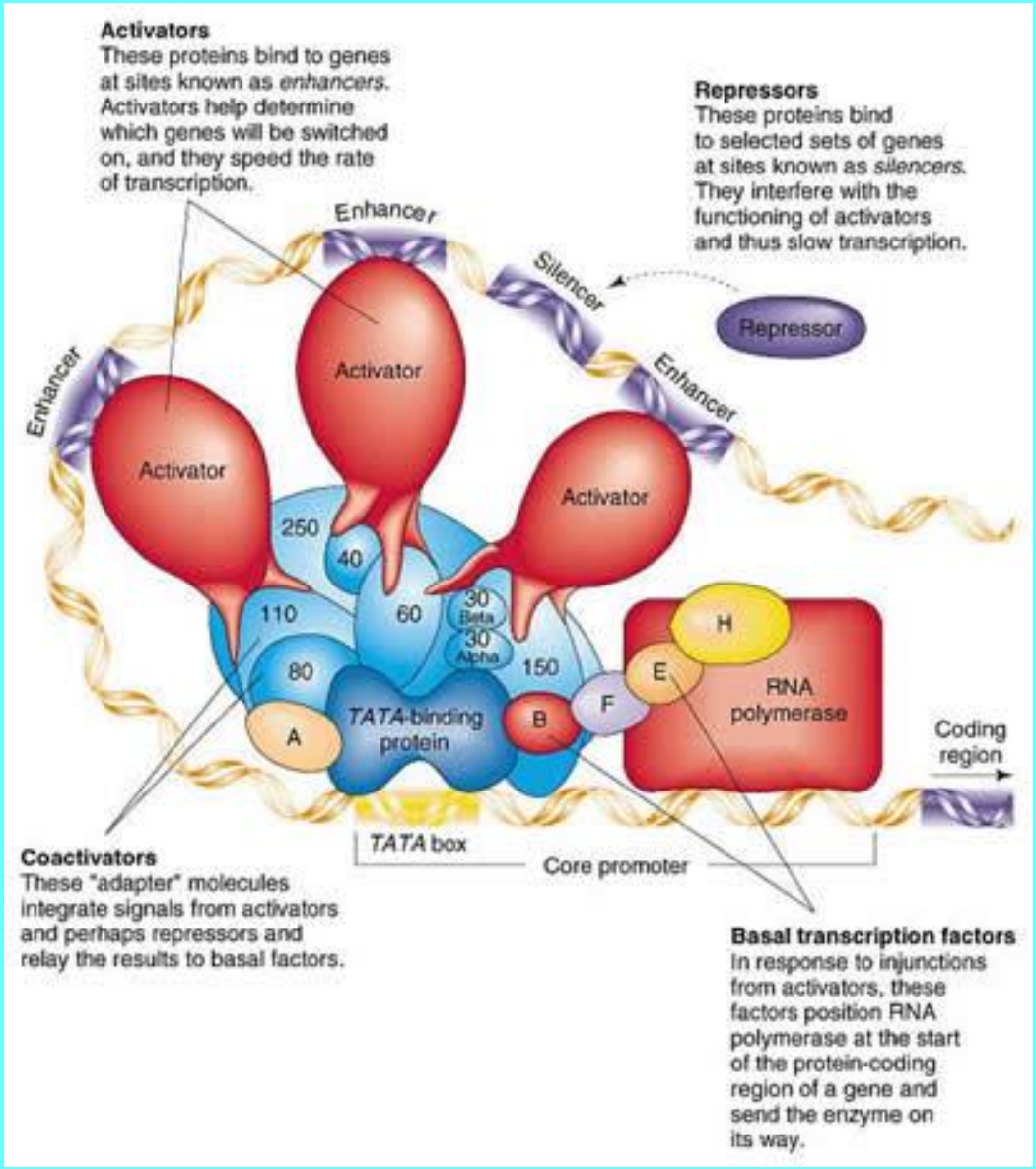
REGULATION OF GENE EXPRESSION IN EUKARYOTES

Regulation of eukaryotic gene expression



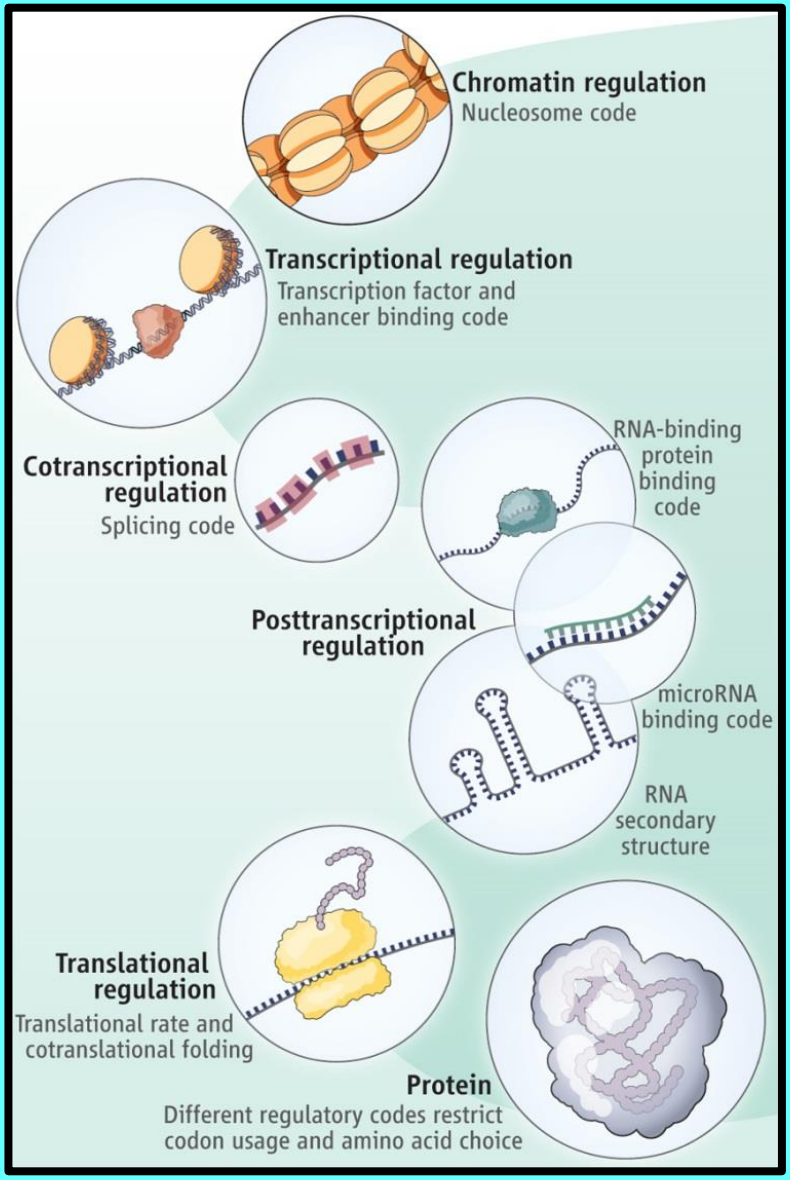
https://cz.pinterest.com/taolog/life-science/

Regulation of eukaryotic gene expression

Complicated network of many different factors which work in dependency to space and time

- **Products of the same gene have different functions in different tissues**
- **Different genes coding similar products are expressed in different phases of ontogenetic development**

Levels of regulatory codes

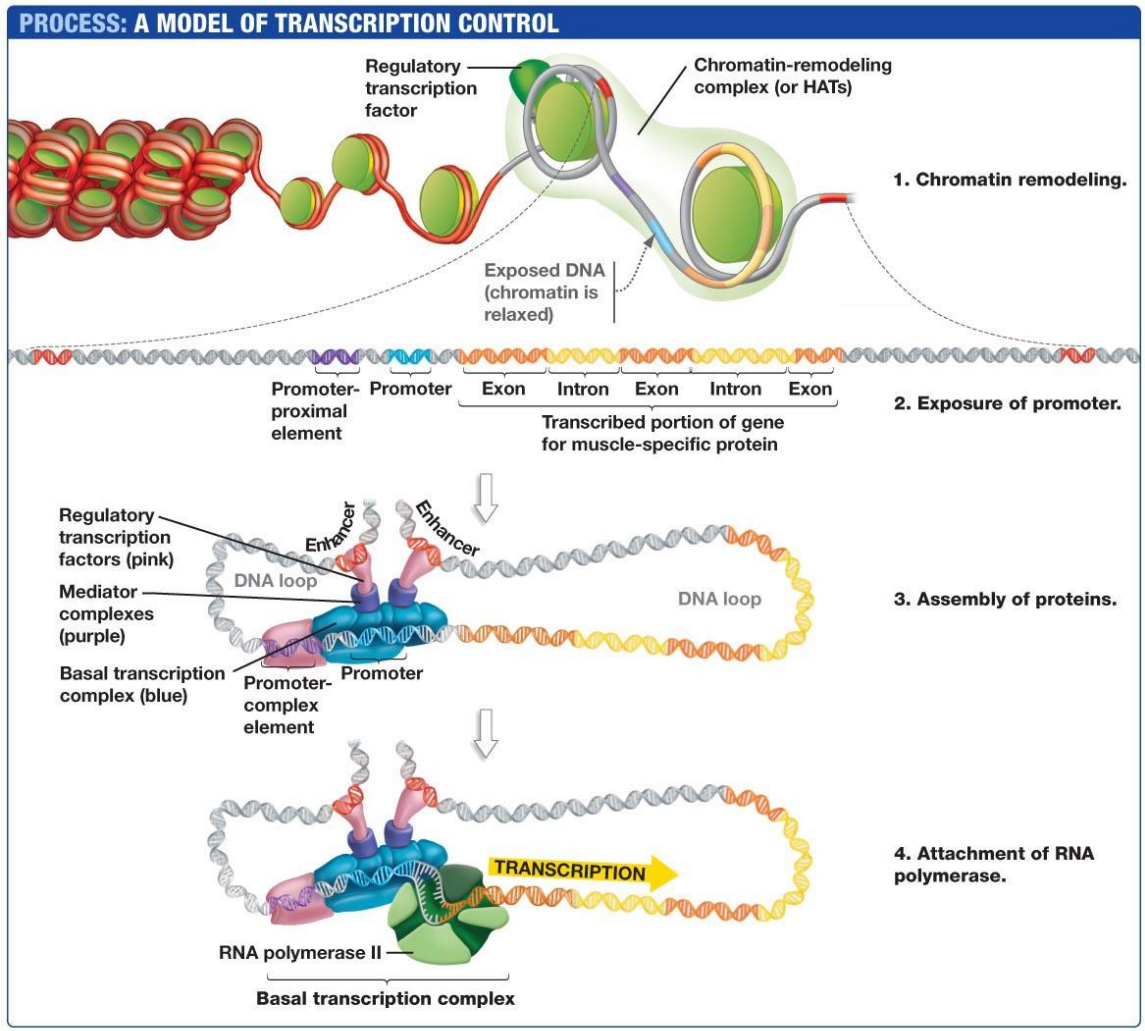


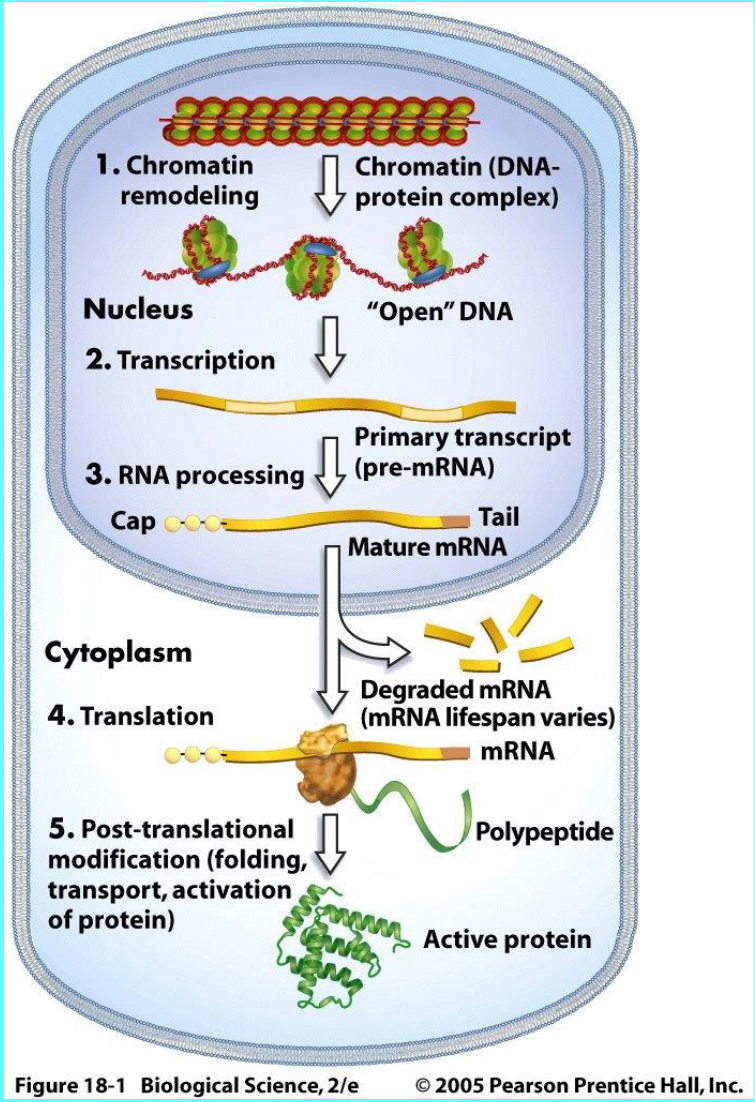
Constraining codes. Regulatory elements within protein-coding regions (such as transcription factor binding) can influence codon choice and amino acid preference that are independent of protein structure or function.

R J Weatheritt, and M M Babu Science 2013;342:1325-1326

Science AAAS

Levels of regulatory codes

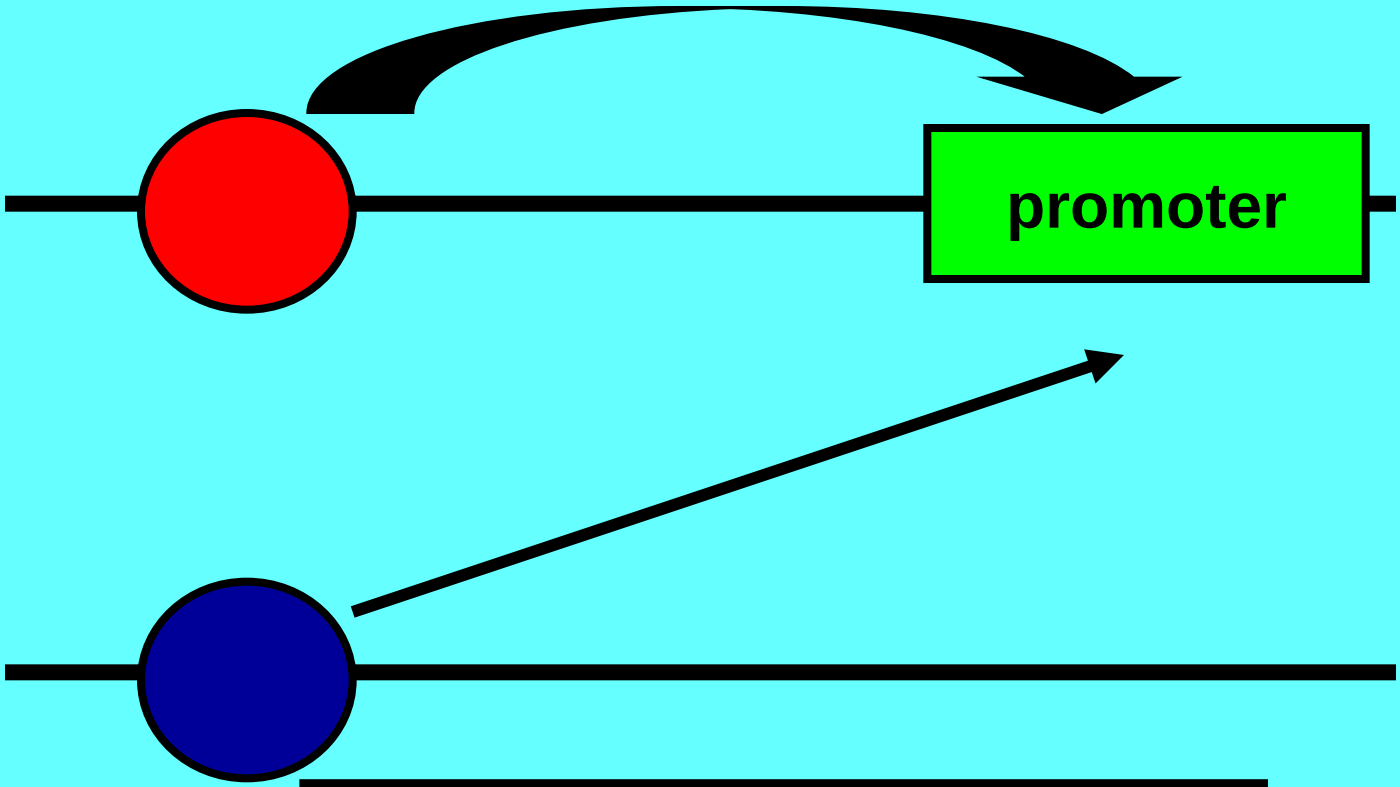




Regulation on the transcription level is driven by protein transcription factors

Cis and trans regulators

cis = on the same molecule

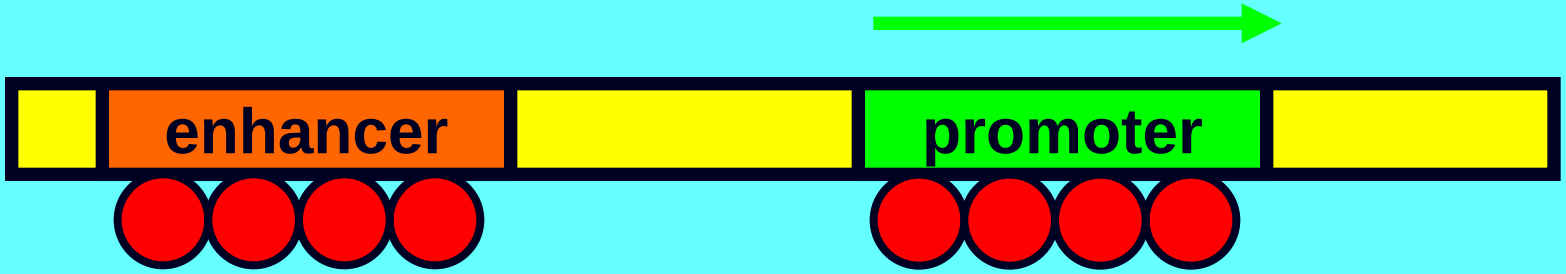


trans = on different molecule

Activation of eukaryotic expression

- Transcription depends on the presence of **activated** transcription factors

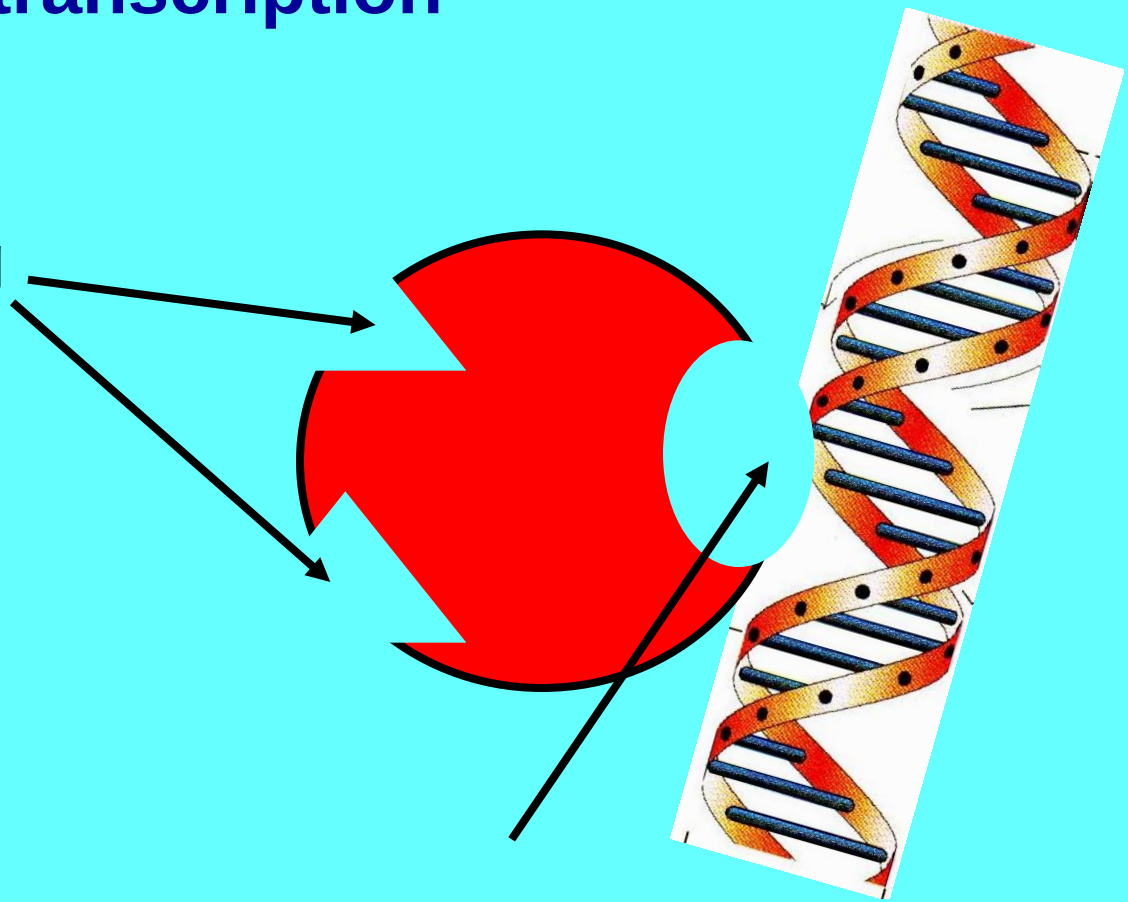
- it is TURN ON, if they are available
- it is TURN OFF, if they are not available



Transcription factors I

They have mostly positive, but also negative effect on transcription

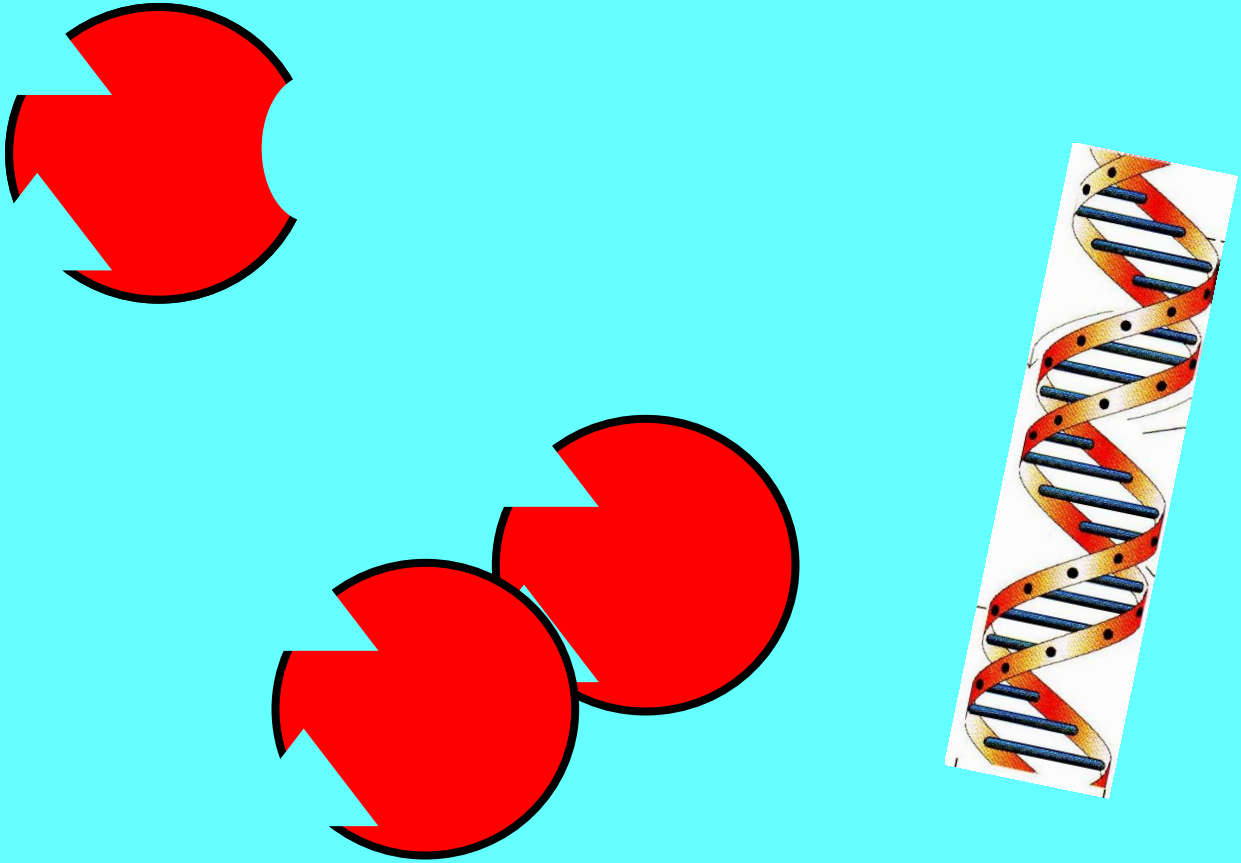
(trans)-activating domains



binding site for DNA

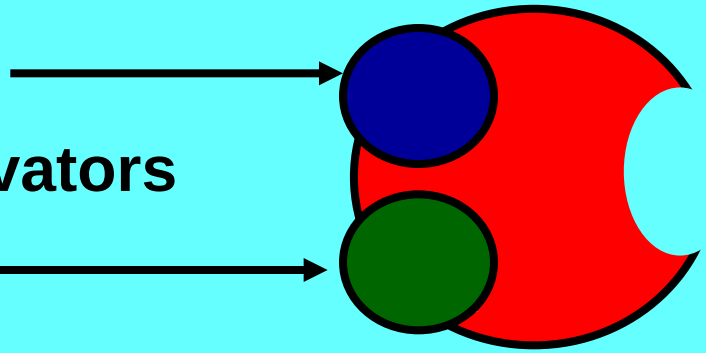
Transcription factors II

Transcription factors without the binding site for DNA bind to the DNA through other transcription factors



Activators of the transcription factors bind to the activating domains

activators

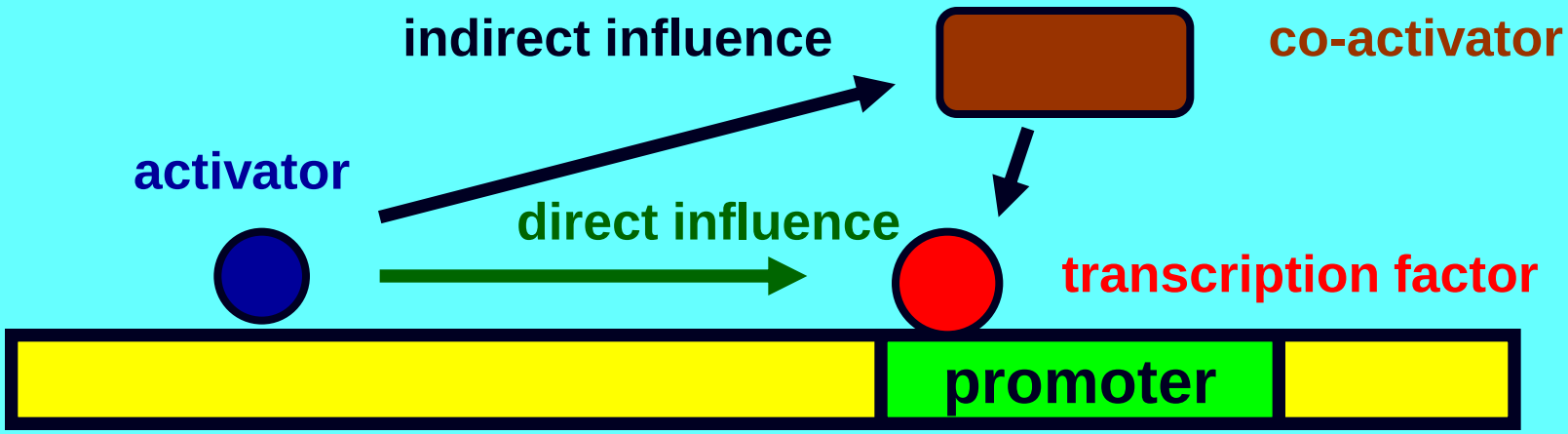


Activators of the transcription factors

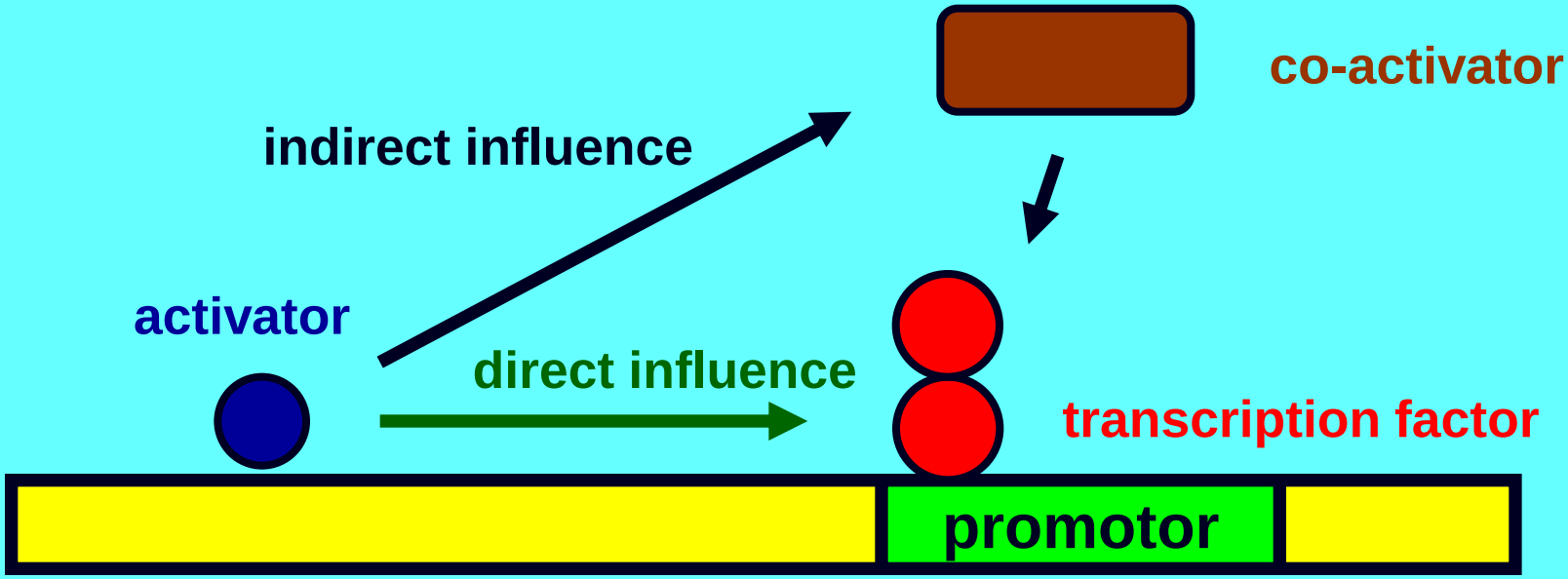
They influence transcription initiation

- They accelerate speed of pre-initiation complex formation
- They stimulate transcription after initiation complex formation
- They change conformation of transcription factor

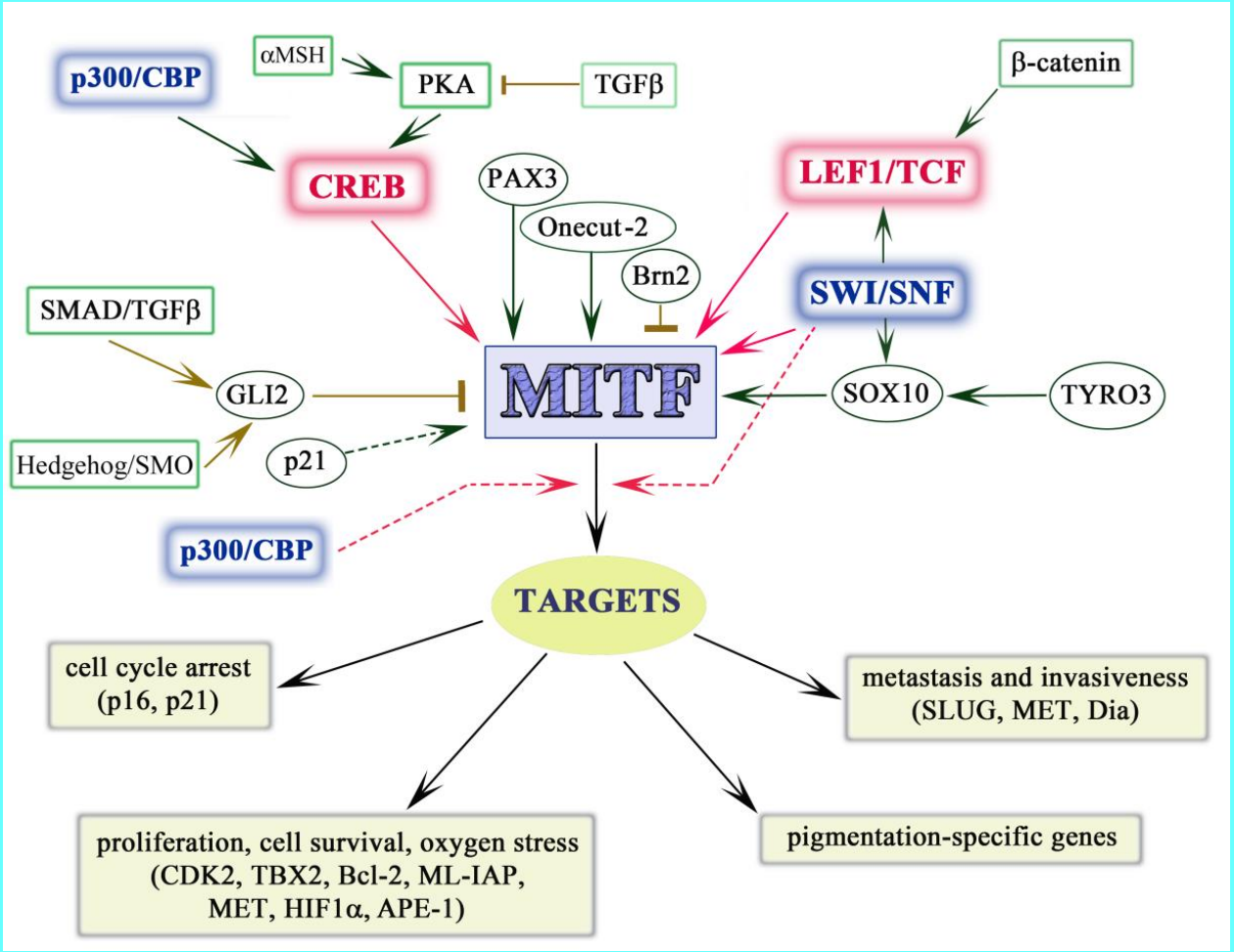
Activators of transcription factors I



Activators of transcription factors II



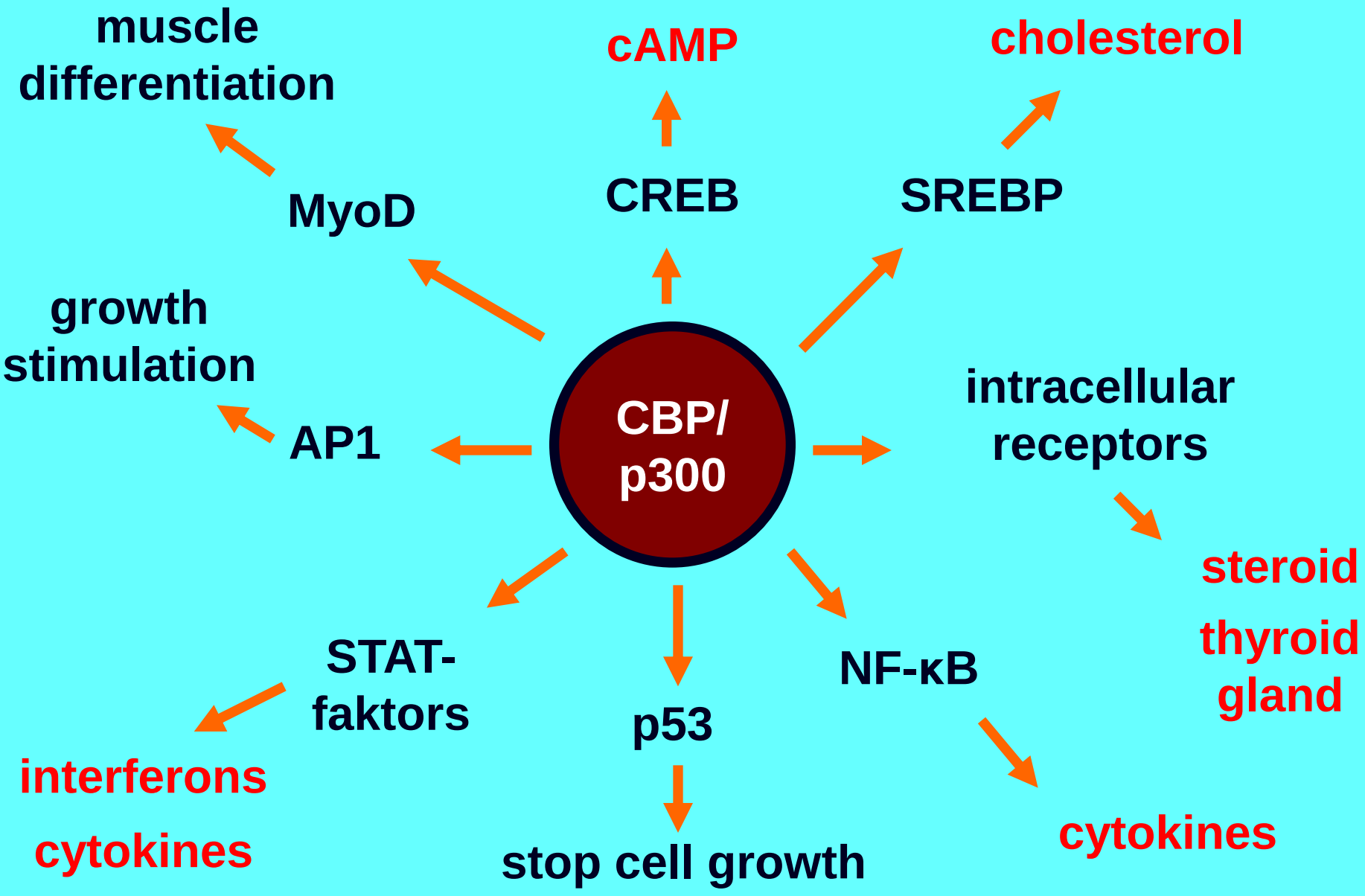
Example - Transcriptional network of MITF-M in melanoma



Transcriptional network of MITF-M in melanoma. The picture shows upstream MITF-M activators and main downstream transcription targets. The blue boxes indicate transcriptional coactivators and red boxes denote two transcription factors probably having more important role for the MITF-M expression in melanoma. Broken lines indicate that not all MITF-M targets may be coactivated by indicated epigenetic coactivators.

Jiri Vachtenheim and Lubica Ondrušova (2013). MITF: A Critical Transcription Factor in Melanoma Transcriptional Regulatory Network, Recent Advances in the Biology, Therapy and Management of Melanoma, Dr. Lester Davids (Ed.), InTech, DOI: 10.5772/55191.

Example - Co-activators CBP and p300

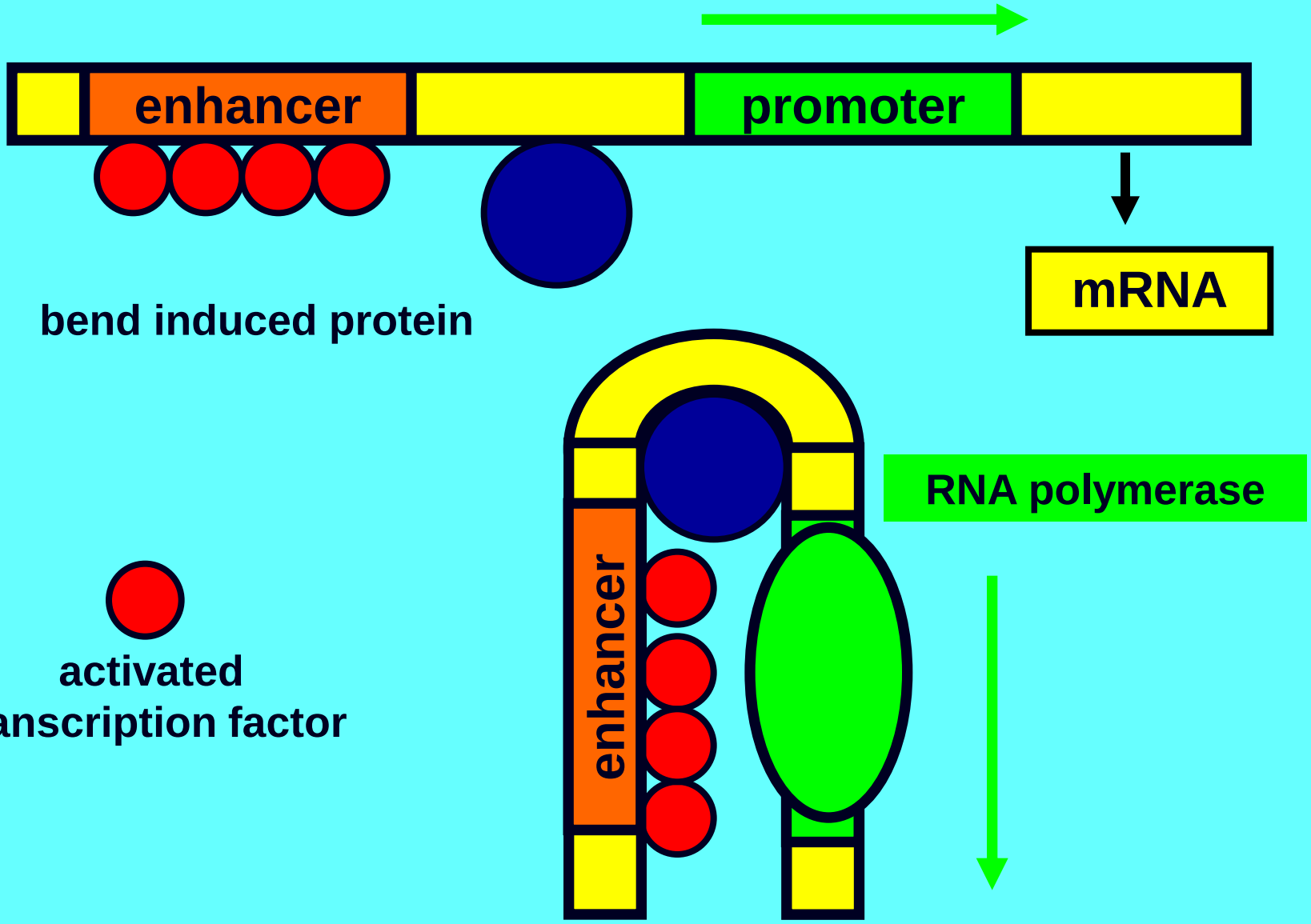


Enhancers and silencers of transcroption

Regulatory sequences which turn up or turn down the transcription

- **They are far hundreds or thousands nucleotides from promoter**
- **In cis position, upstream or downstream of gene**
- **Mechanisms of the enhancers acts through the transcription factors; the result of this process is establishment of RNA polymerase to active state**

Mechanism of enhancer



By which eukaryotic gene expression is influenced?

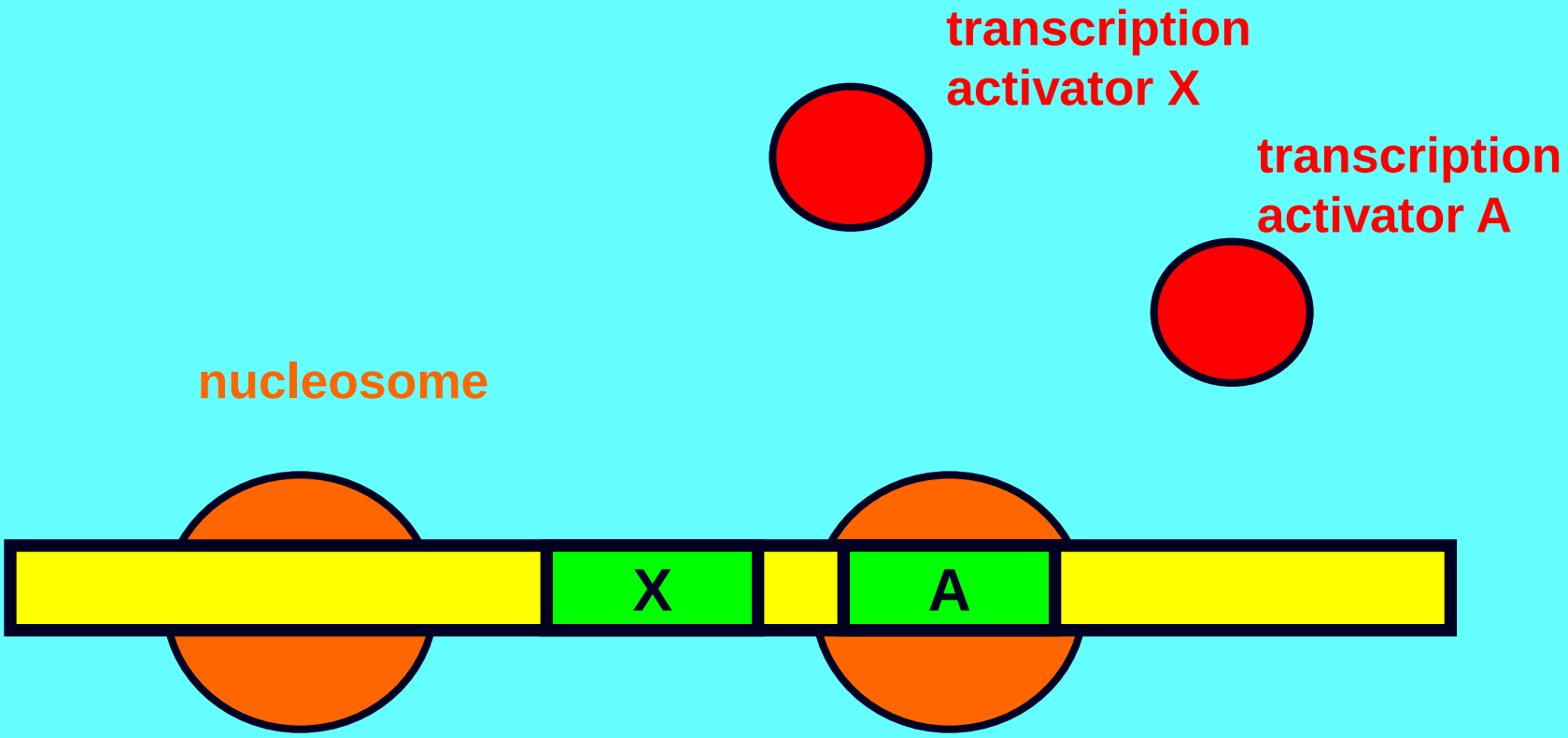
signal IS x IS NOT present

level of activator × level of represor

place of expression – tissue specificity

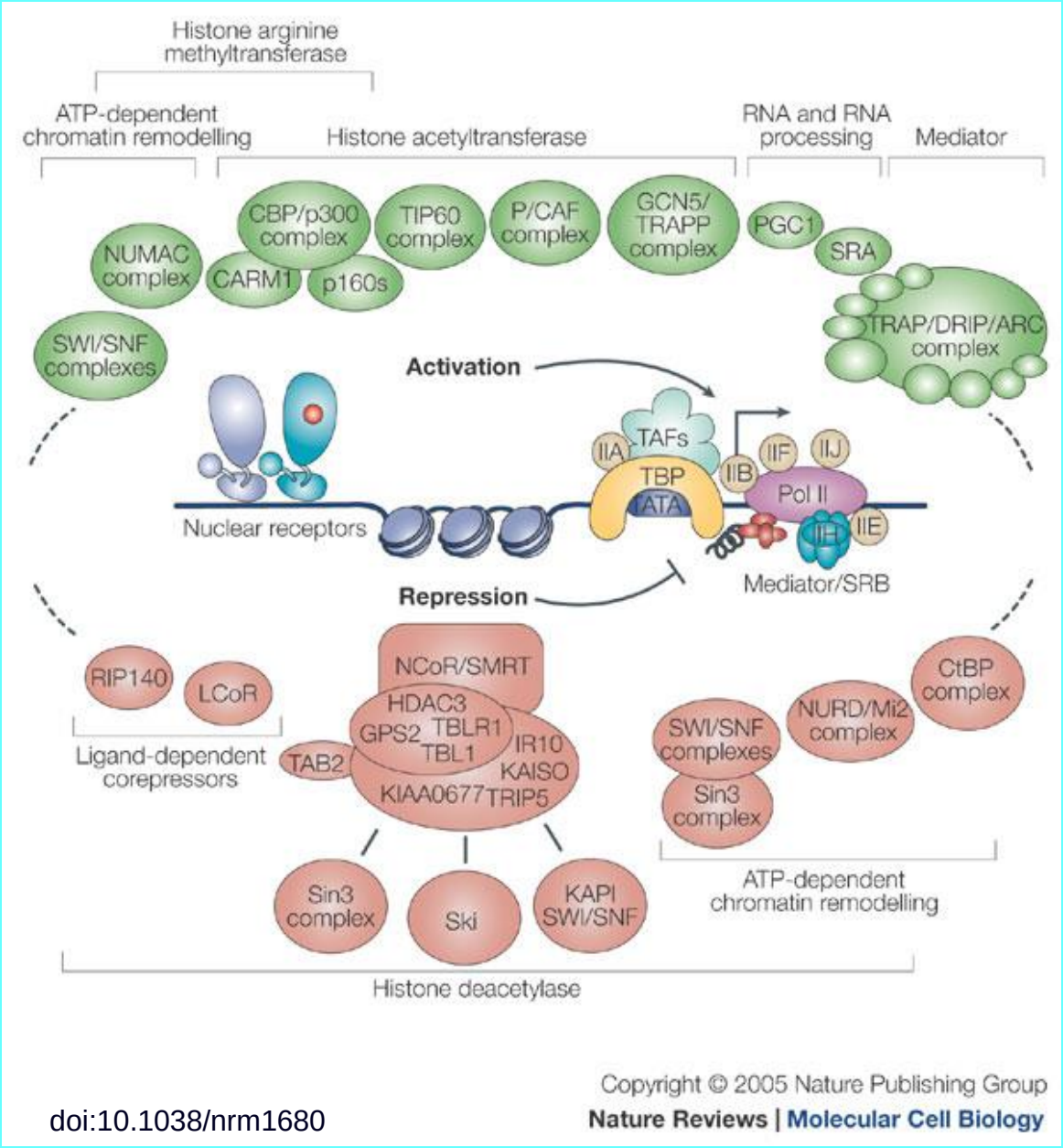
period of expression - ontogenesis

Activators and structure of chromatin



Transcription activation is coupled with change of chromatin structure

Activators and structure of chromatine



Nature Reviews | Molecular Cell Biology

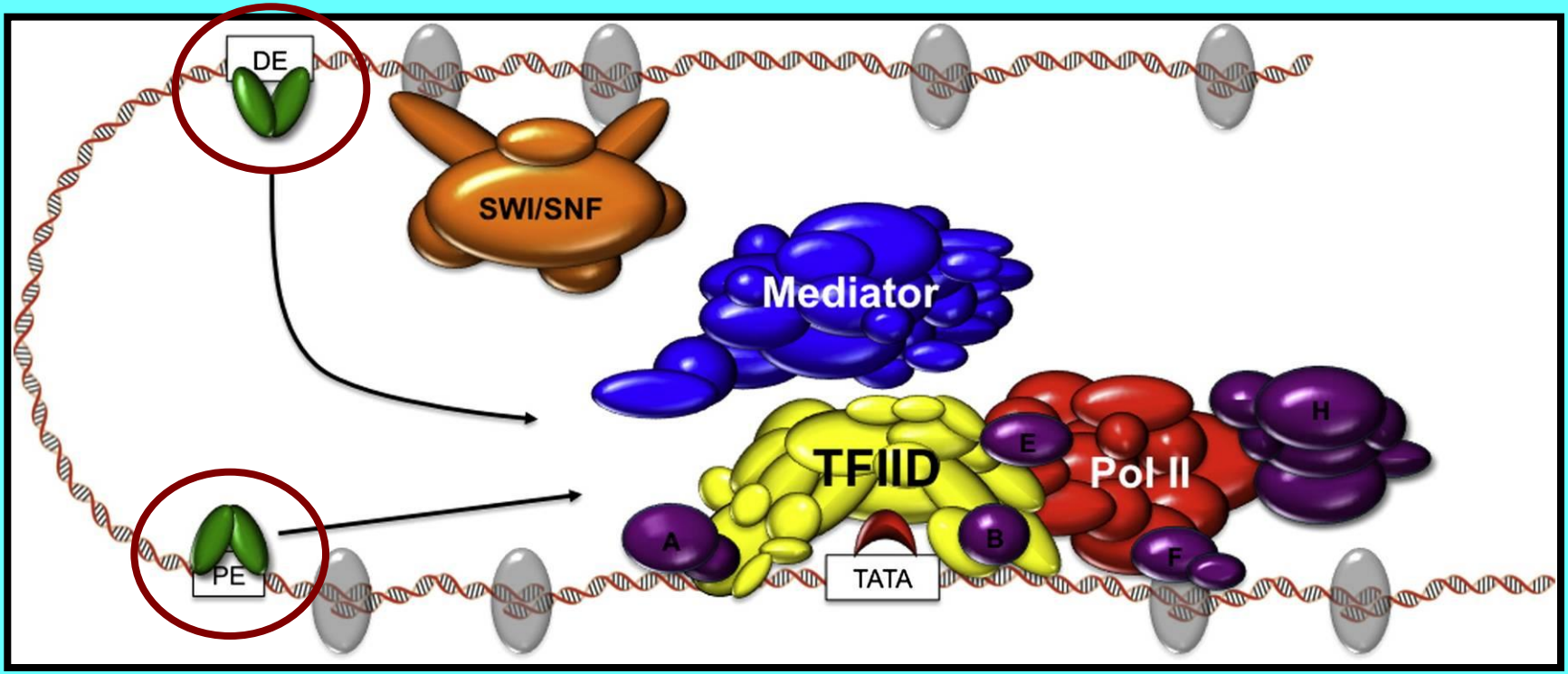
doi:10.1038/nrm1680

How the transcription factors are activated?

- Change of conformation induced by ligand
- Change of conformation after removing of inhibitory protein
- Change of conformation by phosphorylation
  - phosphorylation by proteinkinase
  - dephosphorylation by phosphatase
- Stabilisation of transcription factor active conformation against its degradation

Model for preinitiation complex formation

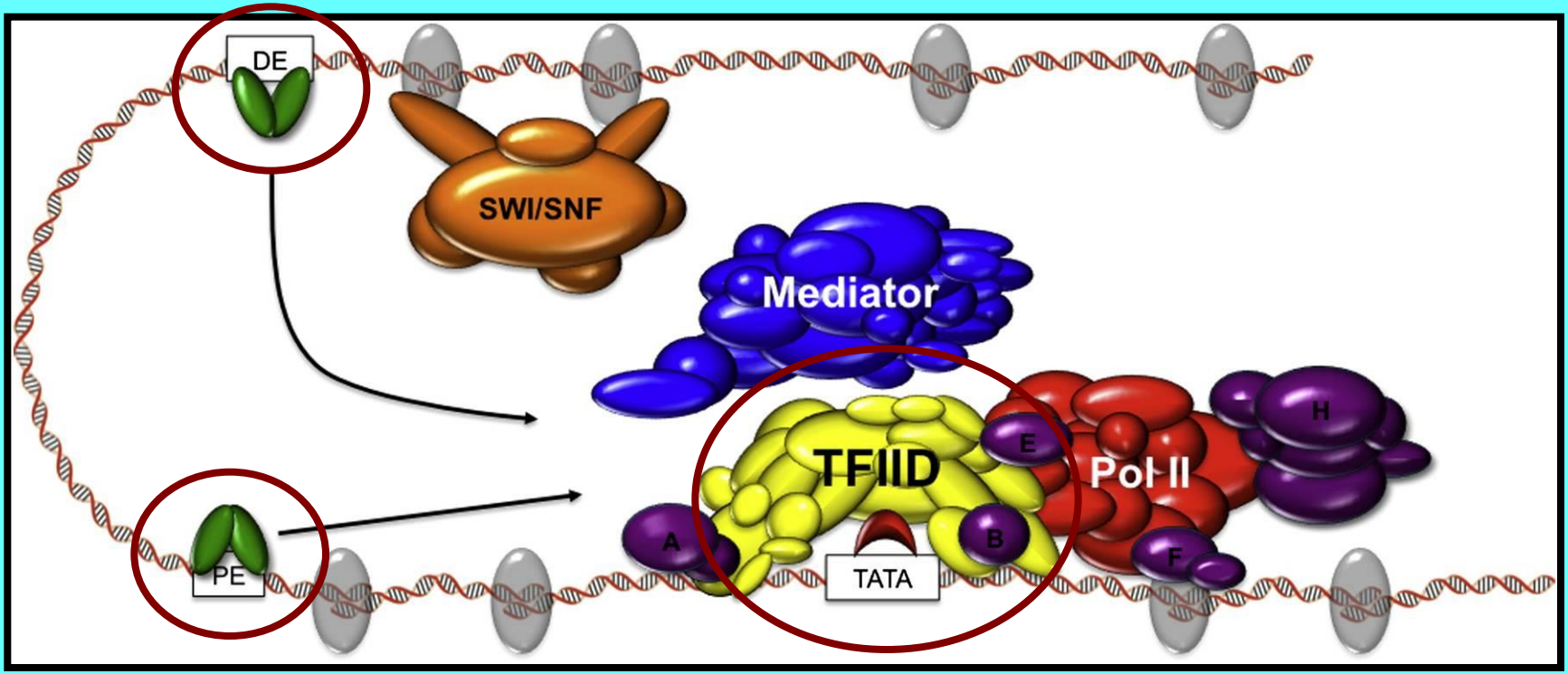
1) Sequence-specific activators (green) bind proximal (PE) and distal (DE) enhancer elements



Joseph A. D'Alessio, J.A., Wright, K.J. and Tjian, R. (2009): Shifting Players and Paradigms in Cell-Specific Transcription. Cell 36, December 24, 924-931

Model for preinitiation complex formation

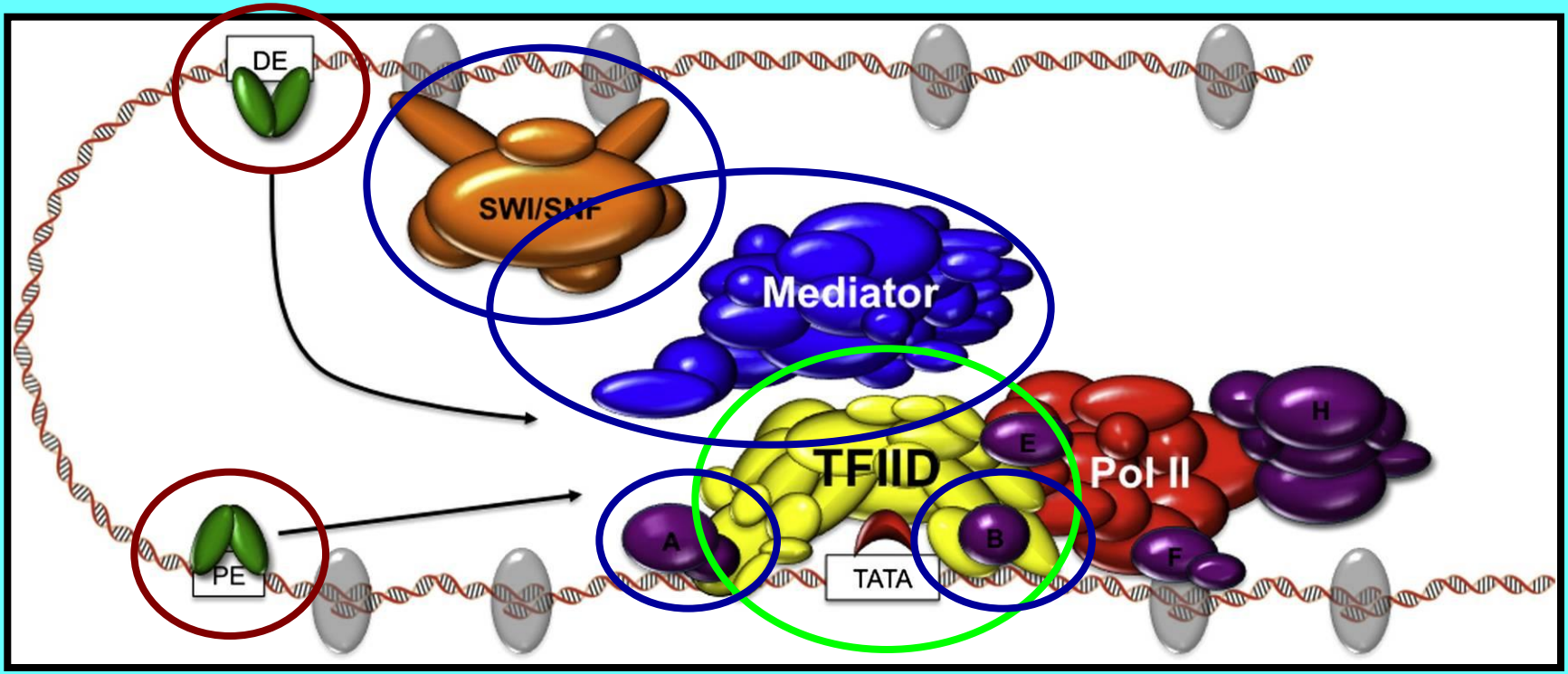
2) It recruits basal factor TFIID (yellow) to the core promoter



Joseph A. D'Alessio, J.A., Wright, K.J. and Tjian, R. (2009): Shifting Players and Paradigms in Cell-Specific Transcription. Cell 36, December 24, 924-931

Model for preinitiation complex formation

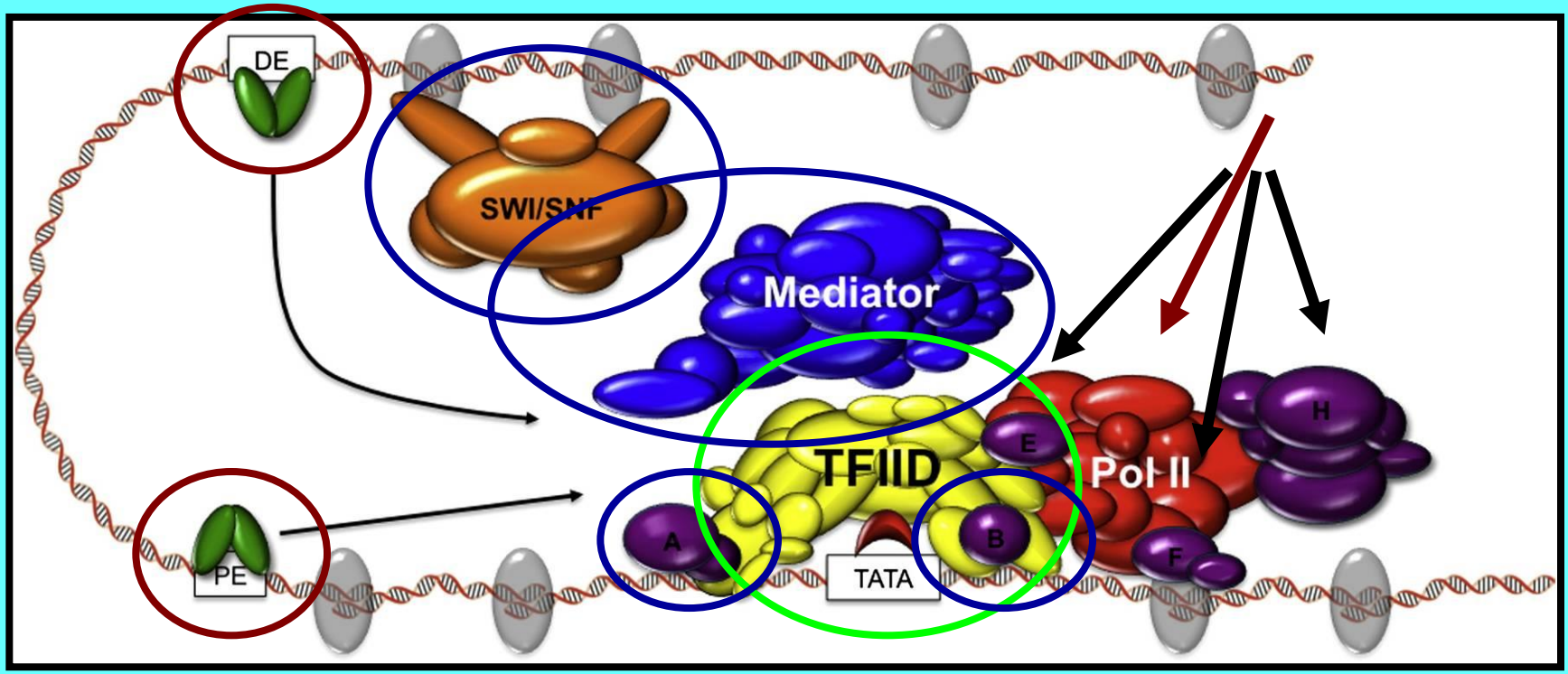
3) recruit chromatin remodelling complexes such as SWI/SNF (orange), co-activators including Mediator (MED, blue), and TFIIA and TFIIB (purple).



Joseph A. D'Alessio, J.A., Wright, K.J. and Tjian, R. (2009): Shifting Players and Paradigms in Cell-Specific Transcription. Cell 36, December 24, 924-931

Model for preinitiation complex formation

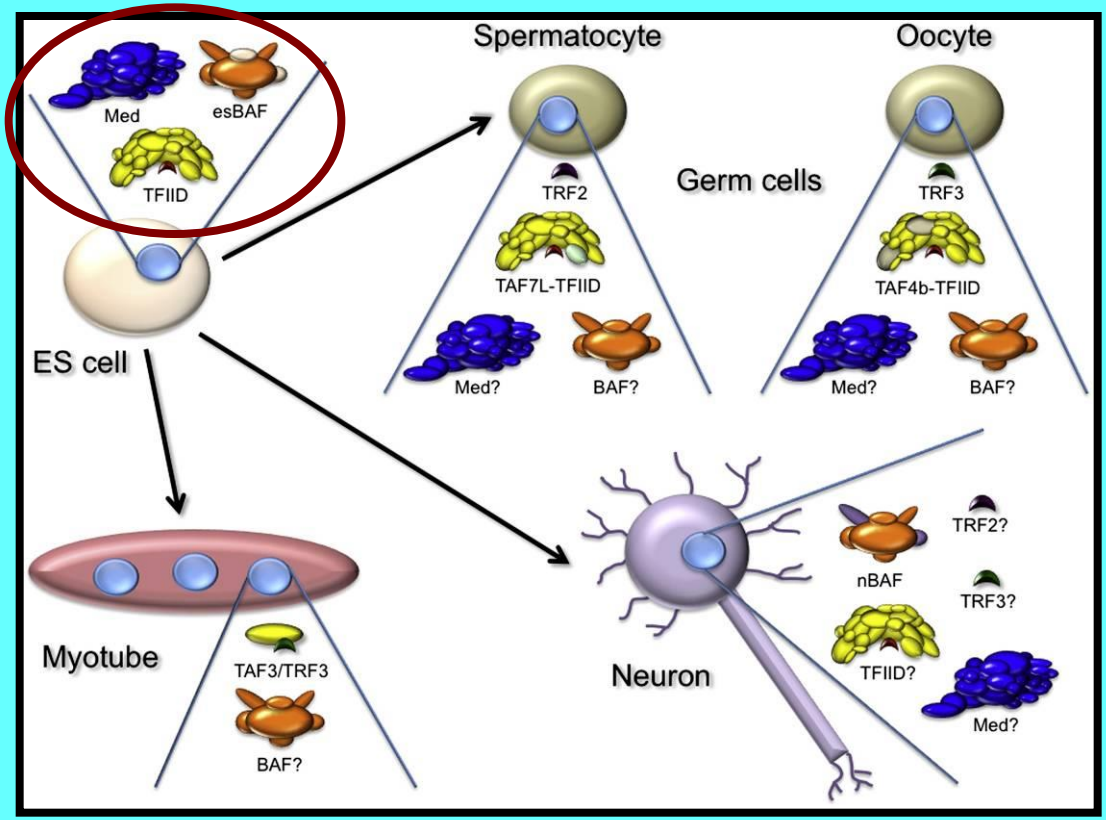
4) The TFIID/TFIIA/TFIIB heterotrimer sequentially recruits TFIIE, TFIIF, PolII (red), and TFIIH (purple), allowing for promoter escape and productive transcriptional elongation



Joseph A. D'Alessio, J.A., Wright, K.J. and Tjian, R. (2009): Shifting Players and Paradigms in Cell-Specific Transcription. Cell 36, December 24, 924-931

Model of changes in transcription factors during ontogenesis

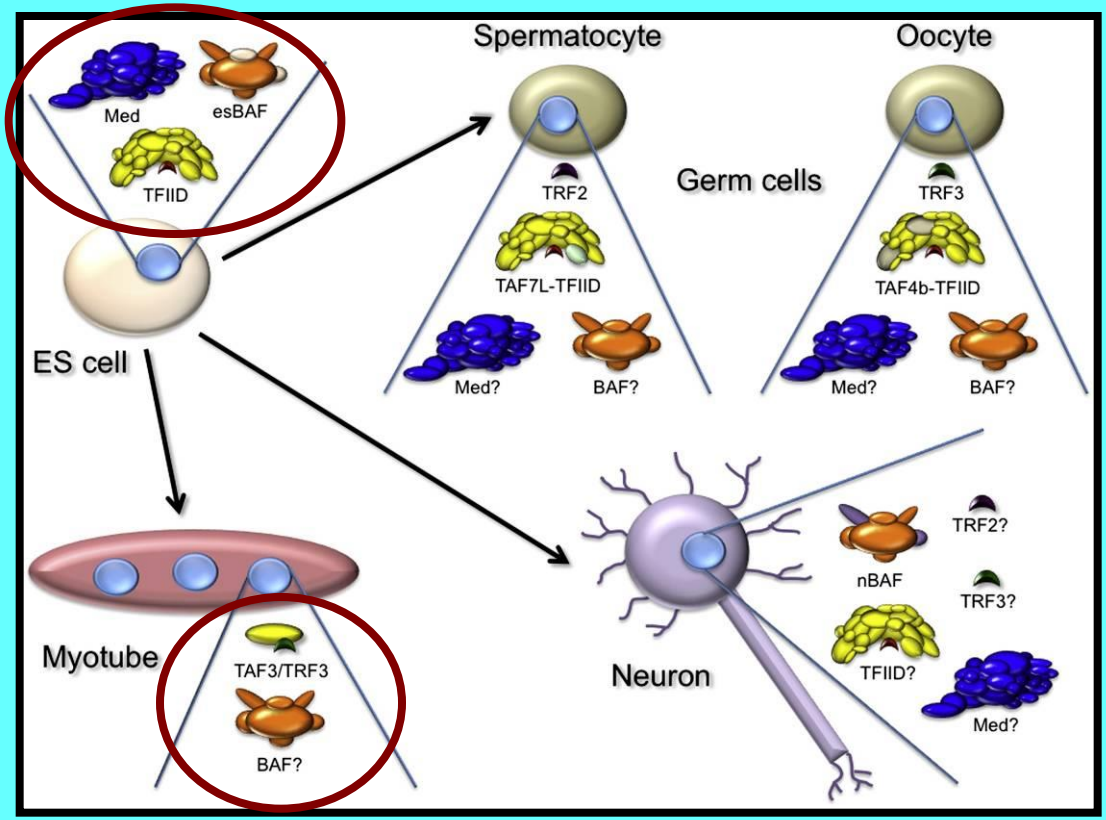
Embryonic stem cells have a „standard“ set of transcription factors



D'Alessio et al. (2009): Cell 36, December 24, 924-931

Model of changes in transcription factors during ontogenesis

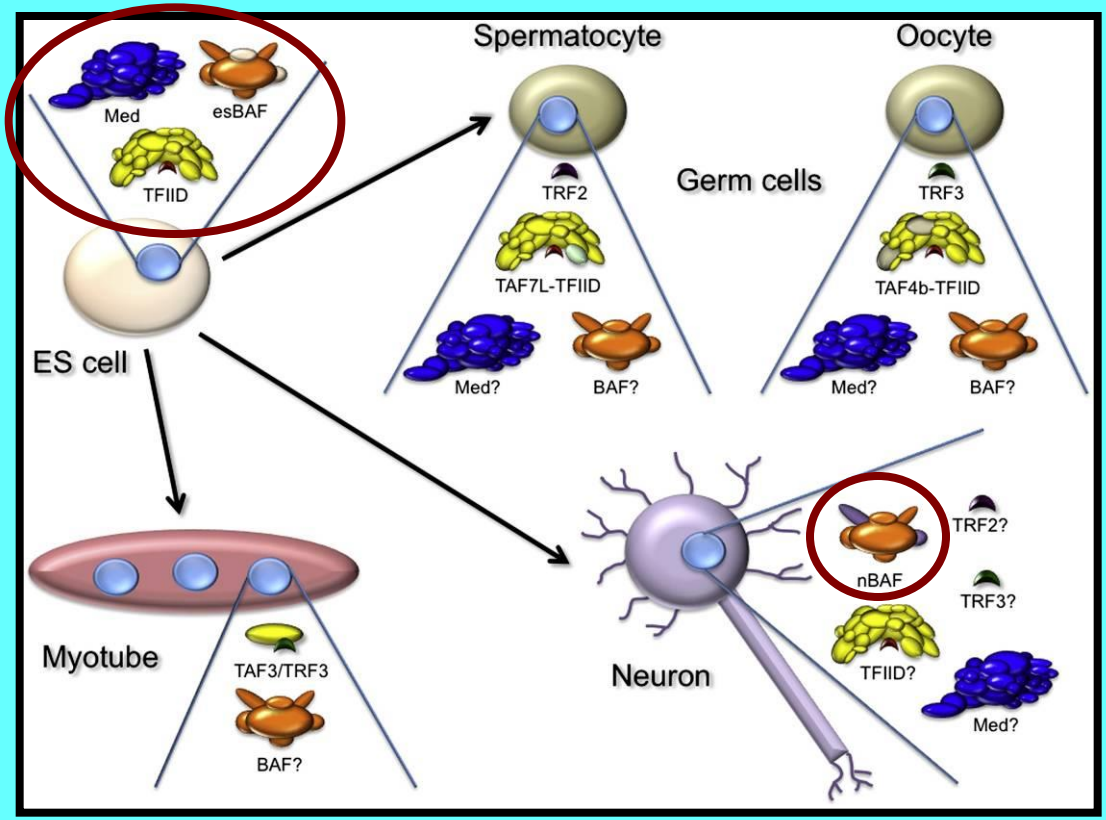
Myotubes may replace TFIID with a novel complex of TRF and TAF3, they have not Med



D'Alessio et al. (2009): Cell 36, December 24, 924-931

Model of changes in transcription factors during ontogenesis

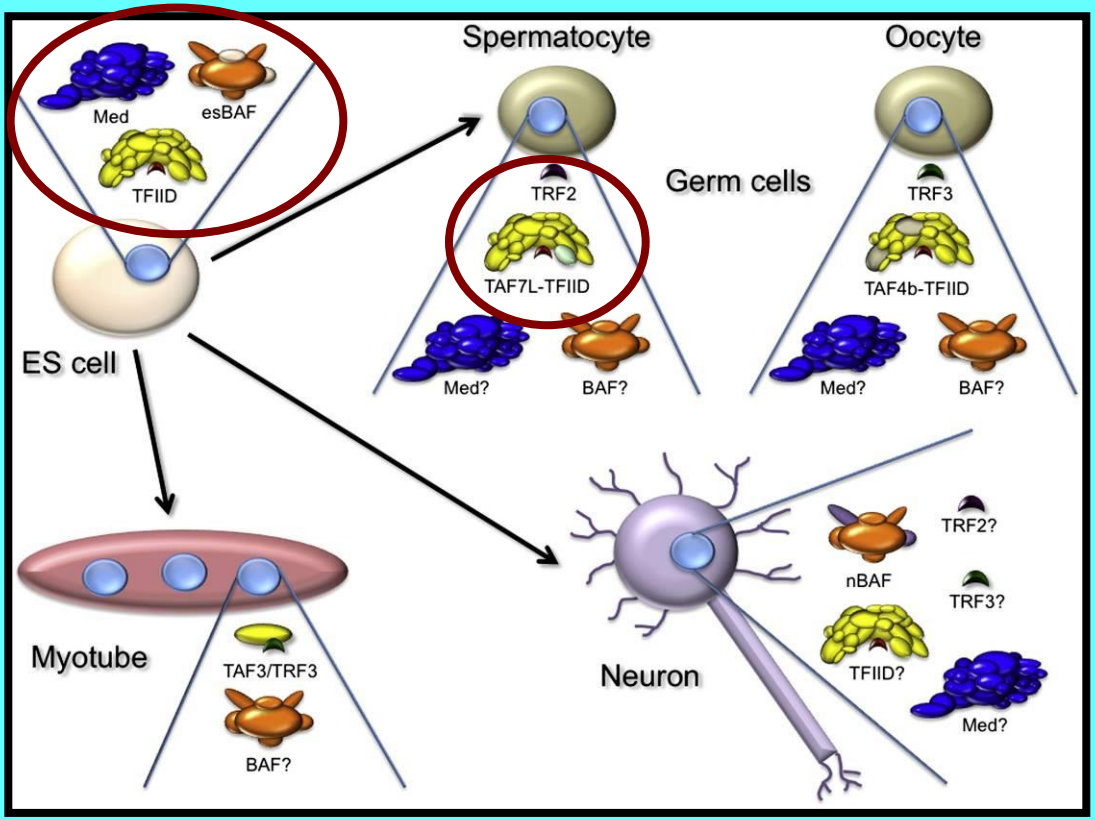
Neurons have new complex BAF, the other factors are not known



D'Alessio et al. (2009): Cell 36, December 24, 924-931

Model of changes in transcription factors during ontogenesis

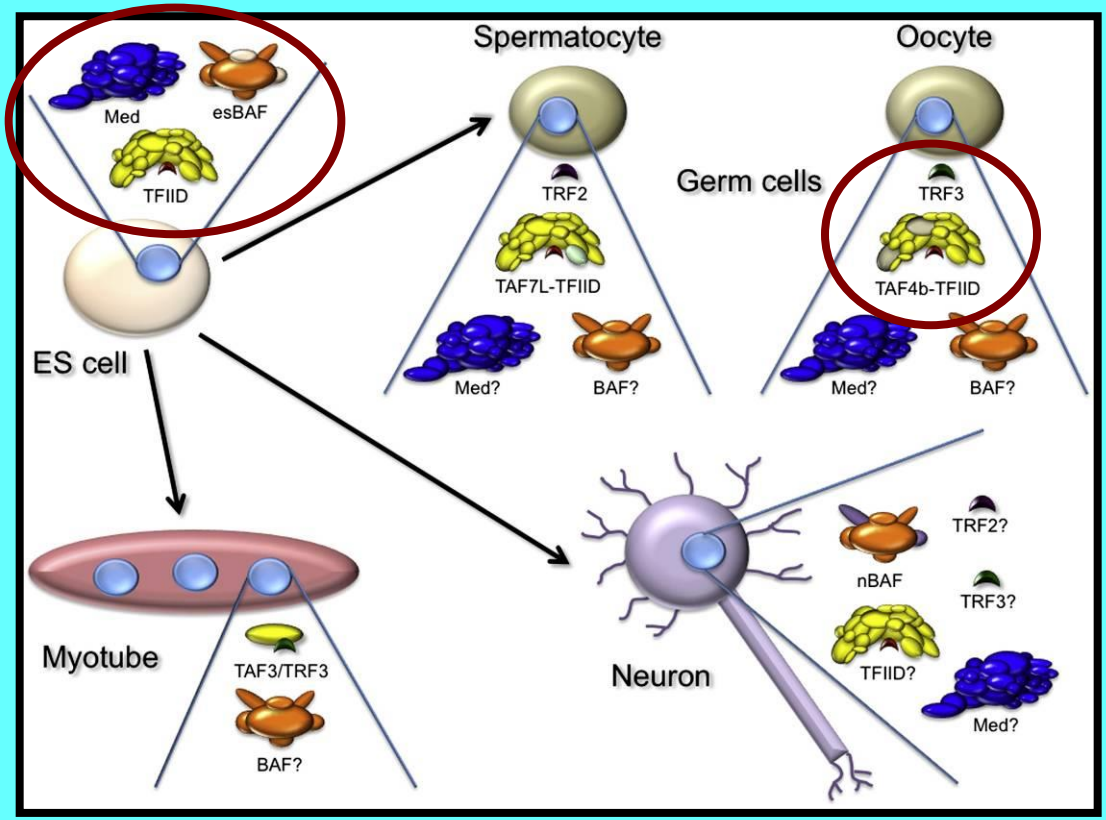
Spermatocytes have elevated levels of TRF2 and of the TAF7 paralog TAF7l (lime), which may or may not be a component of an altered TFIID



D'Alessio et al. (2009): Cell 36, December 24, 924-931

Model of changes in transcription factors during ontogenesis

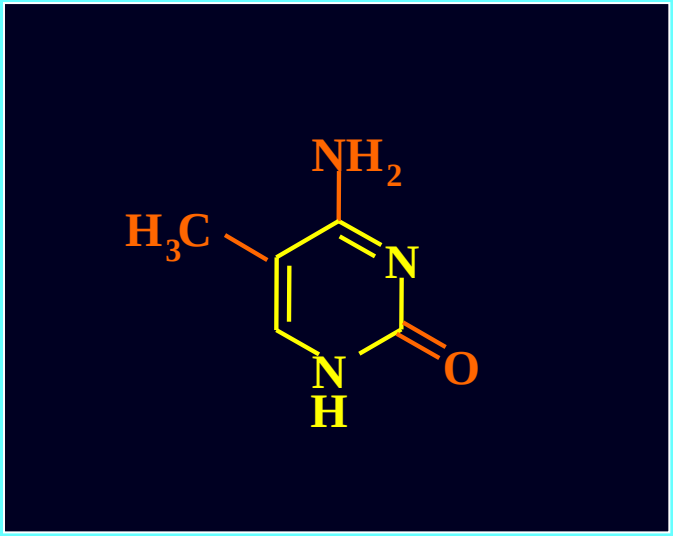
Ovarian cells have elevated levels of TRF3 and of an altered TFIID containing one or more subunits of the TAF4 paralog, TAF4b (tan). Constitutions of Med and BAF are not known.



D'Alessio et al. (2009): Cell 36, December 24, 924-931

Methylation of gene transcription

- The genes which are not expressed in given to developmental stage are methylated
- DNA-metylase



5-metylcytosine

Imprinting

Genomic imprinting is a genetic phenomenon by which certain genes are expressed in a parent-of-origin-specific manner. It is an inheritance process independent of the classical Mendelian inheritance.

Imprinted alleles are silenced such that the genes are either expressed only from the non-imprinted allele inherited from the mother (e.g. H19 or CDKN1C), or in other instances from the non-imprinted allele inherited from the father (e.g. IGF-2).

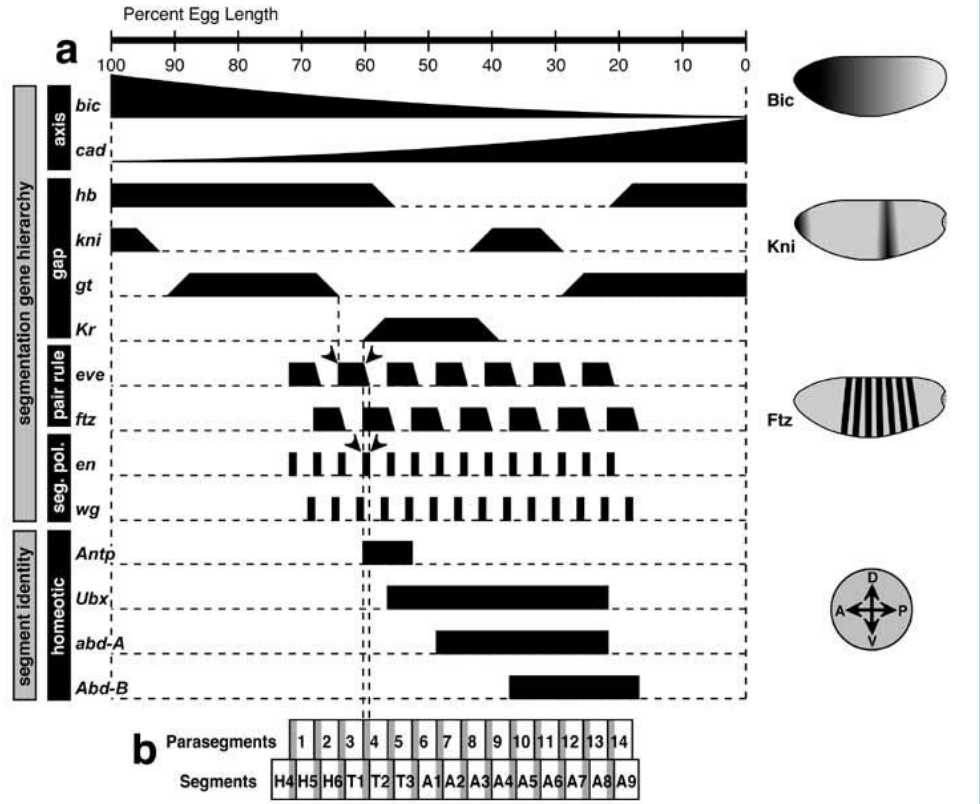
The allele coding for insulin IGF-2 in mouse is expressed if it is coming from father, not from mother.

This phenomenon is done by methylation the allele in oocytes.

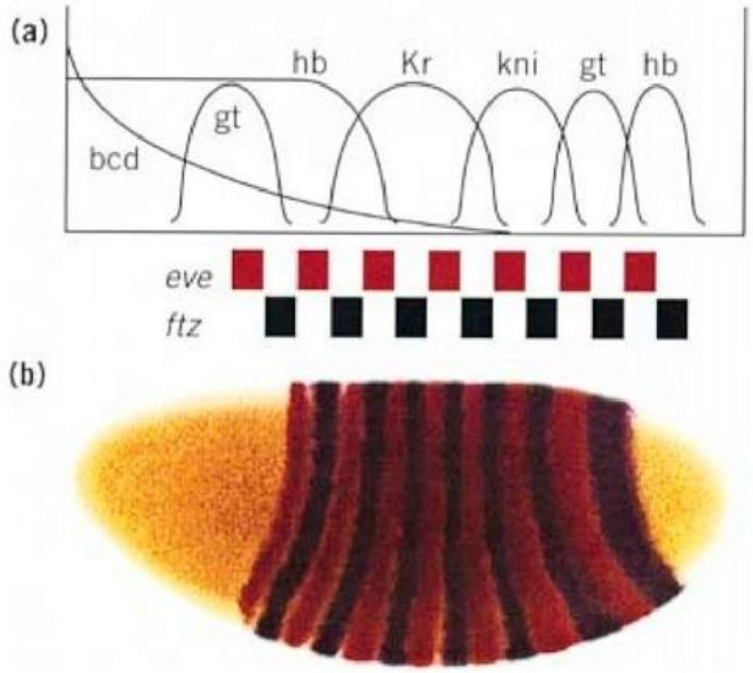
The process of methylation is irreversible.

Changes of gene expression given by the ratio of transcription factors

- **The ratio of individual transcription factors control the expression of particular genes**
- **Well-studied in *Drosophila melanogaster* embryo**



http://www.sdbonline.org/sites/fly/lewheld/id42.htm



http://what-when-how.com/molecular-biology/pair-rule-genes-molecular-biology/

Regulation of gene expression - summary

SUMMARY TABLE 18.1 **Regulating Gene Expression in Bacteria and Eukaryotes**

Level of Regulation	Bacteria	Eukaryotes
Chromatin remodeling	• Limited packaging of DNA • Remodeling not a major issue in regulating gene expression.	• Extensive packaging of DNA • Chromatin must be opened for transcription to begin.
Transcription	• Positive and negative control by regulatory proteins that act at sites close to the promoter • Sigma interacts with promoter.	• Positive and negative control by regulatory proteins that act at sites close to and far from promoter • Large basal transcription complex interacts with promoter. • Mediator complex required.
RNA processing	• None documented	• Extensive processing: alternative splicing of introns addition of 5′ cap and 3′ tail
mRNA stability	• Some RNA interference documented	• For many genes, RNA interference limits life span or translation rate.
Translation	• Regulatory proteins bind to mRNAs and/or ribosome and affect translation rate.	• Regulatory proteins bind to mRNAs and/or ribosome and affect translation rate.
Post-translational modification	• Folding by chaperone proteins • Chemical modification (e.g., phosphorylation) may change activity.	• Folding by chaperone proteins • Chemical modification (glycosylation, phosphorylation) • Ubiquination targets proteins for destruction by proteasome.

MOLECULAR MECHANISMS OF SIGNALISATION

Molecular mechanisms of signalisation

The cell is complex regulated system which reacts to external signals by changes in gene expression. The changes are on the levels of transcription, translational, posttranscriptional, and posttranslational

- **Signals for activation of transcription are <u>extracellular</u>**
- **Intracellular signals follow the extracellular signals**

Signal, signal molecule

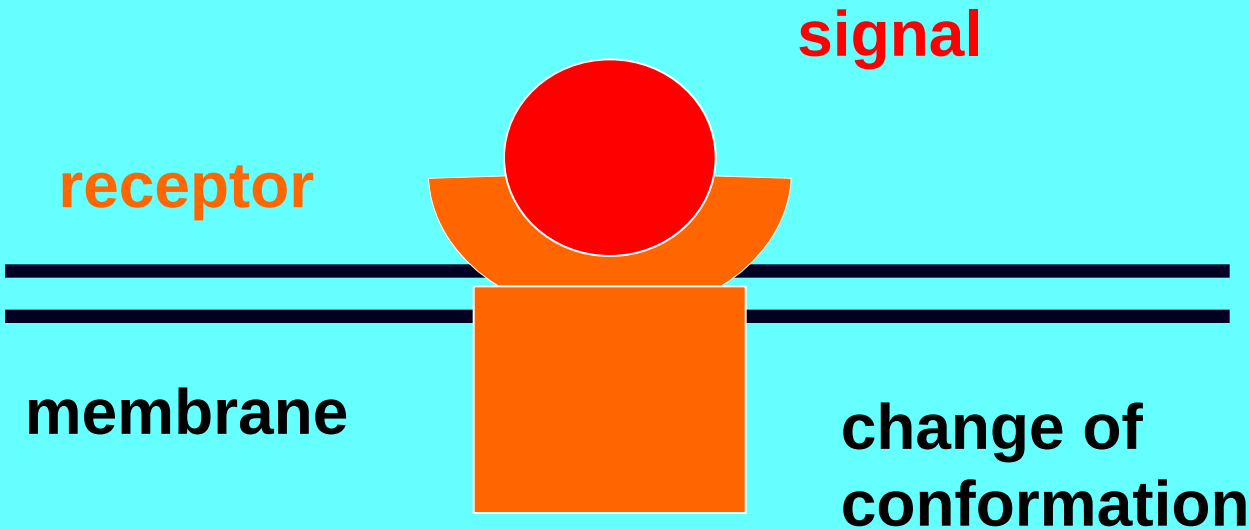
Signal is a physical factor for transmission information

Signal molecule is a small molecule or macromolecule which has function of a signal

Signal molecules

- extracelullar: originated outside cell; receptor and ligand
- intracelullar: originated inside cell, hormones, growth factors, neurotransmitters, cAMP, G-proteins, RAS protein

Extra and intracelullar signalisation



Induction of enzymatic activity in cell

Reaction of the cell to signal

Extracellular signalisation

Transmission of signal from cell, which is originator of the signal, to receptor of recipient cell

Contact depending signalisation

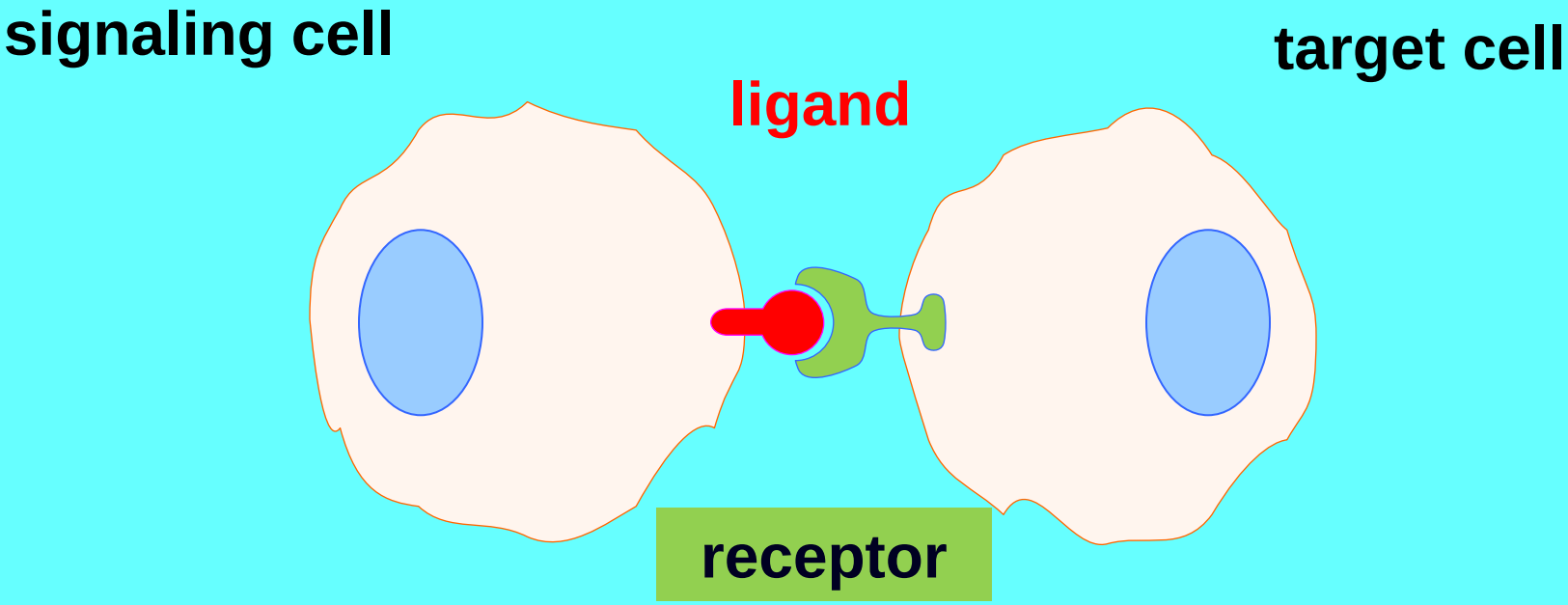
Paracrine signalisation

Endocrine signalisation

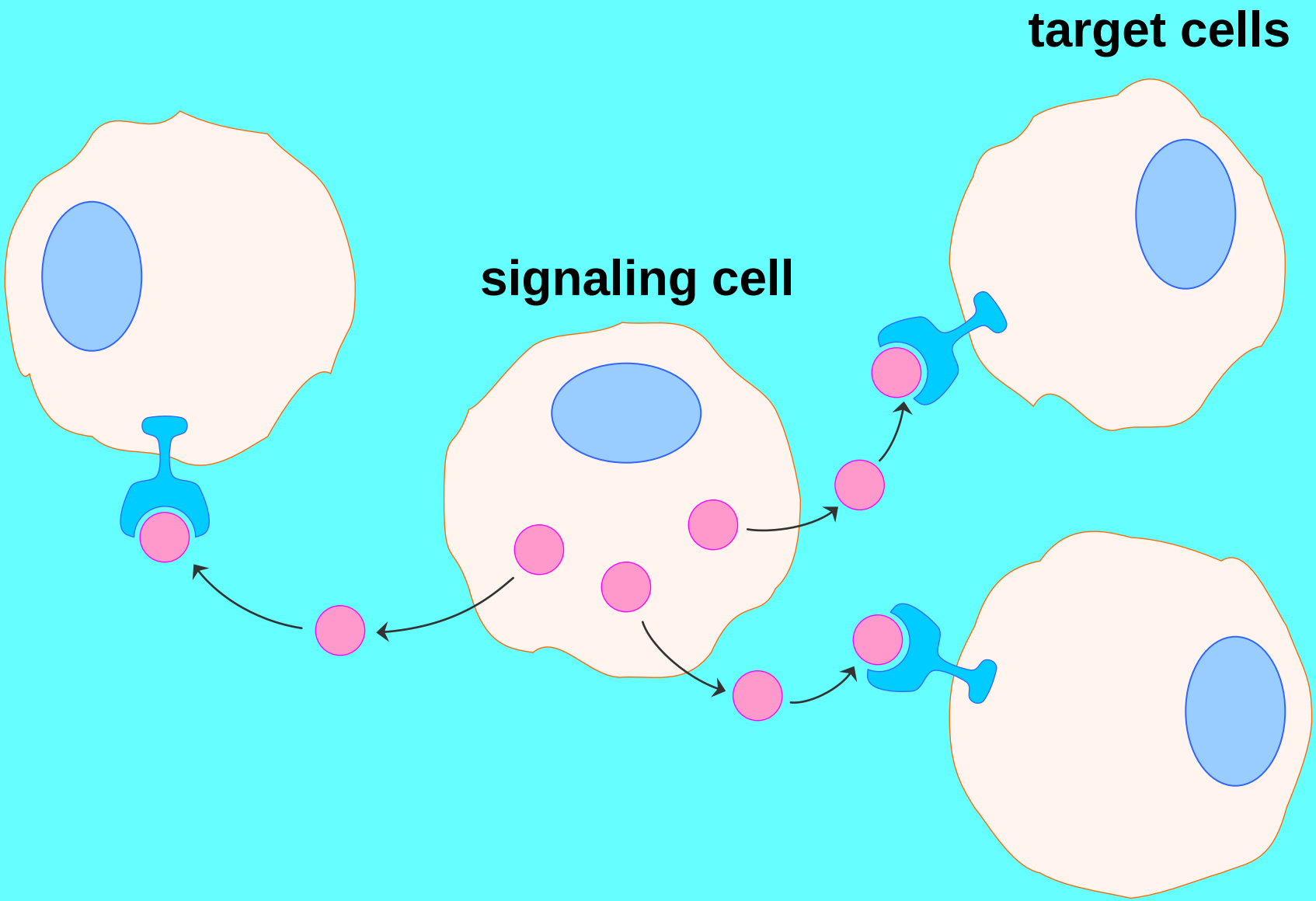
Gap junction signalisation

Autocrine signalisation

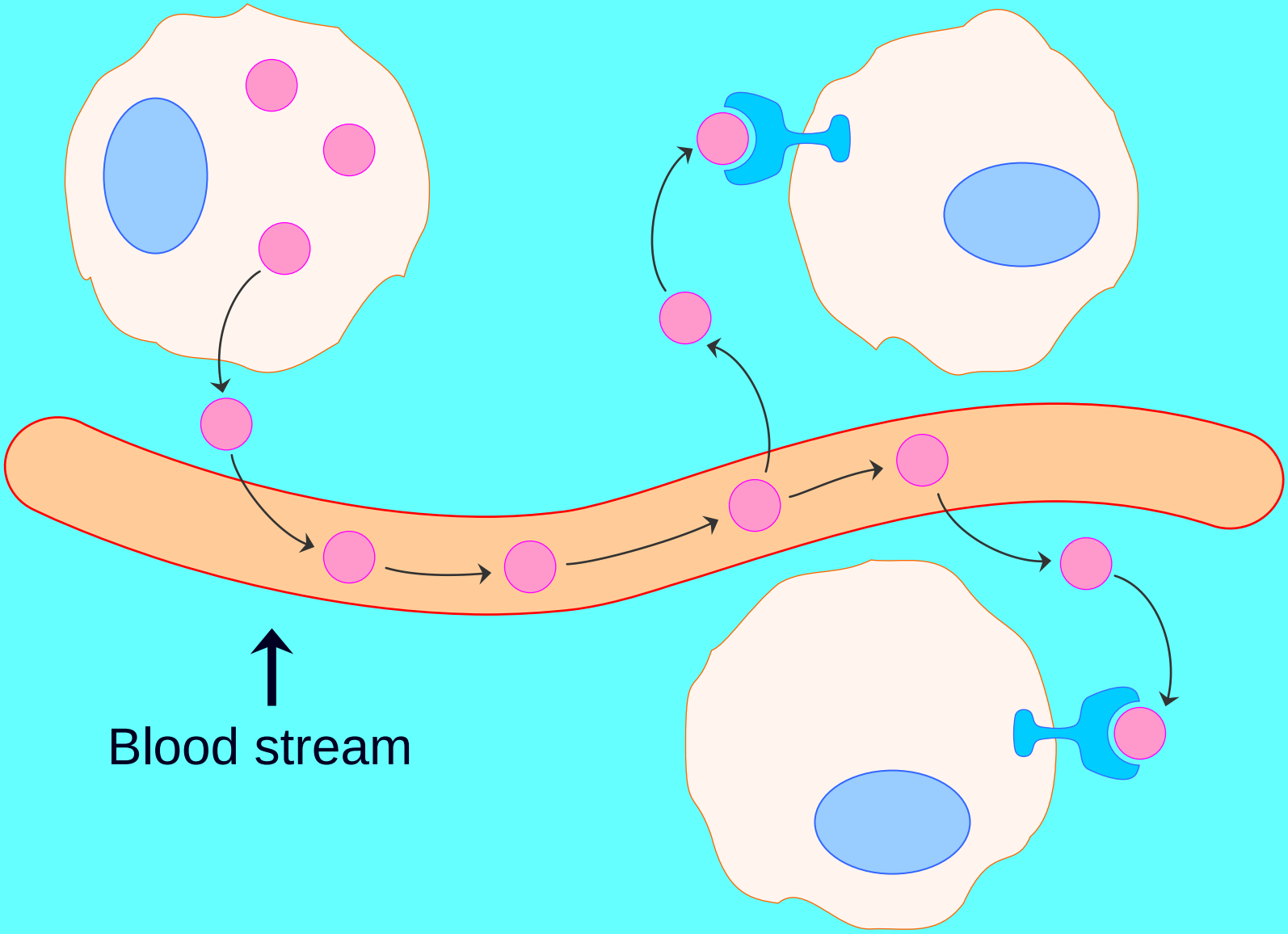
Contact depending signalisation



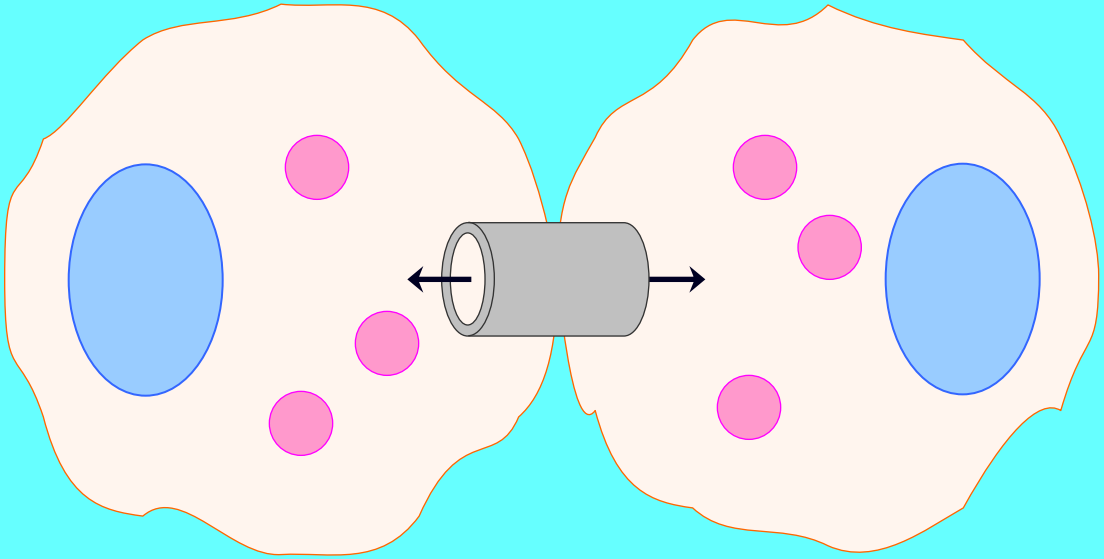
Paracrine signalisation



Endocrine signalisation



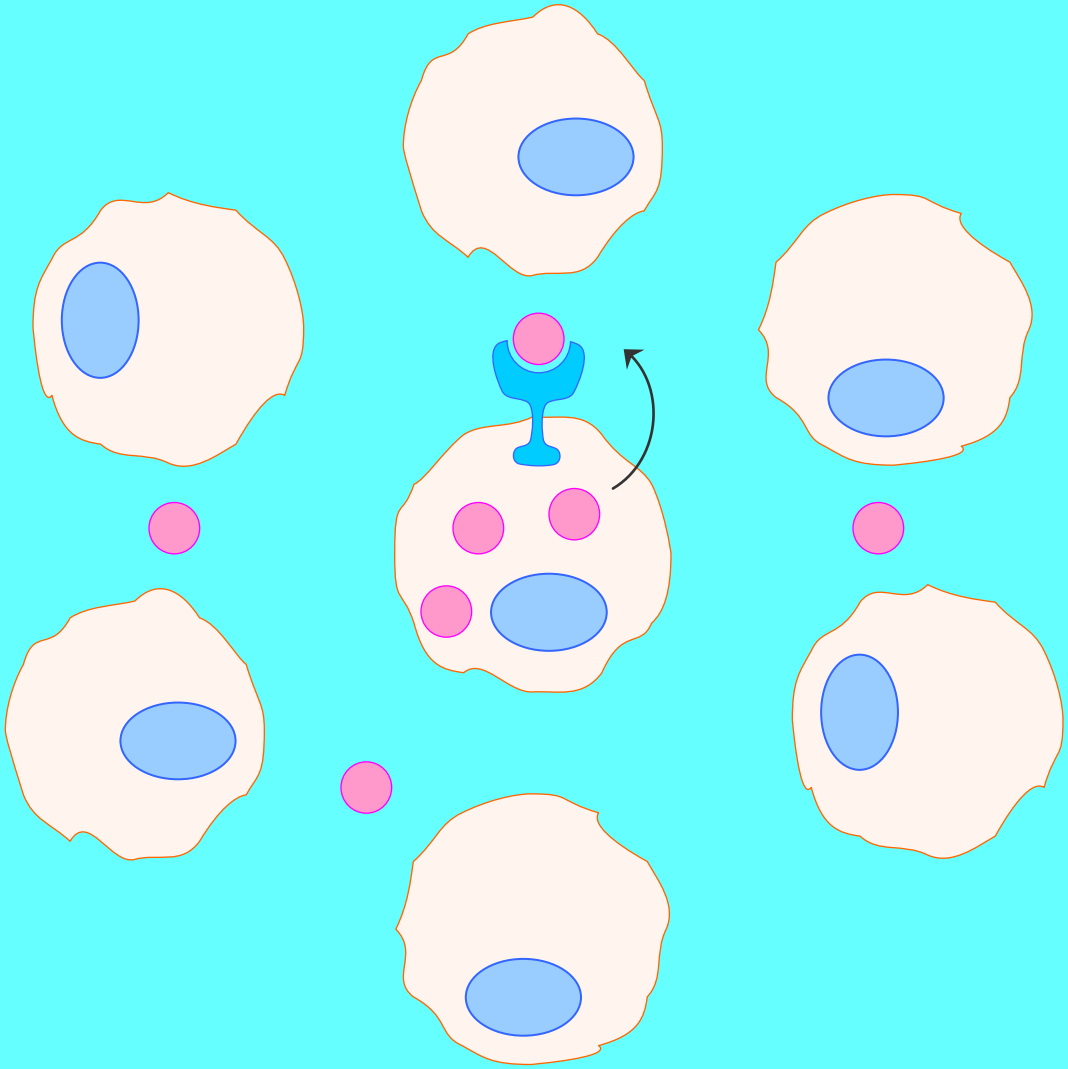
Gap junction signalisation



Transmission of small molecules = Ca^{2+}, cAMP

Autocrine signalisation

Signaling cell is also target cell



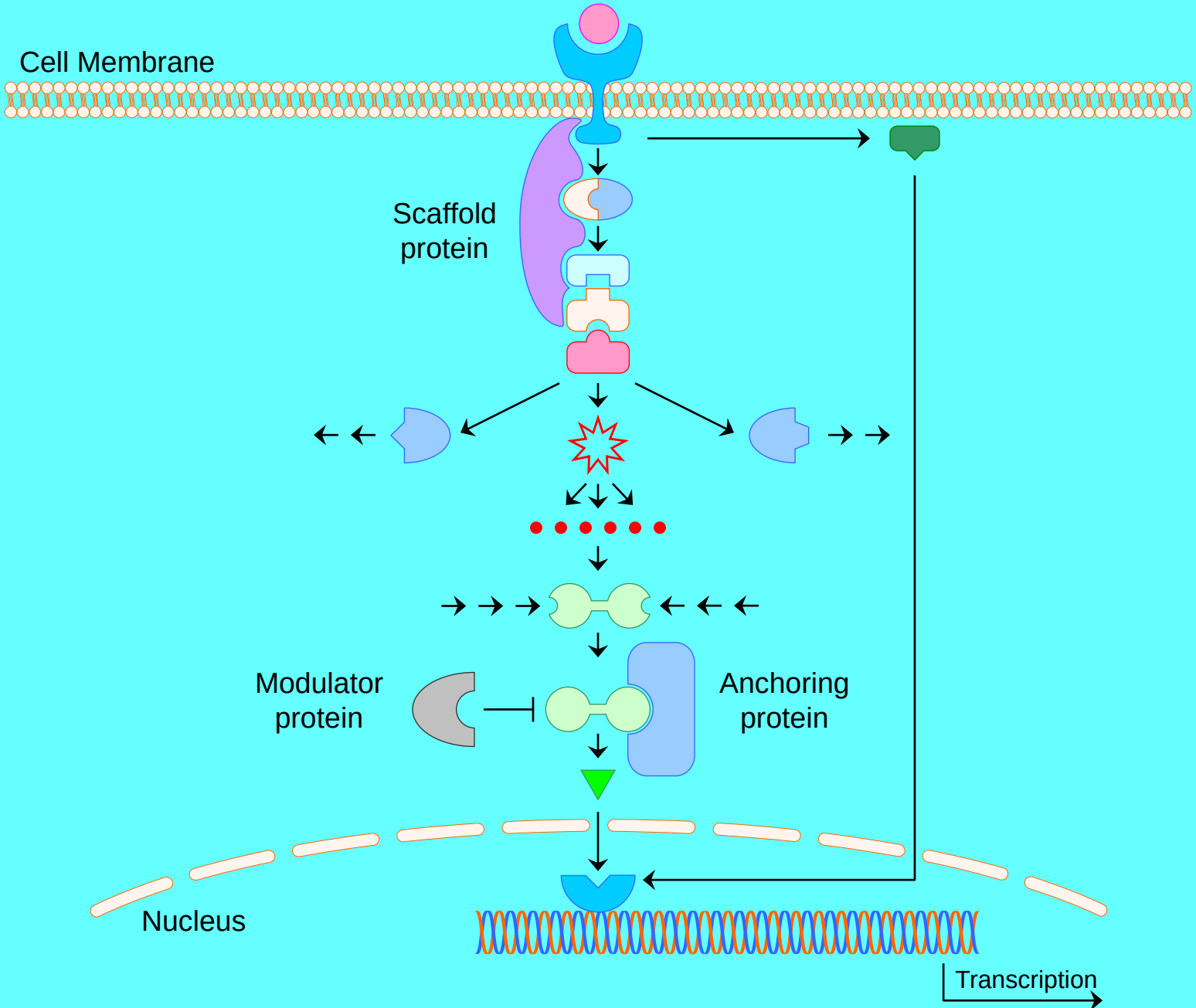
Signal amplification

Extracellular and intracellular signaling create formation of signal molecules cascade, in which the molecules transfer information from one to another

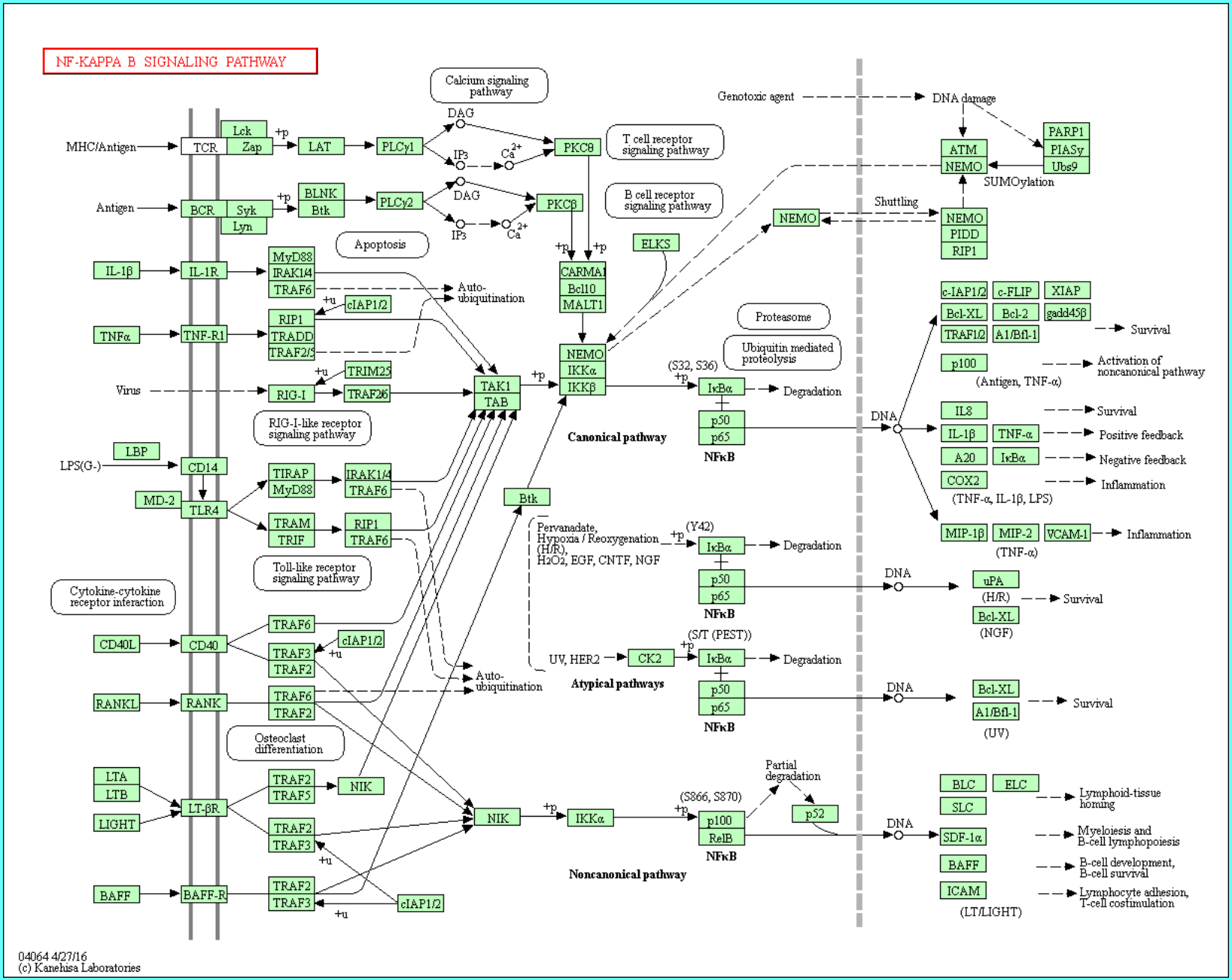
- **first messenger**
- **second messenger**

The second messengers amplify the original (extracellular) signal

Scheme of signal amplification



Interaction of signaling pathways



Enzymes of signal transduction

- serine/threonine proteinkinases
- tyrosine proteinkinases
- mitogene activated protein kinases (MAPK)
- adenylatcyclases
- guanylatcyclases
- phospholipases
- phosphatases

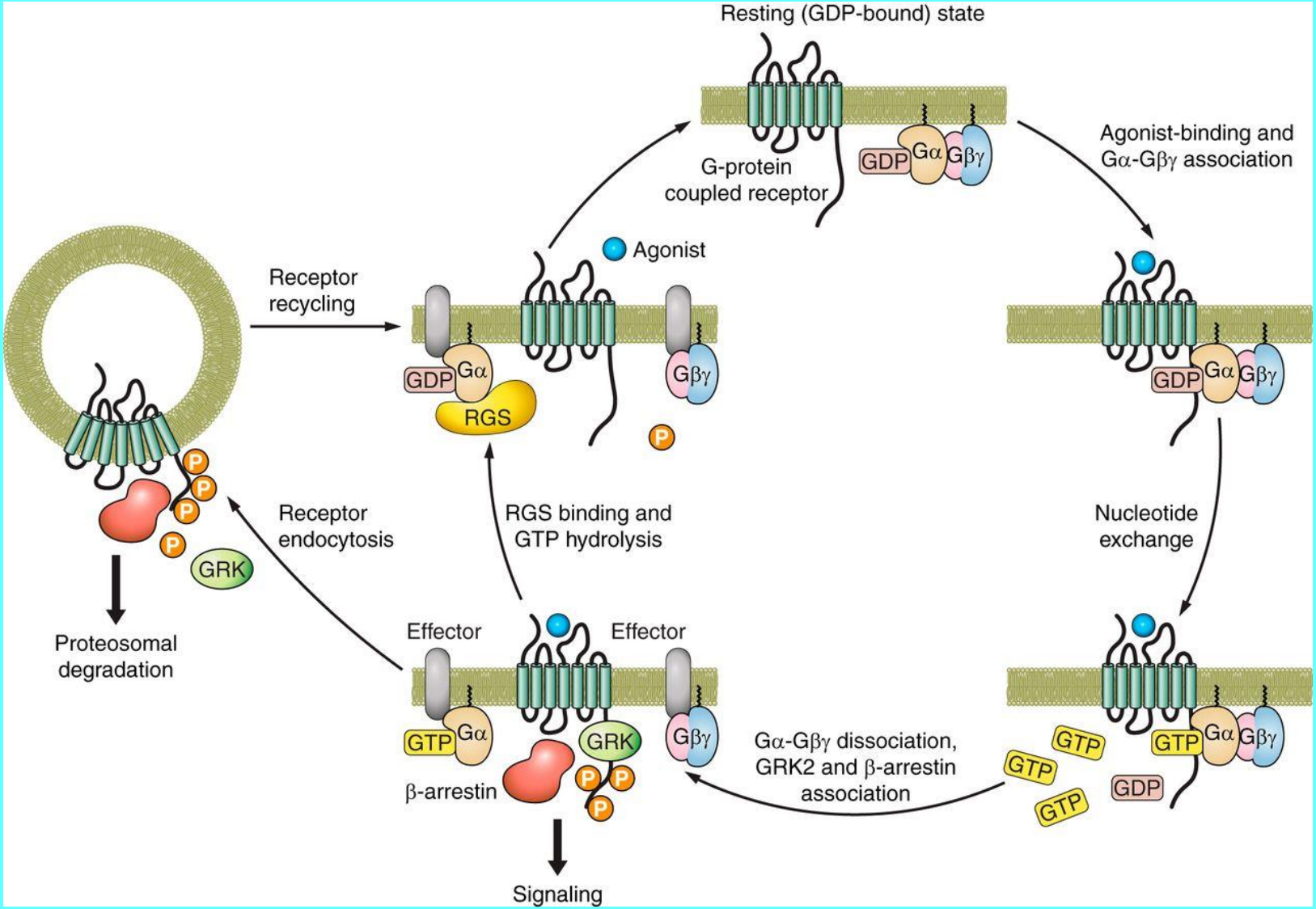
Extracellular signals

- Hormones derived from amino acids = adrenaline, thyroxin, etc.
- Peptide hormones = insulin, glucagon, ...
- Steroid hormones and glucocorticoids
- „Tissue“ hormones = serotonin, gastrin, erythropoietin, etc.

Signalisation via G-protein coupled receptors

- **G-protein = heterotrimer composed from Gα, Gβ and Gγ subunit**
- **Gα-subunit possesses GTPase activity, in non-active state binds GDP**
- **Binding of ligands (e.g., some hormones – serotonin, epinefrin,…) causes exchange of GDP to GTP on Gα-subunit**
- **Gα-subunit disociates from Gβ/γ subunits and both parts activate other enzymes**
- **After a few seconds, Gα-subunit hydrolises GTP on GDP $\Rightarrow$ recreation of heterotrimer**

Signalisation via G-protein coupled receptors



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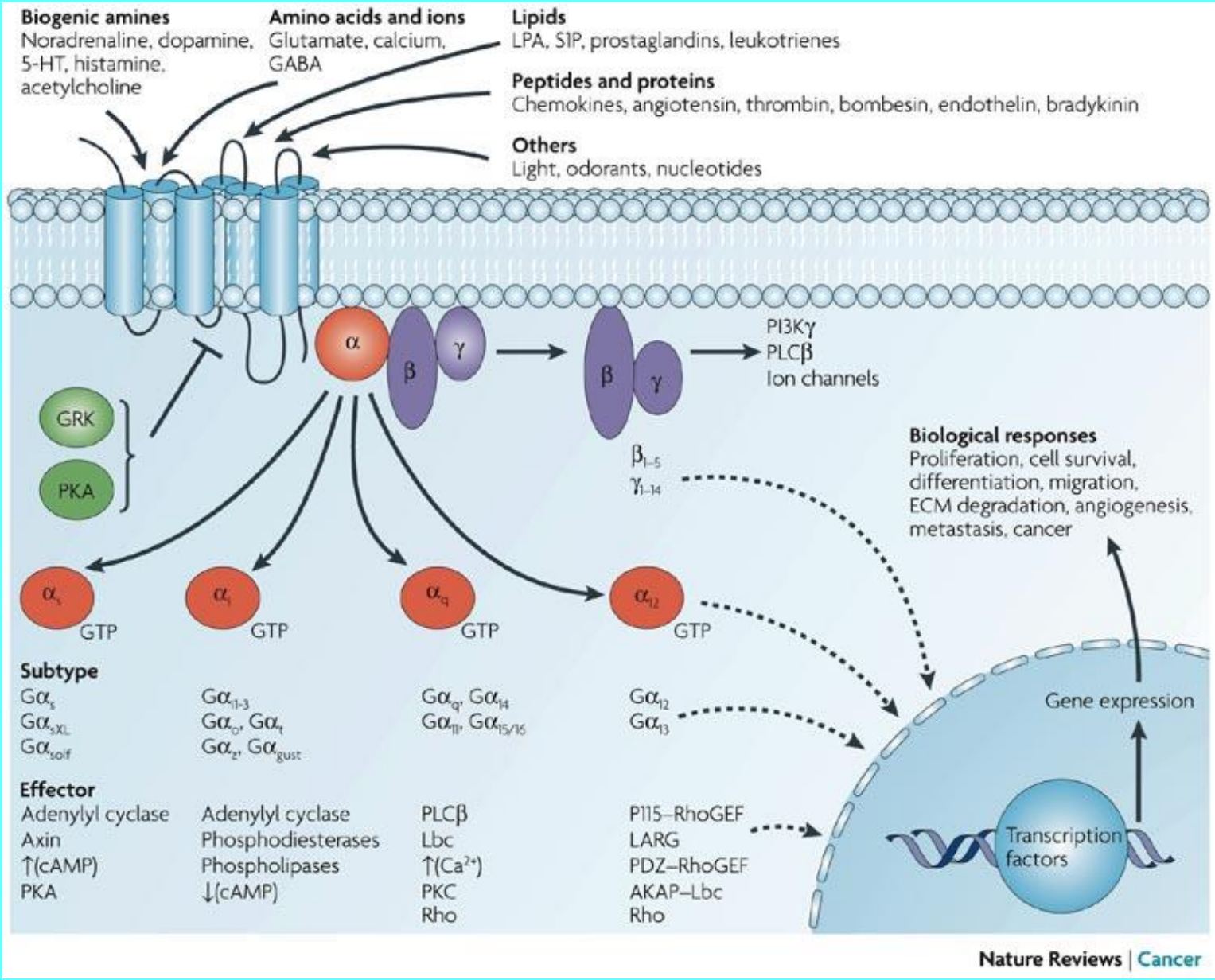
Signalisation via G-protein coupled receptors



Nature Reviews Cancer **7**, 79-94 (February 2007) doi:10.1038/nrc2069

Text to previous picture

Various ligands use G-protein-coupled receptors (GPCRs) to stimulate membrane, cytoplasmic and nuclear targets. GPCRs interact with heterotrimeric G proteins composed of α, β and γ subunits that are GDP bound in the resting state. Agonist binding triggers a conformational change in the receptor, which catalyses the dissociation of GDP from the α subunit followed by GTP-binding to Gα and the dissociation of G_{α} from Gβγ subunits[1]. The α subunits of G proteins are divided into four subfamilies: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$, and a single GPCR can couple to either one or more families of Gα proteins. Each G protein activates several downstream effectors[2]. Typically $G\alpha_s$ stimulates adenylyl cyclase and increases levels of cyclic AMP (cAMP), whereas $G\alpha_i$ inhibits adenylyl cyclase and lowers cAMP levels, and members of the $G\alpha_q$ family bind to and activate phospholipase C (PLC), which cleaves phosphatidylinositol bisphosphate (PIP_2) into diacylglycerol and inositol triphosphate (IP_3). The Gβ subunits and Gγ subunits function as a dimer to activate many signalling molecules, including phospholipases, ion channels and lipid kinases. Besides the regulation of these classical second-messenger generating systems, Gβγ subunits and Gα subunits such as $G\alpha_{12}$ and $G\alpha_q$ can also control the activity of key intracellular signal-transducing molecules, including small GTP-binding proteins of the Ras and Rho families and members of the mitogen-activated protein kinase (MAPK) family of serine-threonine kinases, including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), p38 and ERK5, through an intricate network of signalling events that has yet to be fully elucidated[1, 4, 6]. Ultimately, the integration of the functional activity of the G-protein-regulated signalling networks control many cellular functions, and the aberrant activity of G proteins and their downstream target molecules can contribute to cancer progression and metastasis. 5-HT, 5-hydroxytryptamine; ECM, extracellular matrix; GABA, gamma-aminobutyric acid; GEF, guanine nucleotide exchange factor; GRK, G protein receptor kinase; LPA, lysophosphatidic acid; PI3K, phophatidylinositol 3-kninase; PKA and PKC, protein kinase A and C; S1P sphingosine-1-phosphate.

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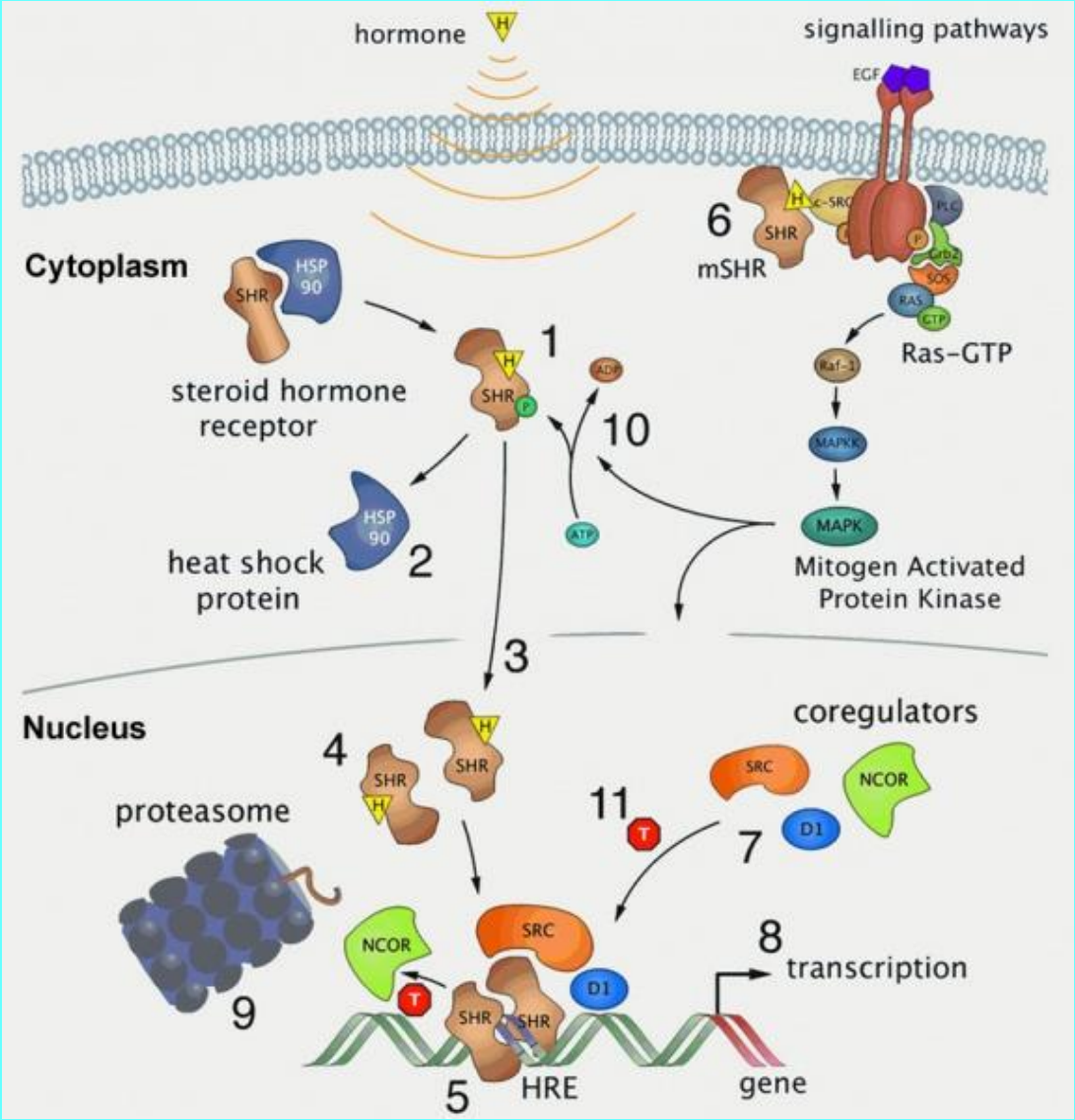
Intracelullar receptors of extracelullar signals

- **Receptors for steroid hormones, thyroxine, retinoic acid and vitamin D**
- **Receptors occurs either in cytoplasm on in nucleus**
- **Ligand (signal) entre to cell, where it bind on receptor, which represents transcription factor, as well**

Steroid hormon signalisation



Steroid Hormone Receptors (SHR) act as hormone dependent nuclear transcription factors.Upon entering the cell by passive diffusion, the hormone (H) binds the receptor, which is subsequently released from heat shock proteins, and translocates to the nucleus. There, the receptor dimerizes, binds specific sequences in the DNA, called Hormone Responsive Elements or HREs, and recruits a number of coregulators that facilitate gene transcription.
This latter step can be modulated by receptor antagonists like tamoxifen (T), and cellular signalling pathways.

Legend

1. hormone binding
2. chaperone interaction
3. nuclear translocation
4. receptor dimerization
5. DNA binding
6. putative membrane-bound receptors
7. coregulator recruitment
8. transcription
9. proteasomal degradation
10. modulation by cellular signalling pathways
11. antagonist resistance