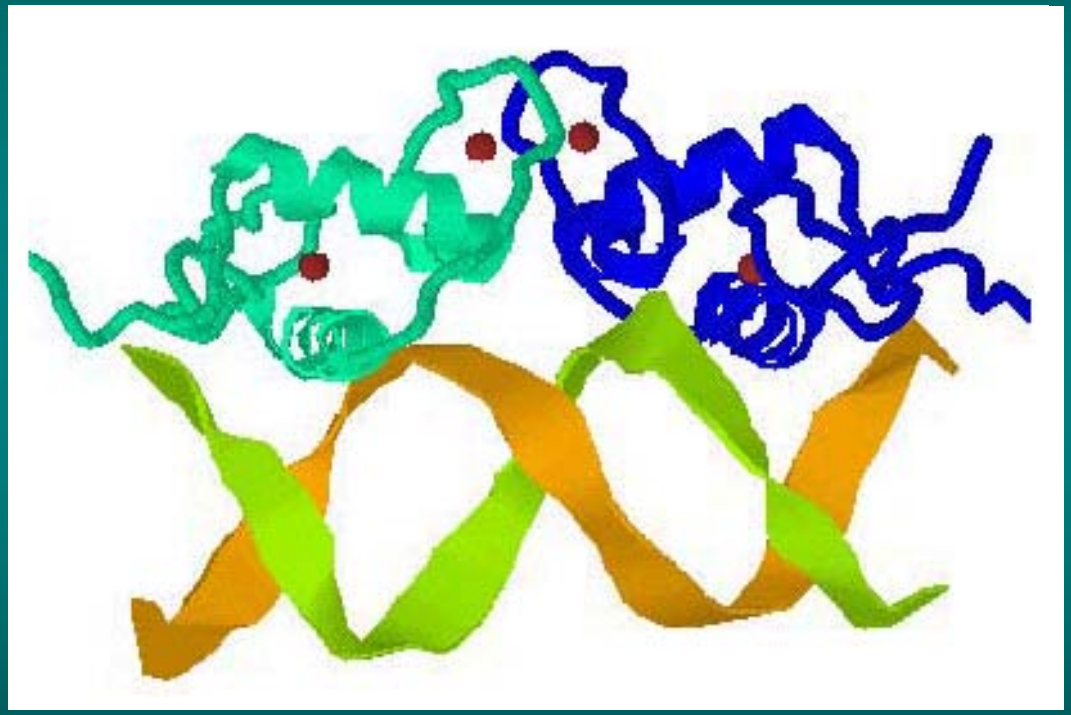
Never work alone....



corepressors
coactivators
heterodimeric partners

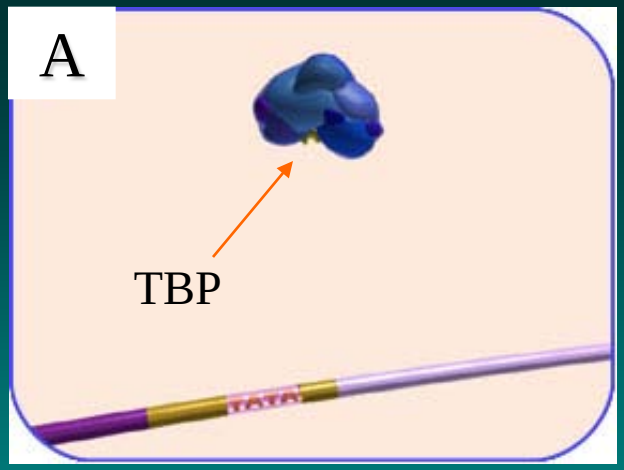
General transcription factors

- Eucaryotic RNA polymerases cannot initiate transcription on their own; they require a set of proteins called general transcription factors (TFIIX).

- General transcription factors are assabled to the promoters before transcription can begin.

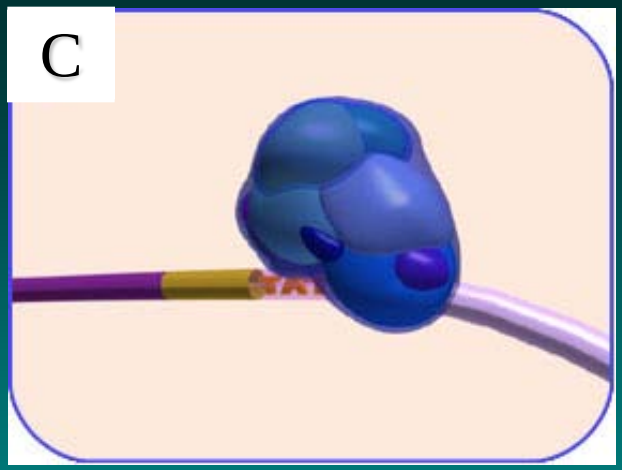
- This process can be speeded up or slowed down and expressions of many genes are regulated at these steps.

- There are small numbers of general transcription factors, but they are abundant in each cells as they assable on the promoters of all genes transcribed by Pol II.

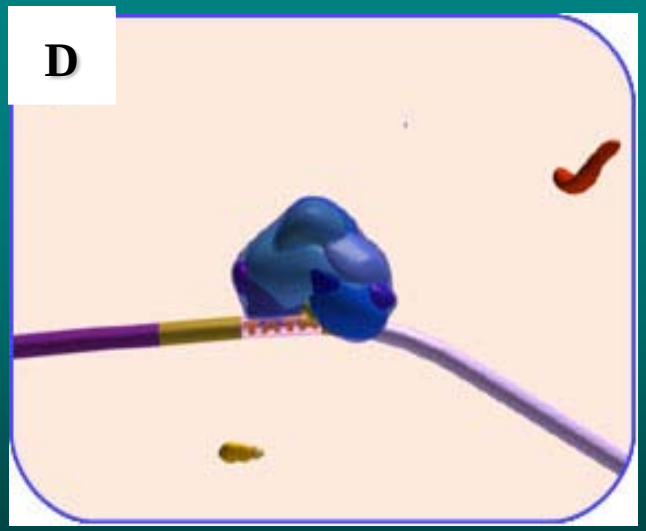


TFIID is the largest of the general transcription factors. Its binding is a rate-limiting step in initiation of transcription.

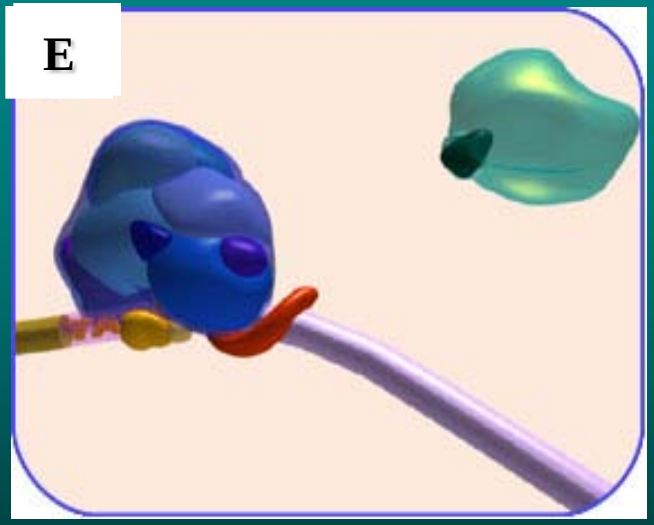
TBP – may bind to DNA in the minor groove, but cannot bind to DNA within nucleosome



When the TBP portion of the TFIID molecule attaches to the TATA-box, its shape causes the DNA to bend.



Two smaller general transcription factors are shown coming into view: *TFIIA* (orange) and *TFIIB* (red).

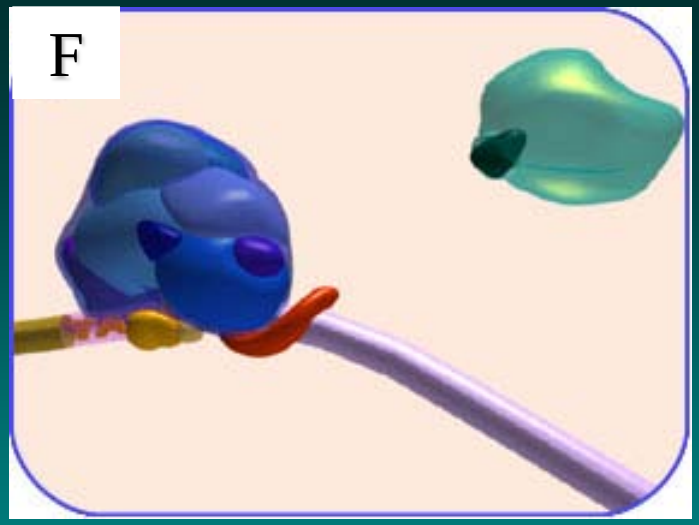


The Poll II complex has been assembled and is approaching the start site for transcription.

TFIIA binds to the TATA-box near TFIID

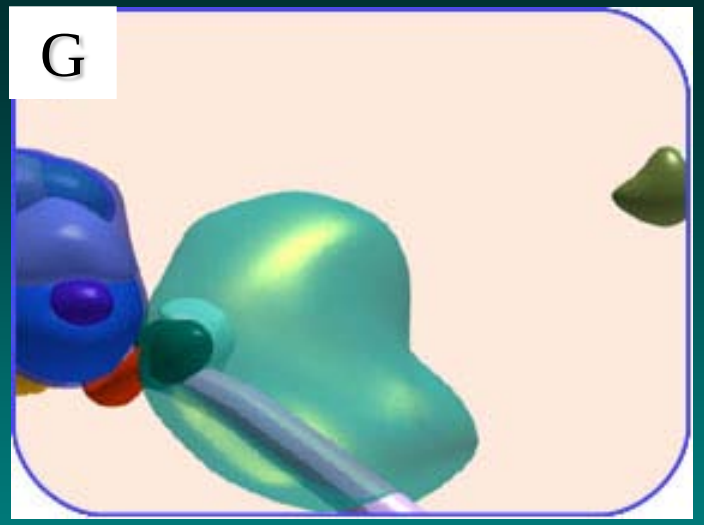
TFIIB binds to the TATA-box and TFIID. It is thought to help the Poll II complex bind correctly.

F



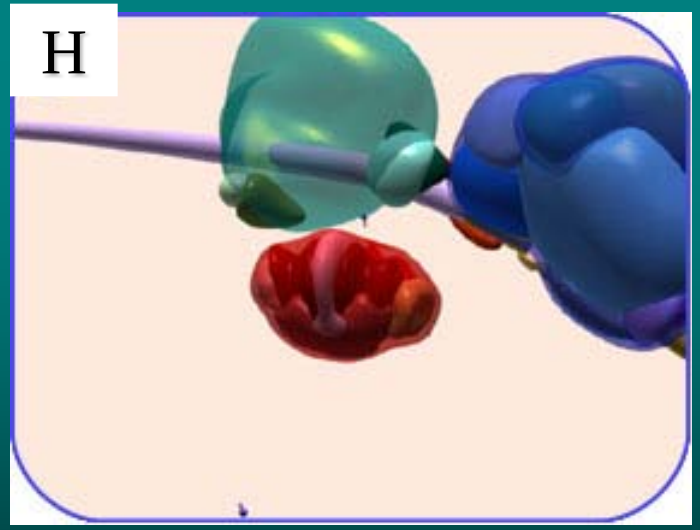
The Poll II complex has been assembled and is approaching the start site for transcription.

G



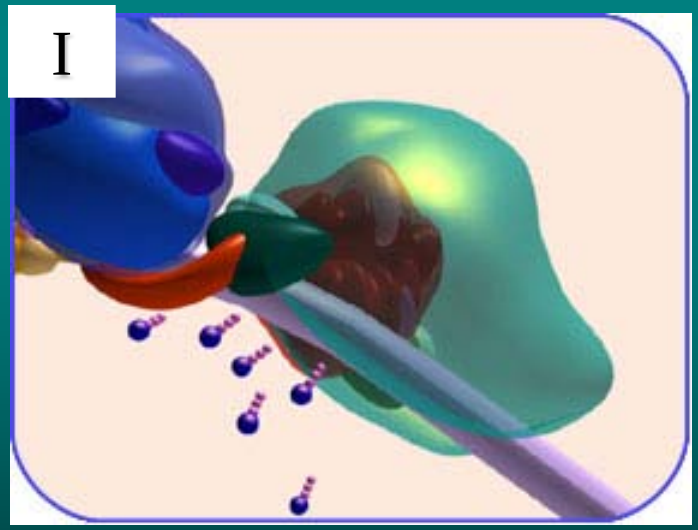
Aided by the general transcription factors, Poll II binds to the DNA strand at the start site for transcription.

H



Additional factors must still bind to the complex in order to start transcription. Here, TFIIE has already bound (green) and TFIIH (red) is preparing to do the same. TFIIH has a subunit which is a kinase phosphorylating pol II.

I

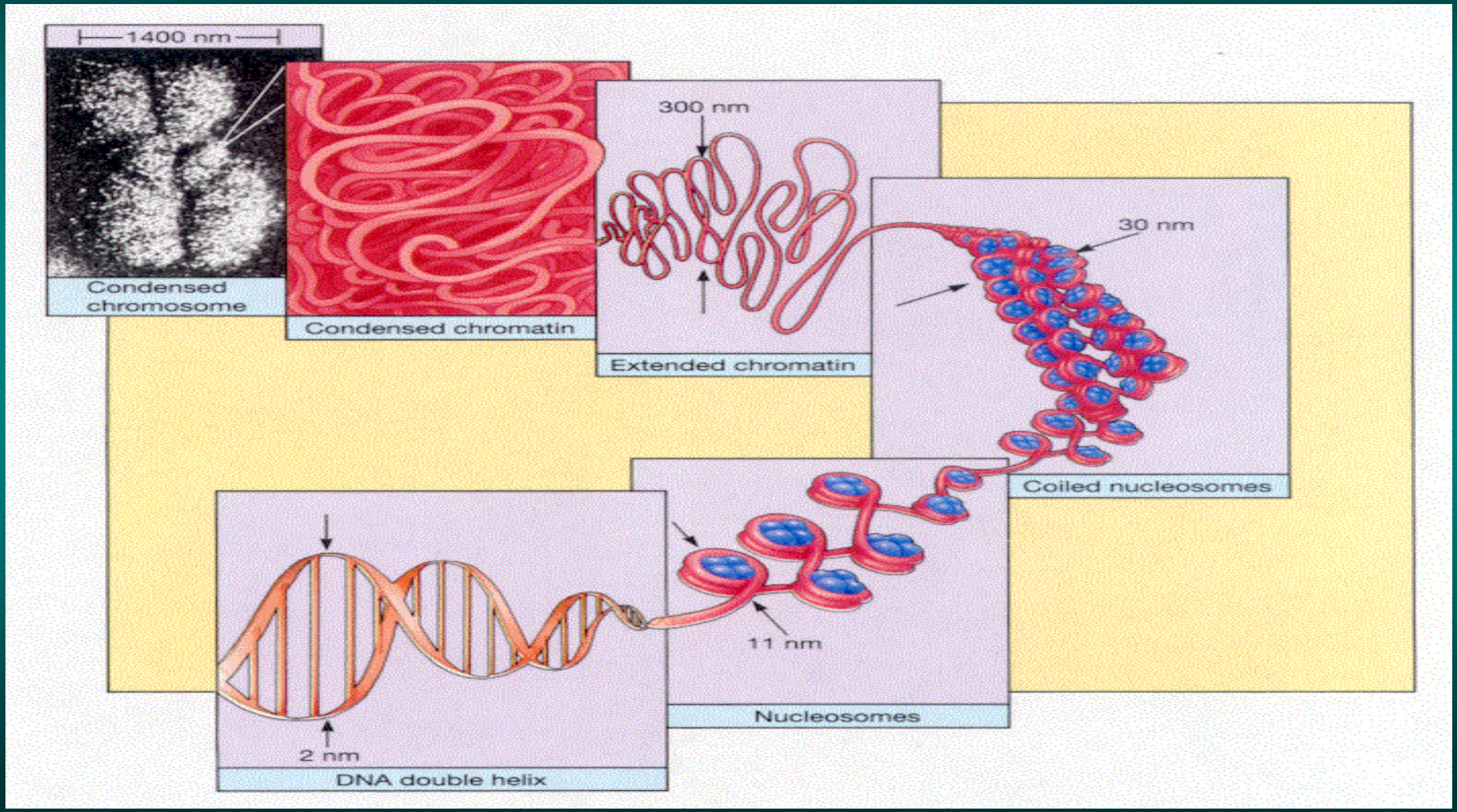


Once all of the general transcription factors are bound, energy, in the form of ATP (blue/pink), is needed to activate the Poll II complex.

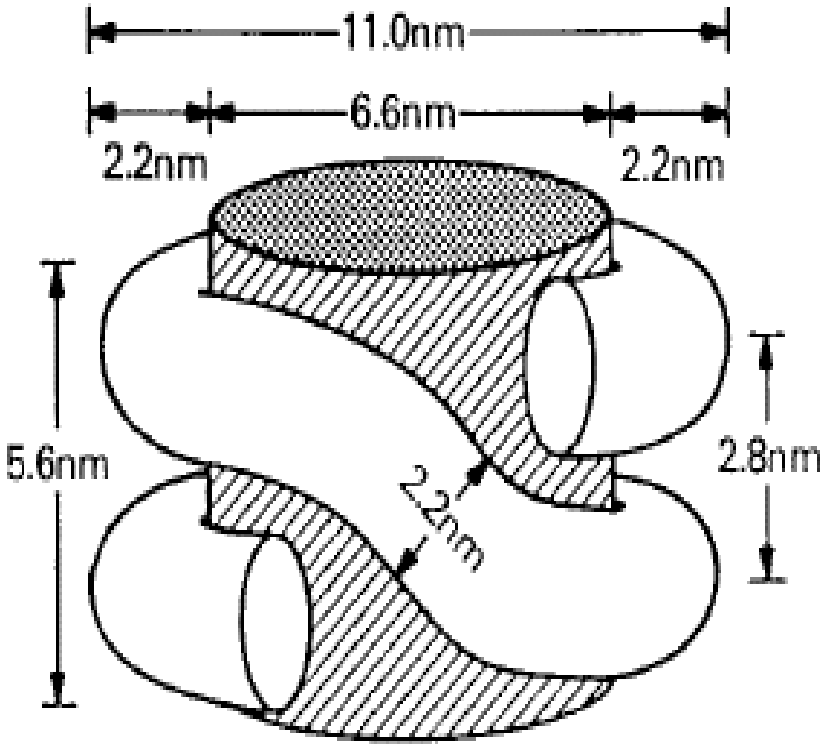
Specific transcription factors

- There are many different transcription factors, specific for some genes.
- Usually, each is present in very small amounts in a cell.
- They recognize a specific DNA sequences using a DNA binding motifs (in the case of nuclear receptors there are zinc fingers). Second domain may recognize general transcription factors and increase their activity.
- These proteins allow individual genes to be turn on and turn off specifically.
- Regulatory sequences are located far from the transcription startpoint (even 50,000 bp).
- Specific transcription factors may be constitutively active or may be inducible by different stimuli.

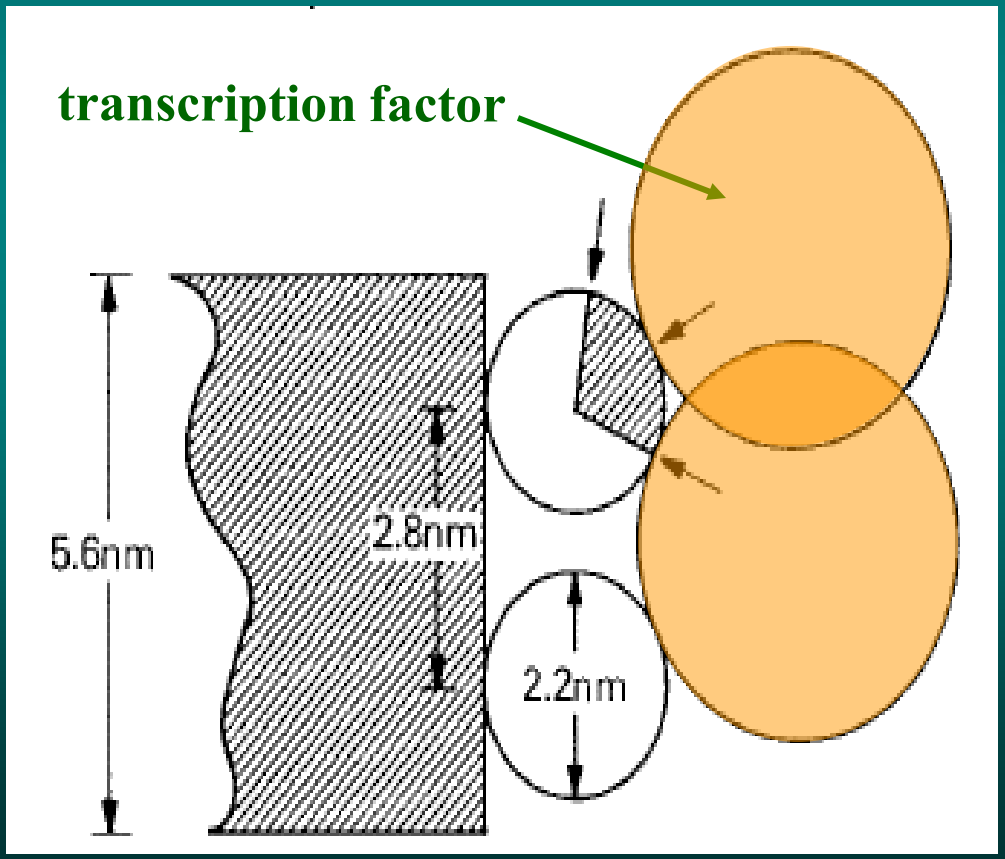
Transcription factors are unable to bind to a promoter that is packed into a nucleosome and is unaccessable for polII. Thus, the binding of gene activating proteins is necessary to relax nucleosome structure.



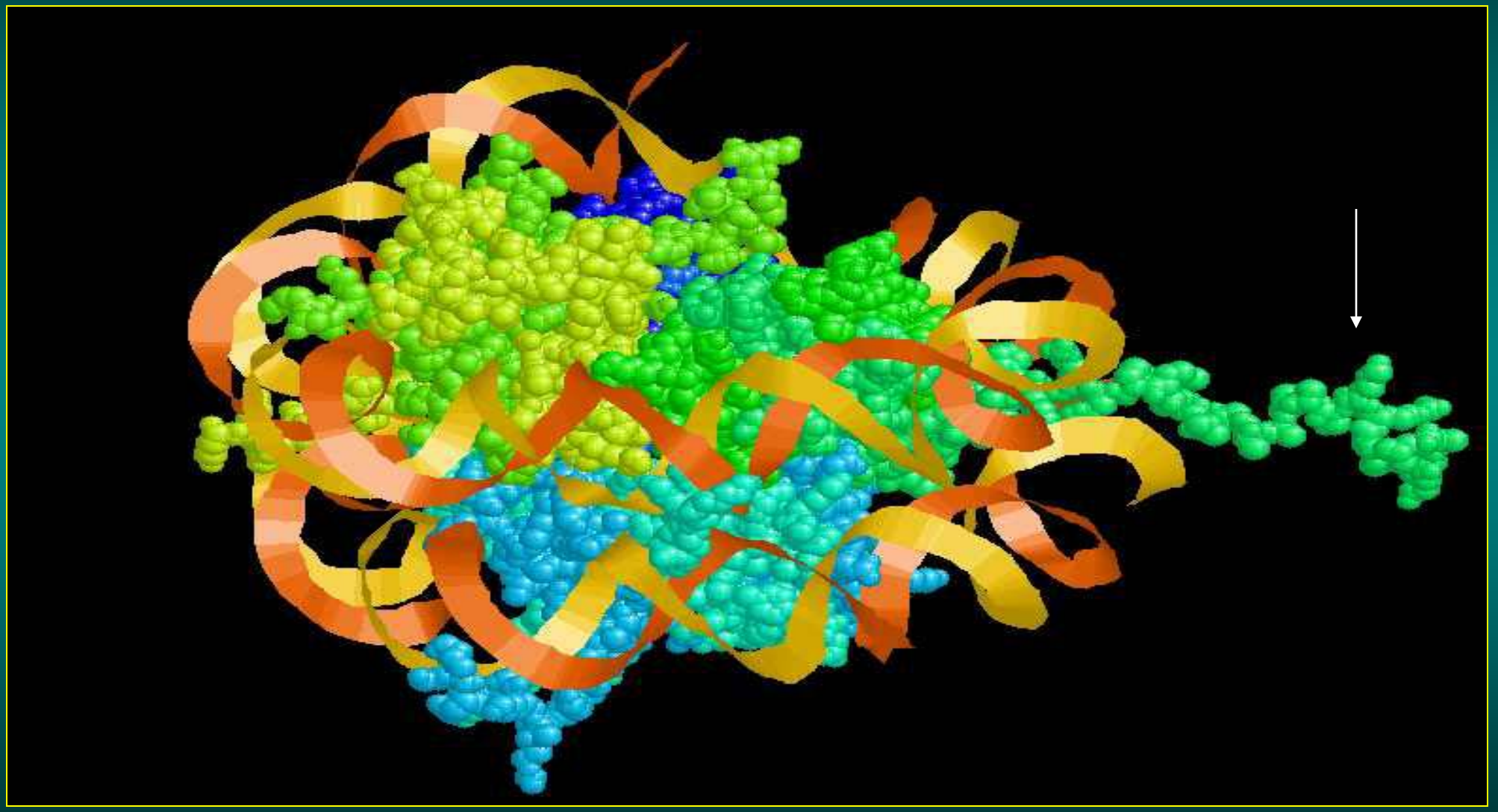
Limited access of transcription factors to DNA in the nucleosome



”crossection” of one side of nucleosome →



Nucleosome

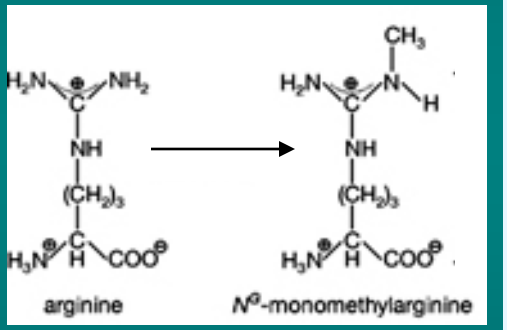


Enzymatic activities affecting chromatin structure and transcriptional activity

Histone tail modifications:

- associated with transcriptional **activation**:

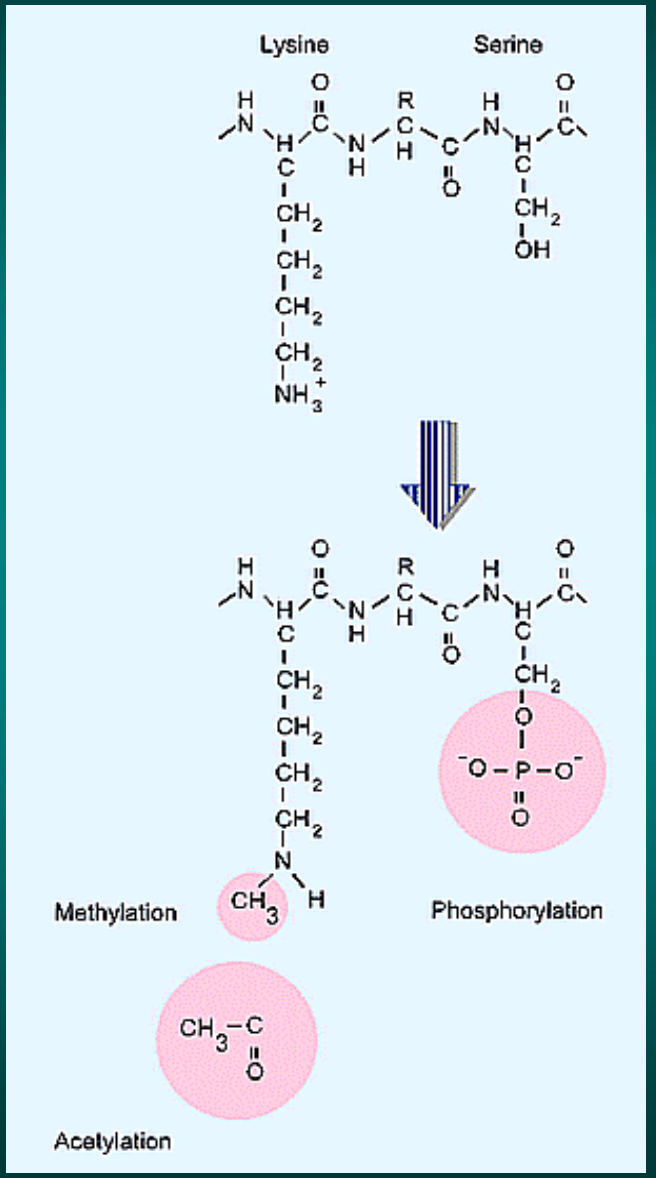
  - methylation (arginine)
  - phosphorylation (serine)
  - acetylation (lysine)



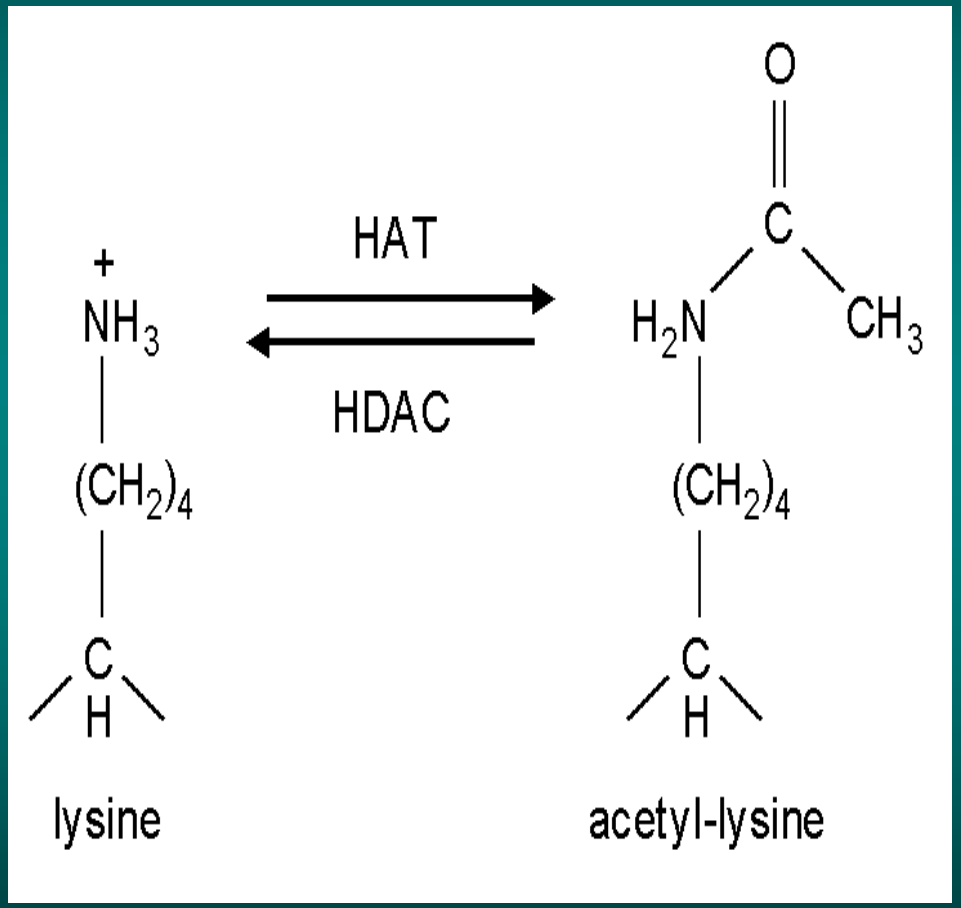
- associated with transcriptional **repression**:

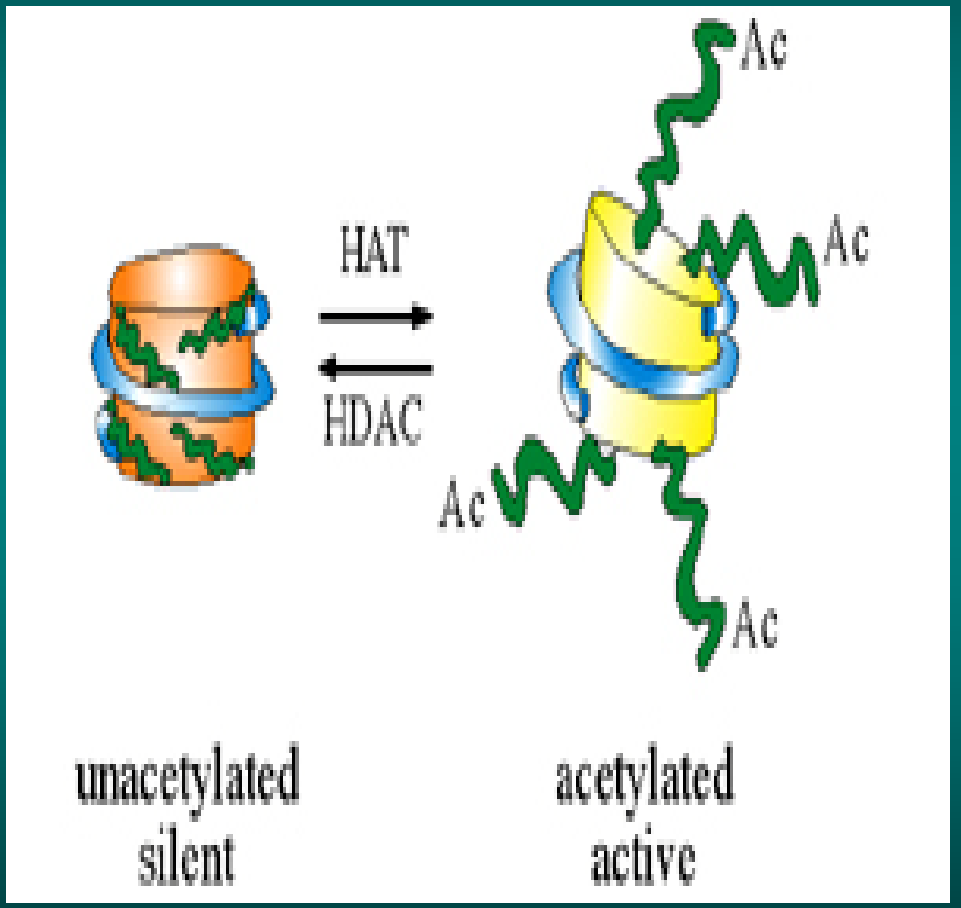
  - de-acetylation (lysine)
  - methylation (lysine)

- modifications are transient and change e.g. during cell cycle

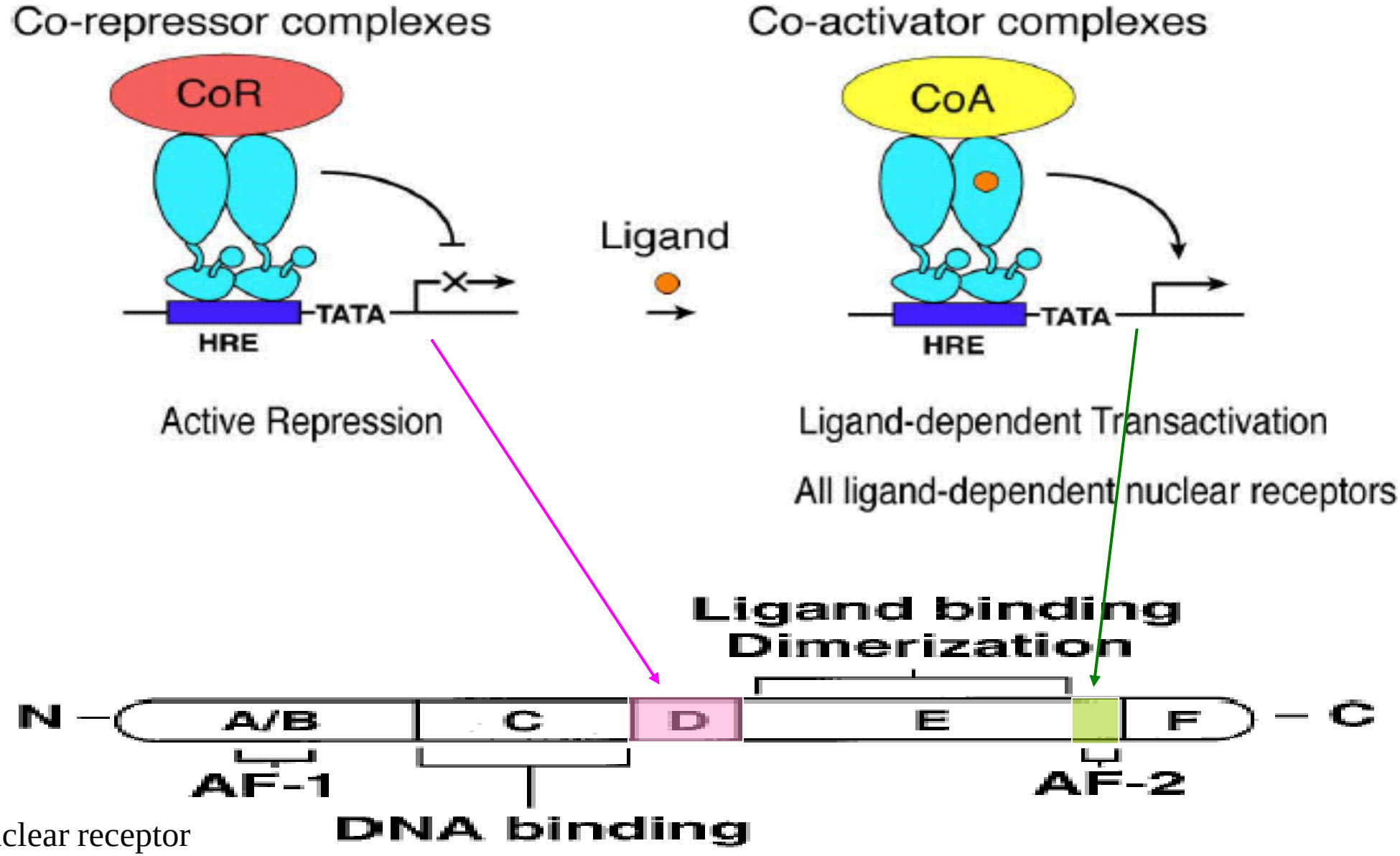


Acetylation of histones





Ligand-induced exchange of co-activators for co-repressors



nuclear receptor

p160/Src (steroid receptor coactivator) family

• **T**hree distinct but related family members, with each family member having a number of splice variants. These include SRC-1/NCoA-1, SRC-2/TIF-2/GRIP-1/NCoA-2, and SRC-3/pCIP/ACTR/AIB1/TRAM1/RAC3.

• A distinctive structural feature of the p160 coactivators is the presence of multiple LXXLL signature motifs, which interact with the nuclear receptor in an agonist and AF-2-dependent manner.

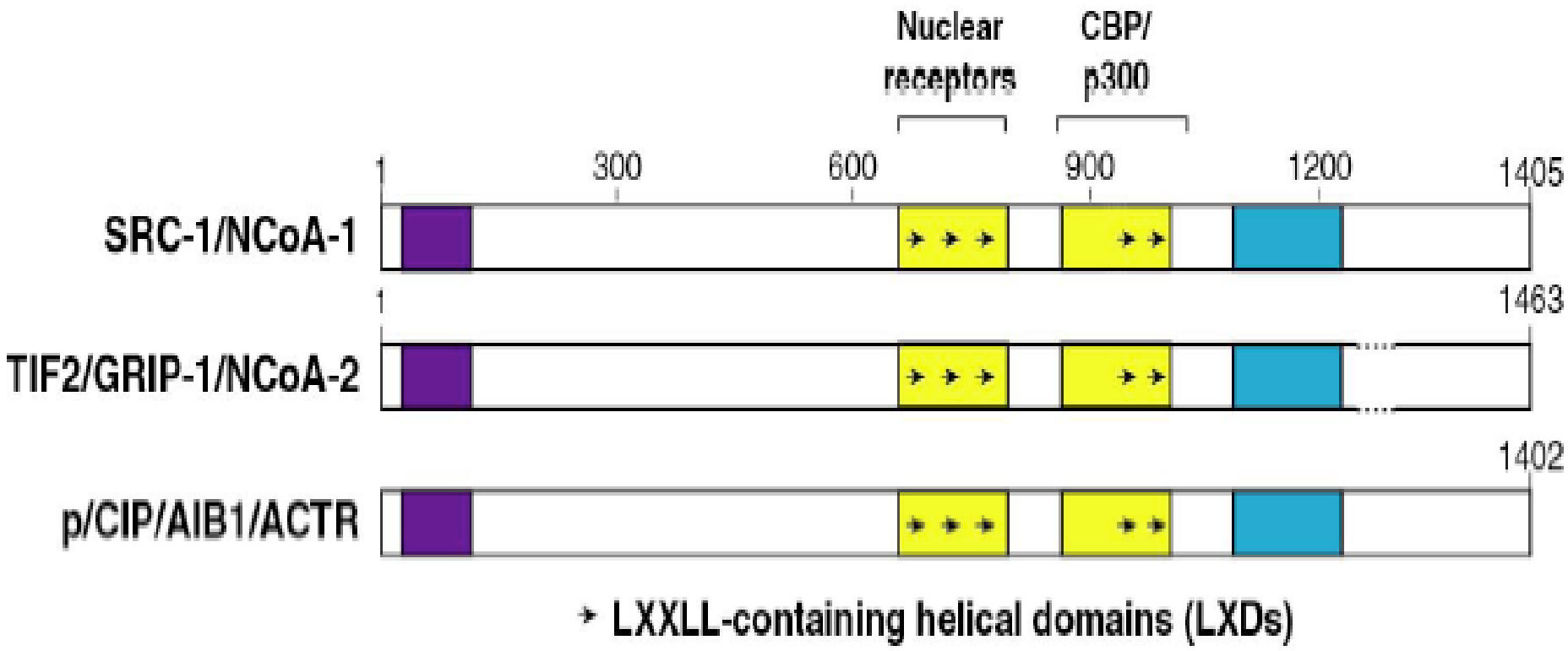
• SRC-1 knockout mice show a partial resistance to hormones and a reduced growth and development of various steroid target organs.

• SRC-3 gene is amplified in 5–10% of breast tumors and 7–8% of ovarian cancer samples.

• These factors can interact with CBP and p300 transacetylases and with CARM1, which can methylate histone H3 in vitro.

• SRC-1 also mediates transactivation by a series of other non-receptor-type transcription factors, including AP-1, NFkB, SRF, and p53.

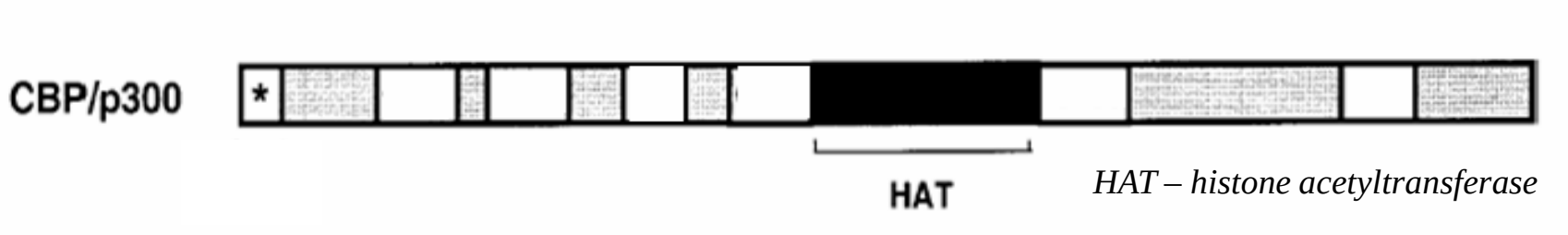
The p160/SRC-1 family of nuclear receptor coactivators



CBP and p300

• **CBP** (mediates cAMP signaling) and **p300** (binds E1A adenoviral protein) contain intrinsic acetyltransferase activity and appear to be the **predominant acetyltransferases for histones**.

• For nuclear receptors, the interaction with CBP and p300 is ligand- and AF2-dependent, although this direct interaction does not appear to be essential for many nuclear receptors.



• These proteins can acetylate both free histones and nucleosomal histones in vitro. In addition, they are able to acetylate nonhistone proteins, including p53, HNF-4, E2F, and MyoD and components of the general transcriptional machinery such as TFIIE.

• CBP and p300 complement the activities of many transcription factors, including p53, NFκB, AP-1, HIF-1.

TRAP/DRIP family

- TRAP family comprises at least nine polypeptides.

- These proteins are devoid of any HAT activity.

- TRAP (thyroid receptor associated protein)/DRIP (D receptor intereacting protein) complex is recruited to the LBD AF2 core (in response to ligand binding) via an LXXLL motif.

- TRAP/DRIP can directly connect to the RNA polymerase II core machinery.

- Consistent with such a pivotal role in NR-mediated transcriptional regulation, null mutation of the gene encoding a NR box-containing TRAP/DRIP subunit (TRAP220) results in embryonic lethality attributable to a variety of pleiotropic abnormalities, including:

  * defects in cell cycle regulation
  * and increased apoptosis.

Corepressors (NCoR and SMRT)

- NCoR (nuclear receptor coreporessor) and SMRT (silencing mediator of thyroid and retinoid receptors) harbor repression domains that associate with various histone deacetylases (HDACs) - histone hypoacetylation correlates with gene repression.

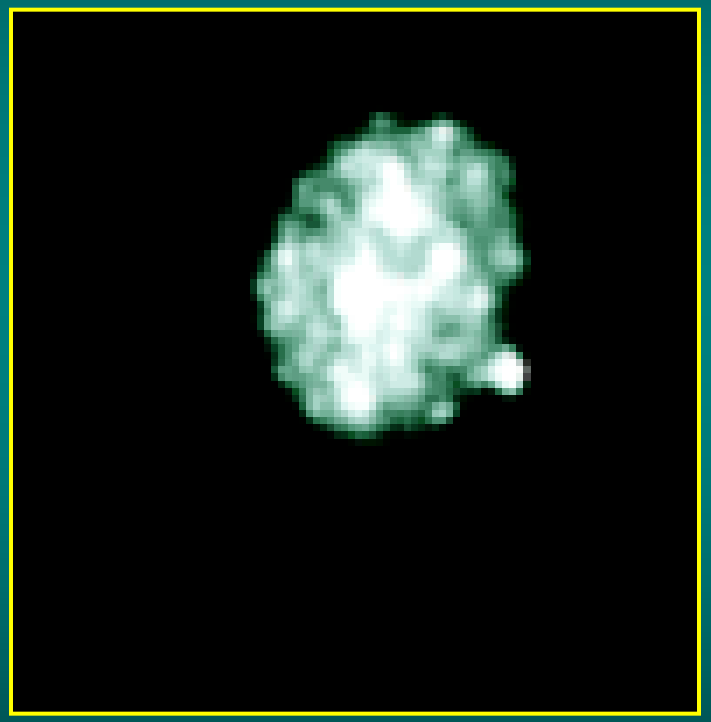
- NCoR and SMRT are also known to mediate transcriptional repression from a wide variety of other non-receptor-mediated pathways. These include AP-1, NFκB, and many others.

- MEK-1 kinase pathway was demonstrated to regulate SMRT phosphorylation and nuclear export, resulting in inhibition of its corepressor function.

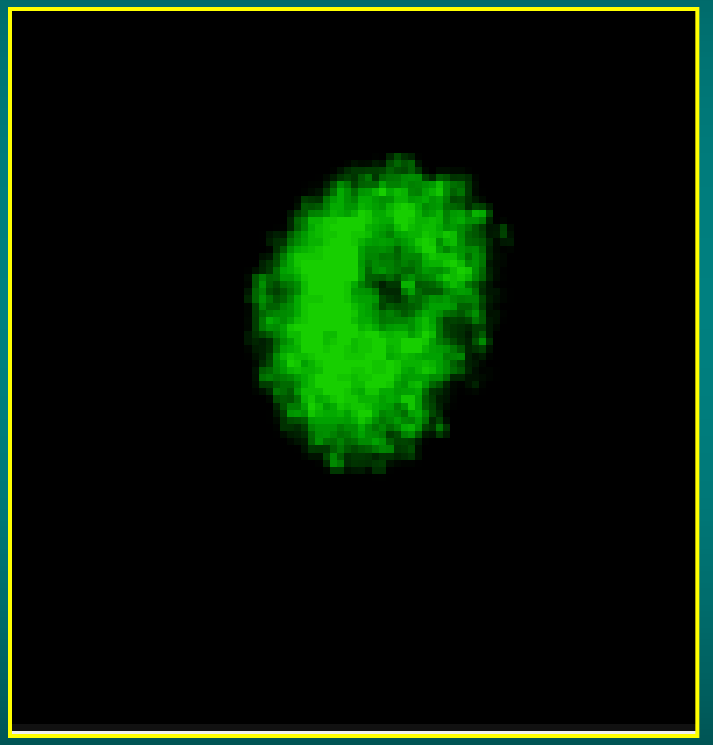
- NCoR-deficient embryos exhibit abnormalities in erythrocyte, thymocyte, and neural development and generally die around embryonic age (E)16.

HDAC – histone deacetylase

NCoR and SMRT associate with a multiprotein complex, consisting (among others) of histone deacetylases, HDAC1 or HDAC2.

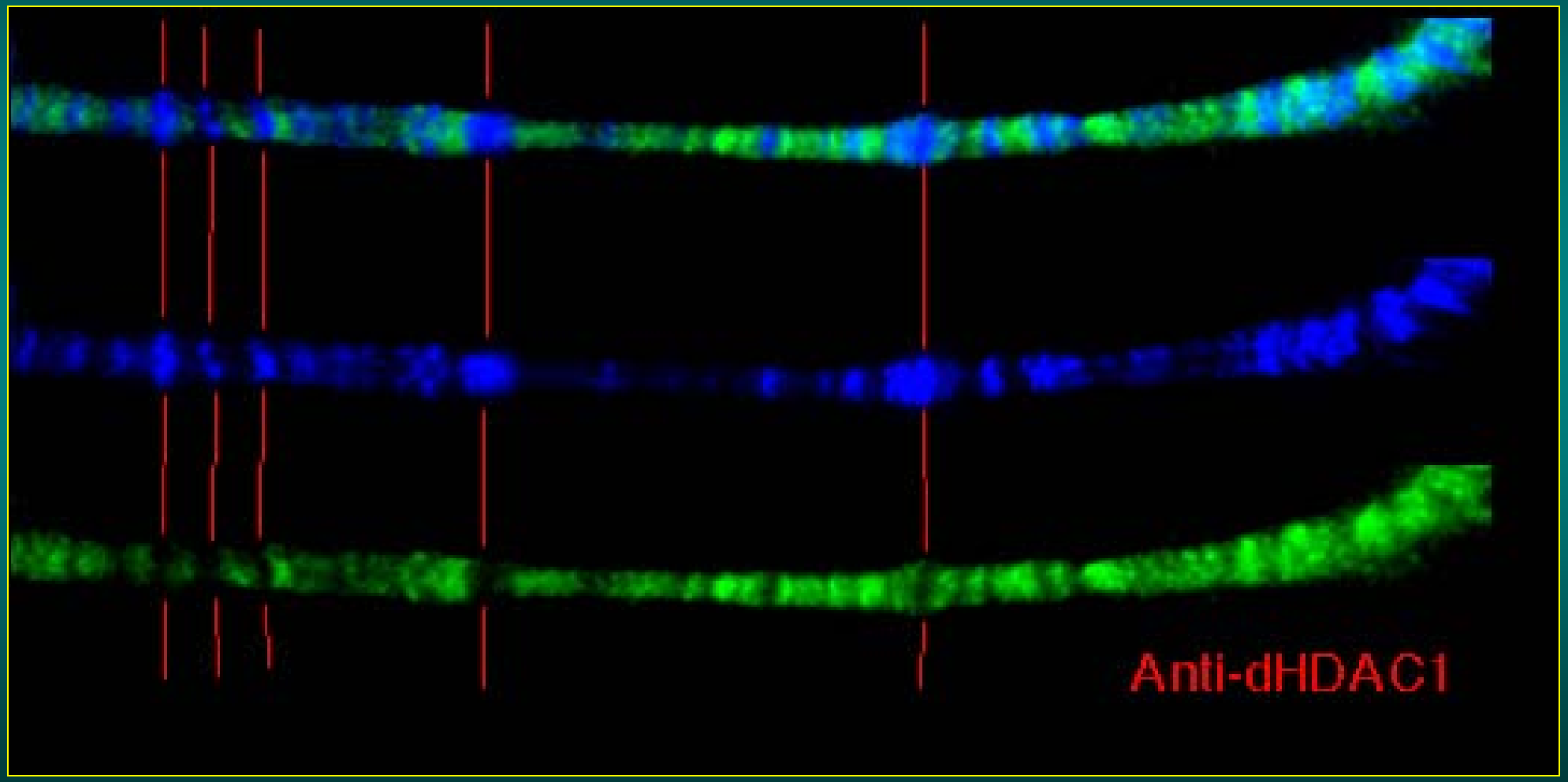


Hoechst



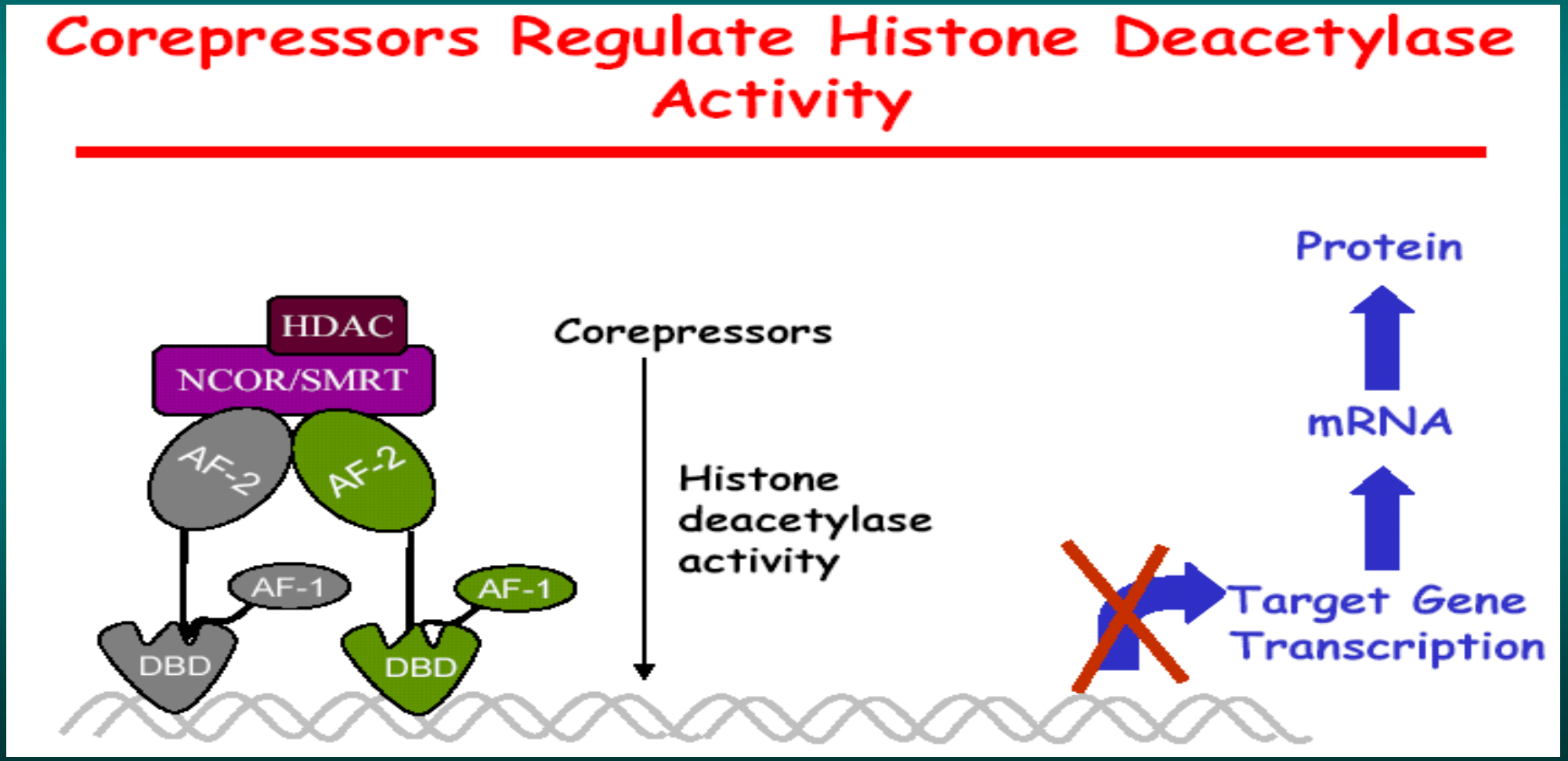
HDAC

Drosophila chromosome immunostained with anti-HDAC1 antibody (green), with DAPI as the blue counterstain



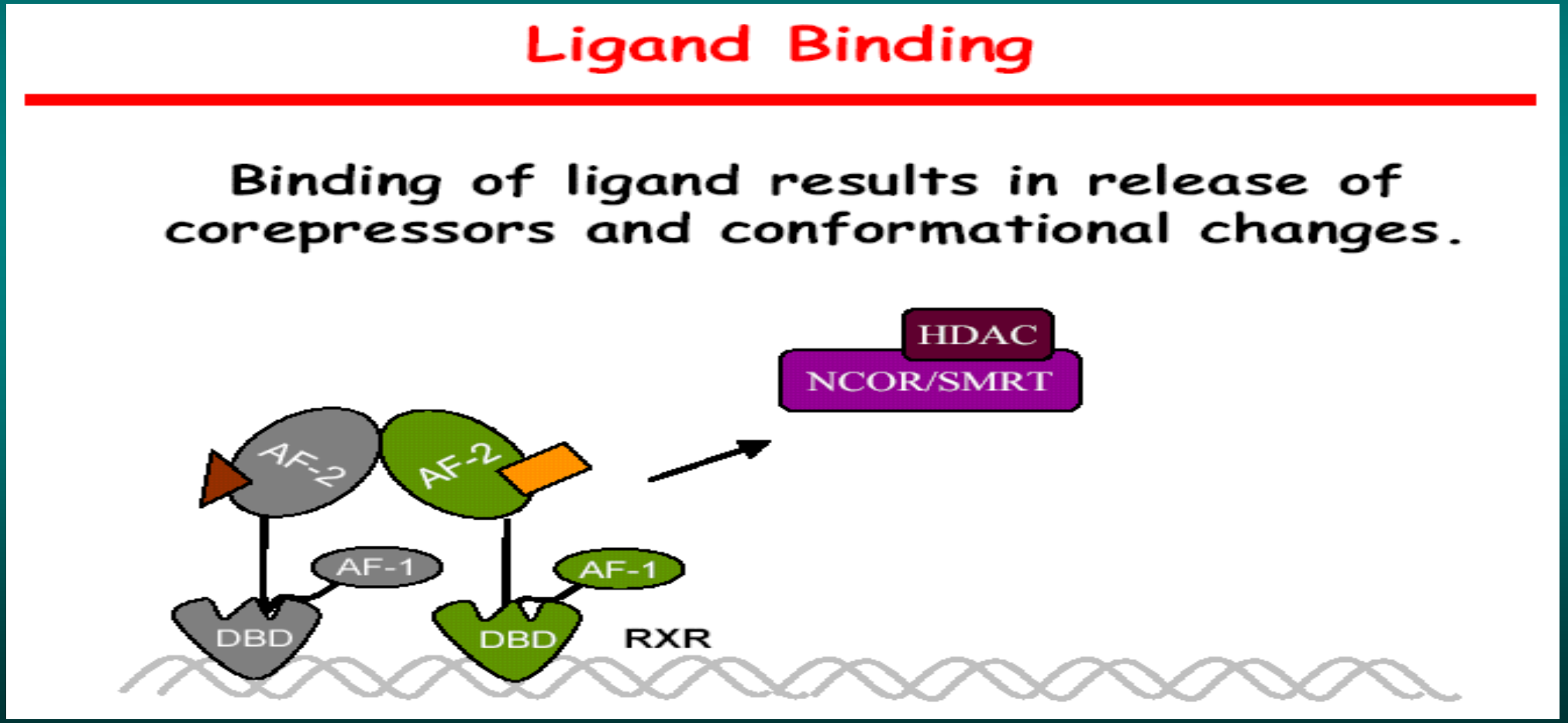
• **Some unliganded NRs** (TR and RAR) **and antagonist-bound NRs** (e.g., tamoxifen-bound ERα)**, can bind to their cognate DNA response elements.**

• **However, in this situation they interact with corepressors such as NCoR/SMRT to recruit HDAC complexes and repress transcription of their target genes.**

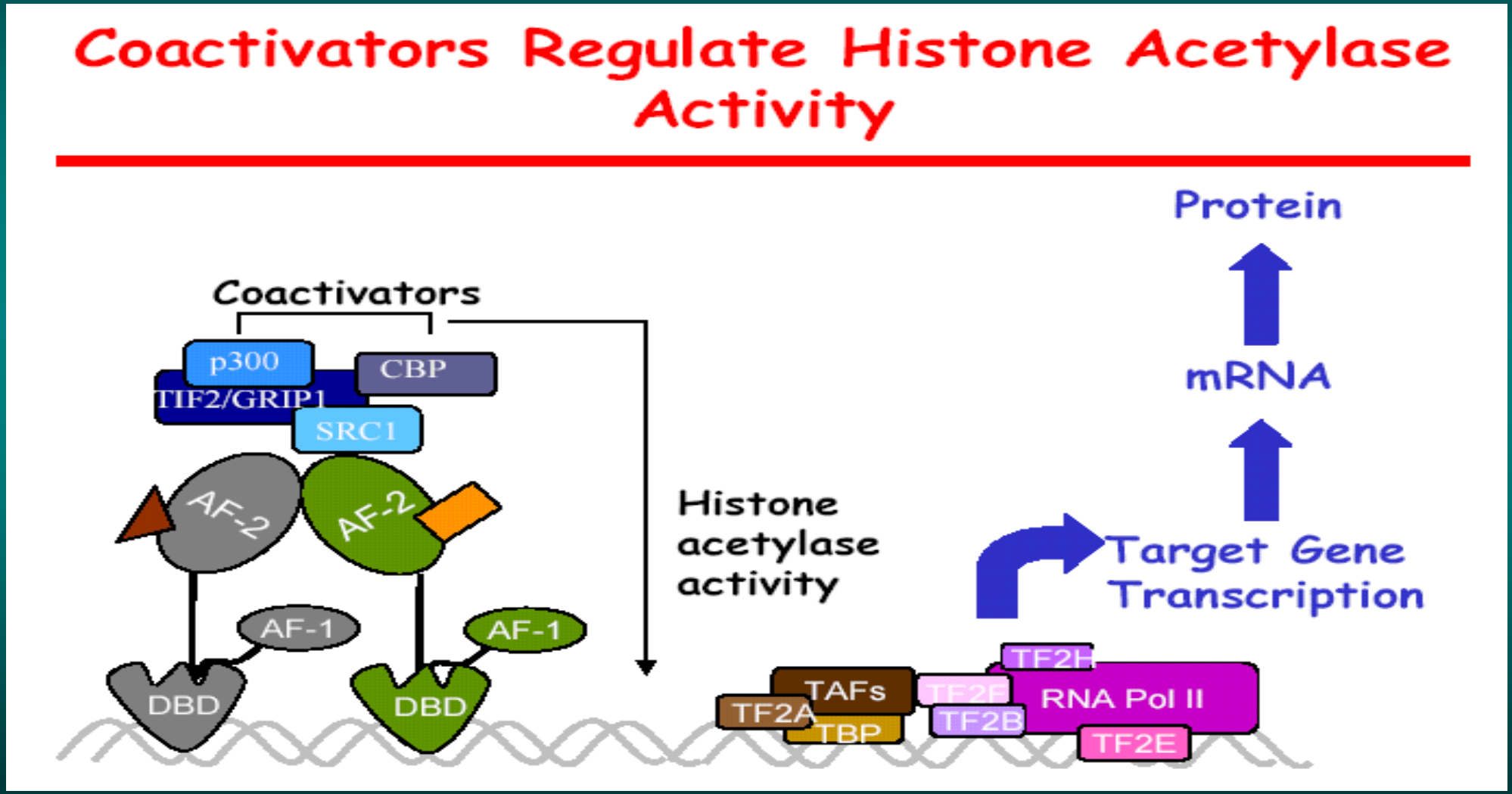


• Compactation of chromatin structure due to recruitment of HDAC complexes by corepressors is involved in transcriptional silencing by unliganded receptors.

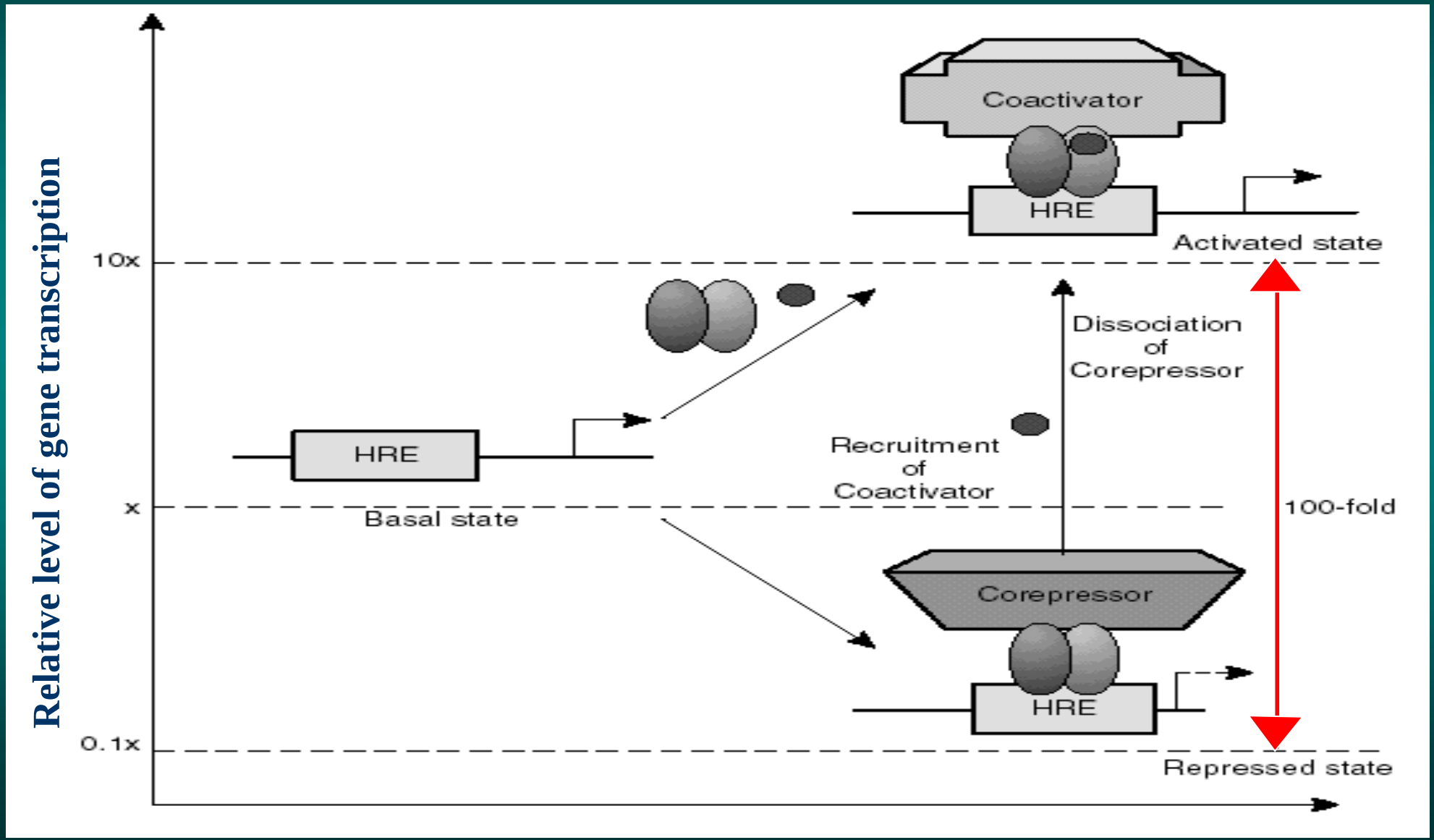
• Ligand binding allows release of corepressors and enables the receptor to recruit coactivators and stimulate transcription.



• **Ligand binding allows release of corepressors and enables the receptor to recruit coactivators and stimulate transcription.**

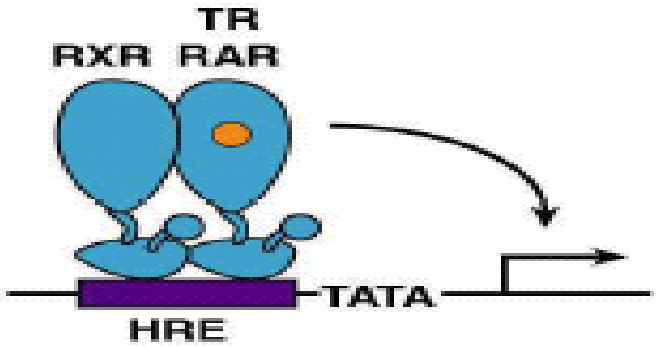


Repression and activation of transcriptional regulation by nuclear hormone receptors

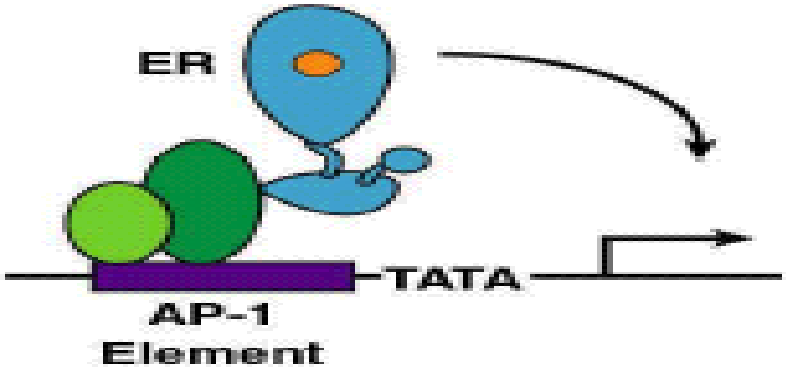


Activation

Ligand-dependent Transactivation

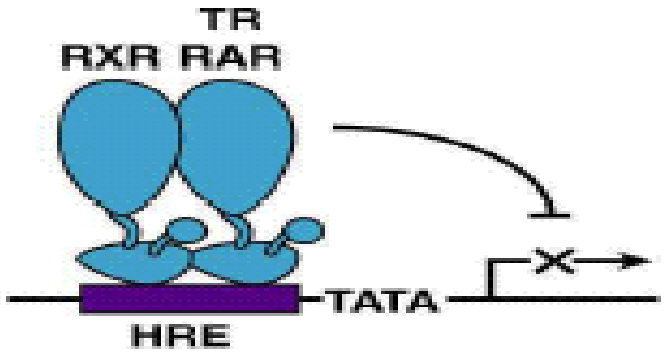


Ligand-dependent Coactivation

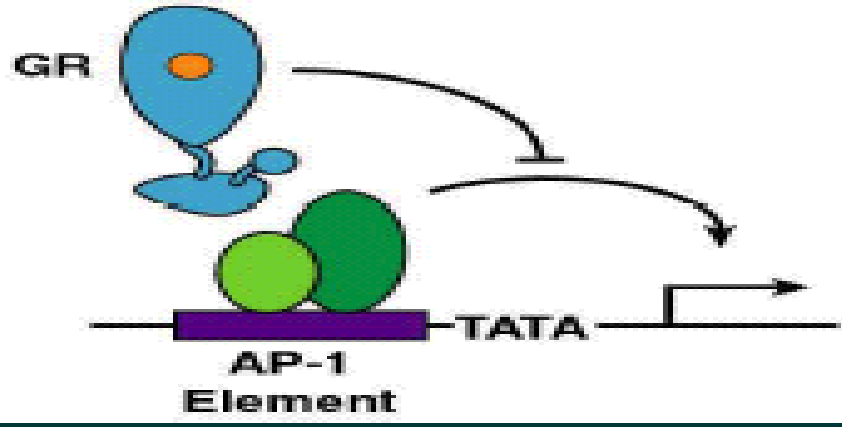


Repression

Active Repression

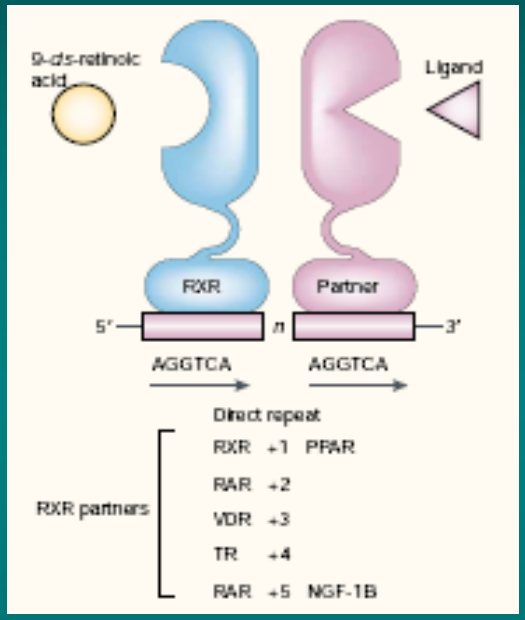


Ligand-dependent Transrepression



RXR (retinoid X receptor)

- **RXR is the only heterodimeric partner for all other nuclear receptors**
- **RXR may form homodimers and bind to DR1 sequences**



- **There are three different proteins of RXR in mammals:**
  - *** RXRα** (ubiquitous expression)
  - *** RXRβ** (ubiquitous expression)
  - *** RXRγ (**skeletal muscles, heart, central nervous system)
- **Usually it is not a limiting factor for formation of heterodimers**
- **Its natural ligand is 9-cis retinoic acid**
- **Description of natural ligand of RXR was the first example of so called ”reversed endocrinology” (first: cloning of receptor, then identification of ligand and study on its physiological role)**
- **RXR can be also activated by non-cyclic terpenoids: methoprene acid** (insecticide) **and phytane acid** (component of chlorophil, present in diet)**, but with low affinity**

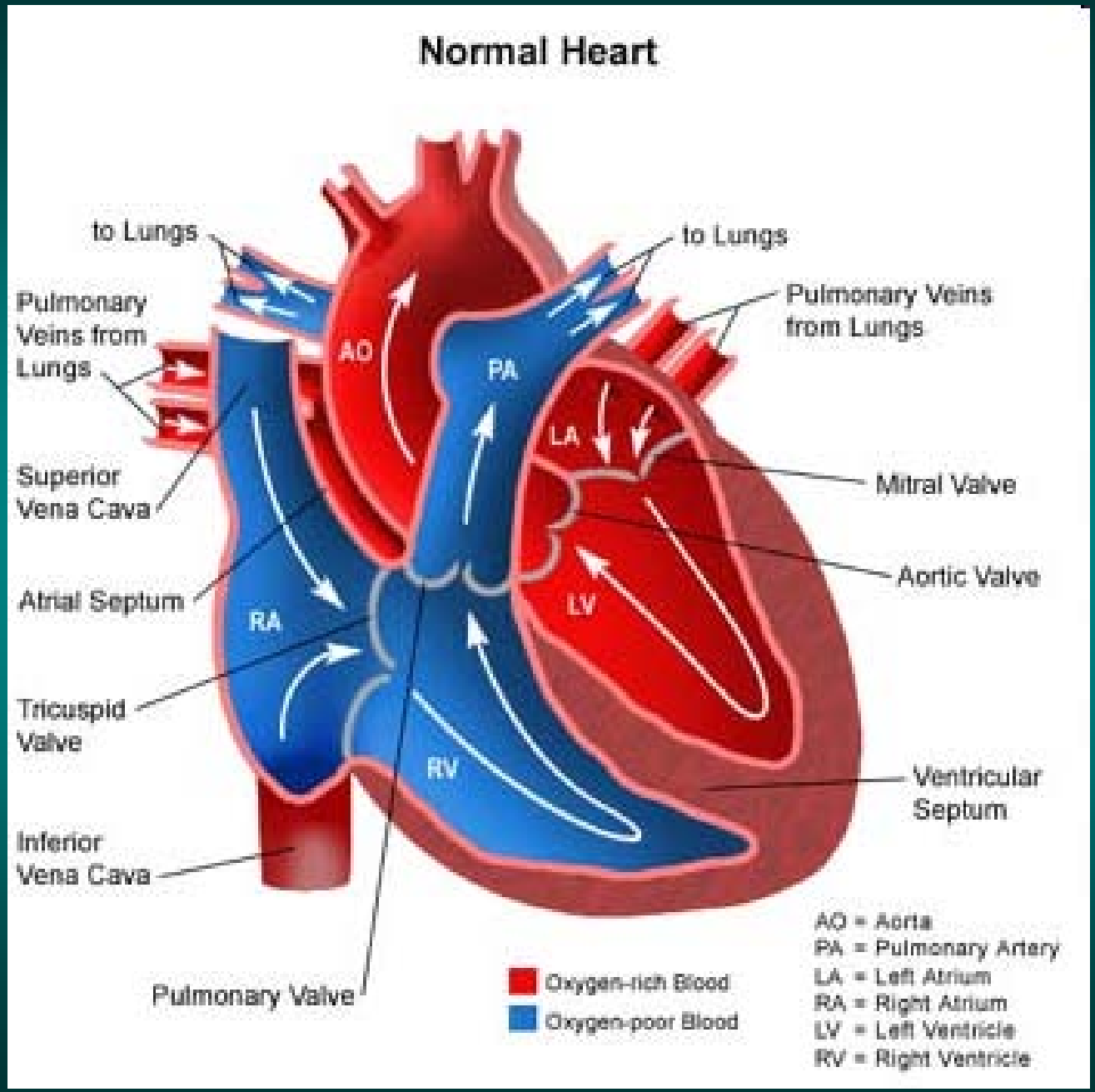
RXR

- RXR of *Cnidaria* can bind 9-cis retinoic acid

- In *Drosophila melanogaster* RXR (here: USP) is a heterodimeric partner for other nuclear receptors (natural ligand: terpenoid juvenile hormone).

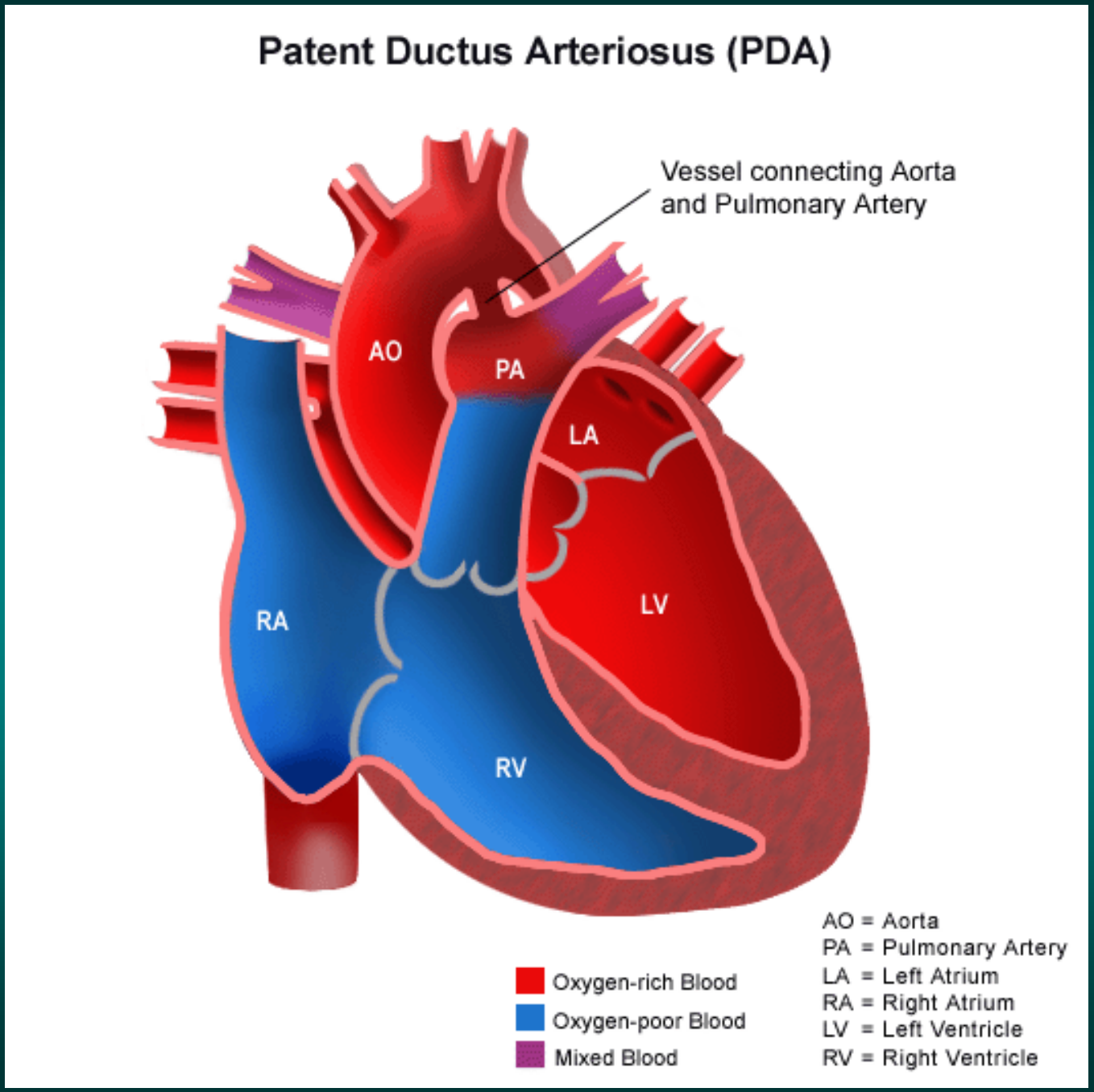
Cyanosis

- Eight out of every 1,000 babies born in the United States have a congenital (present at birth) heart defect.
- Heart is completely formed by eight weeks into the pregnancy. Congenital heart defects happen during this crucial first eight weeks of development.
- The vast majority of congenital heart defects have no known cause. Some heart problems do occur more often in families, so there may be a genetic link to some heart defects.
- Congenital heart problems range from simple to complex. Some heart problems can be watched by the baby's physician and managed with medicines, while others will require surgery, sometimes as soon as in the first few hours after birth.



Normal heart

- The human heart consists of four chambers as seen in the picture above. They are the right atrium, left atrium, left ventricle and right ventricle.
- The blood enters the heart from the superior and inferior vena cava into the right atrium. Then it goes to the right ventricle and is them pumped to the lungs via the pulmonary artery.
- Once oxygenated it comes back via the pulmonary veins into the left atrium. It then fills the left ventricle below as it flows down through the mitral valve.
- The blood flows back to the entire body, by entering the aorta.

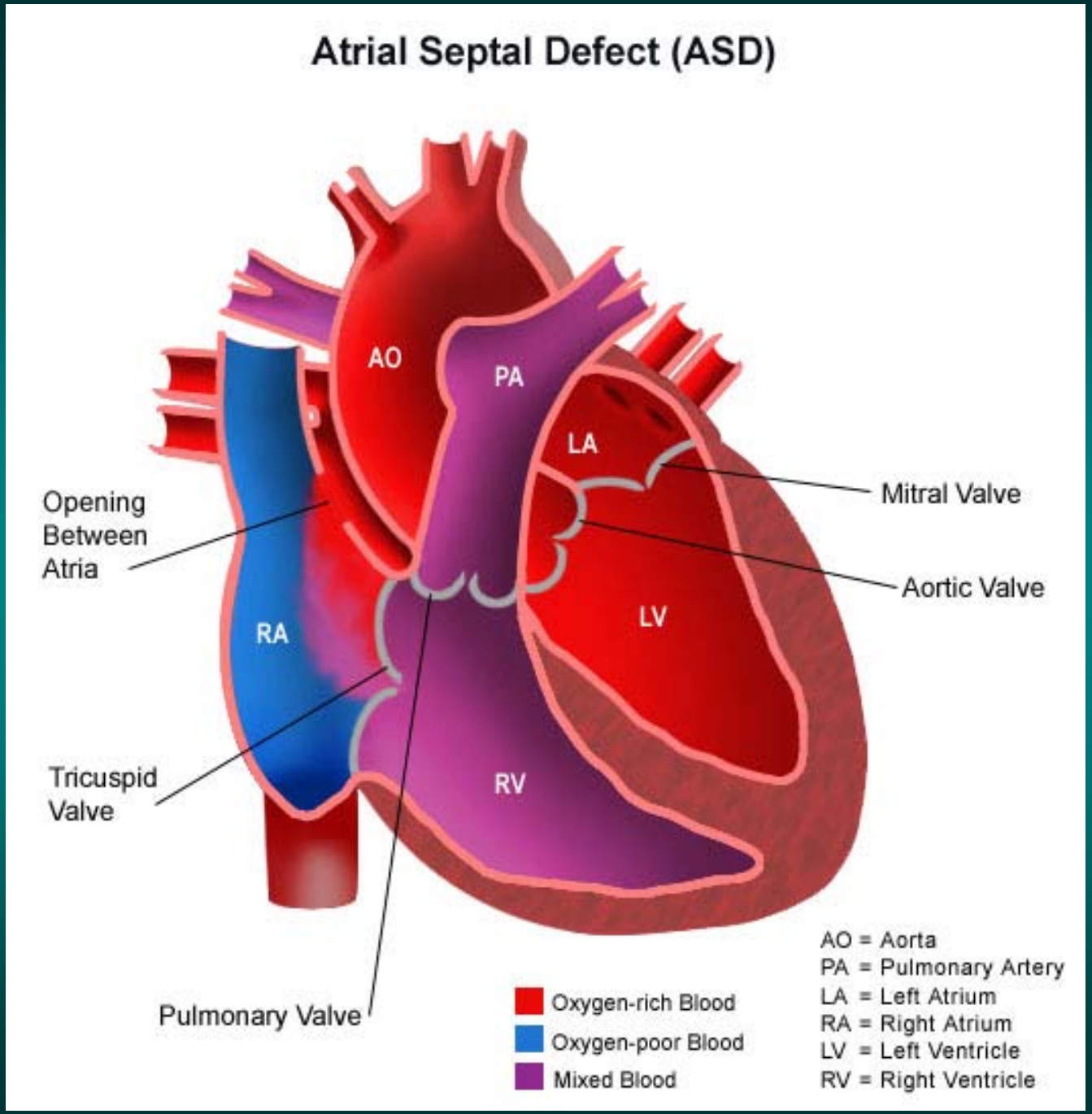


Patent ductus arteriosus (PDA)

- This defect, which normally occurs during fetal life, short circuits the normal pulmonary vascular system and allows blood to mix between the pulmonary artery and the aorta.

- Prior to birth, there is an open passageway between the two blood vessels, which closes soon after birth. When it does not close, some blood returns to the lungs. Patent ductus arteriosus is often seen in premature infants.

Przetrwały przywód tętniczy Botalla

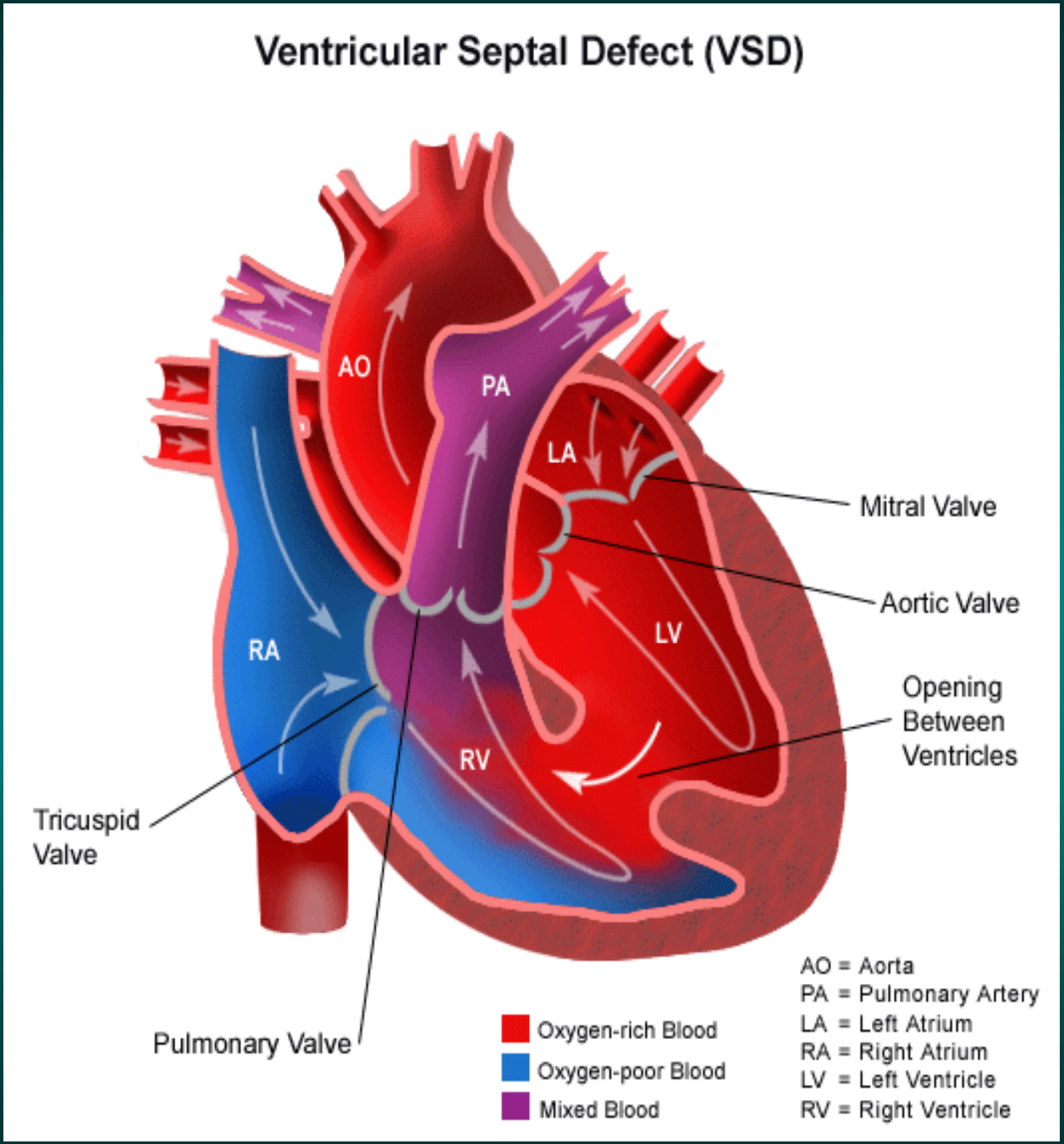


Atrial septal defect (ASD)

- In this condition, there is an abnormal opening between the two upper chambers of the heart - the right and left atria - causing an abnormal blood flow through the heart.

- Some children may have no symptoms and appear healthy. However, if the ASD is large, permitting a large amount of blood to pass through the right side, symptoms will be noted.

Ubytek przegrody międzyprzedsionkowej

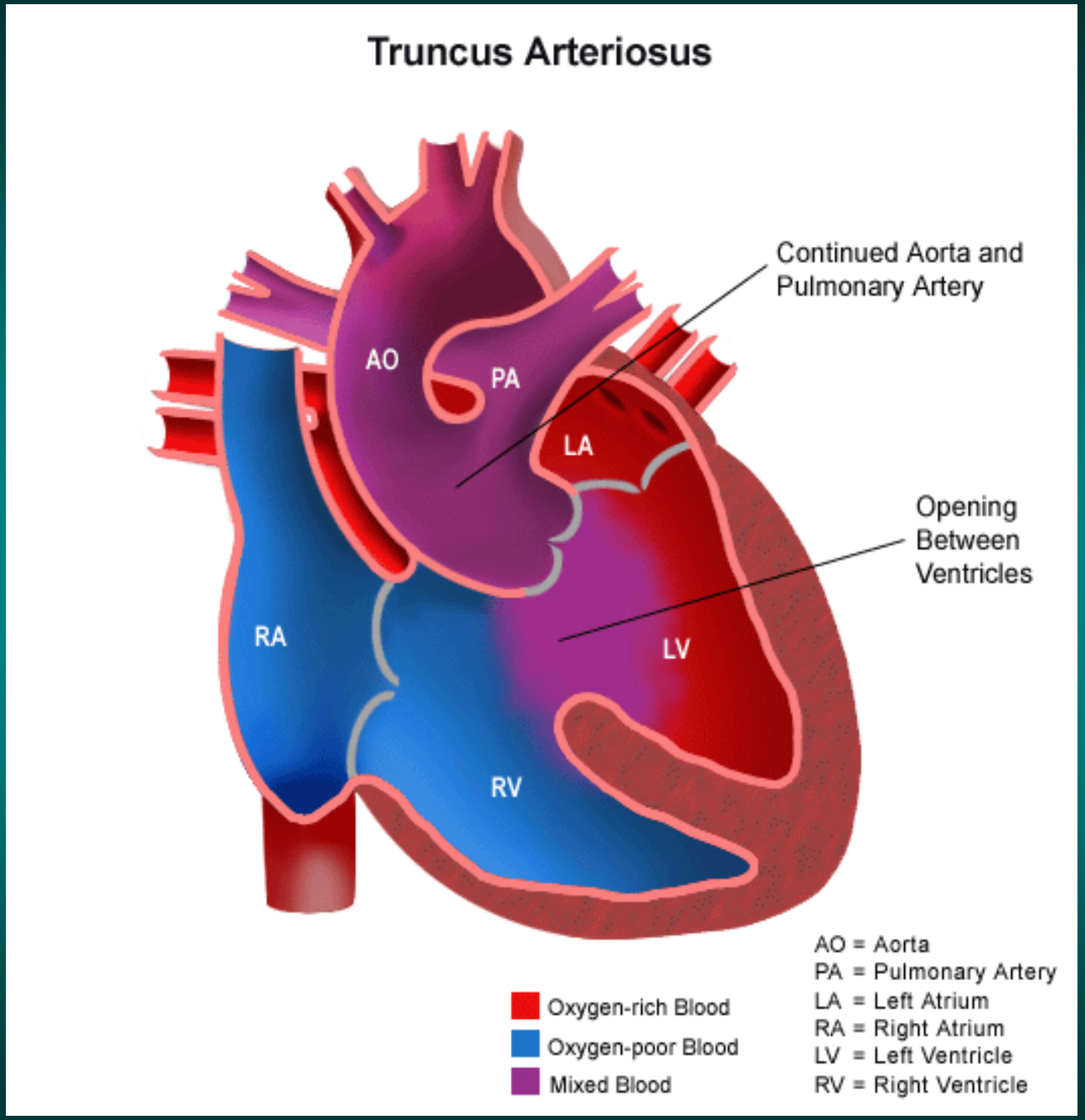


Ventricular septal defect (VSD)

- In this condition, a hole in the ventricular septum (a dividing wall between the two lower chambers of the heart - the right and left ventricles) occurs.

- Because of this opening, blood from the left ventricle flows back into the right ventricle, due to higher pressure in the left ventricle. This causes an extra volume of blood to be pumped into the lungs by the right ventricle, which can create congestion in the lungs.

Ubytki przegrody międzykomorowej

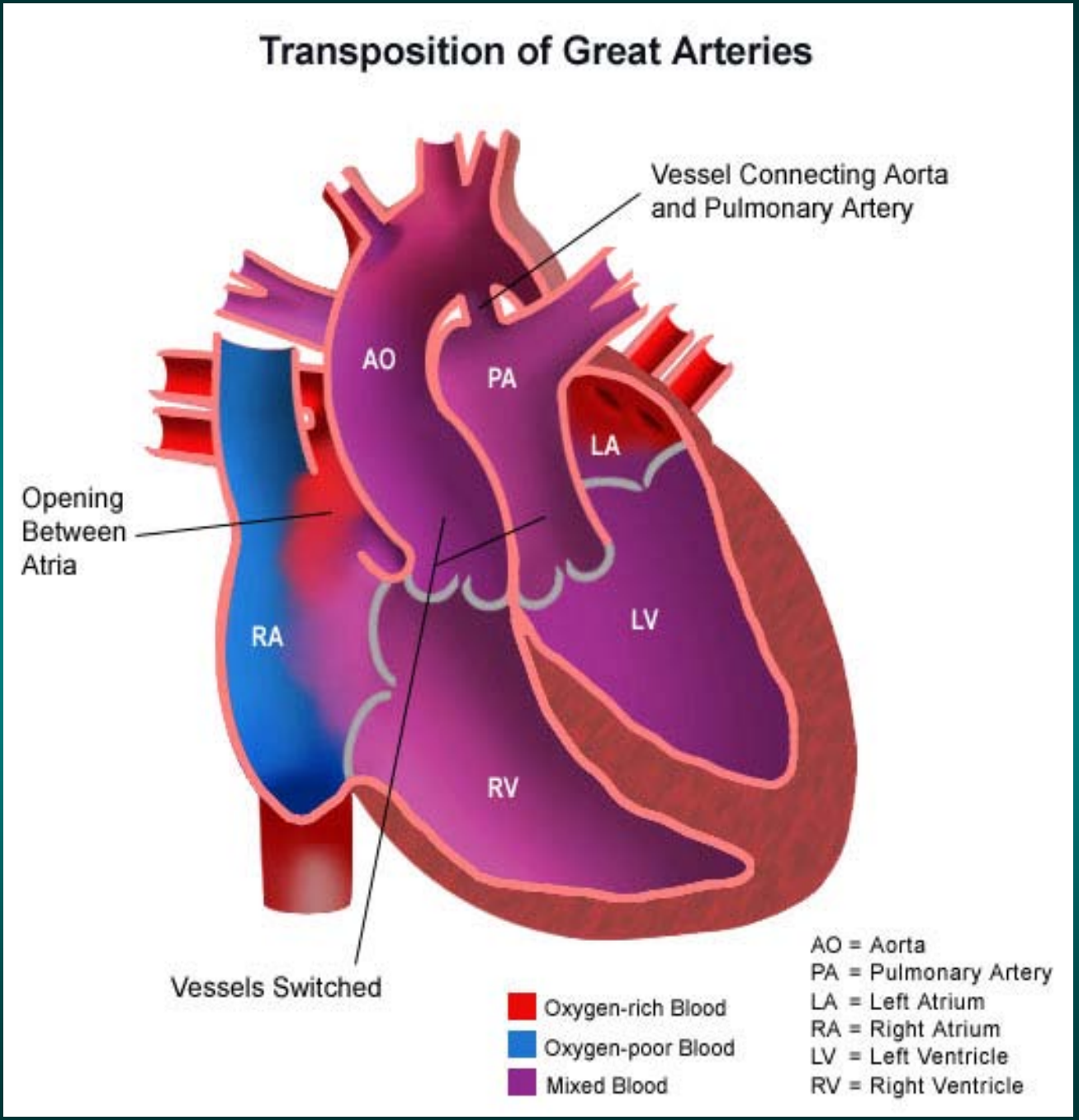


Truncus arteriosus

- The aorta and pulmonary artery start as a single blood vessel, which eventually divides and becomes two separate arteries.

- Truncus arteriosus occurs when the single great vessel fails to separate completely, leaving a connection between the aorta and pulmonary artery.

Przetrwały pień tętniczy



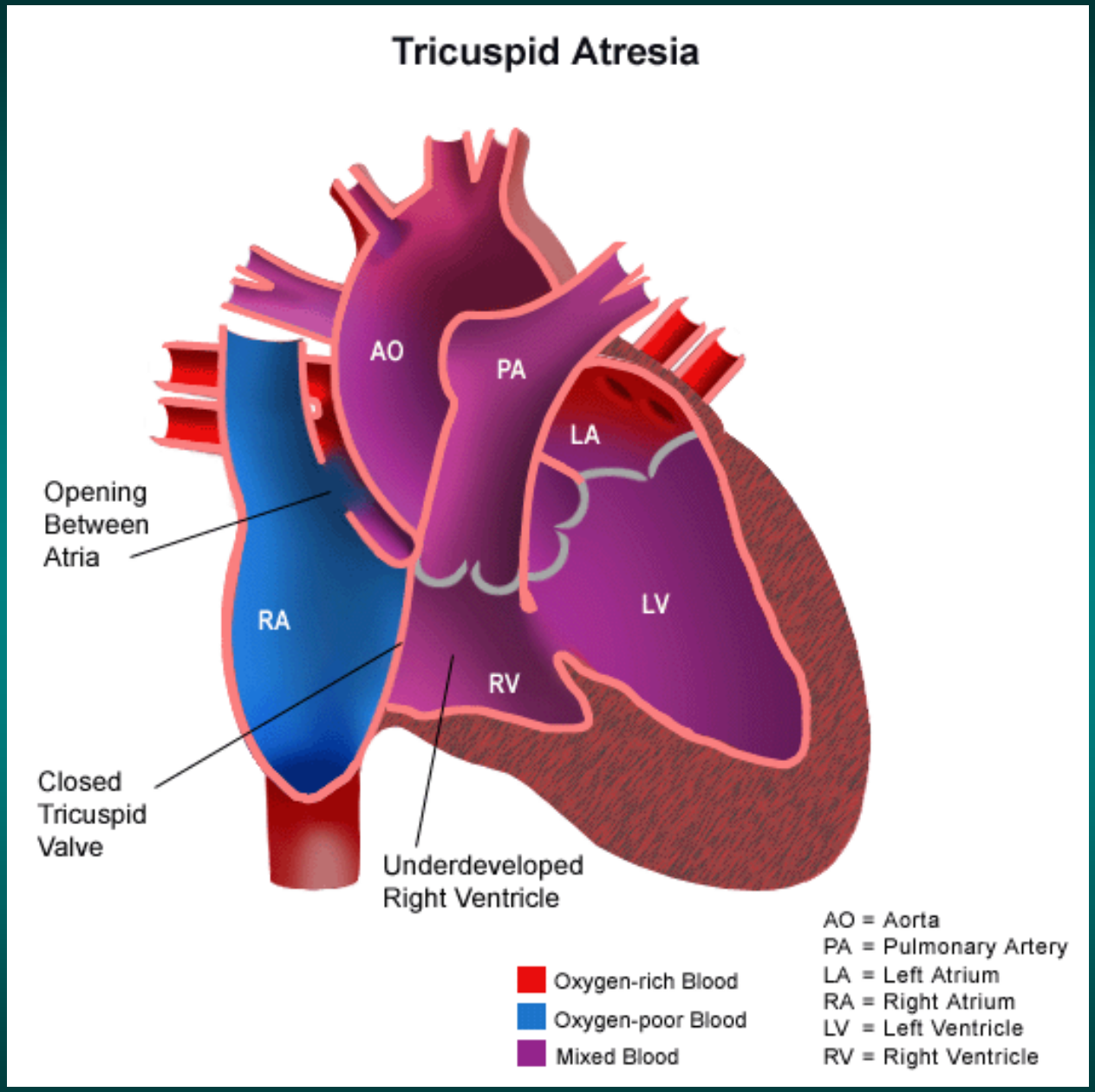
Transposition of the great arteries

- With this congenital heart defect, the positions of the pulmonary artery and the aorta are reversed, thus:

- The aorta originates from the right ventricle, so most of the blood returning to the heart from the body is pumped back out without first going to the lungs.

- The pulmonary artery originates from the left ventricle, so that most of the blood returning from the lungs goes back to the lungs again

Przestawienie naczyń aorty i tętnicy płucnej



Tricuspid atresia

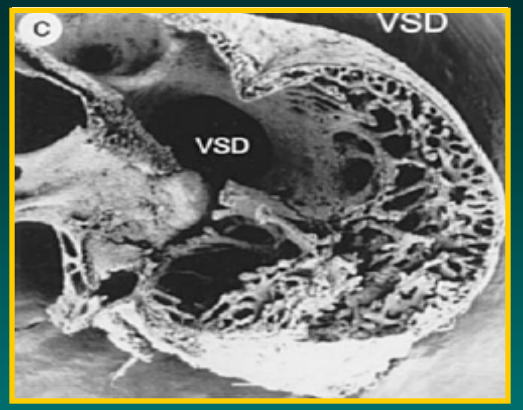
- In this condition, there is no tricuspid valve, therefore, no blood flows from the right atrium to the right ventricle.

- Tricuspid atresia defect is characterized by a small right ventricle, a large left ventricle, diminished pulmonary circulation, or cyanosis, bluish color of the skin and mucous membranes caused from a lack of oxygen.

- A surgical shunting procedure is often necessary to increase the blood flow to the lungs.

Niedrożność zastawki trójdzielnej

4 days old puppies



Ventricular septal defect in embryo

• **V**entricular septal defects are one of the most common congenital cardiac defects in human infants.

• The ontogeny of atrioventricular septation is complex. Multiple primordia contribute to the formation of the interatrial and interventricular septa, including endocardial cushion tissue and myocardium. The muscular portion of the interventricular septum arises from ingrowth and folding of the myocardial wall of the developing ventricle, whereas the membranous portion of the interventricular septum is derived primarily from endocardial cushion tissue.

• Environmental influences, such as alcohol exposure, may cause ventricular septal defects. No other genes have been shown to affect the ventricular septum exclusively, but many, such as NFATc, endothelin-1, BMP6 and BMP7, and RXRα have been shown to affect ventricular septation as part of a larger constellation of congenital cardiac abnormalities.

• Retinoid signaling may be critical for the maintenance of normal cardiac morphogenesis.

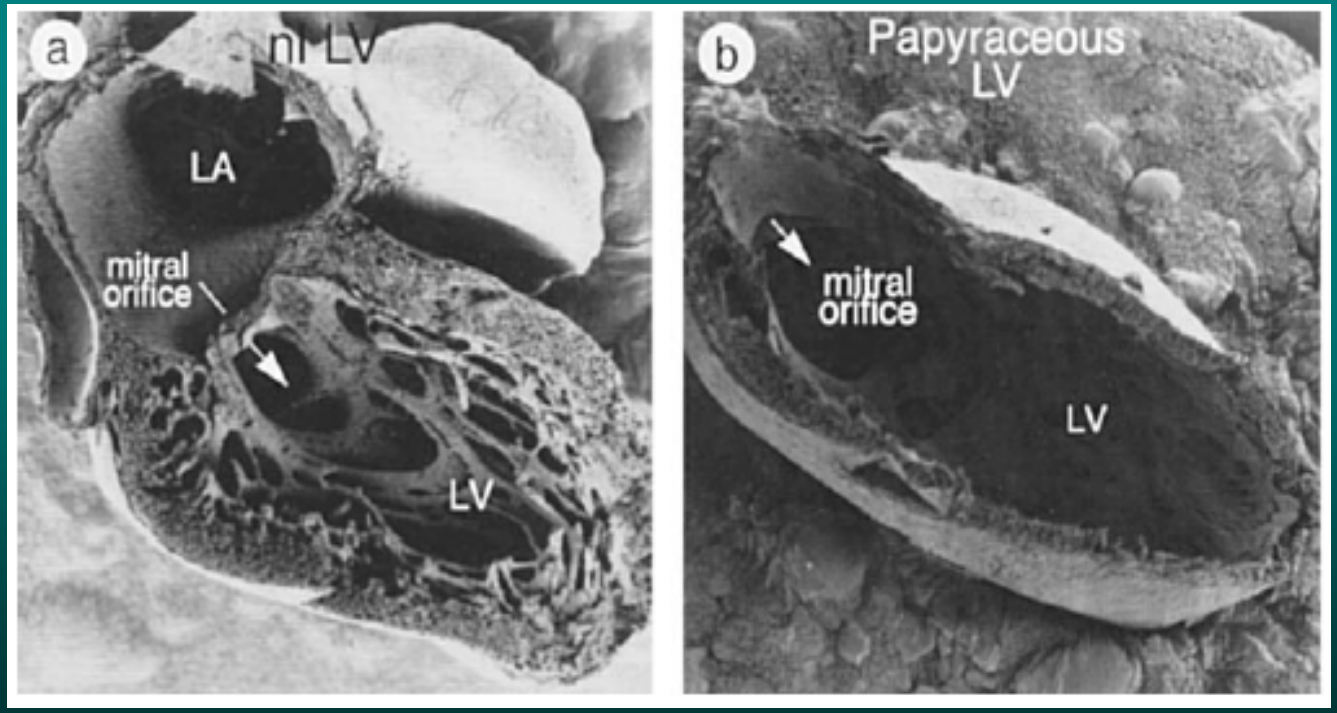
• A gene knockout of the RXRα gene results in severe cardiac muscle defects and a consequent decrease in cardiac function leading to embryonic heart failure and lethality around embryonic day 14.5. This defect is similar to a subset of the cardiovascular alterations seen in the setting of vitamin A deficiency, suggesting that RXRα-dependent pathways are critical in the maintenance of normal cardiac morphogenesis.

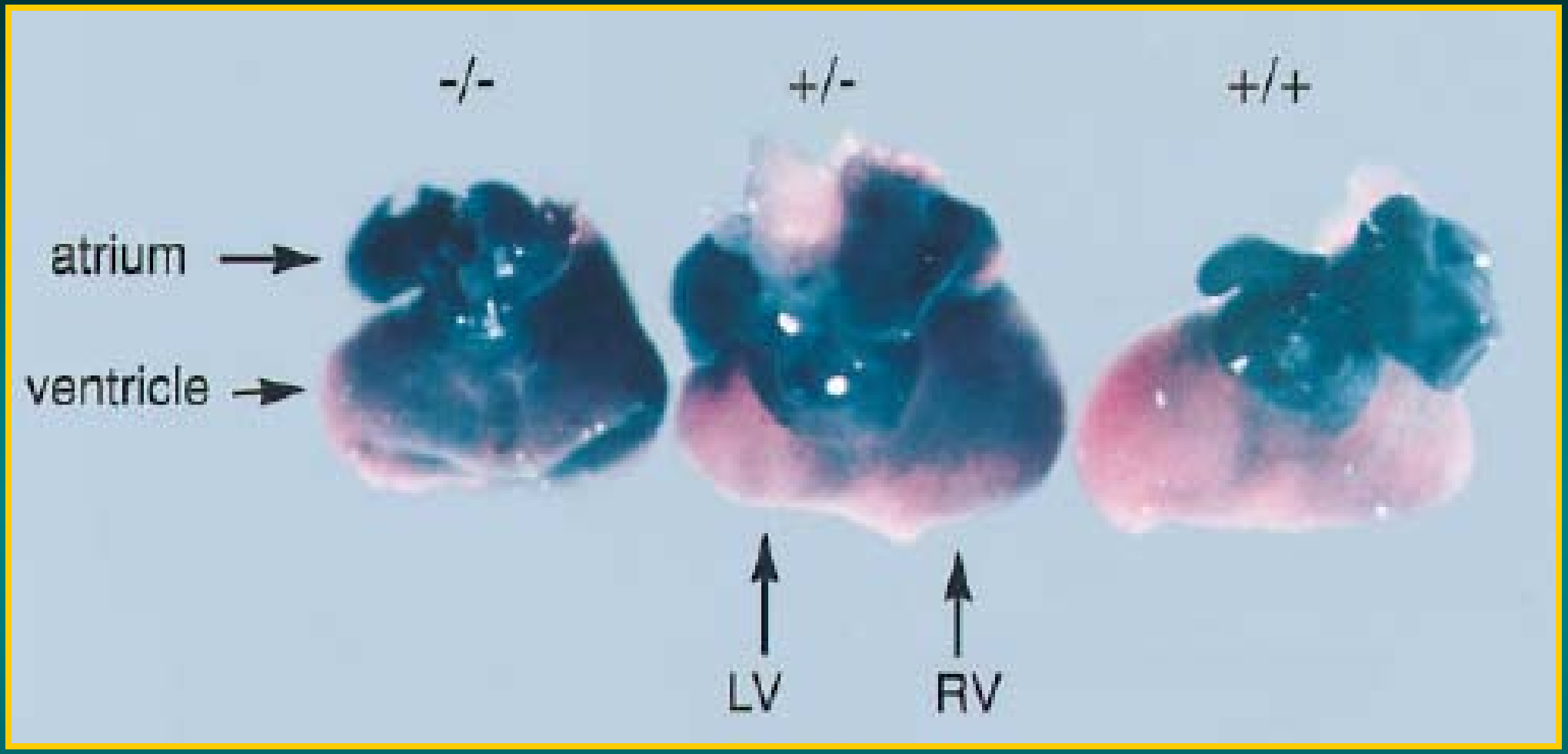
• Mice that are heterozygous for RXRα also display a number of cardiac abnormalities and show multiple defects to virtually every compartment of the developing heart, implying a central role for RXRα in cardiac morphogenesis.

Cardiac muscle defects in RXRα deficient mice:

LV, left ventricle;

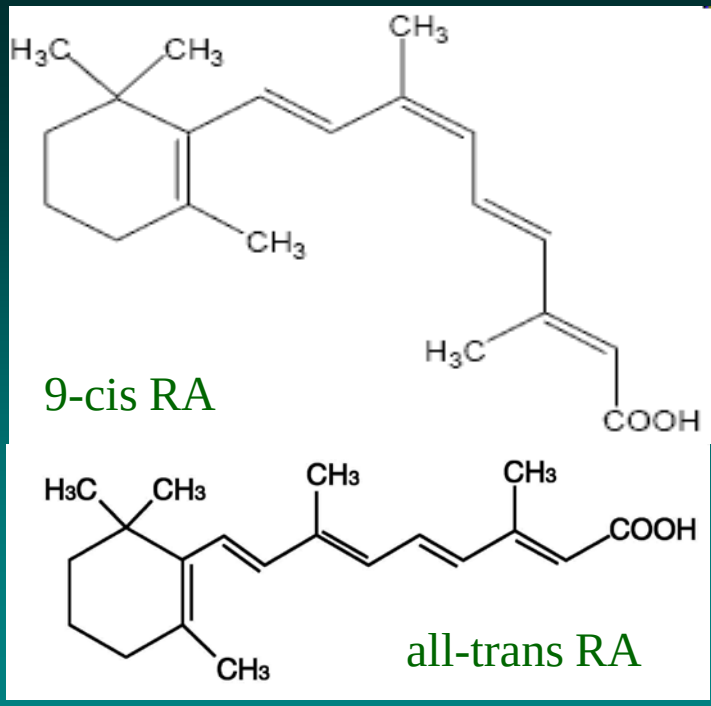
LA, left atrium.





In wild-type animals myosin light chain-2a (MLC-2a) transcript is restricted to the atrium, while the ventricle does not stain. In homozygous mutant embryos, the MLC-2a transcript is inappropriately expressed in the ventricle especially in the left ventricle. Heterozygous animals display an intermediate level of expression of MLC-2a.

ATRA and 9-cis RA in clinical use



- ATRA and 9-cis RA are used in the treatment of cancers.

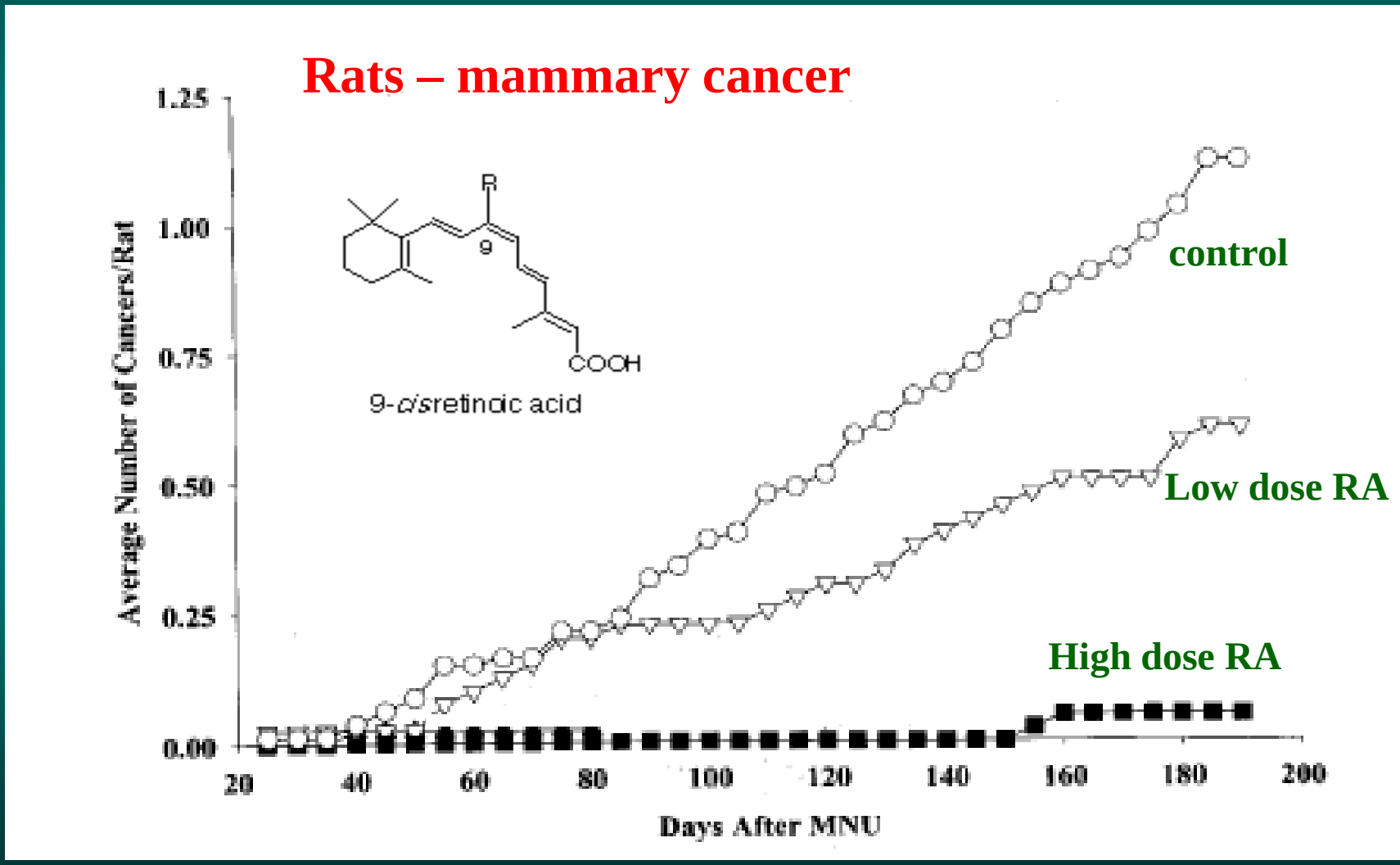
- Preclinical studies suggest potential roles of these agents for
  - * direct induction of differentiation,
  - * growth inhibition without differentiation,
  - * induction of apoptosis.

- Their activity as differentiating agents seems to be the most significant.

- When neoplastic cells are exposed to a differentiating agent, they stop abnormal replication and become mature cell, which are no longer capable of multiple divisions and undergo apoptosis.

- Compared with chemotherapy, differentiation therapy may produce fewer severe side effects. However, when treatment with this therapy is discontinued, the effect on the cancer cell may be lost.

9-cis retinoic acid



What Is Kaposi Sarcoma?

- A sarcoma is a cancer that develops in connective tissues such as cartilage, bone, fat, and muscle.

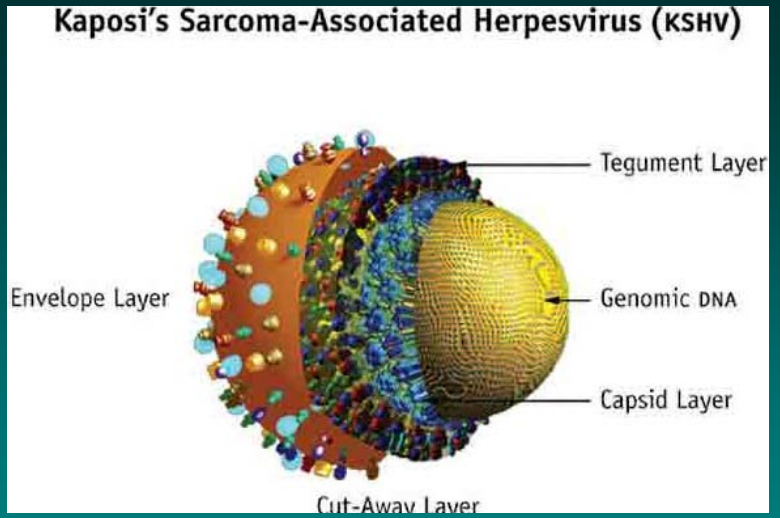
- Kaposi sarcoma (KS) was named for Dr. Moritz Kaposi who first described it in 1872.

- For decades KS was considered a rare disease that mostly affected elderly men of Mediterranean or Jewish heritage, organ transplant patients, or young adult African men.

- In the last 25 years, however, the vast majority of KS cases have developed in association with human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS).

- With the use of new treatments for AIDS the number of KS in the western countries cases due to HIV infection is decreasing.

Causes of Kaposi Sarcoma



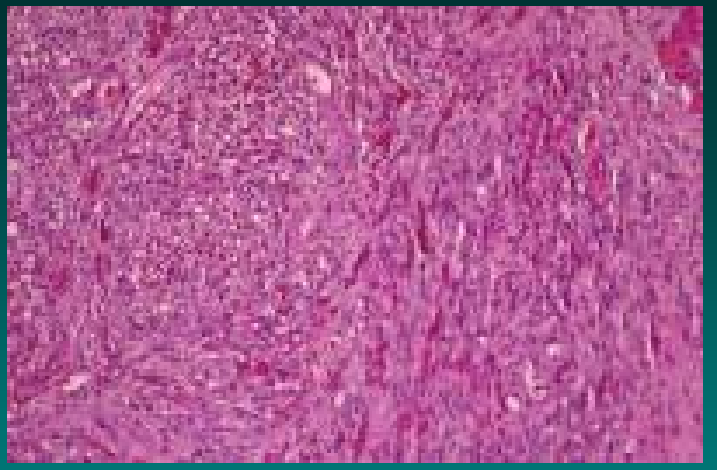
- Kaposi sarcoma is caused by a virus called the Kaposi sarcoma-associated herpesvirus (KSHV) - or human herpesvirus 8 (HHV-8).

- HHV-8 is related to other herpes viruses, such as the viruses that cause cold sores and genital herpes, as well as Epstein-Barr virus and cytomegalovirus (CMV).

- The percent of people in the US infected with this virus is not clearly known. Studies have found infection rates ranging from 3.5% to 25% in reports from different parts of the country. In Africa, this number is over 50% in certain areas.

- Most people infected with this virus do not get KS. A very small number will get the endemic, low-grade KS. But people who are immunosuppressed, such as those with AIDS, develop KS much more readily if they are infected with this virus.

What Is Kaposi Sarcoma?



- Disease typically causes tumors to develop in the tissues below the skin surface, or in the mucous membranes of the mouth, nose, or anus.

- These lesions appear as raised blotches or nodules that may be purple, brown, or red. Sometimes the disease causes painful swelling, especially in the legs, or skin around the eyes.

- Although the skin lesions of KS may be disfiguring, they usually are not life threatening or disabling. In most cases, the lesions cause no symptoms. In some, the lesions may be painful, especially if they cause swelling of nearby unaffected skin.

- If the disease also involves the lungs, liver, gastrointestinal tract, or lymph nodes, other symptoms may develop. KS in the gastrointestinal tract, for example, can produce bleeding, while tumors in the lungs may cause difficulty breathing.

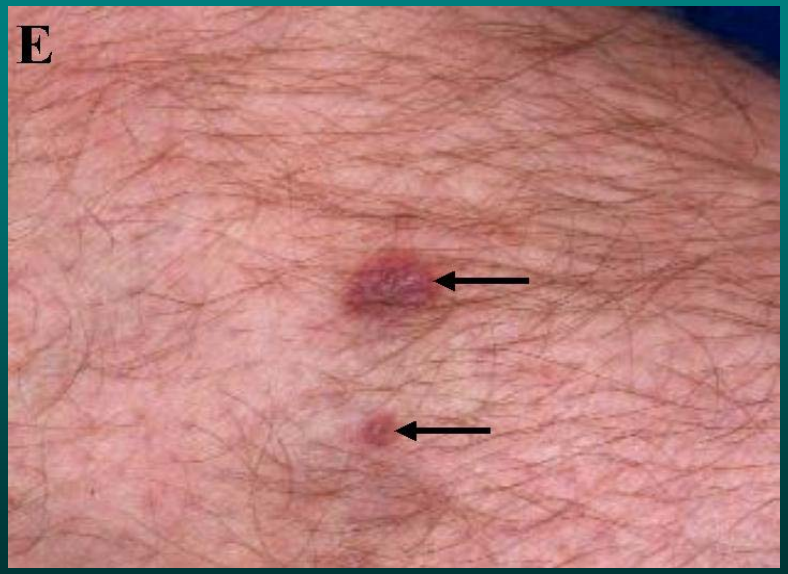
Classic Kaposi Sarcoma

- Classic Kaposi sarcoma usually develops in Jewish men of Eastern European origin or among men of Mediterranean heritage between the ages of 50 and 70.

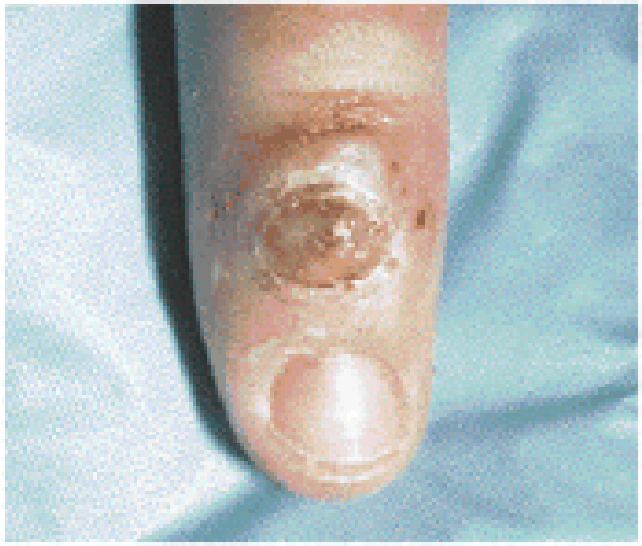
- Classic KS is quite rare, even in these ethnic and age groups. Ten to fifteen men are affected for every woman with classic KS.

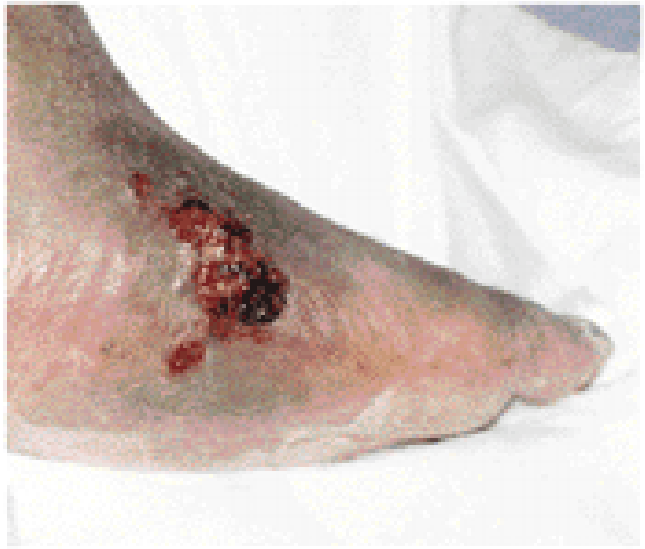
- Patients typically have one or more lesions on the legs, ankles, or the feet. The lesions slowly enlarge, and new lesions may develop over the course of 10 to 15 years.

- Pressure from the lesions can block lymph vessels causing swelling that may be painful. Lesions can also develop in the gastrointestinal tract, lymph nodes, and elsewhere in the body, although they rarely cause symptoms.



Classic Kaposi Sarcoma





African (endemic) Kaposi Sarcoma

- African (or endemic) Kaposi sarcoma is a form of the disease that develops in people living in equatorial Africa.

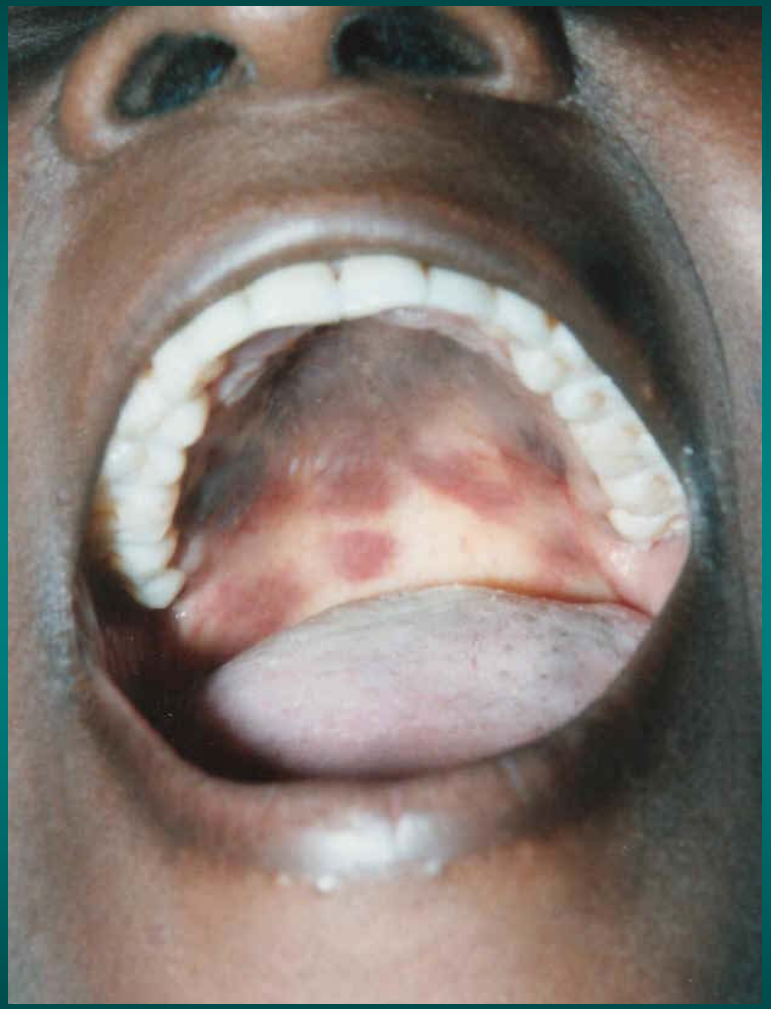
- This disease is fairly common: it accounts for 9% of all the cancers seen among Ugandan men, for example.

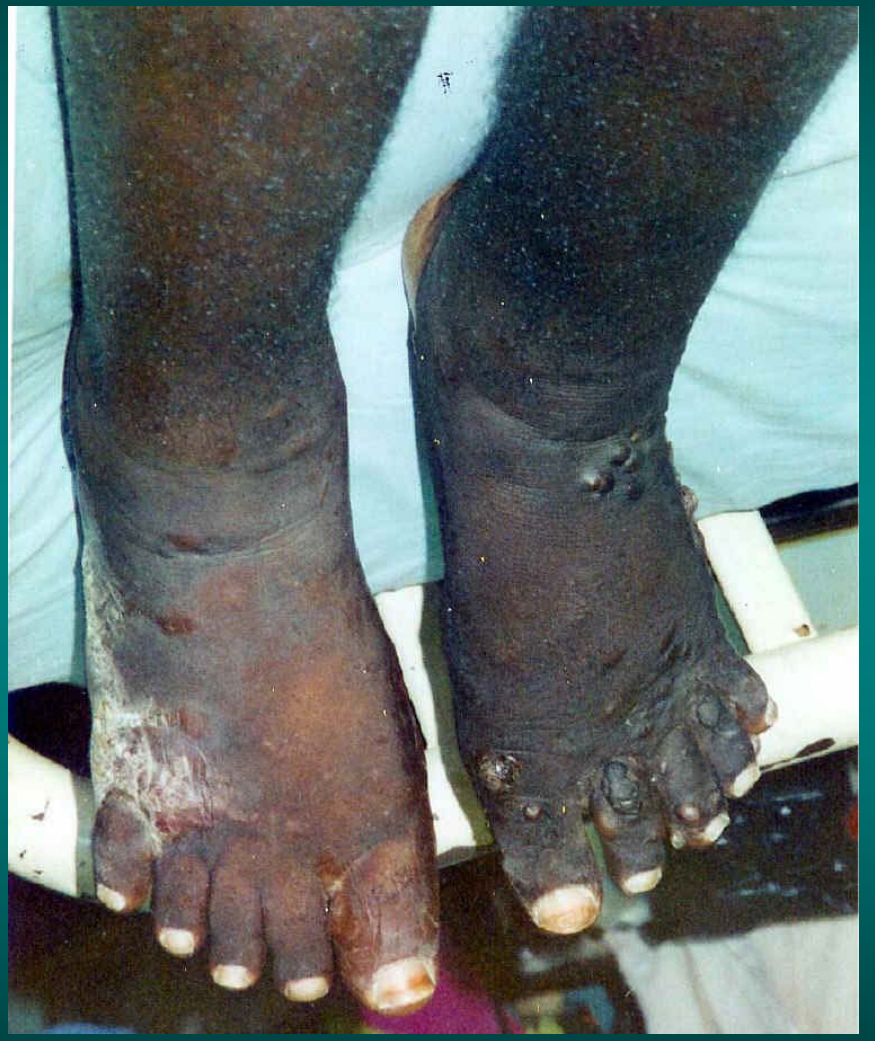
- In many cases, this disease is identical to classic KS, although it usually strikes at a much younger age. It affects many more men than women.

- Typically, African (endemic) KS causes skin lesions that do not produce symptoms and do not spread to other parts of the body. However, more aggressive cases do occur, and some tumors may penetrate from the skin to the underlying bone.

- Another form of the disease strikes children before puberty, affecting 3 times as many boys as girls, and usually involves the lymph nodes and other organs. In most cases, it leads to death within 3 years.

African (endemic) Kaposi Sarcoma



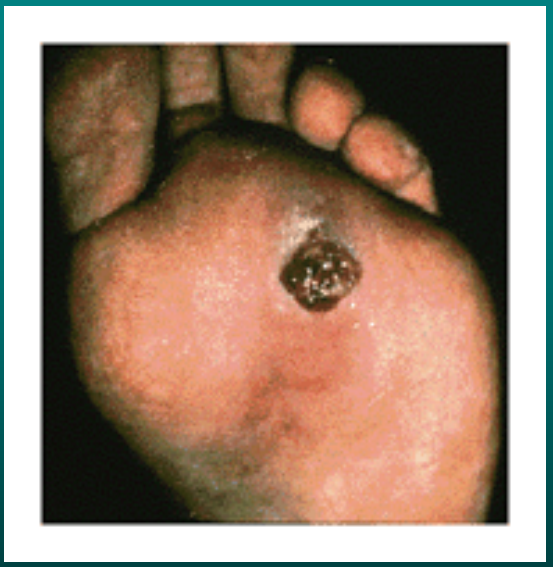


Transplant-related (acquired) Kaposi Sarcoma

- Refers to the form of the disease developed by people whose immune systems have been suppressed following an organ transplant.

- Kaposi sarcoma is 150 to 200 times more likely to develop in transplant patients than among the general population.

- Often, transplant-related KS affects only the skin. In some cases, though, the disease can spread to the mucous membranes or other organs.



AIDS –related (epidemic) Kaposi Sarcoma

- AIDS-related (or epidemic) Kaposi sarcoma arises in people who are infected with HIV. It was in part the unusual and sudden appearance of this form of KS in so many young men at the start of the AIDS epidemic that led scientist to realize that a new disease had emerged.

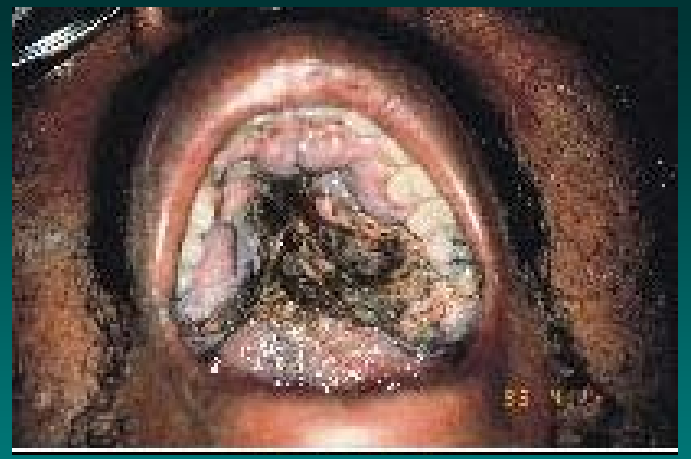
- In most cases, epidemic KS causes widespread lesions that erupt at many places on the body soon after AIDS develops.

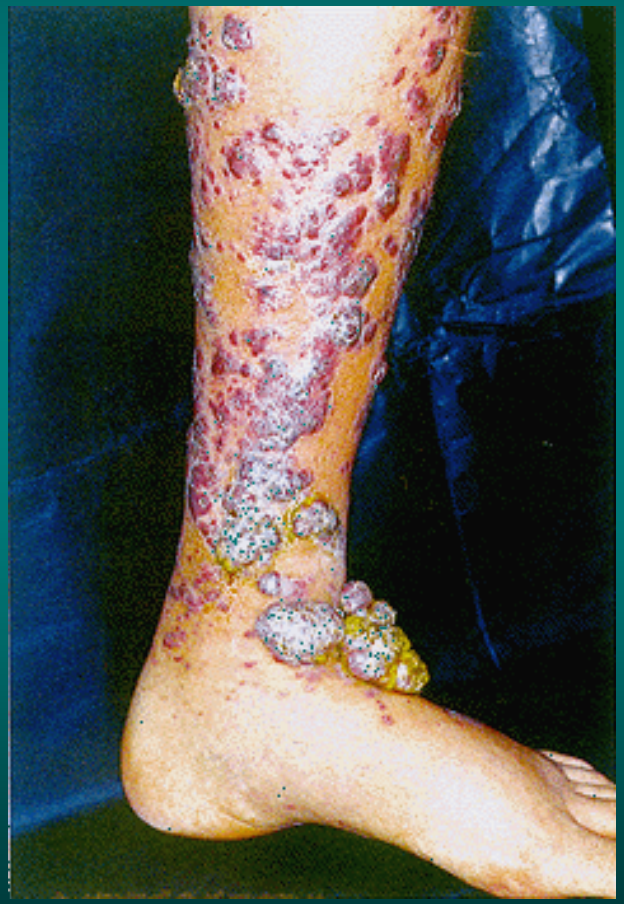
- Lesions of epidemic KS may arise on the skin and the mouth and may affect the lymph nodes and other organs, usually the gastrointestinal tract, lung, liver, and spleen.

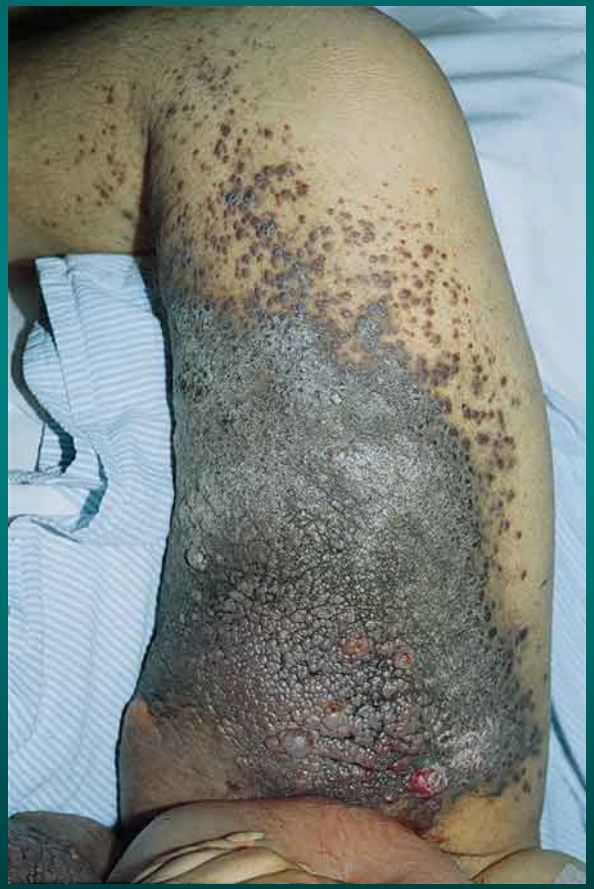
- At the time of diagnosis, some people with epidemic KS experience no symptoms, especially if their only lesions develop on the skin. However, many -- even those with no skin lesions - will have swollen lymph nodes, unexplained fever, or weight loss.

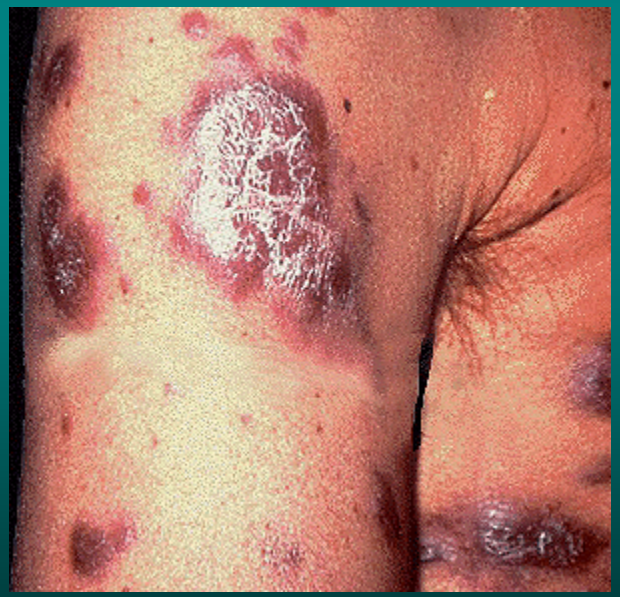
- Eventually, in almost all cases, epidemic KS spreads throughout the body. Extensive lung involvement by KS can be fatal. More often, however, patients die of other AIDS-related complications such as infections.

AIDS –related (epidemic) Kaposi Sarcoma









AIDS –related (epidemic) Kaposi Sarcoma

- Treatment for AIDS-related KS often significantly relieves the pain and discomfort that accompany the lesions. However, it is important to be aware that treatment will not produce a cure, and there is little evidence showing that treatment for AIDS-related KS prolongs life.

- Local lesions sometimes improve with injections of vinblastine, a chemotherapy drug, directly into the KS lesion. This method, known as intralesional chemotherapy, is an especially good choice for lesions that develop in the mouth. Because the drug is injected into the lesion instead of into a vein, it does not spread throughout the body and does not cause side effects in organs and tissues such as the bone marrow or digestive system.

- In many cases, local KS tumors of the skin, mouth, or anus improve significantly when treated with low-dose external radiation therapy.

- Patients with about 25 or fewer small skin or mouth lesions may be treated with local therapies such as a cream containing 9-cis-retinoic acid.

9-cis retinoic acid and Kaposi sarcoma

	Study 1 (TID, QID)[1]		Study 2 (BD)[2]	
	Panretin N= 134	Vehicle N=134	Panretin N=62	Vehicle N=72
Clinical Complete Response (CCR) %	0.7	0.0	1.6	0.0
Partial Response (PR) %	34.3	17.9	35.5	6.9
Stable Disease %	50.0	59.0	43.5	58.3
Progressive Disease %	14.9	23.1	19.4	34.7
Overall Response %	35.1	17.9 p=0.002	37.1	6.9 p= 0.00003

1. Protocol-specified dose regimen was application three times a day (TID) escalating to four times a day (QID) after two weeks, with downward adjustments for toxicity
2. Protocol-specified dose regimen was application twice a day (BD) only, with downward adjustments for toxicity

• III phase clinical trial confirmed usefulness of 9-cis RA gel (alitretinoin, Panretin) in apical treatment of skin lessions.

Arch Dermatol 2003;139:178-186; EMEA 2007

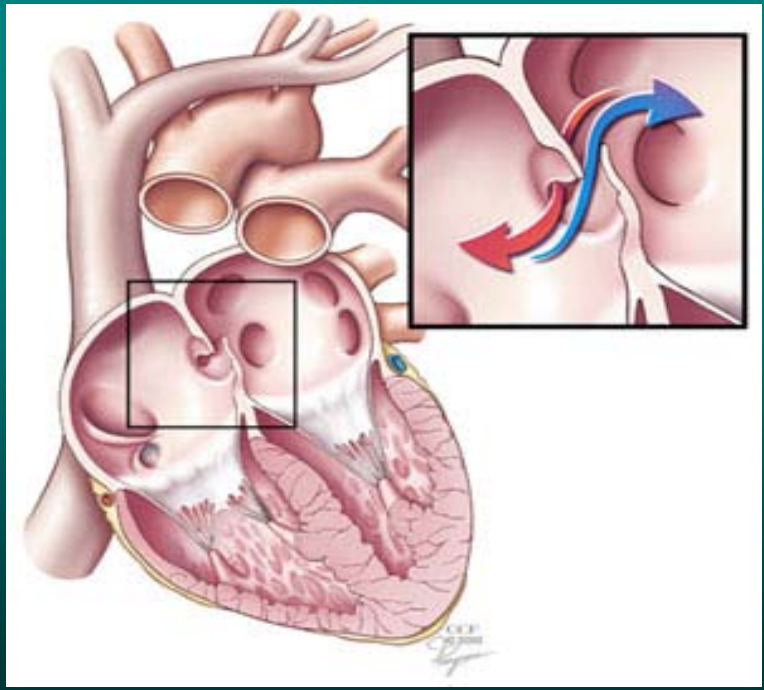
Thank you and see you next week...

What would be profitable to remember in June:

- Major coactivators and corepressors of nuclear receptors

- RXRα: general characteristic and effect on development of the heart

- Kaposi sarcoma and retinoids.



Slides can be found in the library and at the Heme Oxygenase Fan Club page:

https://biotka.mol.uj.edu.pl/~hemeoxygenase