

真核生物基因表达调控的特点

(一) 真核生物基因表达调控的环节更多

原核生物基因表达在同一空间进行，而真核基因转录发生在细胞核，翻译则在胞浆，两个过程是分开的，因此其调控增加了更多的环节和复杂性。

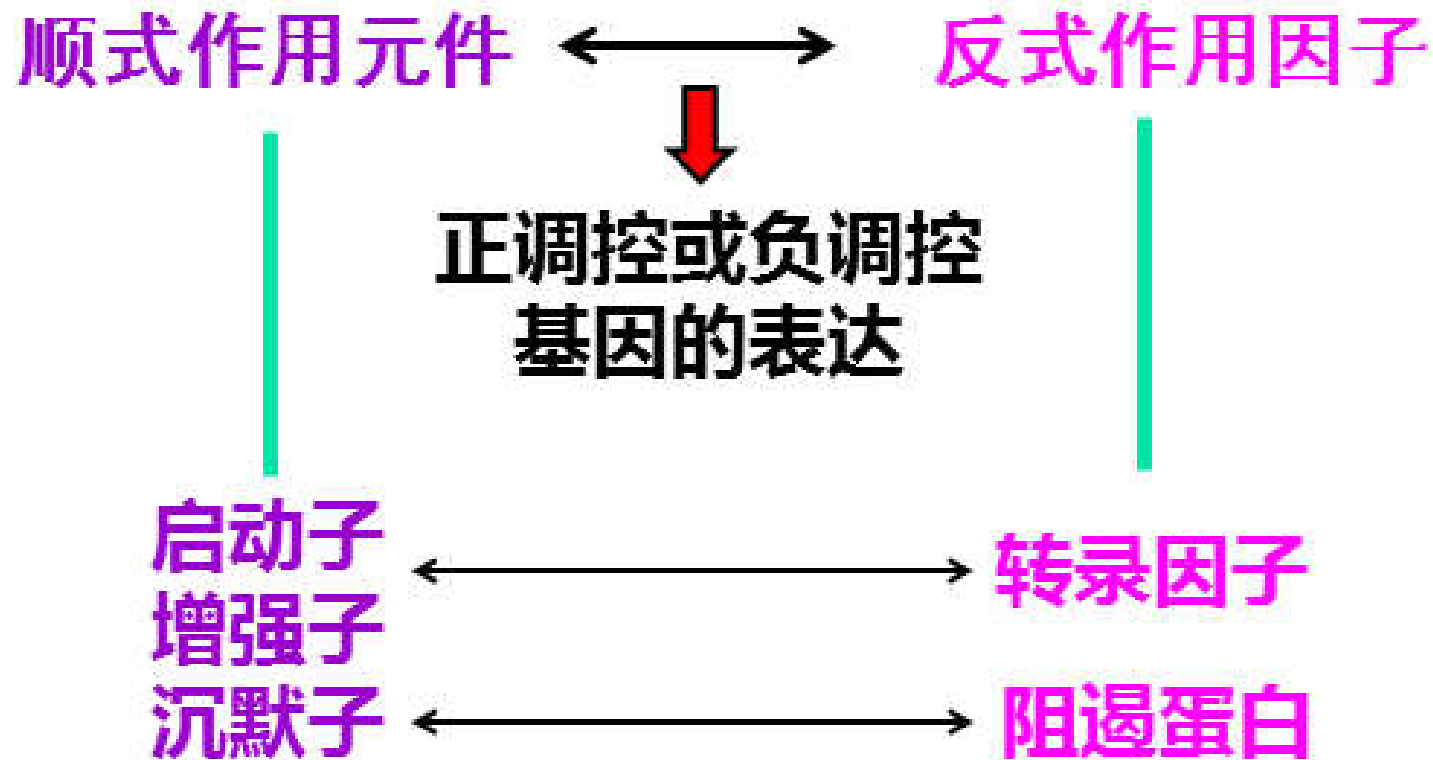
(二) 真核基因转录与染色质的结构变化相关

真核基因组DNA绝大部分都在细胞核内与组蛋白等结合成染色质，染色质的结构、染色质中DNA和组蛋白的结构状态都影响转录。

(三) 真核基因转录调控以正调控为主

多数真核基因在没有转录因子作用时是不转录的，需要表达时就要有激活因子来促进转录，虽然也有负调控元件，但并不普遍。

Chapter 10 Regulation of transcription in eukaryotes



1. Eukaryotic *cis*-acting elements

1.1 Enhancer (增强子)

缺失后相应基因
转录效率显著降低



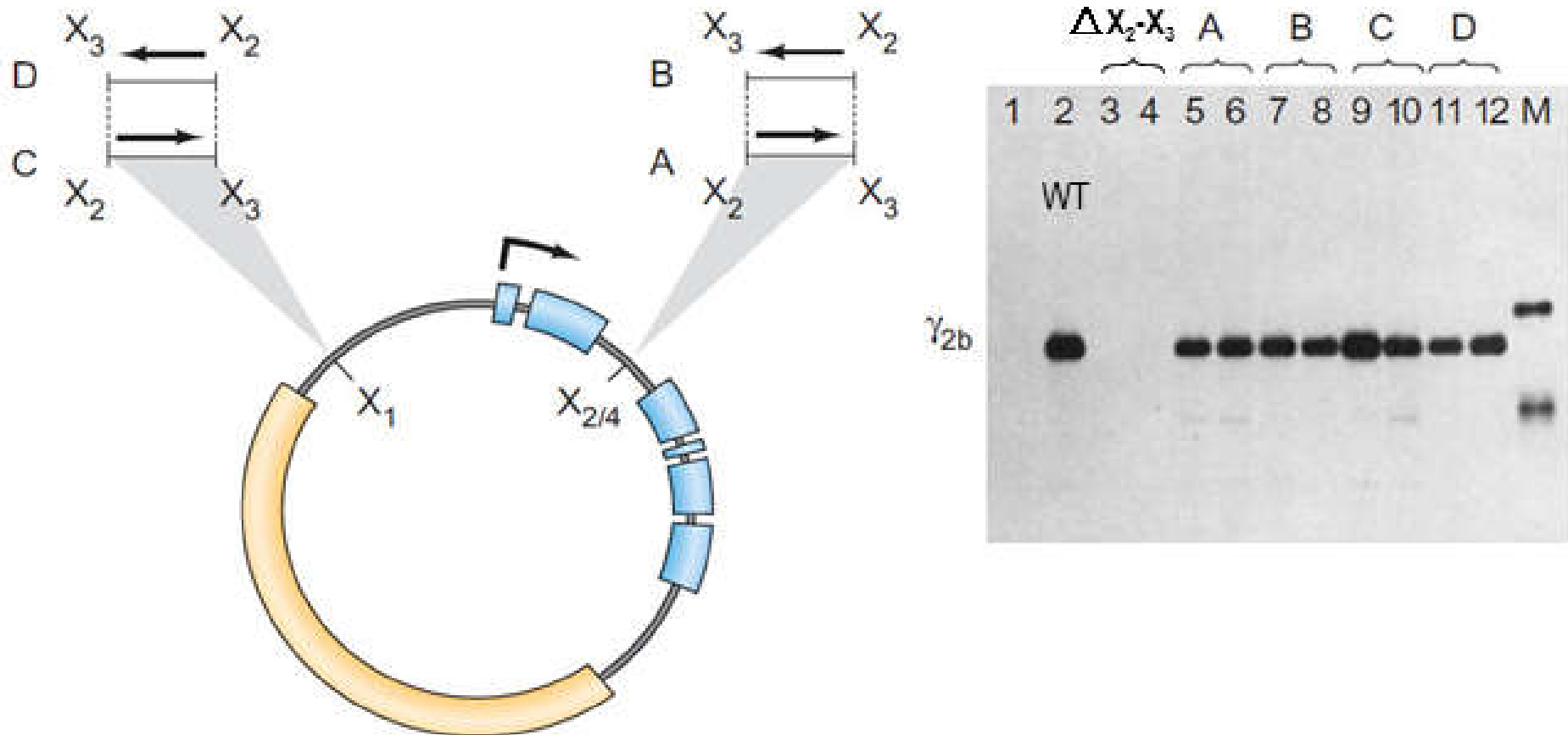
UPE ? X



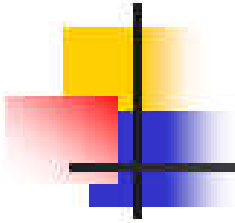
Structure of the SV40 virus early control region

Enhancers are position and orientation-independent (无位置和方向依赖性) **DNA elements** that stimulate the transcription of associated genes.

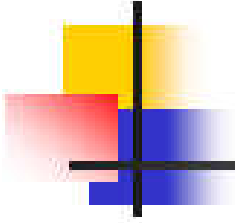
(3) They are able to function over long distances
whether from an upstream or downstream
position relative to the start site.



Fragment X₂-X₃ has the enhancer of immunoglobulin γ 2b H-chain gene.



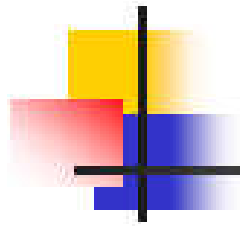
- (1) Enhancers exert strong **activation of transcription of a linked gene** from the correct start site.
- (2) They activate transcription when **placed in either orientation** with respect to linked genes.
- (3) They **are able to function over long distances** whether from an upstream or downstream position relative to the start site.
- (4) They exert **preferential stimulation of the closest** of two tandem promoters.



(5) Enhancers are also *tissue-specific* in that they *rely on tissue-specific DNA-binding proteins* for their activities.

不同类型的细胞表达不同的激活因子，与相应的增强子结合后激活不同的基因，继而产生不同的蛋白质。

造成不同组织细胞基因差异表达的原因之一。

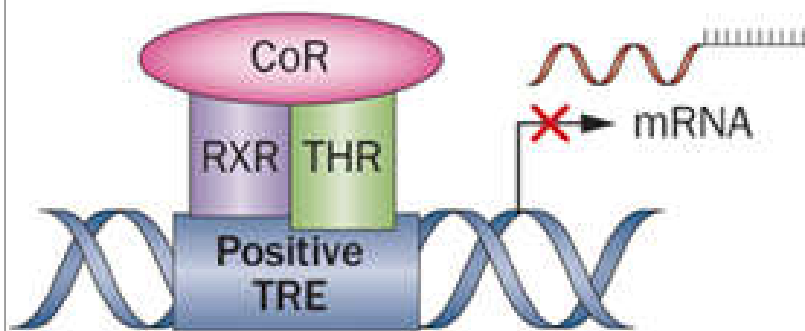


1.2 Silencer (沉默子)

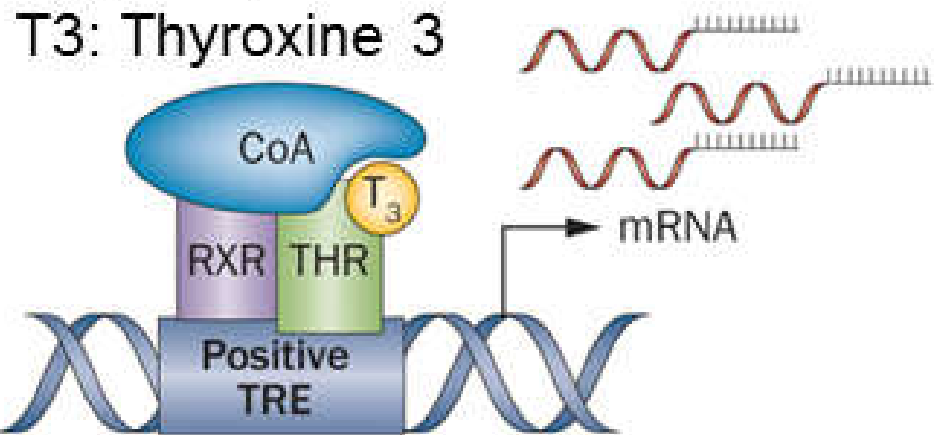
silencers are position and orientation-independent (无位置和方向依赖性) **DNA elements** that depress the transcription of associated genes.

- Sometimes a DNA element can act as either an enhancer or a silencer depending on what is bound to it.

T_3 -ligand-independent repression



T_3 -ligand-dependent transactivation

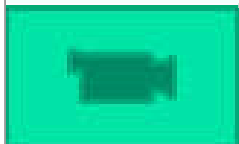


THR: thyroid hormone receptor (甲状腺激素受体)

TRE: thyroid hormone response element

RXR: retinoic acid X receptor (维甲酸 X 受体)

CoR: corepressor; CoA: coactivator





2. Transcription factor/ activator domains

Transcription factors have structures composed of at least two **functional domains**:

DNA-binding domain (DNA结合域)

识别和结合DNA特定序列的功能区域

Transcription-activating domain (转录激活域)

激活转录的功能区域

Many TFs also have:

dimerization domain (二聚体域)

A few TFs have:

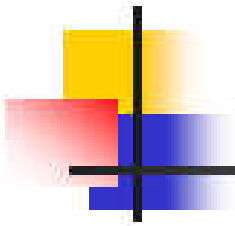
ligand-binding domain (配体结合域)



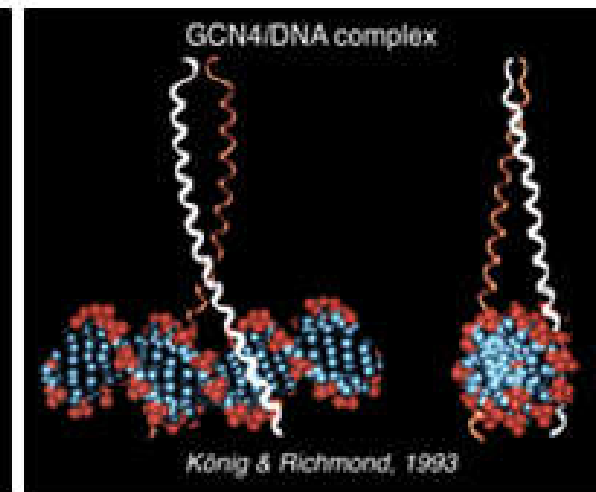
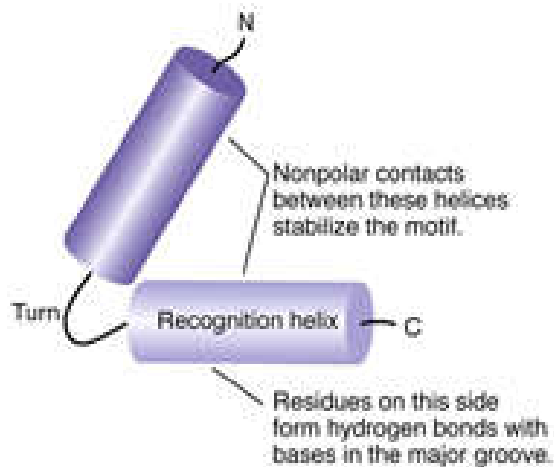
2.1 DNA-binding domains

TFs use their DNA-binding domain to bind to a specific DNA motifs, e.g. upstream promoter elements (UPEs), enhancers.

Transcription factor	Binding motif
Sp1	GGGCGG
AP-2	GCCNNNGGC
Oct-1	ATGCAAAT
GATA-1	TGATAG

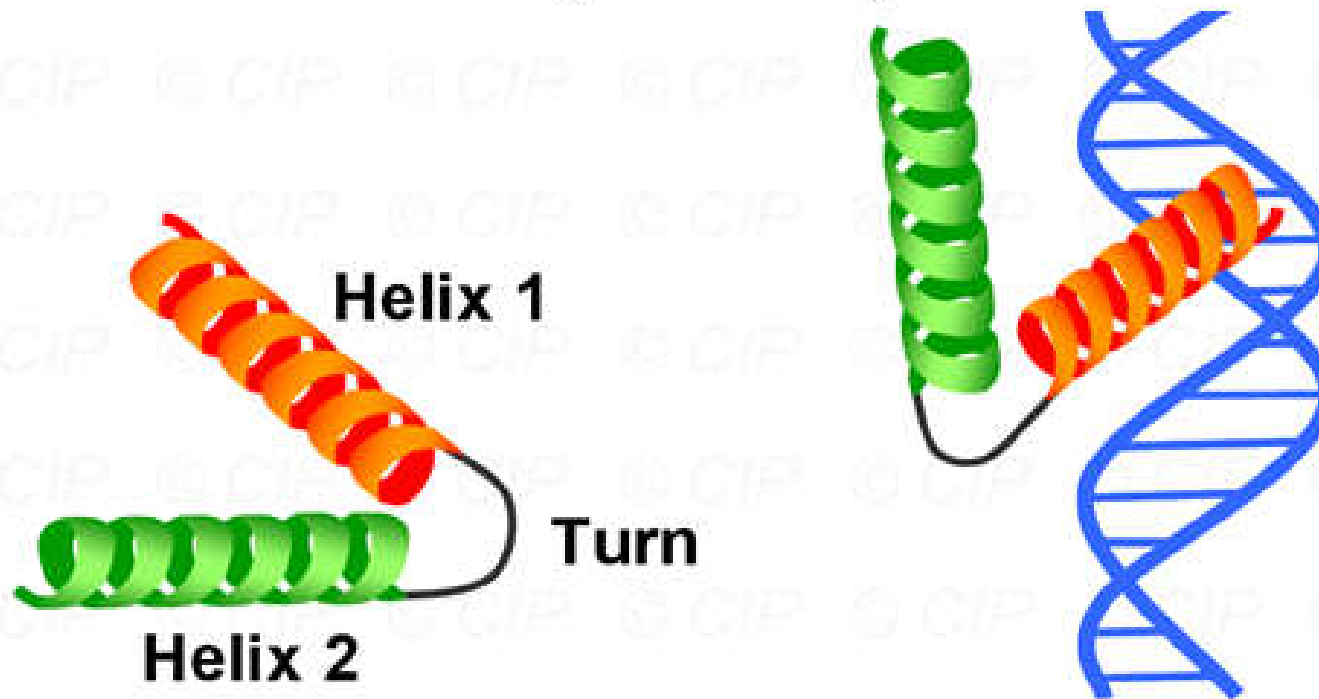


- **DNA-binding domains consist of:**
 - **Helix-turn-helix domain (螺旋-转角-螺旋结构域)**
 - **Zinc finger domain (锌指结构域)**
 - **Basic domain (碱性结构域)**



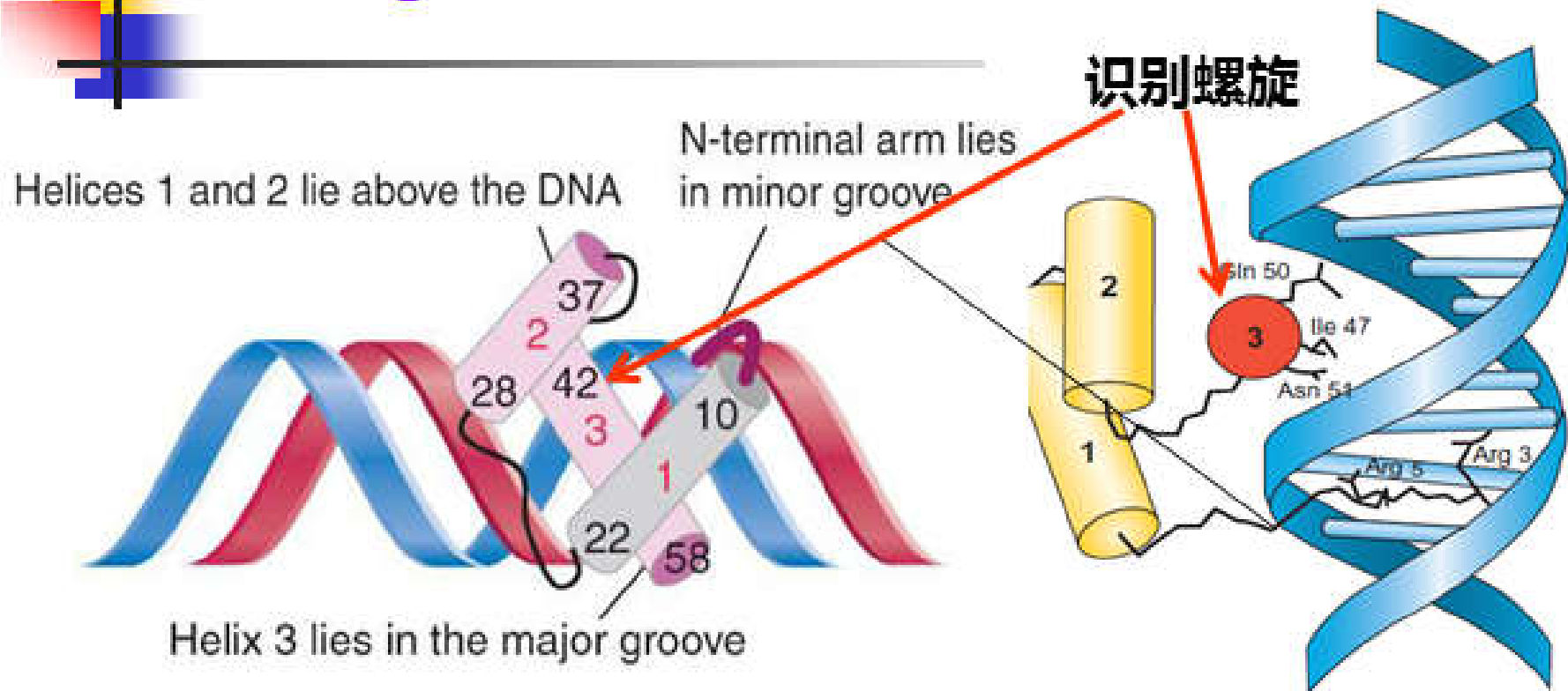
2.1.1 Helix-turn-helix domain

Helix-turn-helix domain is made of two α -helices connected by a short β -turn.



The recognition helix (识别螺旋) can fit tightly into the **major groove** of DNA.

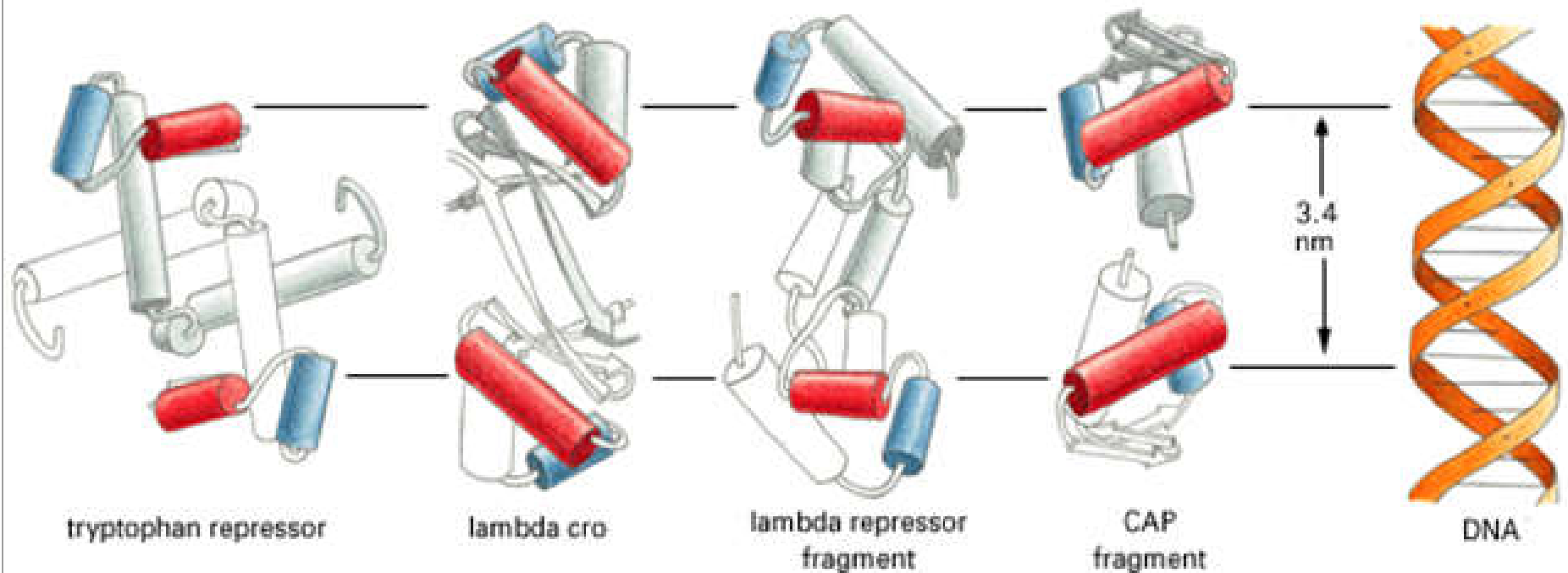
Examples of Helix-turn-helix domains



同源域

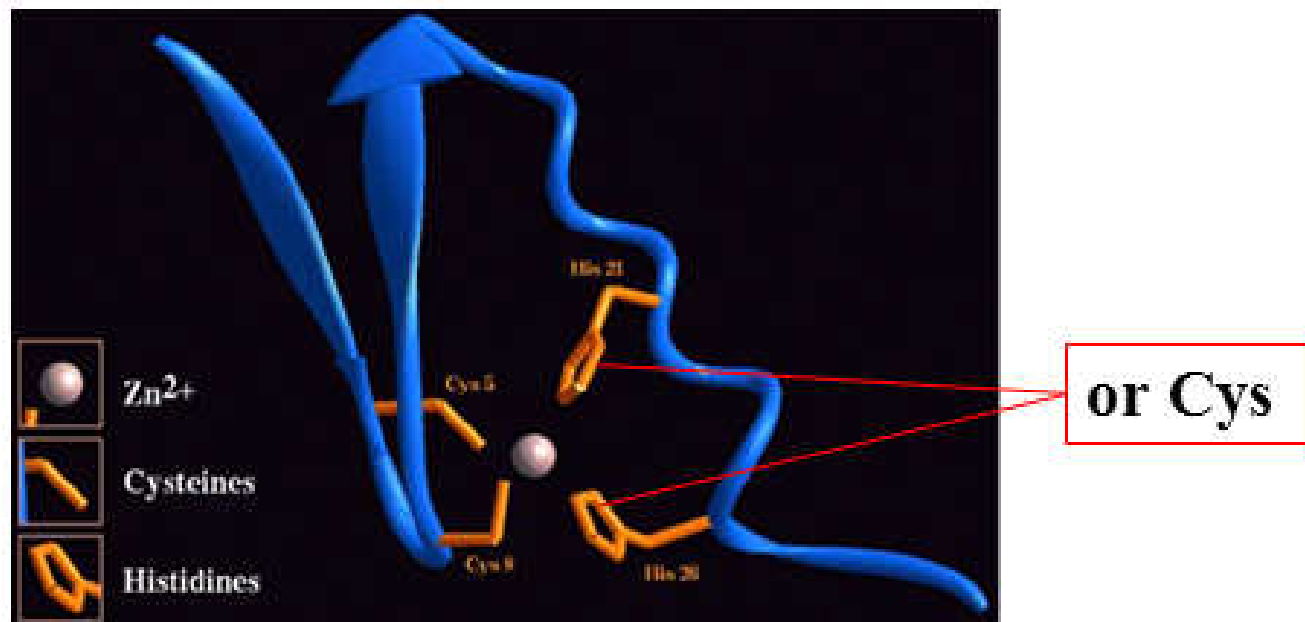
- Each **homeodomain** contains three α -helices. Helices 2 and 3 form the helix-turn-helix motif.
- The third helix serving as the recognition helix lies in the major groove.

e.g. *lac* and *Trp* repressors, Phage λ *cro* repressor, CRP.

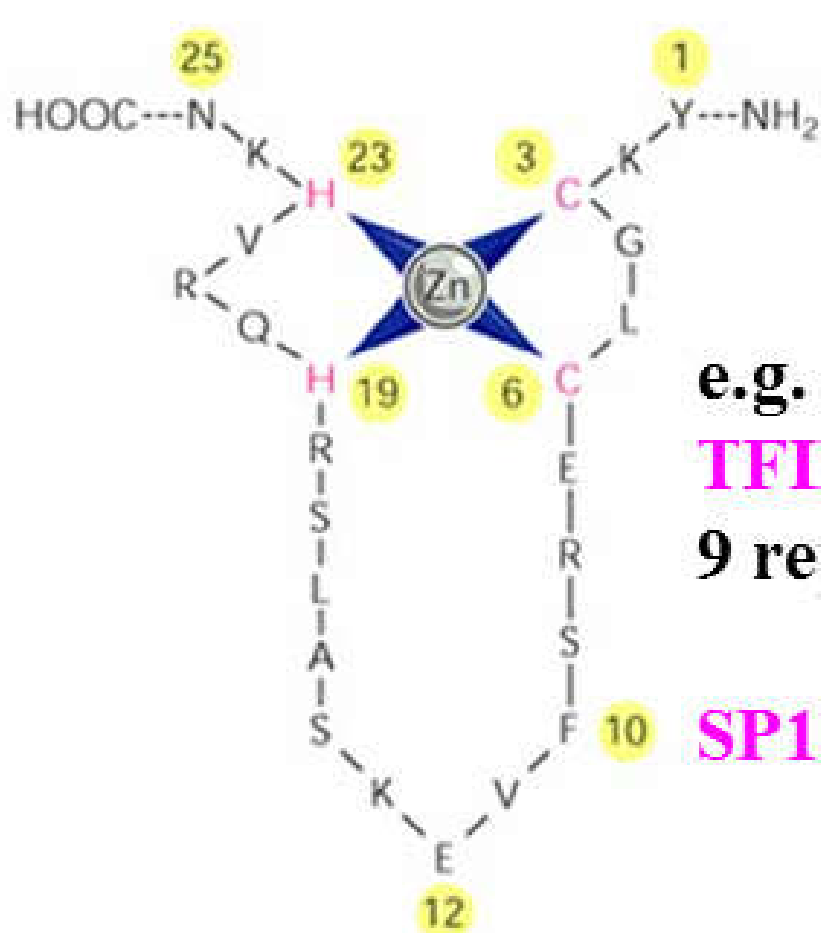


2.1.2 Zinc finger domain

- Zinc fingers are composed of **an antiparallel β -sheet** (two β -strands), followed by an **α -helix**.
- The **β -sheet contains two cysteines**, and the **α -helix contains two histidines or cysteines**.
- These **Cys (and His)** are **coordinated to a zinc ion**, which helps form the finger shaped structure.

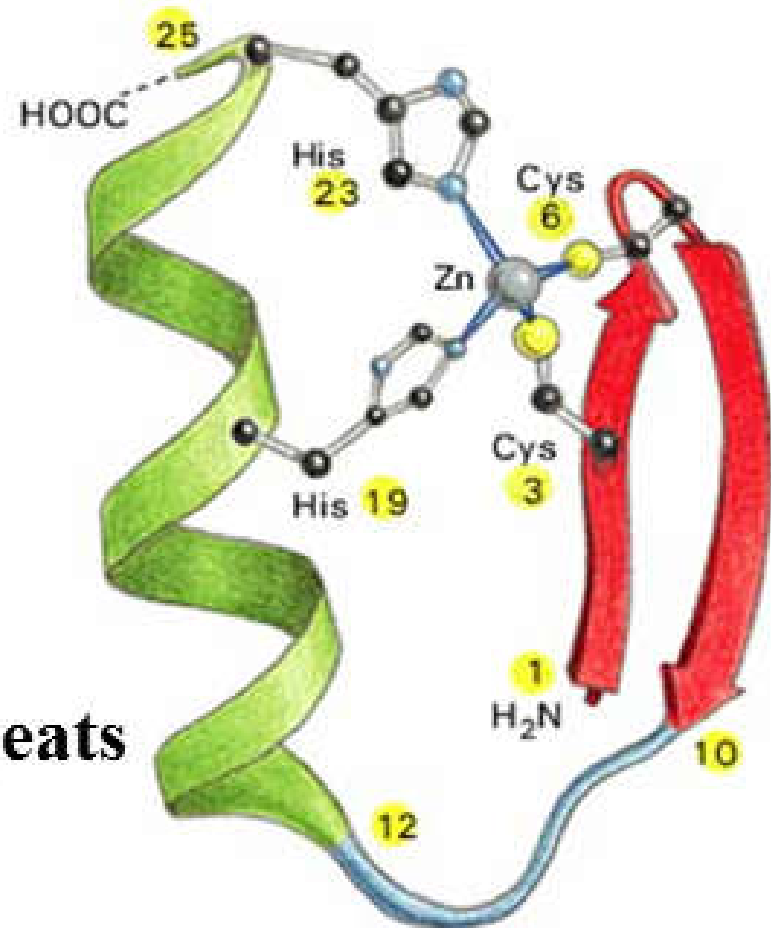


(1) C₂H₂ zinc finger



e.g.
TFIIIA:
9 repeats

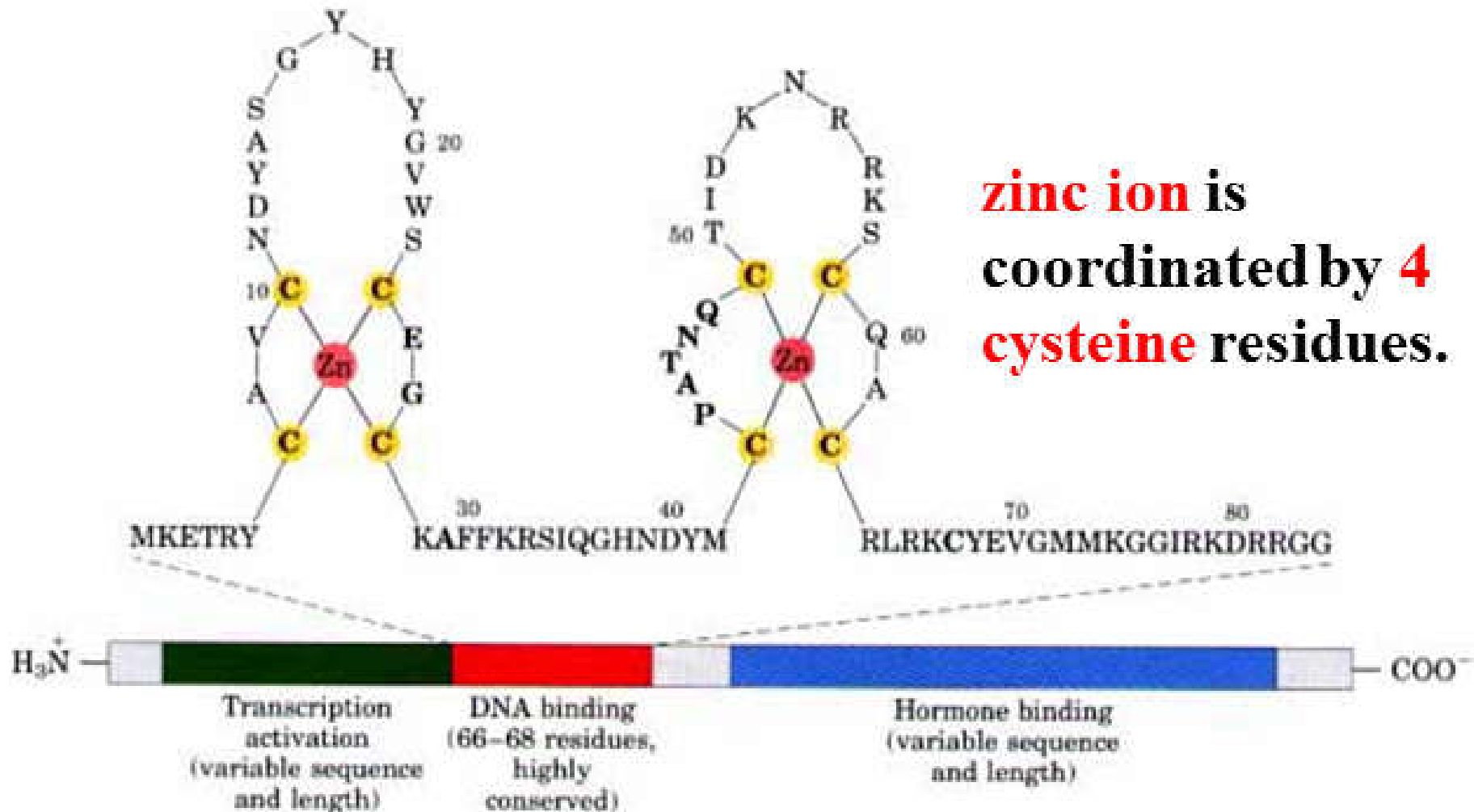
SP1: 3 repeats



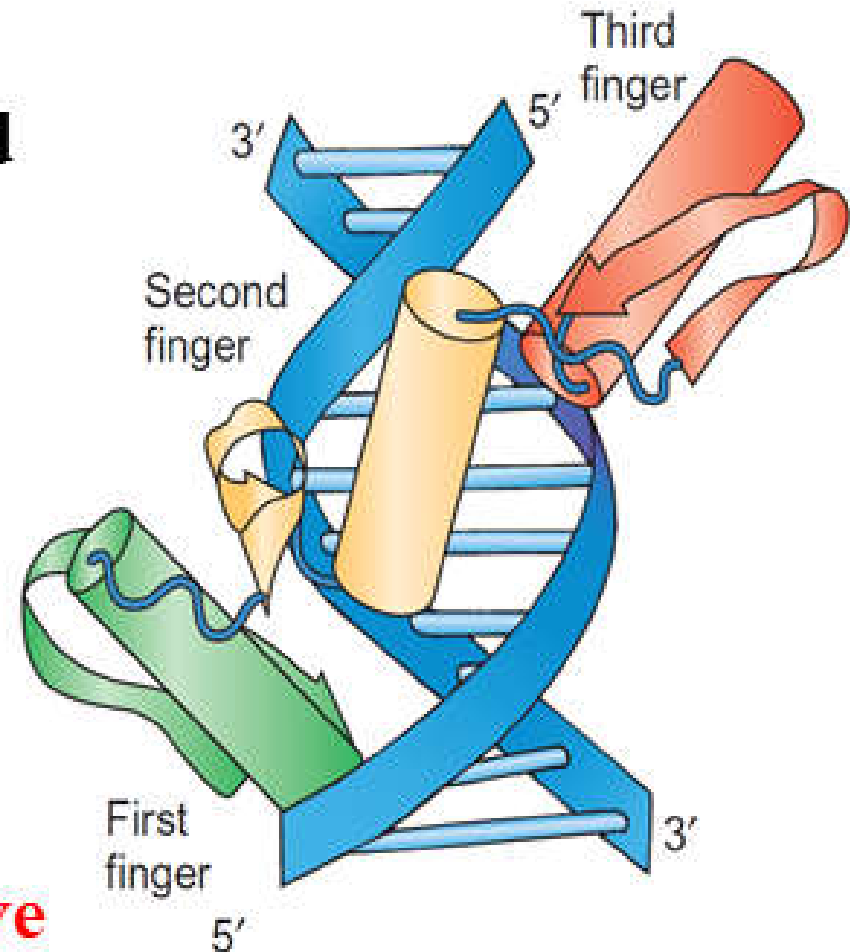
A loop of 12 amino acids anchored by **two cysteine** and **two histidine** residues that tetrahedrally co-ordinate **a zinc ion**.

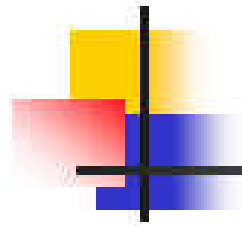
(2) C₄ zinc finger:

>100 steroid hormone receptor (类固醇激素受体)



- Usually, **three or more zinc fingers** are required for DNA binding.
- Binding between each finger and its DNA-binding site relies on direct amino acid-base interactions of **amino acids in the α -helix and bases in the major groove of the DNA.**



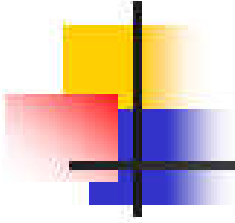


2.1.3 Basic domain

Rich in basic amino acid residues [arginine (R), lysine (K), and histidine (H)].

A **basic domain is found in a number of DNA-binding proteins and is generally associated with dimerization domains:**

- **Leucine zipper (ZIP, 亮氨酸拉链) motif**
- **Helix-loop-helix (HLH, 螺旋-环-螺旋) motif**



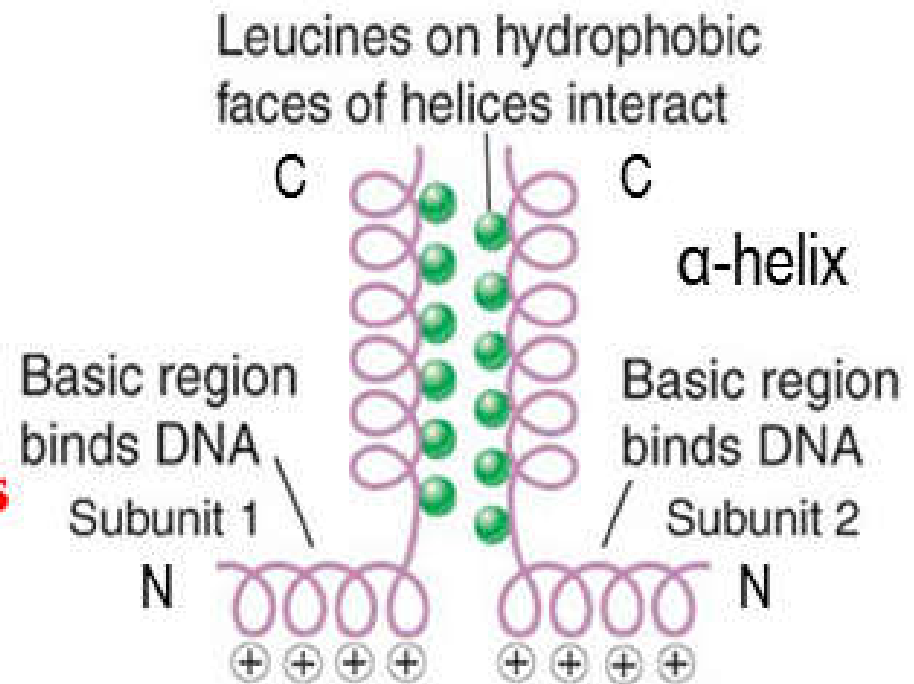
These are referred to as:

- **Basic leucine zipper (bZIP) protein**
- **Basic helix-loop-helix (bHLH) protein.**

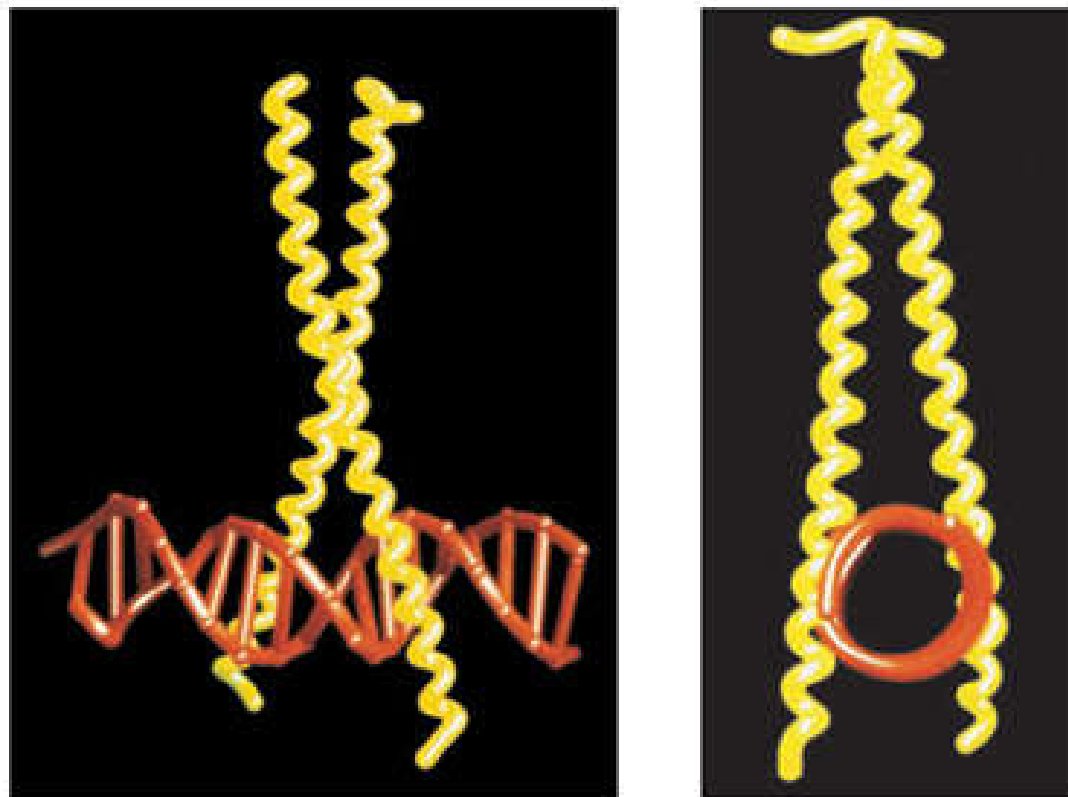
Dimerization (二聚化) of the proteins brings together two basic domains which can then interact with DNA.

(1) Basic leucine zippers (bZIP)

- **bZIP protein** contains a hydrophobic (疏水的) **leucine** residue at every 7th position in a region that is often at the **C-terminal** part.
- bZIP transcription factors contain **basic domains N-terminal to the ZIP**.
- These **Leu** are all on **one face of the α -helices** and **responsible for dimerization**.

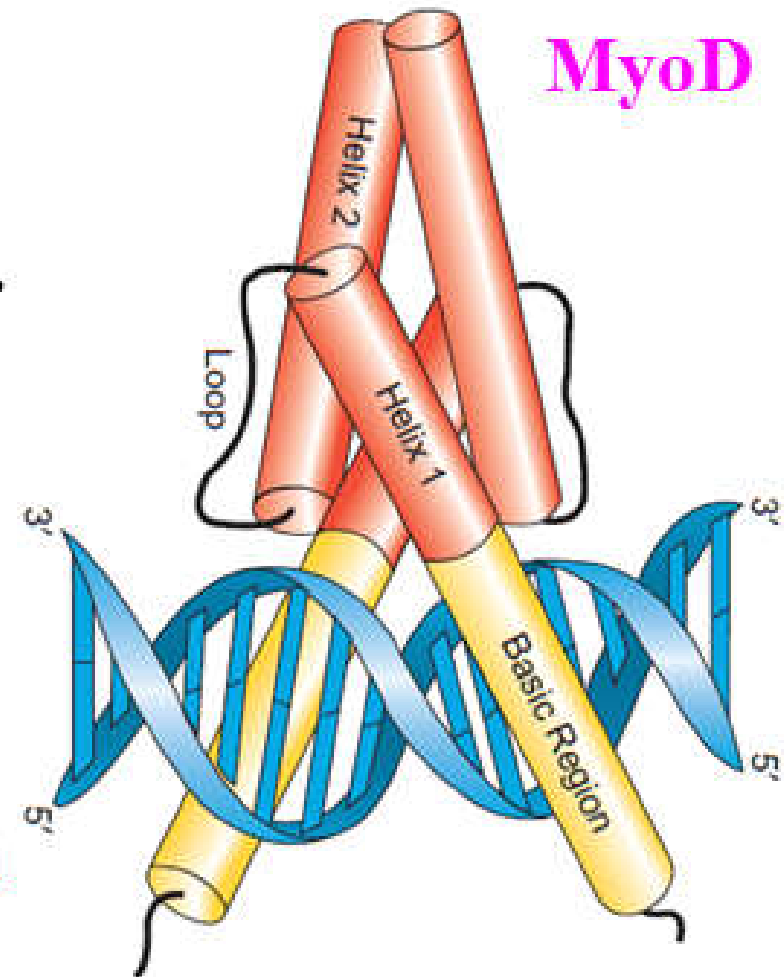


- **bZIP not only brings the two monomers together, it also places the two basic parts of the domain in position to grasp the DNA like a pair of forceps (镊子), with the basic motifs fitting into the DNA major groove.**



(2) Basic helix-loop-helix (bHLH)

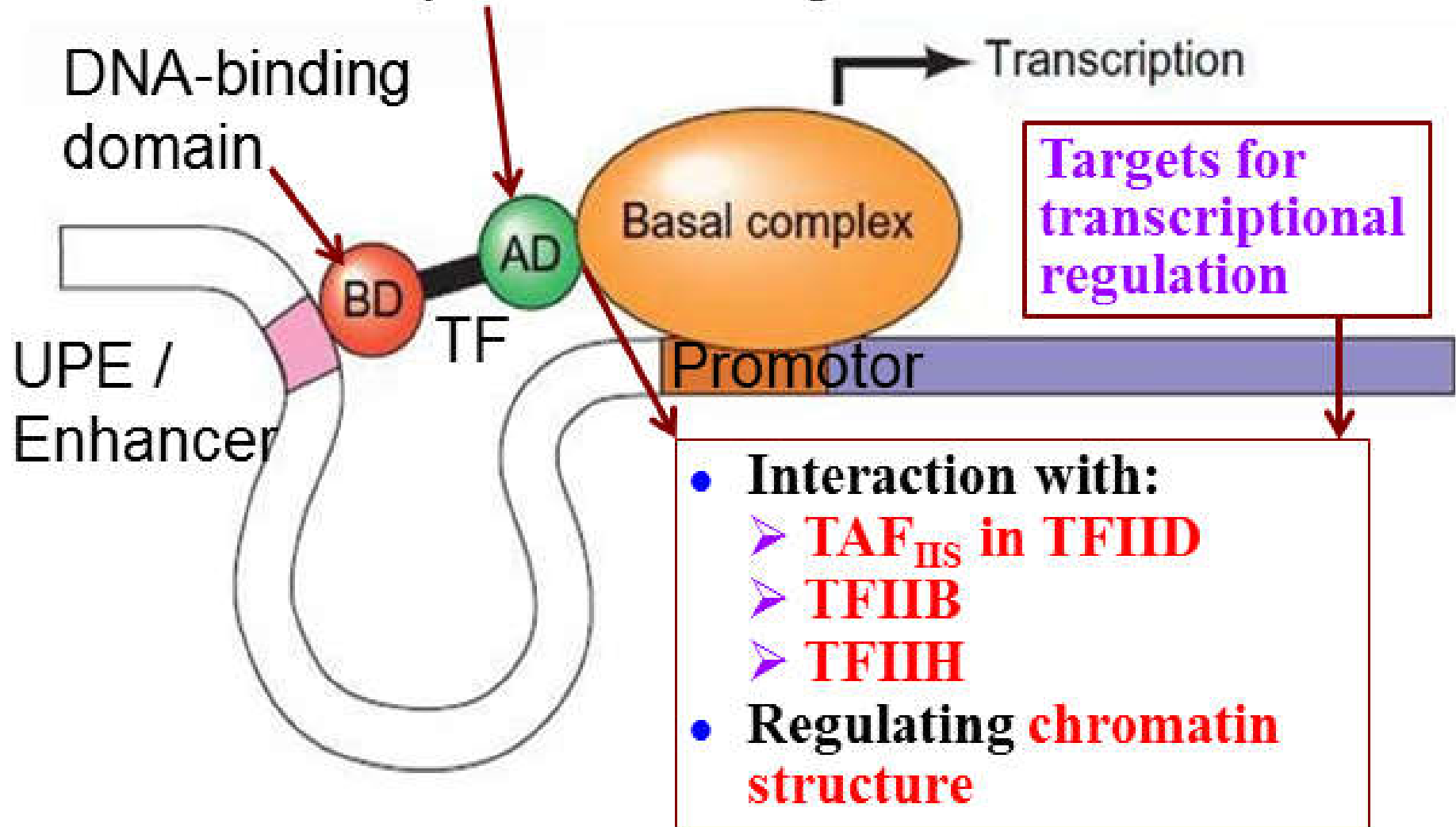
- Hydrophobic residues on one side of the **C-terminal α -helix** allow dimerization.
- A **nonhelical loop** of polypeptide chain separates two α -helices in each monomeric protein.
- HLH motif is often found adjacent to a **basic domain** that requires dimerization for DNA binding.

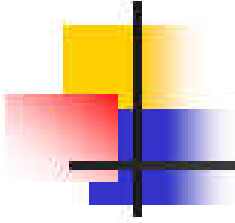


Similar to bZIP

2.2 Transcription-activating domains

Transcription-activating domain





Acidic activation domains
(酸性激活域)

Glutamine-rich domains
(富含谷氨酰胺结构域)

Proline-rich domains
(富含脯氨酸结构域)

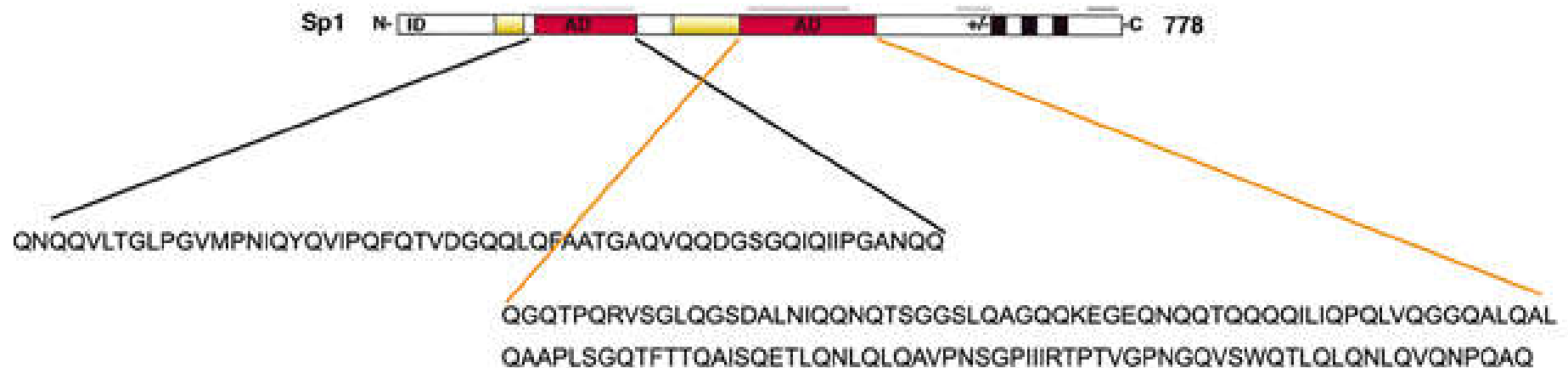


2.2.1 Acidic activation domain (酸性激活域)

- **Rich in acidic amino acids** [aspartic acid (D), glutamic acid (E)]
- **Exists in many transcription activation domains:**
 - yeast **Gcn4** and **Gal4**;
 - mammalian **glucocorticoid receptor** (糖皮质激素受体)
 - herpes virus activator (疱疹病毒激活蛋白) **VP16**

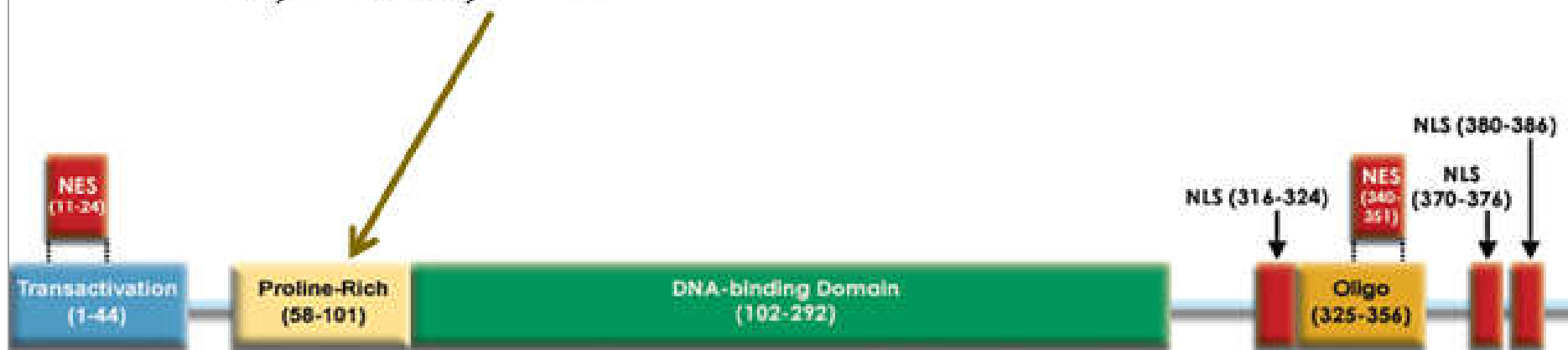
2.2.2 Glutamine-rich domain (富含谷氨酰胺结构域)

- Rich in glutamine (Q)
- the proportion of glutamine residues seems to be more important than overall structure.
- Exists in the transcription factor SP1.

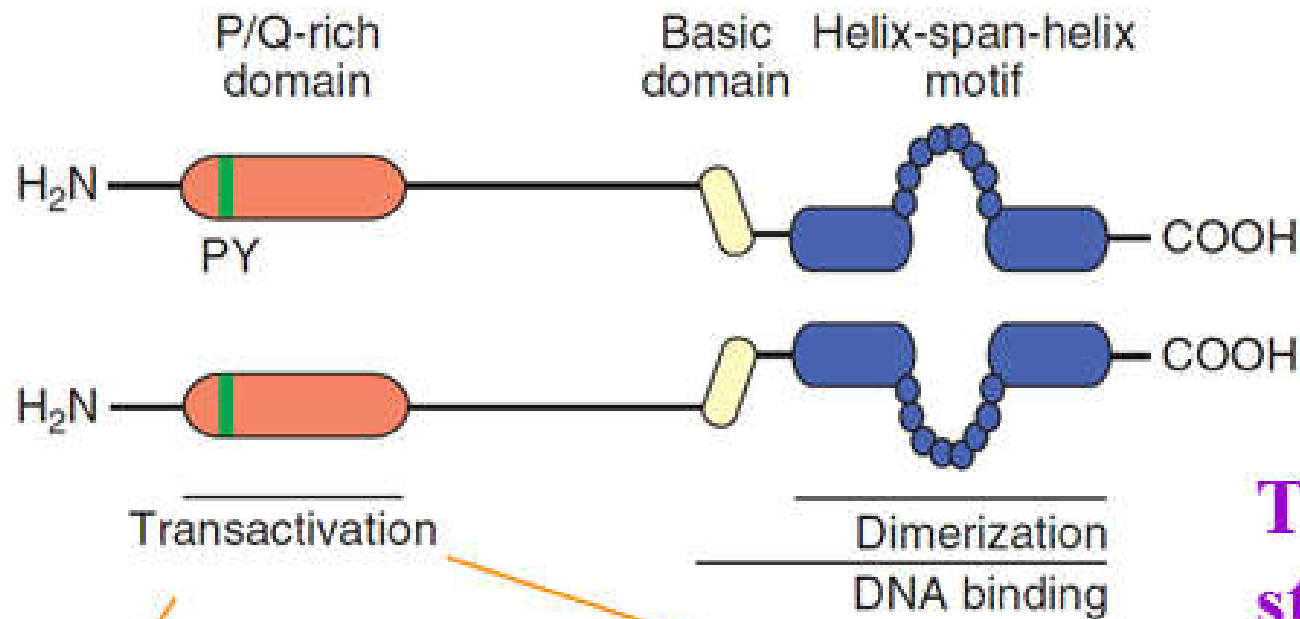


2.2.3 Proline-rich domain (富含脯氨酸结构域)

- Proline-rich (P)
- continuous run of proline residues can activate transcription
- Exists in transcription factors c-jun, AP-2, Oct-2, P53.



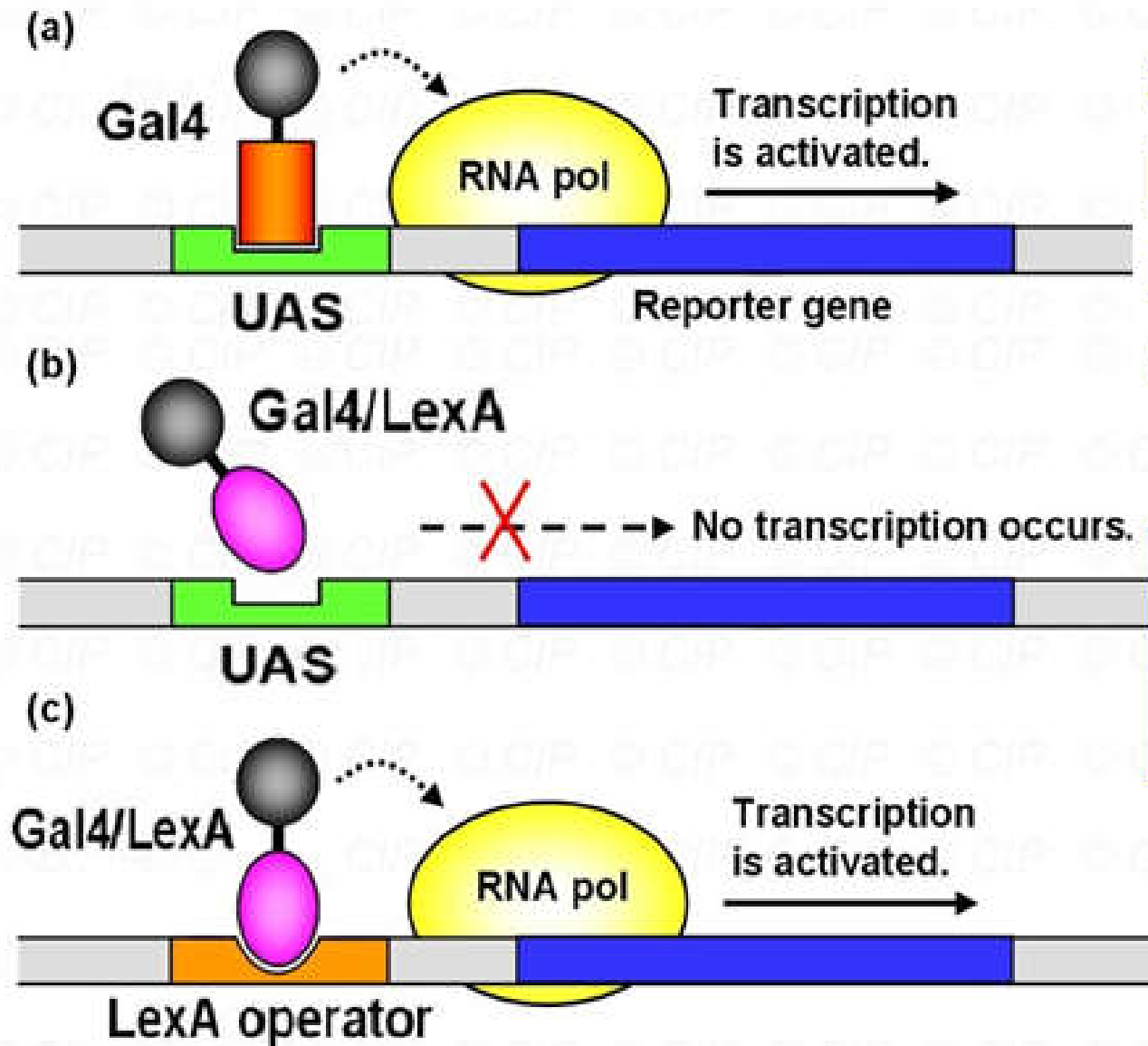
Most activators have one of transcription-activating domains, but some have more than one.



The protein structure of an AP-2 dimer

PQLGTVGQSPYTSAPPLSHTPNADFQPPYFP
PPYQPIYPQSQDPYSHVNDPYSLNPLHAQPQ
PQHPGWPGQRQSQESGLLHTHRGLPHQ

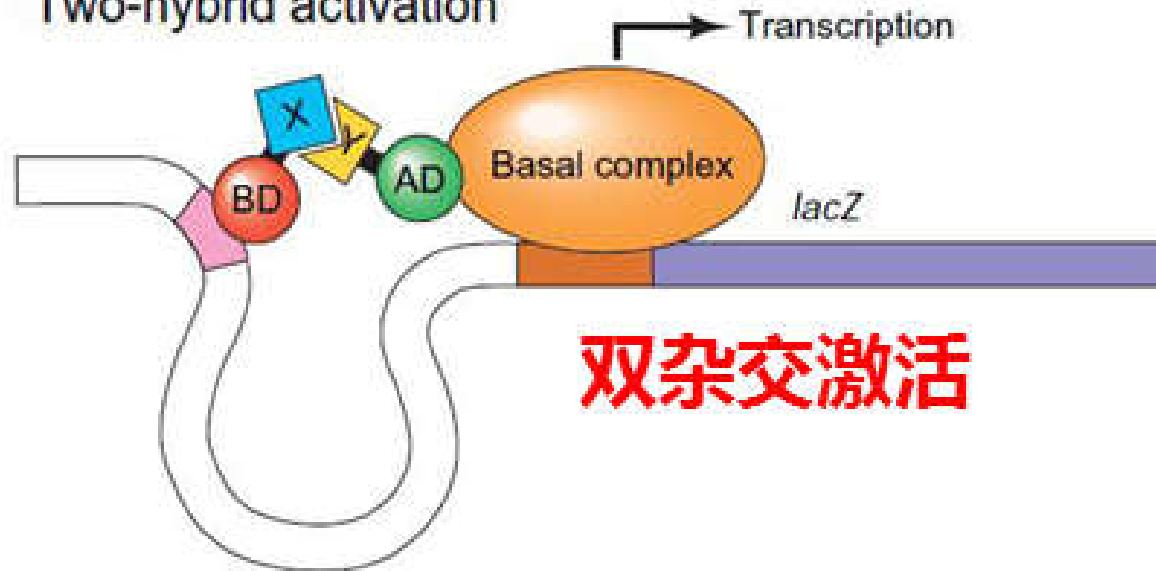
2.3 Independence of the domains of activators



**Domain swap
experiments
结构域交换实验**

The DNA-binding and transcription-activating domains of activator proteins are independent modules (组件).

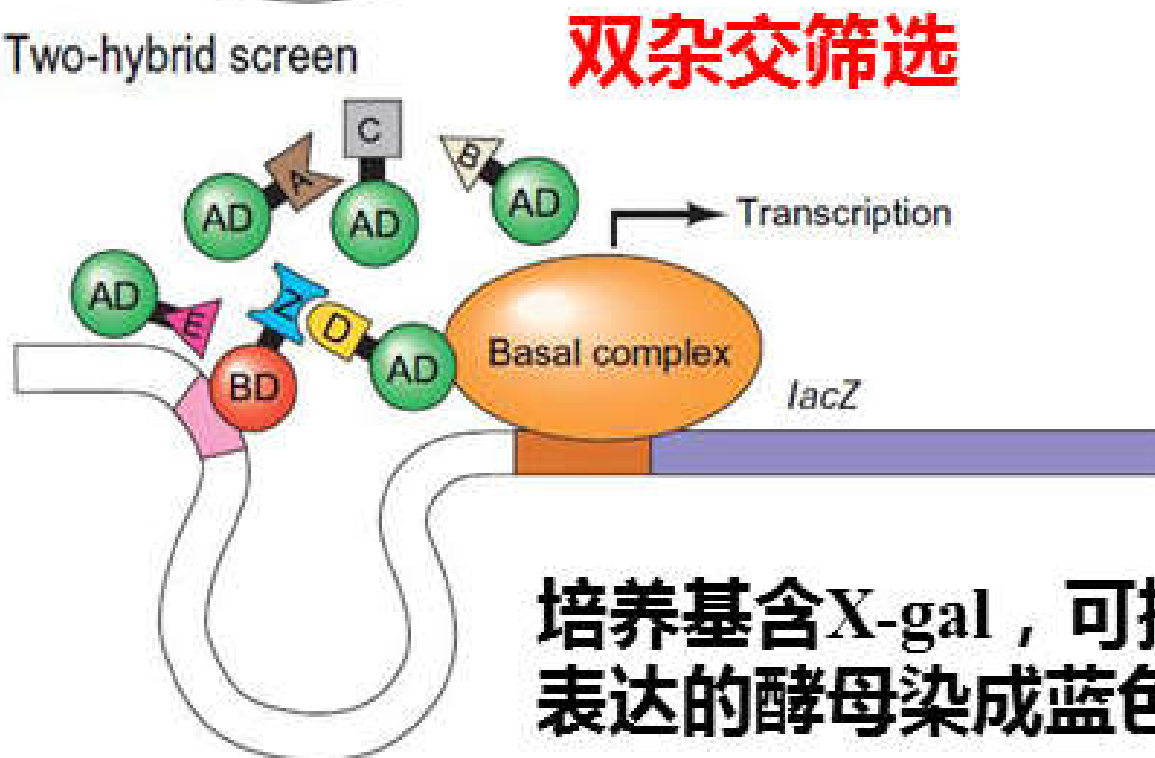
Two-hybrid activation



双杂交激活

双杂交技术是研究蛋白质-蛋白质相互作用的方法之一。

Two-hybrid screen



双杂交筛选

每个酵母细胞集落都表达BD-Z融合蛋白和一种AD-?融合蛋白

培养基含X-gal，可把激活表达的酵母染成蓝色

An activator has independent domains

转录激活因子直接与DNA结合

转录激活结构域

An activator must have a DNA-binding domain

DNA结合结构域

转录因子之间常通过蛋白质-蛋白质相互作用来调控基因转录。

An activator may use a coactivator

共激活因子无DNA结合域，不直接与DNA结合

共激活因子 辅助激活因子

An coactivator have no DNA-binding domain

转录因子

3. Activity regulation of transcription factors

非活性条件

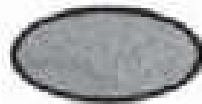
无蛋白质



非活性蛋白



非活性蛋白



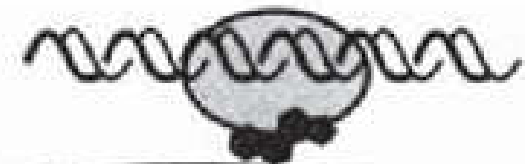
非活性蛋白



蛋白质合成



有活性条件



类固醇激素受体



CREB; STAT1

配体结合



单泛素化

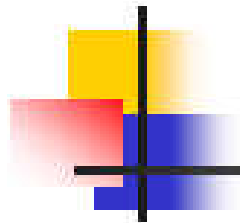
蛋白质磷酸化



蛋白质修饰

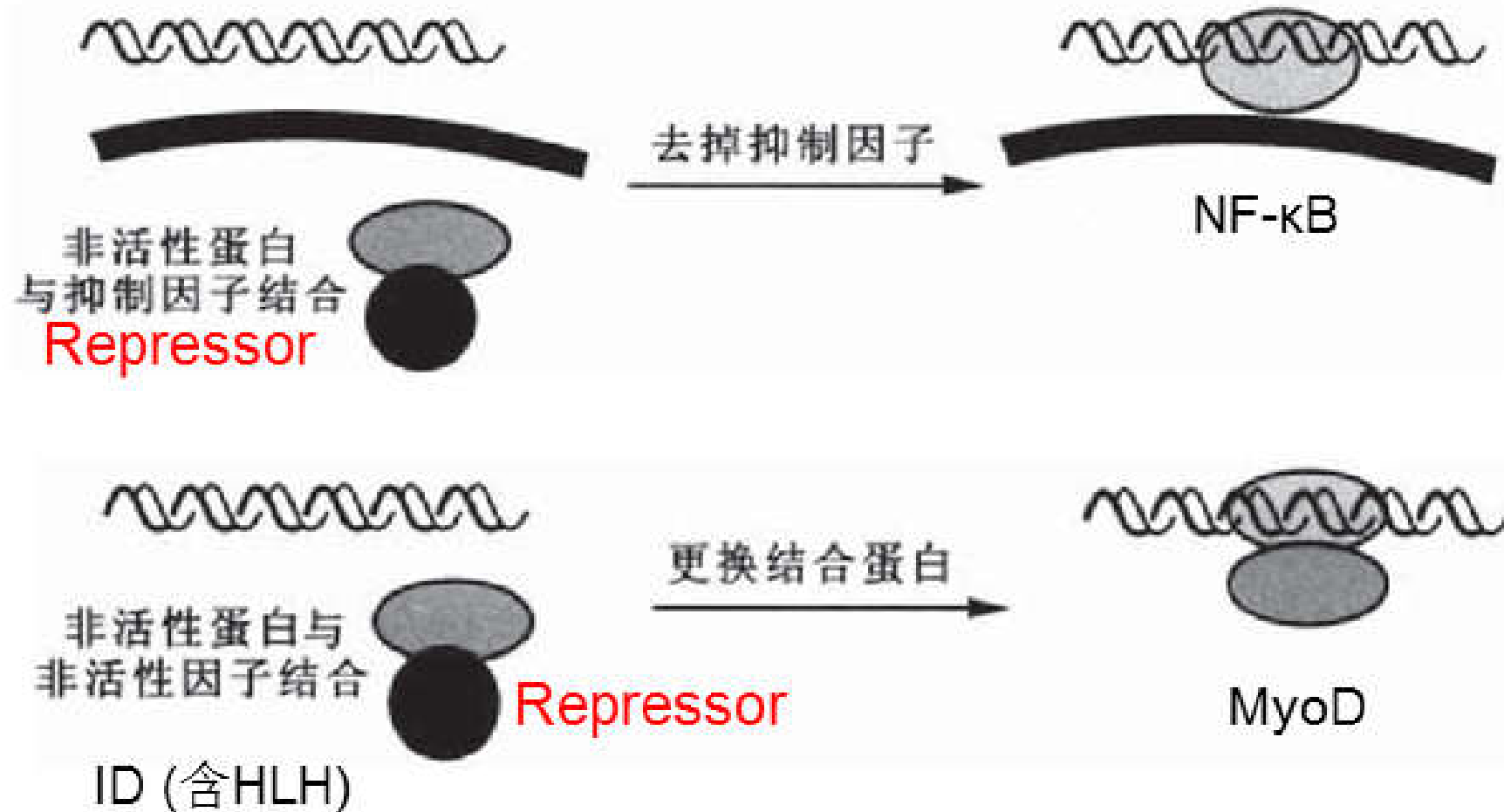
蛋白质去磷酸化





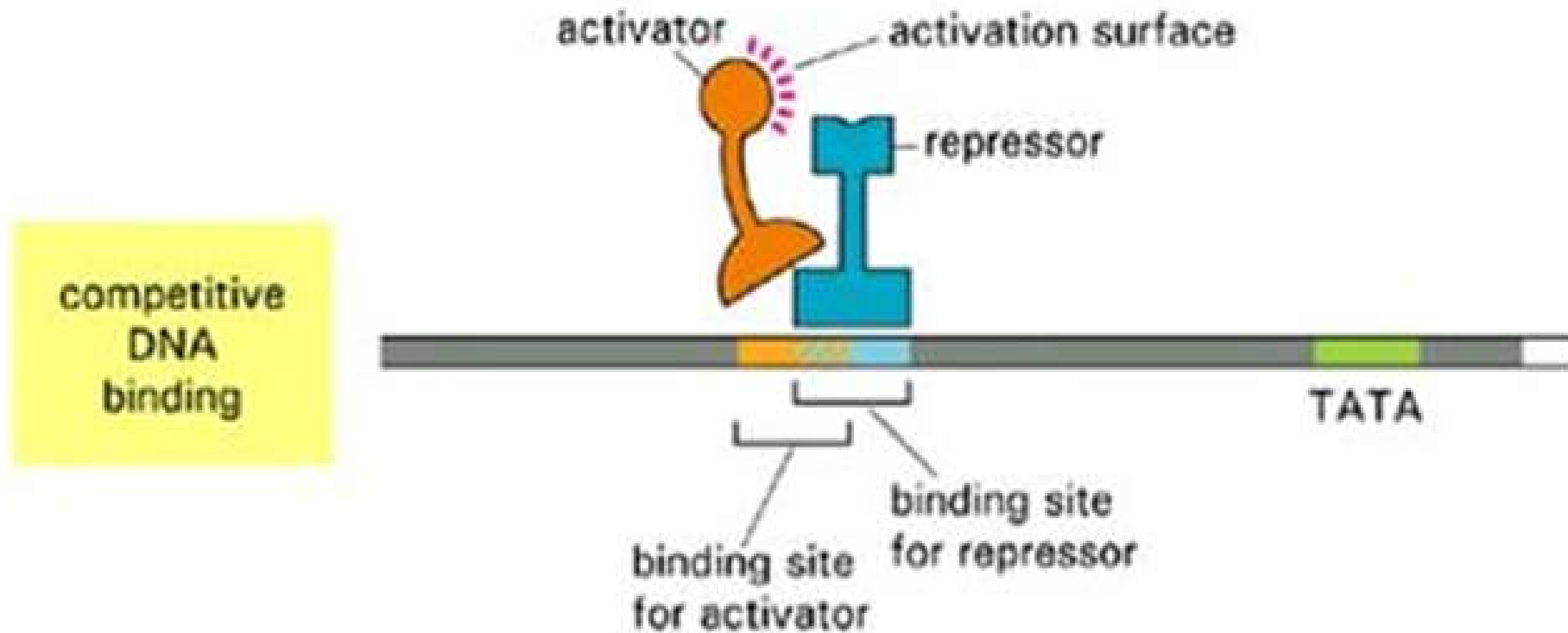
非活性条件

有活性条件

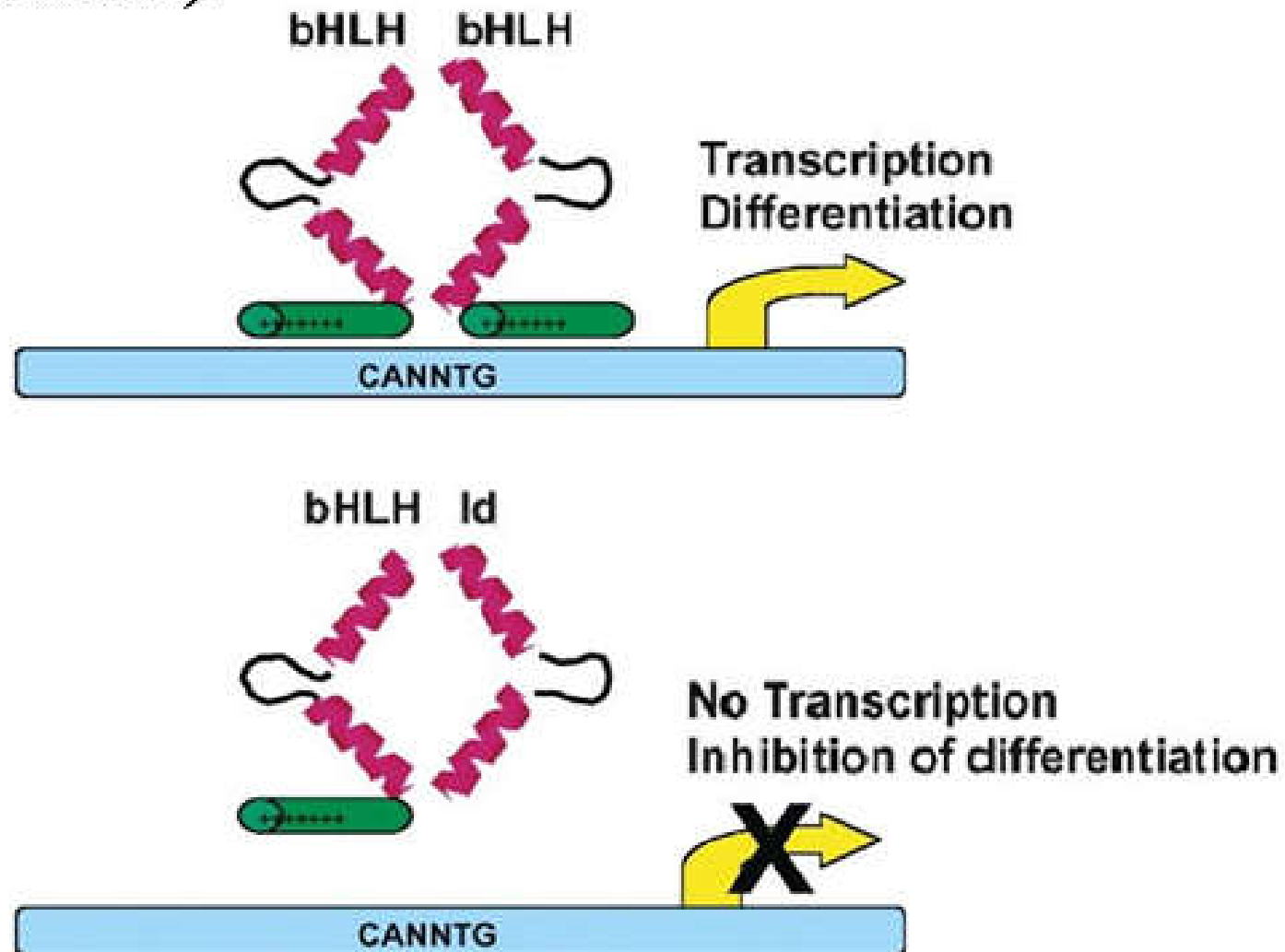


Repression of transcription

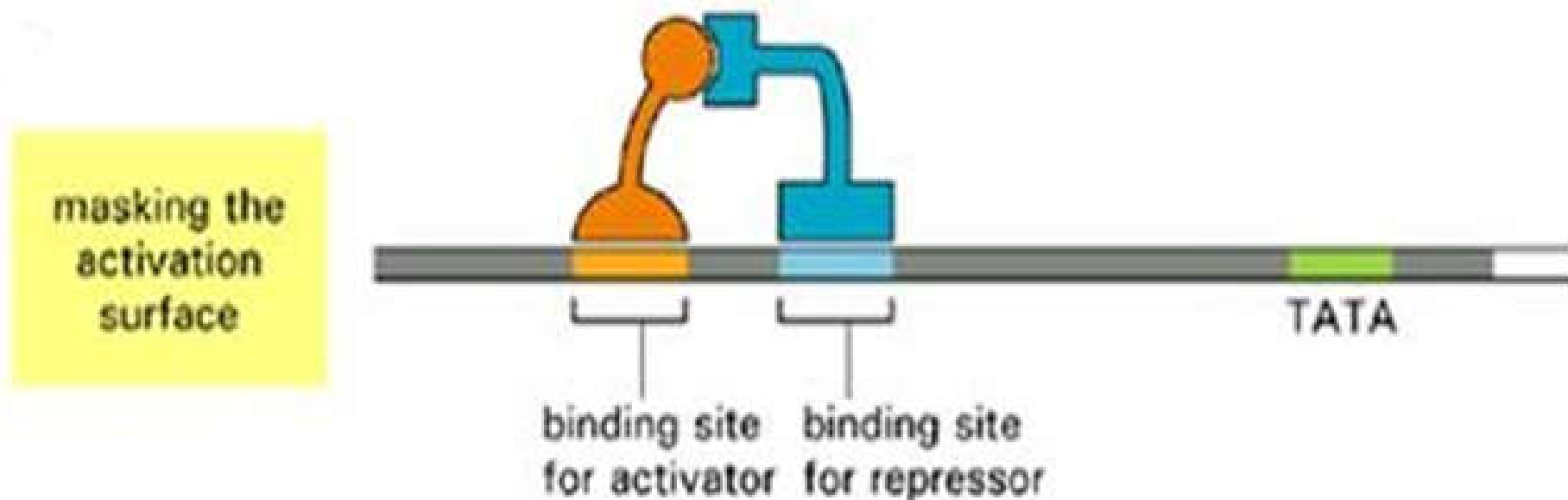
(1) Blocking the activator DNA-binding site (as with prokaryotic repressors)



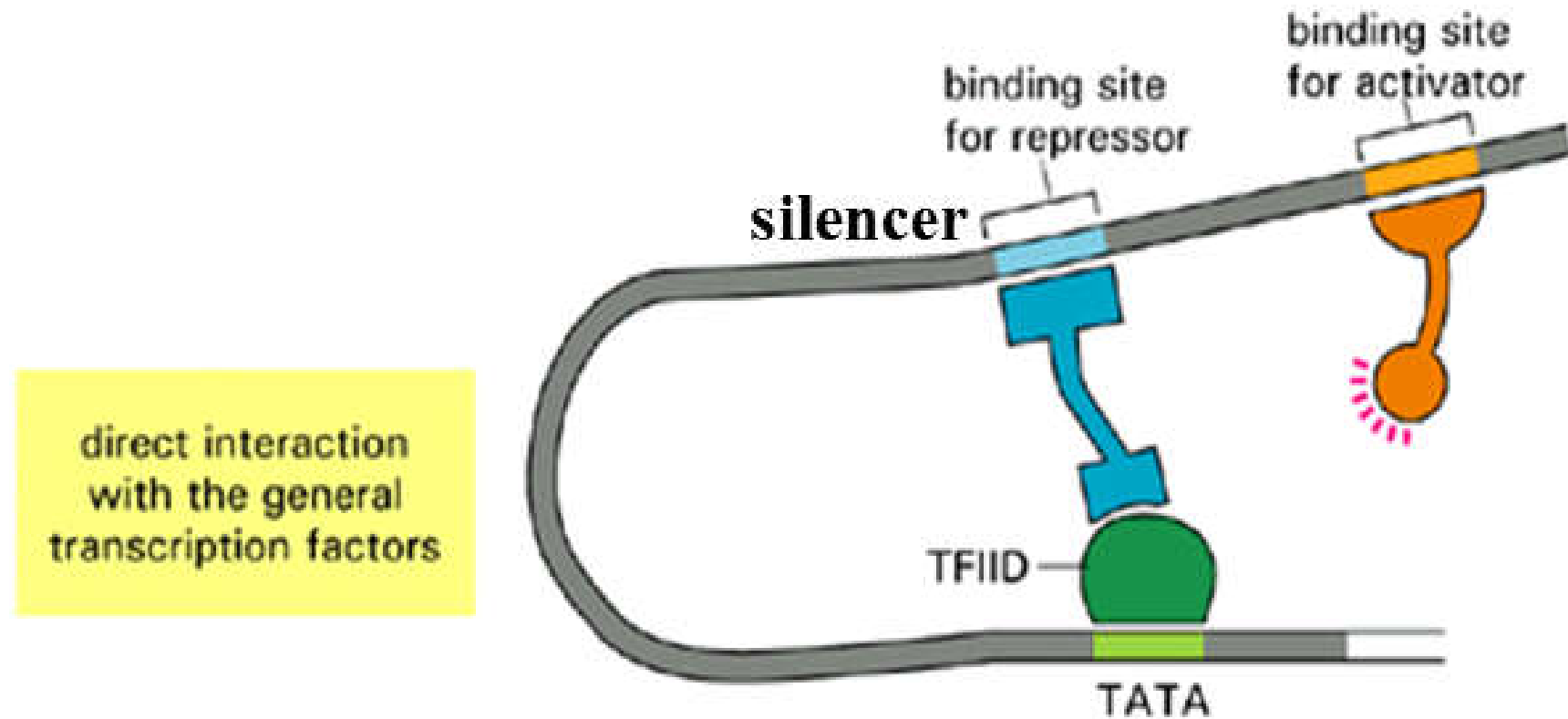
(2) Formation of a non-DNA-binding complex (e.g. Id protein lacks a DNA-binding domain).



(3) Masking of the activation domain without preventing DNA binding (e.g. Gal80 masks the activation domain of the yeast TF Gal4).



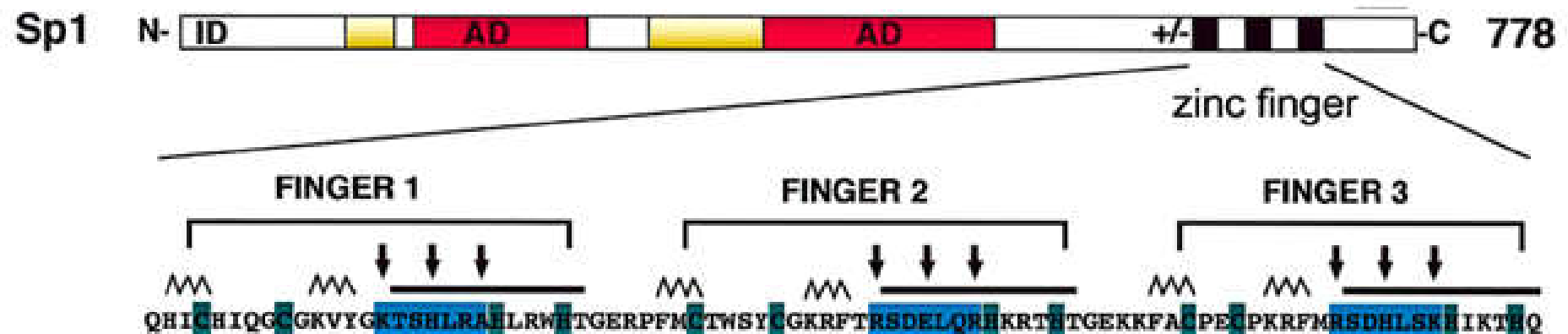
(4) A specific domain of the repressor is directly responsible for inhibition of transcription (e.g. WT1)



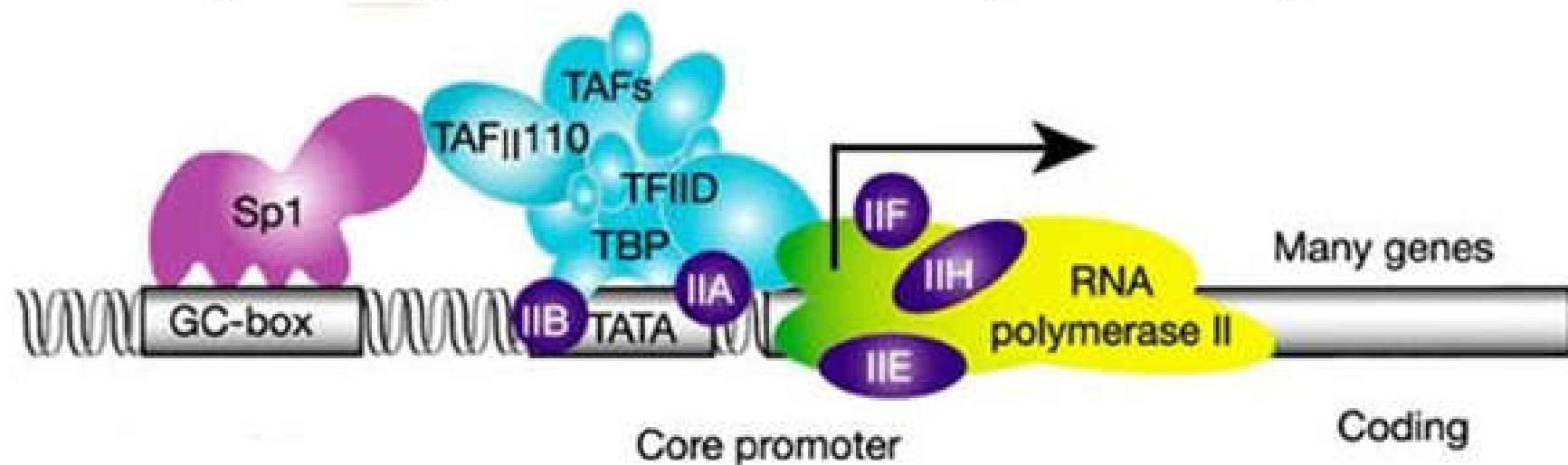
4. Examples of transcriptional regulation

4.1 Constitutive TF: SP1

- Contains **three zinc finger motifs** and **two glutamine-rich activation domain**



- **binding to GC box.**
- Interacting with TAF_{II}110 (TAF4), thus regulating the basal transcription complex.

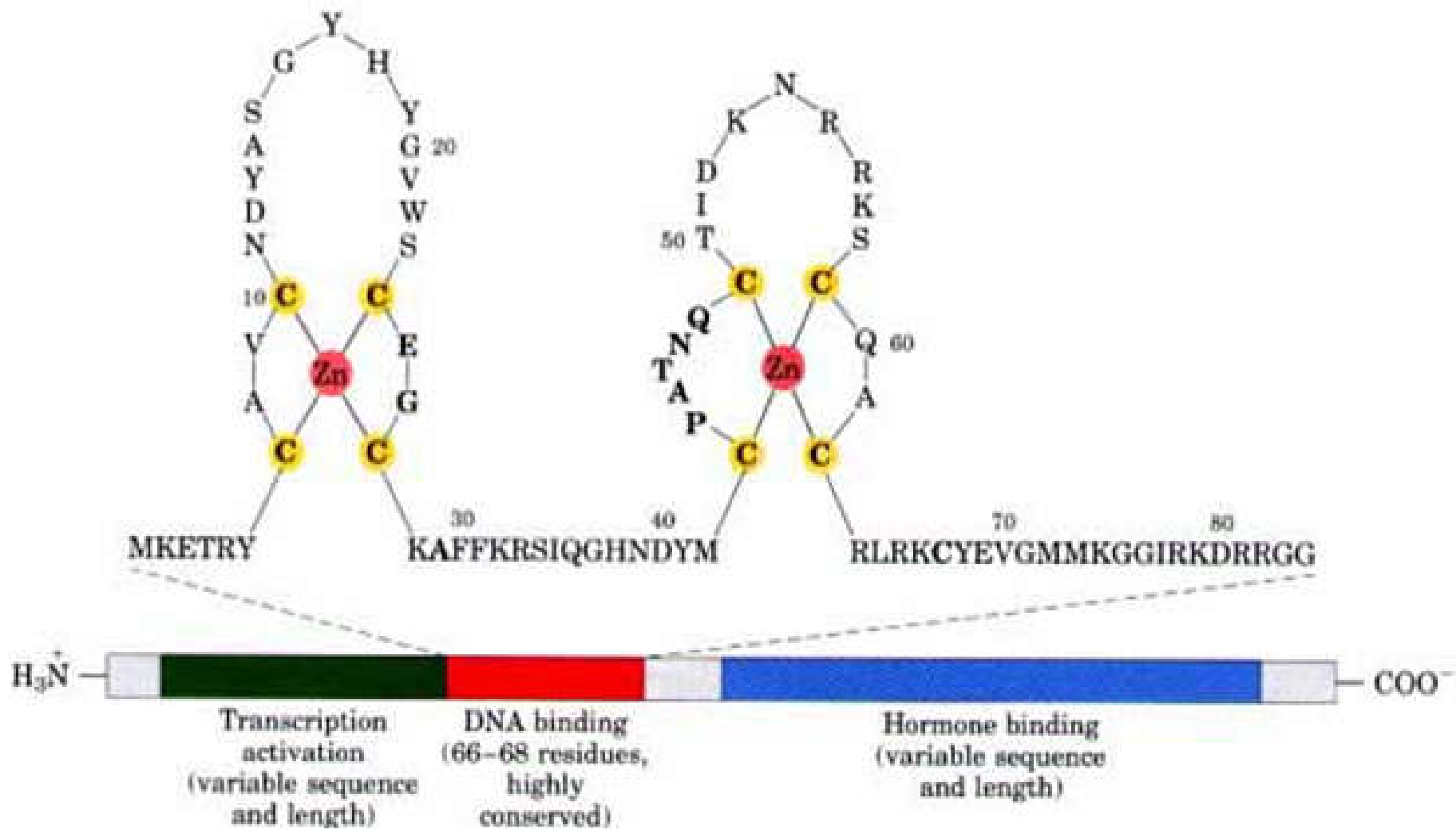


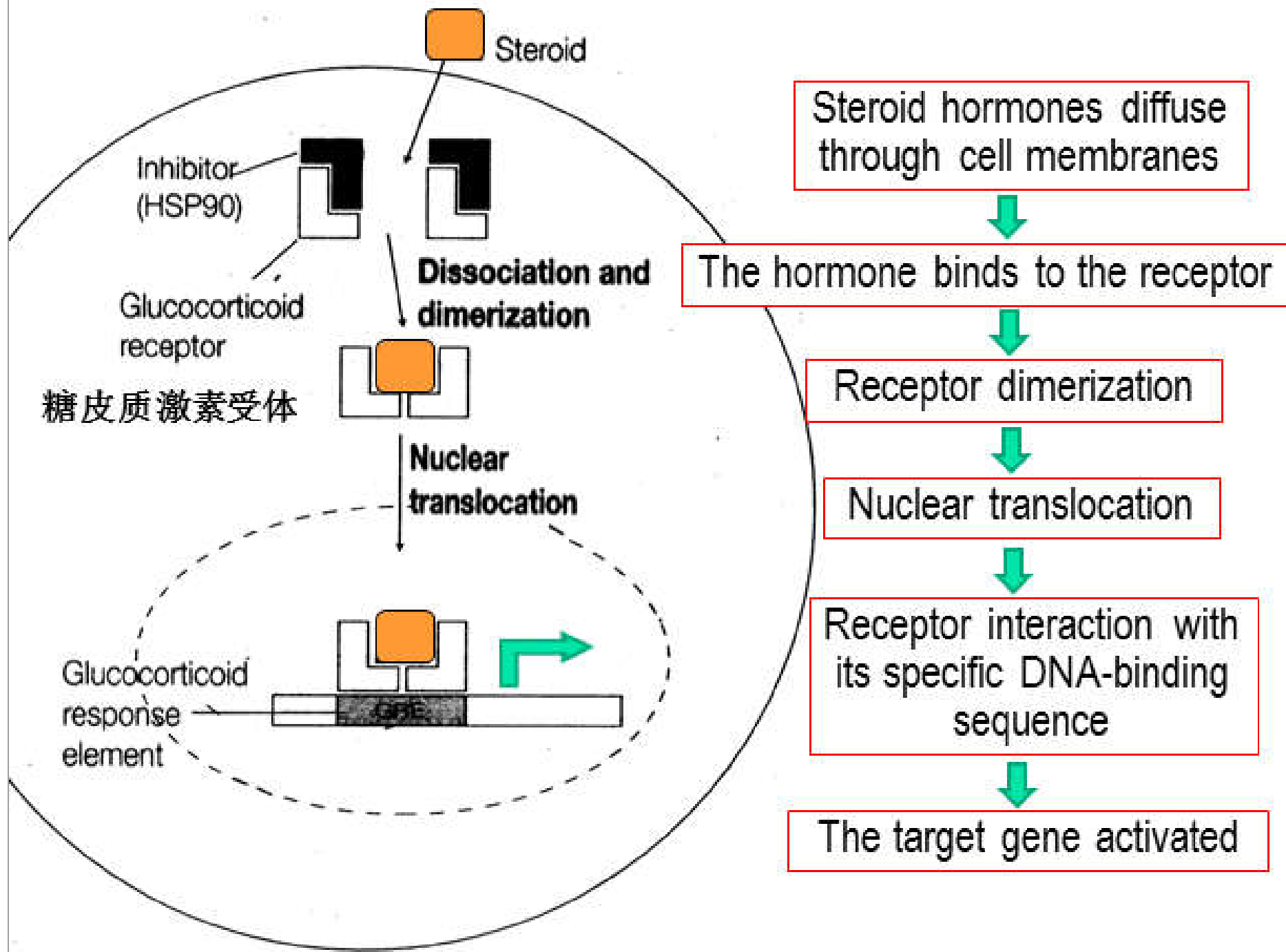
- binding site is in the promoter of many **housekeeping genes**
- It is a **constitutive transcription factor** present in all cell types.



4.2 Hormonal (激素) regulation: steroid hormone receptors (类固醇激素受体)

- Many transcription factors are activated by **hormones** which are secreted by one cell type and **transmit a signal** to a different cell type.
- **Steroid hormones: lipid soluble** and can diffuse through cell membranes to interact with transcription factors called **steroid hormone receptors**.
- e.g. glucocorticoid (糖皮质激素), estrogen (雌激素), retinoic acid (视黄酸) and thyroid hormone (甲状腺激素).

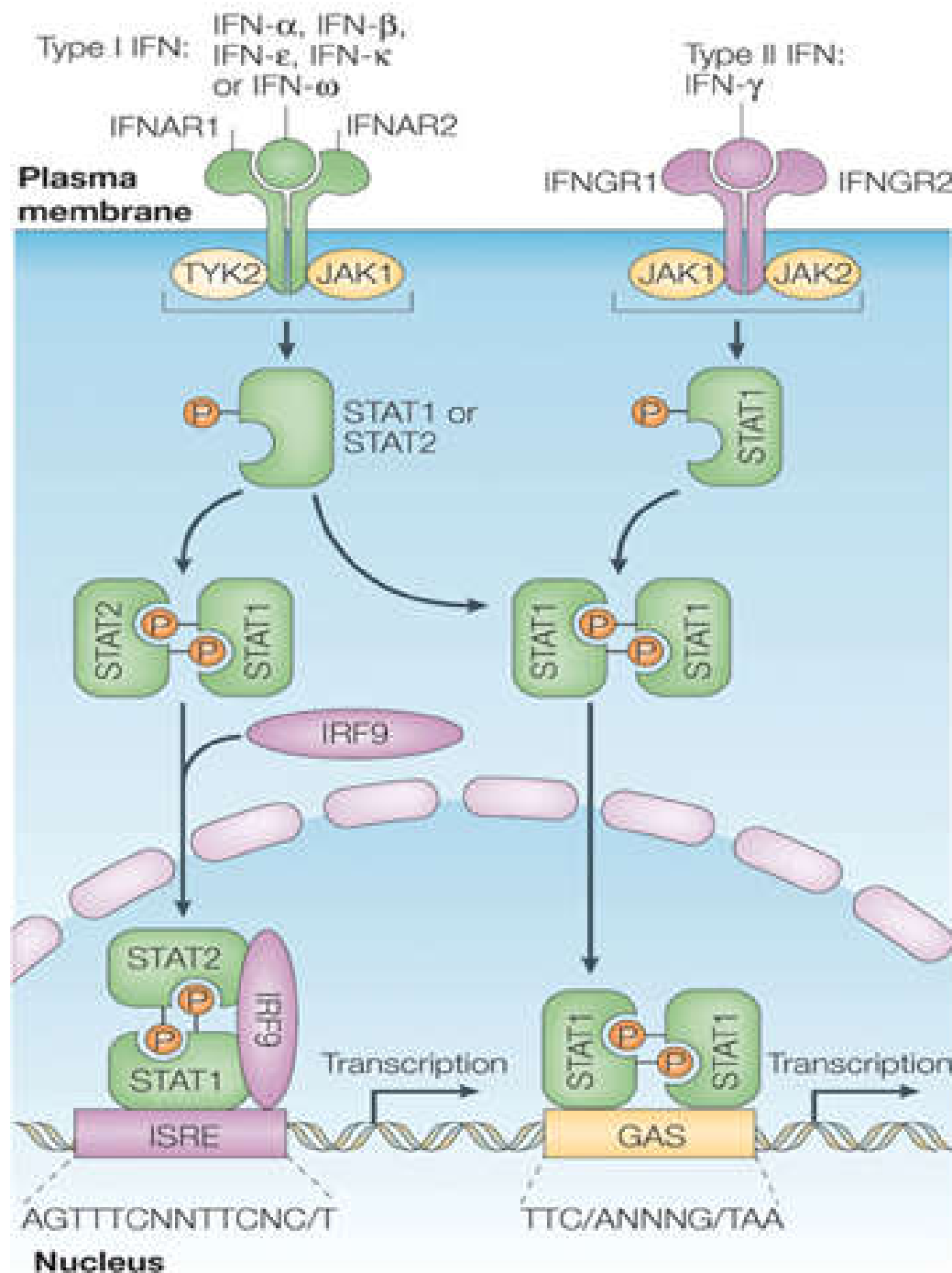






4.3 Regulation by phosphorylation: STAT

- For **water soluble** hormones that do not diffuse into the cell.
- The hormones binds to cell-surface receptors and pass a signal to proteins within the cell through **signal transduction (信号转导)**.
- Signal transduction often involves protein **phosphorylation**.



Peptide hormones binds to cell-surface receptors

Signal transduction

STAT phosphorylation and dimer formation

Nuclear translocation

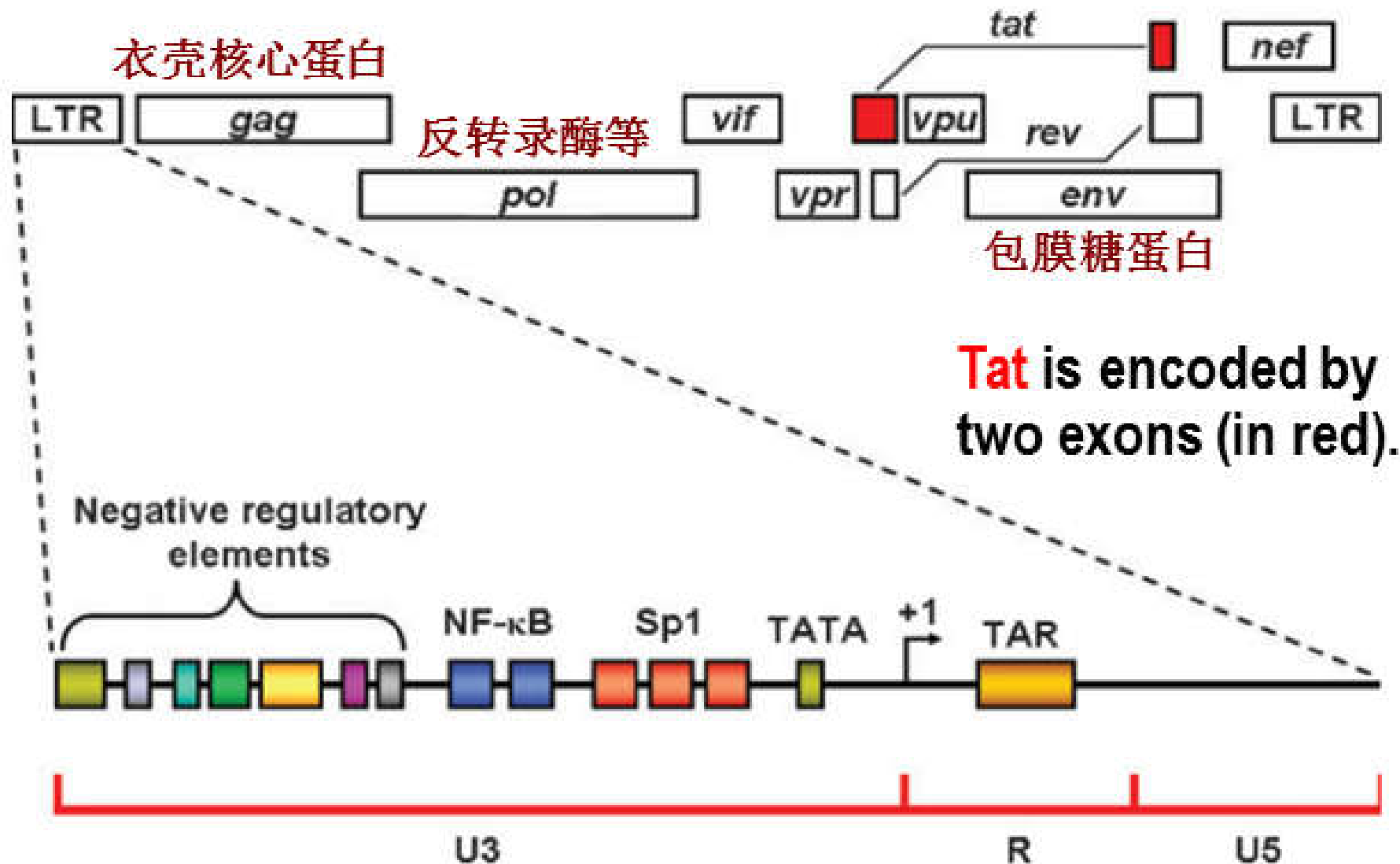
STAT dimer interaction with its specific DNA-binding sequence

The target gene activated



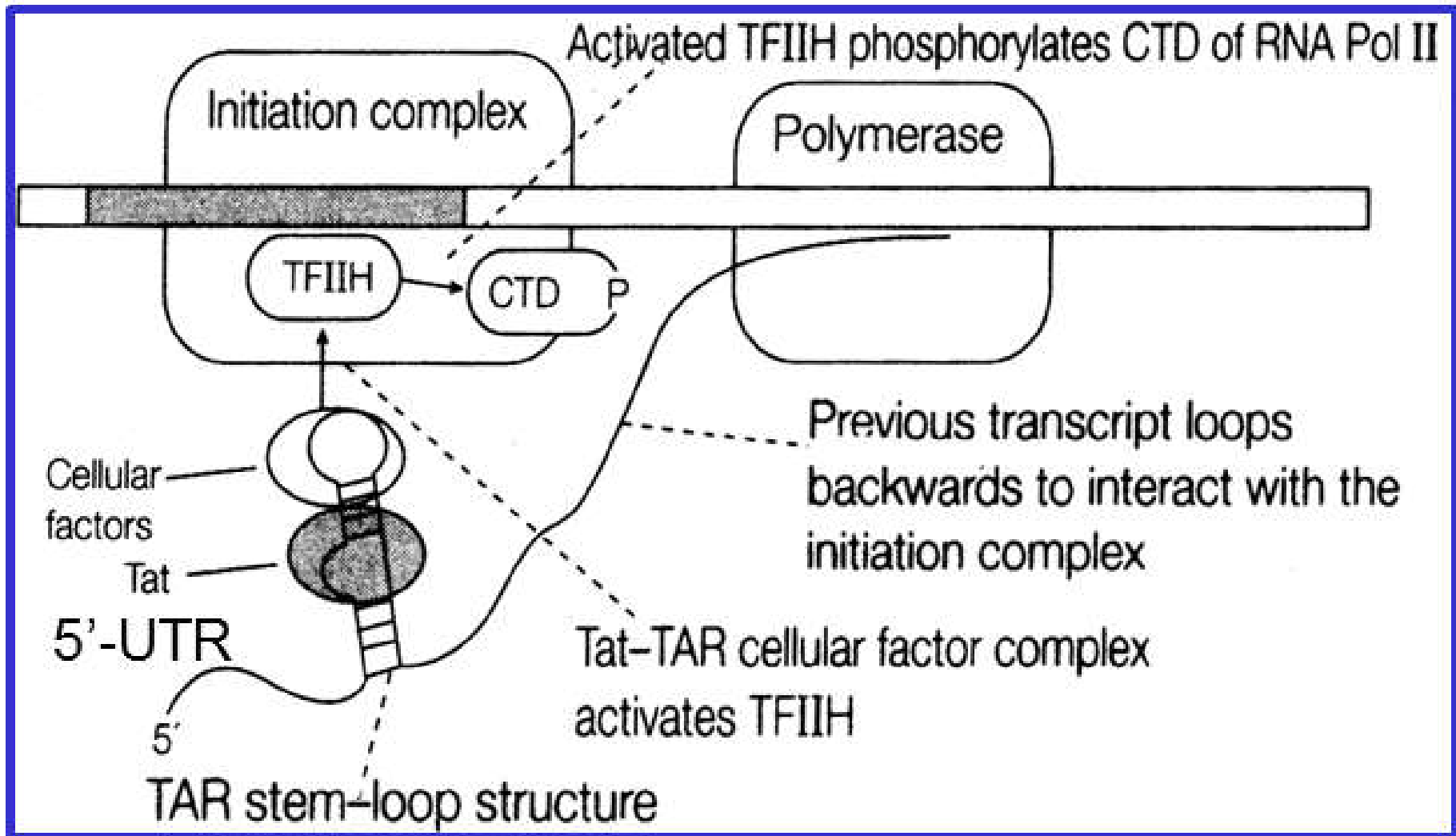
4.4 Transcription elongation: HIV Tat

Human immunodeficiency virus (HIV) encodes an activator protein called **Tat, which is required for productive HIV gene expression.**

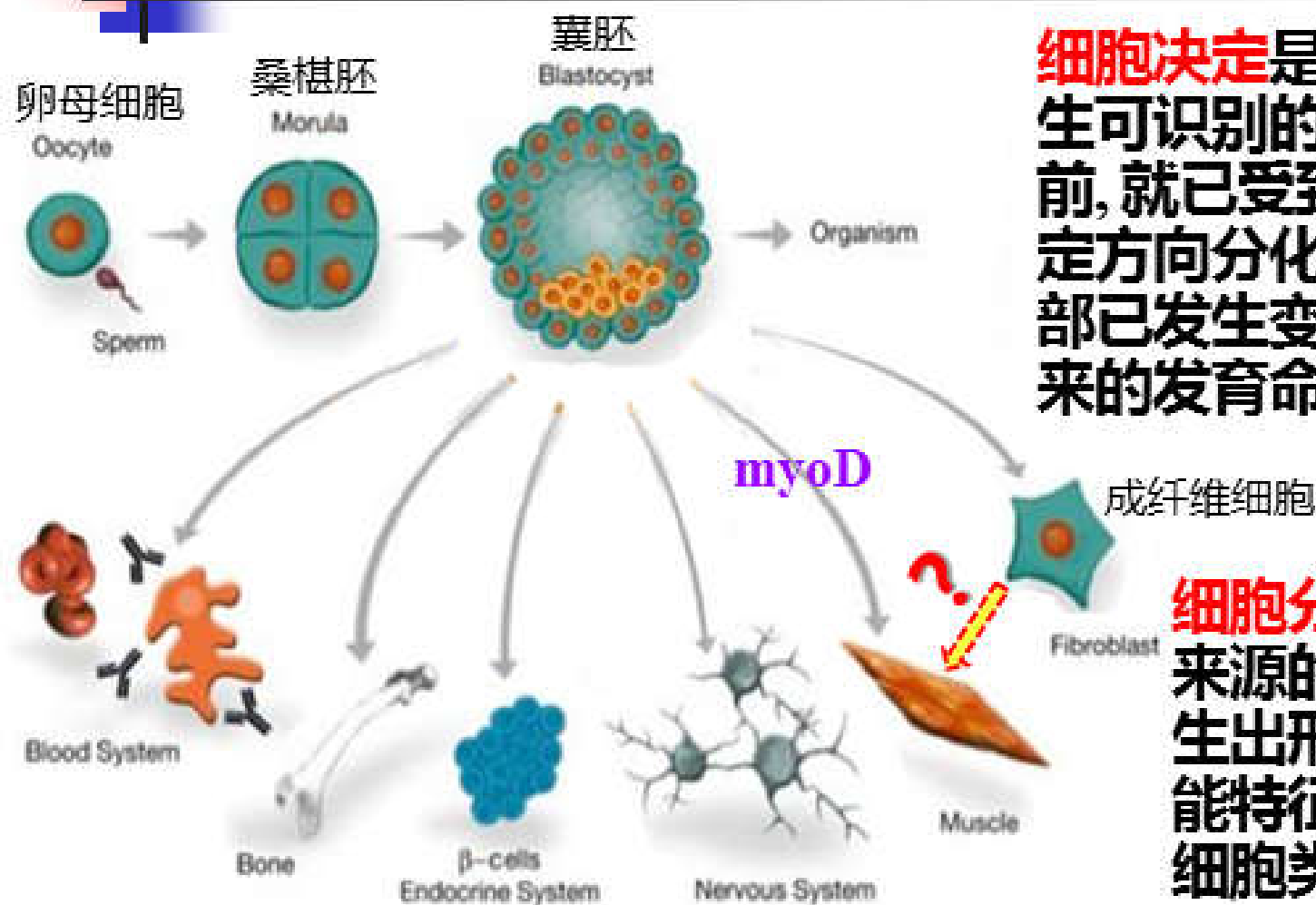


Tat binds to an RNA stem-loop structure called **TAR**, which is present in the 5'-UTR of all HIV RNAs just after the HIV transcription start site.

- **The interaction of Tat and TAR may result in the activation of the kinase activity of TFIIH, leading to phosphorylation of the CTD of RNA Pol II.**



4.5 Cell determination: myoD



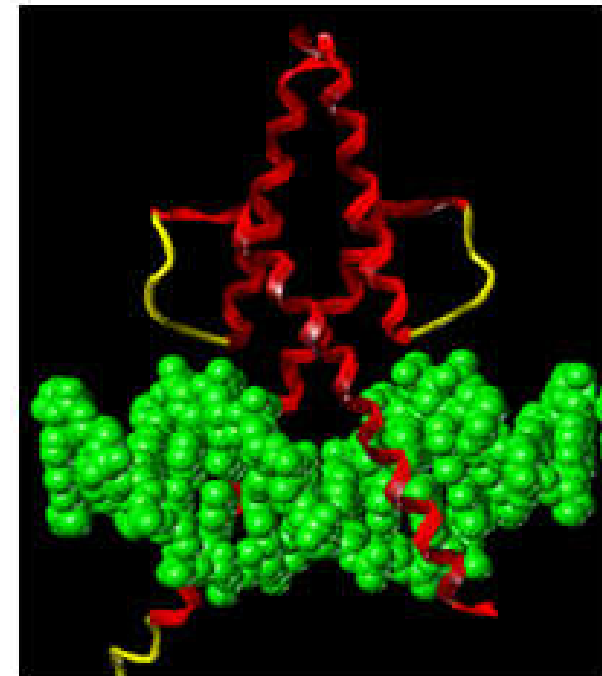
细胞决定是指细胞在发生可识别的形态变化之前,就已受到约束而向特定方向分化,这时细胞内部已发生变化,确定了未来的发育命运。

细胞分化是指同一起来源的细胞逐渐产生出形态结构、功能特征各不相同的细胞类群的过程。

细胞决定可看作分化潜能逐渐限制的过程, 决定先于分化。

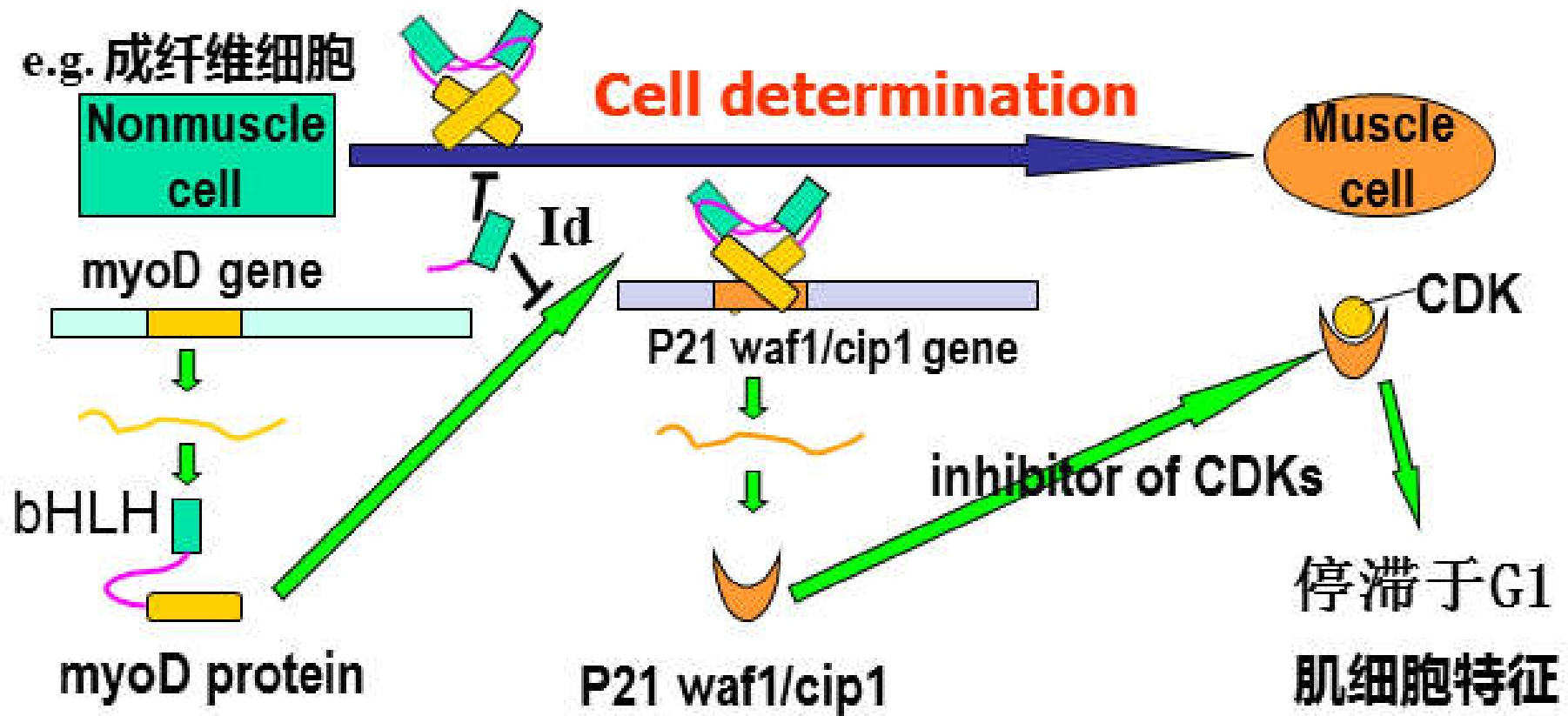
- *myoD*, *myogenin*, *myf5* and *mrf4* can regulate gene expression in cell determination, converting fibroblasts (成纤维细胞) into muscle.

- The encoded proteins are all members of the **bHLH transcription factor family**.



(1) Activates muscle-specific gene expression directly

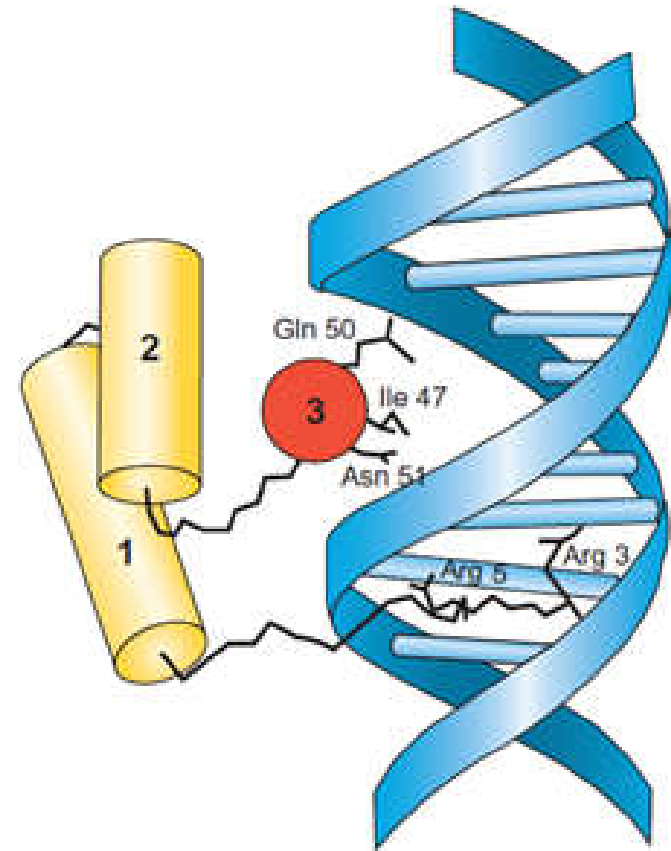
Id lacks a DNA-binding domain, but contains the HLH dimerization domain.



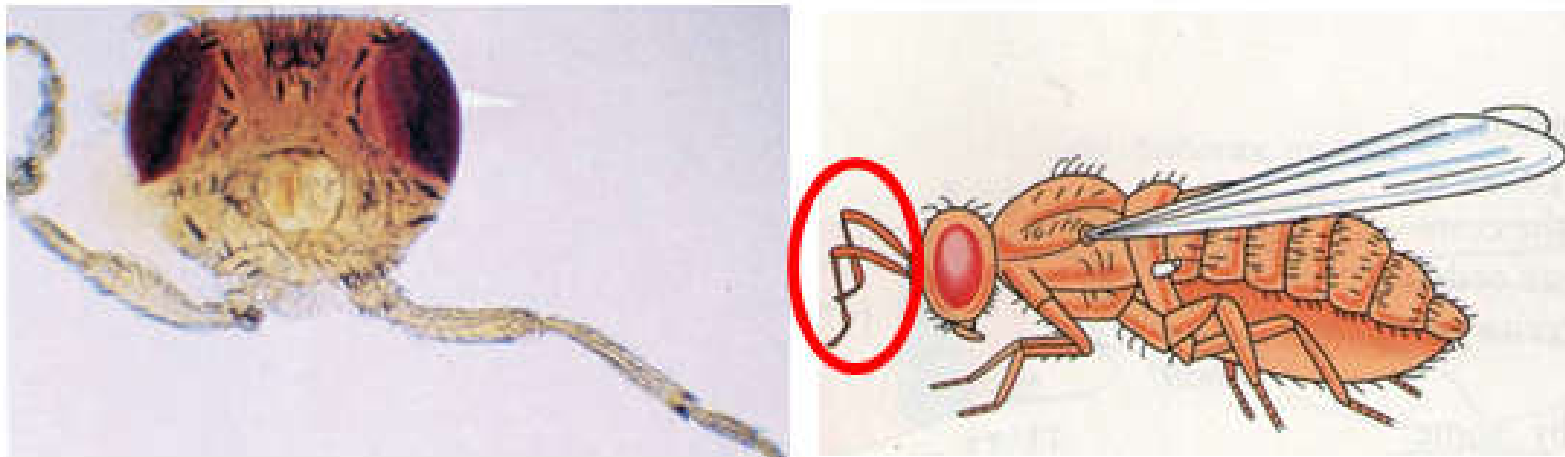
(2) Activates expression of p21waf1/cip1 expression

4.6 Embryonic development: homeodomain proteins

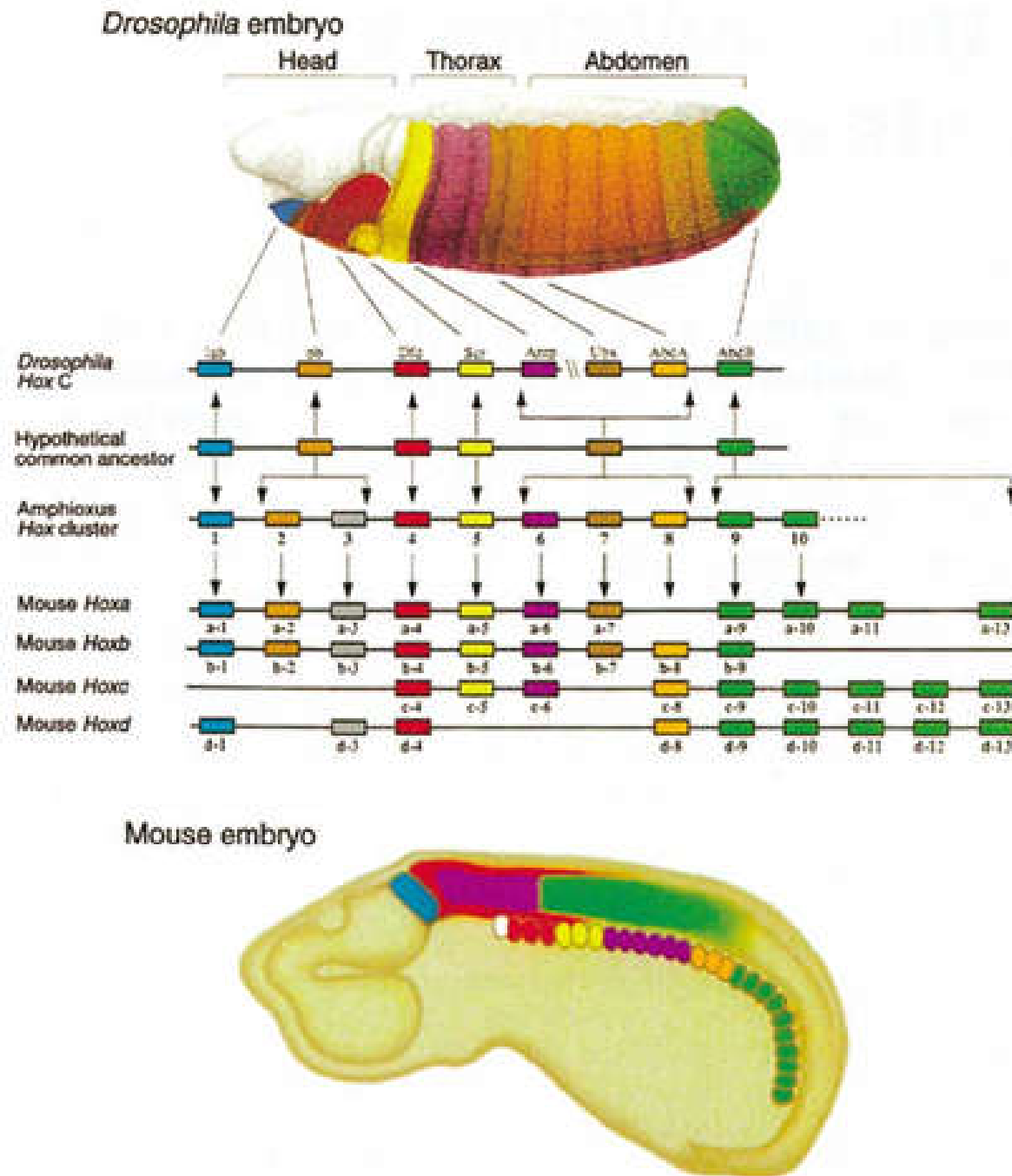
- Homeodomain protein is a **HTH DNA binding protein** which was first found in *Drosophila* (果蝇).
- It is encoded by homeobox (同源框), or homeotic gene (同源异型基因, *HOX*) in *Drosophila*.



- **Homeotic genes are responsible for the correct specification of body parts.**



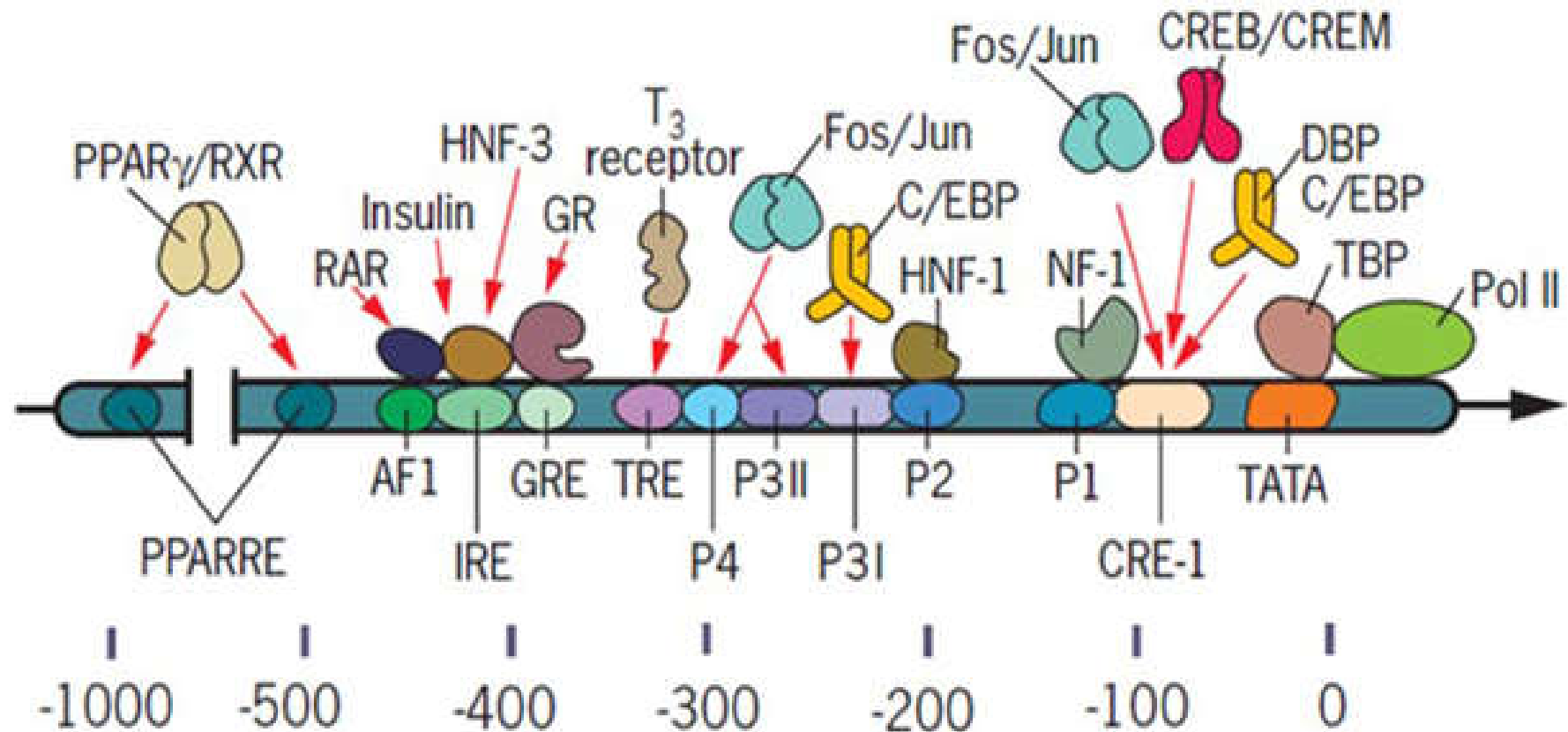
***Antennapedia* 触角足**

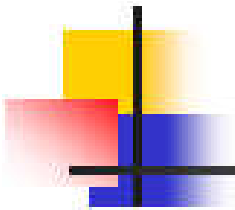


- The homeobox sequence is conserved between a wide range of eukaryotes.
- The homeobox genes are arranged in gene clusters in which homologous genes are in the same order.

(磷酸烯醇式丙酮酸羧激酶)

参与调控大鼠PEPCK基因的诸多转录因子





Summary

- 1. Definition and features of enhancers and silencers**
- 2. Functional domains in transcription factors (especially the structures of DNA-binding domain and transcription-activating domain)**
- 3. Targets for transcriptional regulation (how to regulate transcription)**
- 4. Repression of transcription**
- 5. Mechanisms of transcriptional regulation of SP1, steroid hormone receptors, STAT proteins, HIV Tat, myoD and homeodomain proteins**