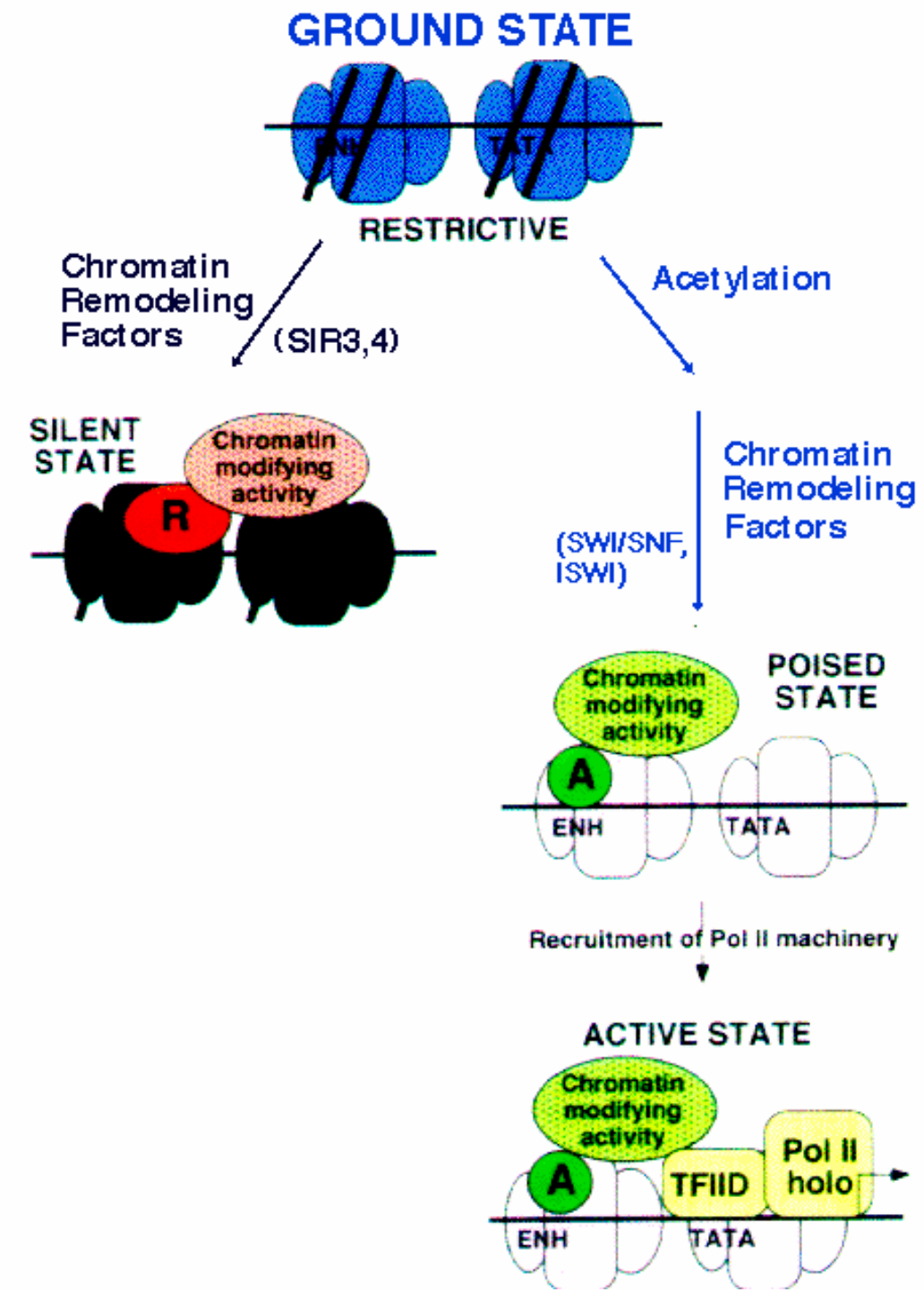
Inaktive / Aktives Chromatin

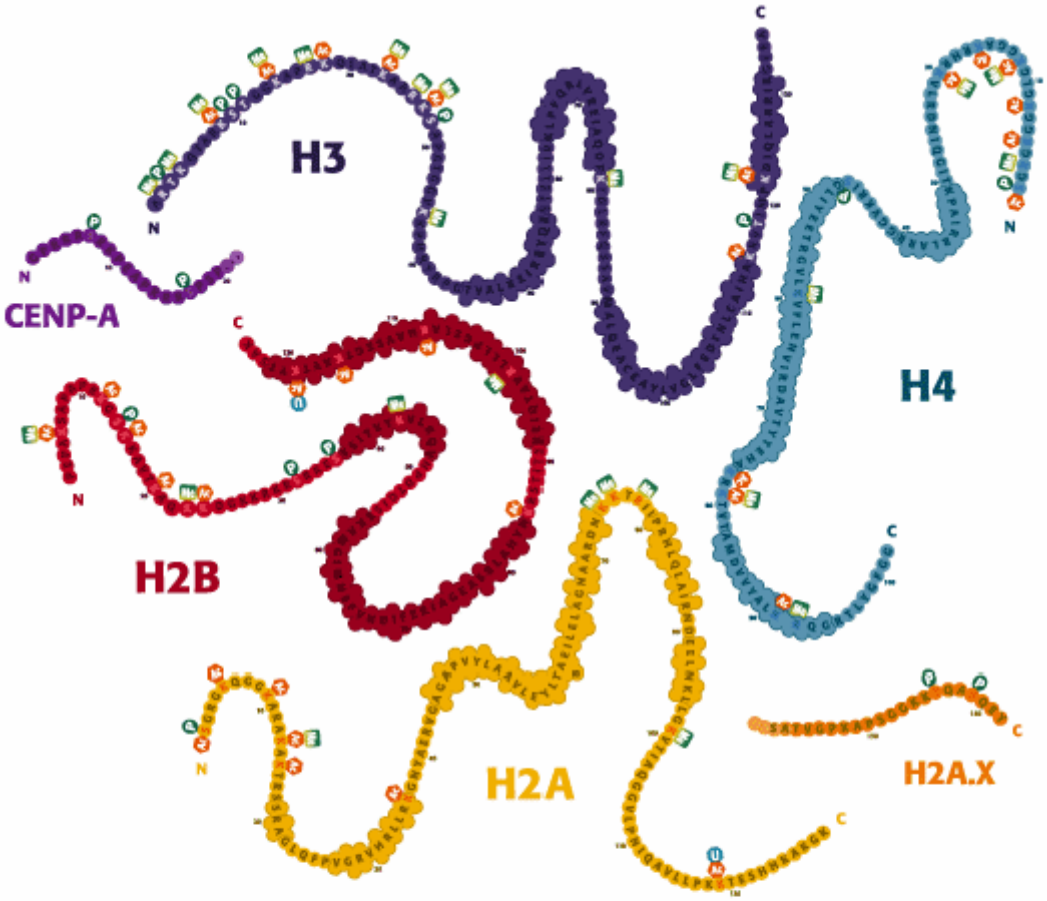



DNA
Lysine
H4 N-terminus
20
lys
16
lys
12
lys


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


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


GROUND STATE
ENH
TATA
RESTRICTIVE
Chromatin
Remodeling
Factors
(SIR3,4)
Acetylation
SILENT
STATE
Chromatin
modifying
activity
R
(SWI/SNF,
ISWI)
Chromatin
Remodeling
Factors
Chromatin
modifying
activity
POISED
STATE
A
ENH
TATA
Recruitment of Pol II machinery
ACTIVE STATE
Chromatin
modifying
activity
A
TFIID
Pol II
holo
ENH
TATA



Histone Modification Map

Sequence of the four human core histones with published post-translational modifications indicated. The N-terminal sequence of the H3 variant CENP-A and the C-terminal sequence of the H2A variant H2A.X are also shown.

The enlarged and darkened sections of the sequences represent the alpha helices in the structured domains of the proteins (Luger et al, 1997. Nature 389:251-260). For clarity, interactions between histone proteins are not indicated.

To keep up with the most up-to-date modification sites, please visit **www.histone.com/modification_map.htm.**

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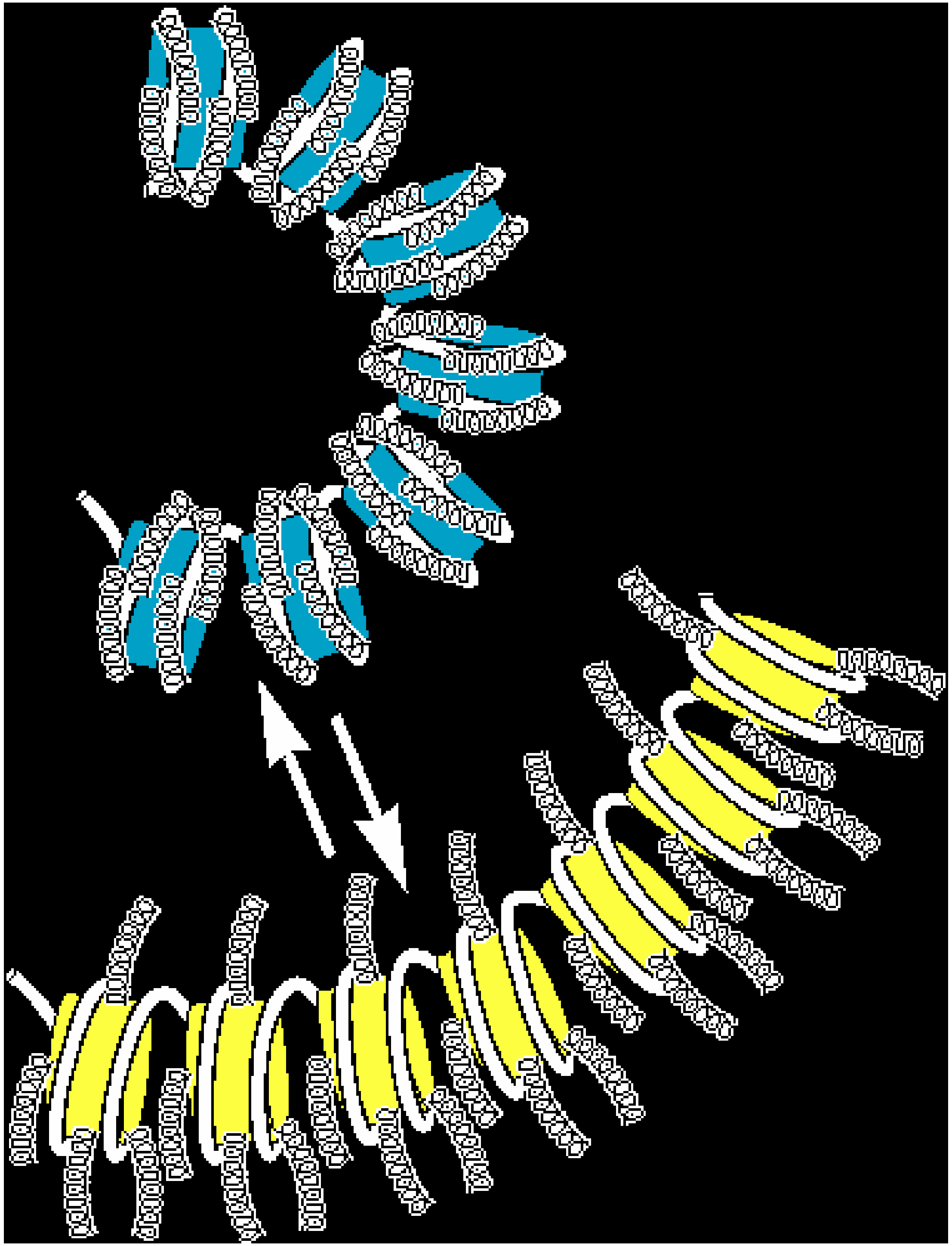
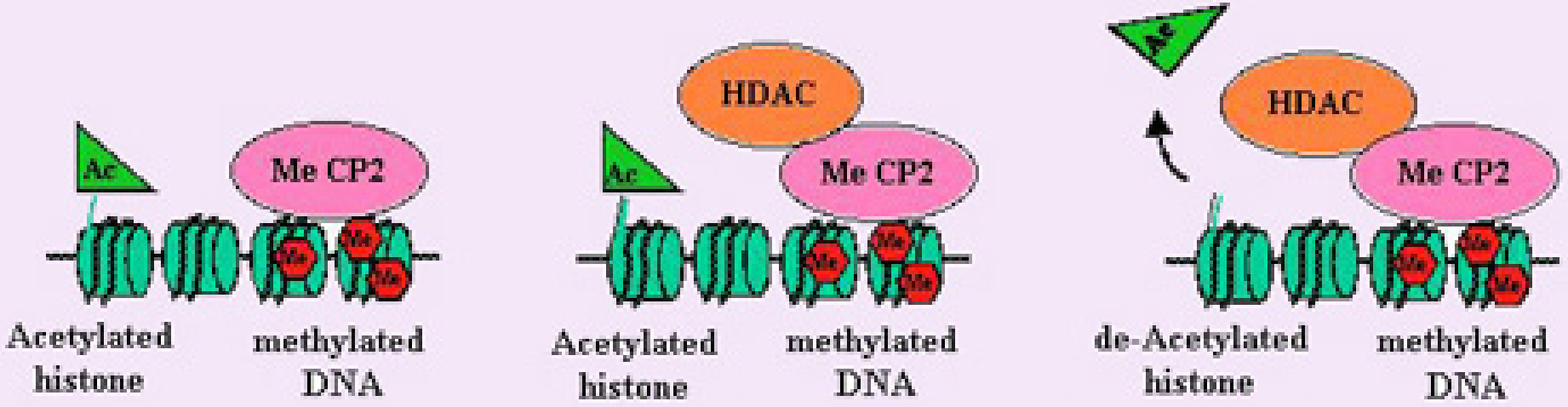
Histone Modification Map Key

acetylation	phosphorylation
methylation	ubiquitination



DNA methylation induces Histone de-acetylation



Me CP2

binds methylated DNA

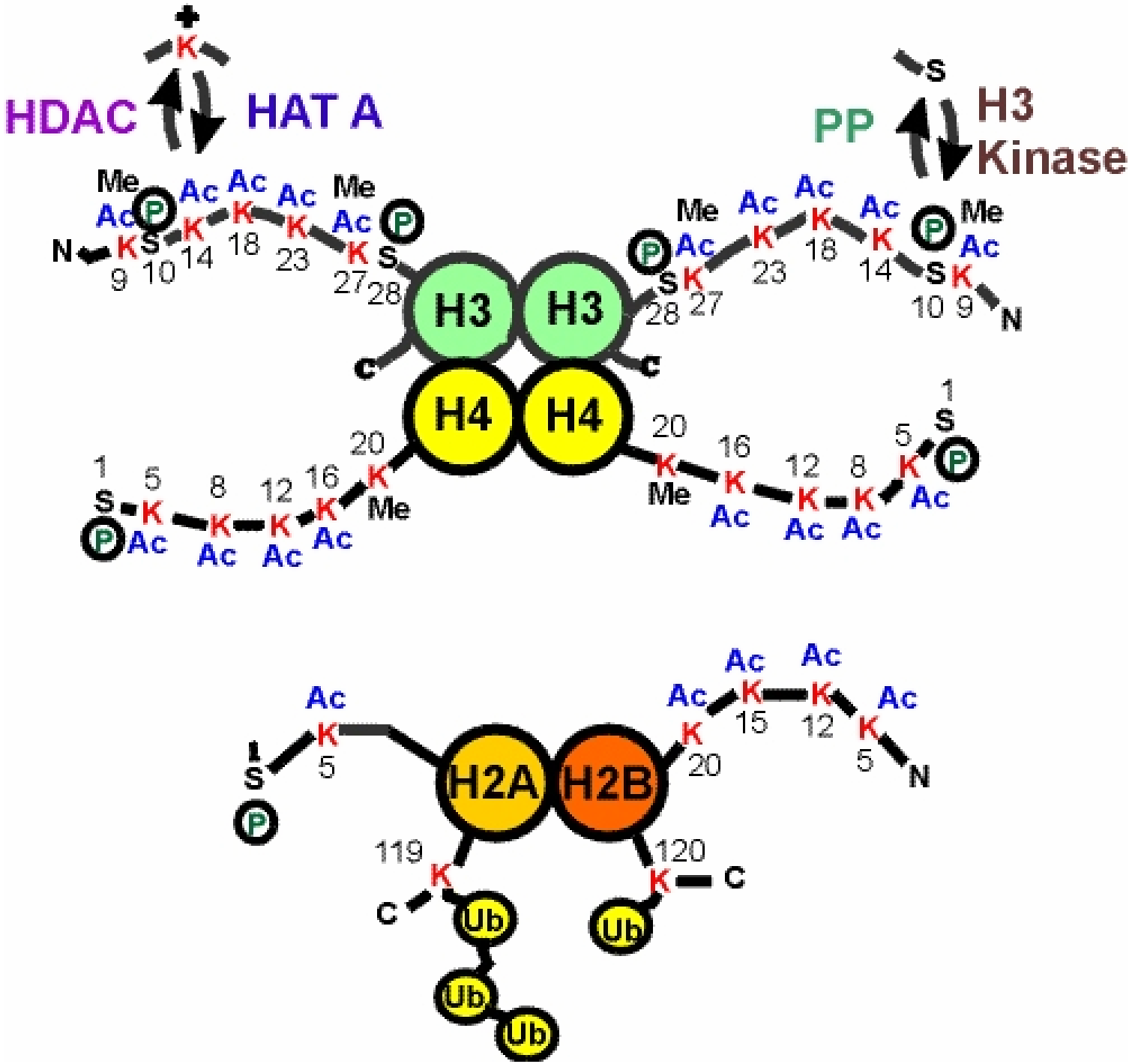
→

then recruits

HDAC

→

which de-Acetylates Histones


HDAC
HAT A
PP
H3
Kinase
H3
H3
H4
H4
H2A
H2B
Ub
Ub
Ub