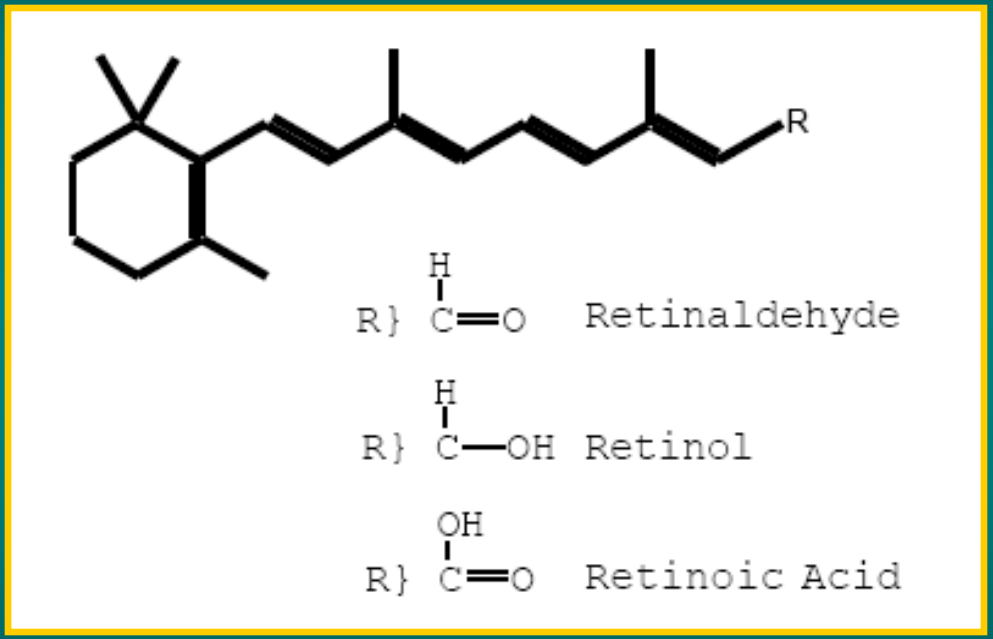

RAR
retinoic acid
receptor

Retinoids

• Retinoids comprise a family of polyisoprenoid lipids which include retinol (Vitamin A), retinaldehyde (retinal) and retinoic acids (all transRA, 13-cisRA, 9-cisRA).

• Retinoids are indispensable for:

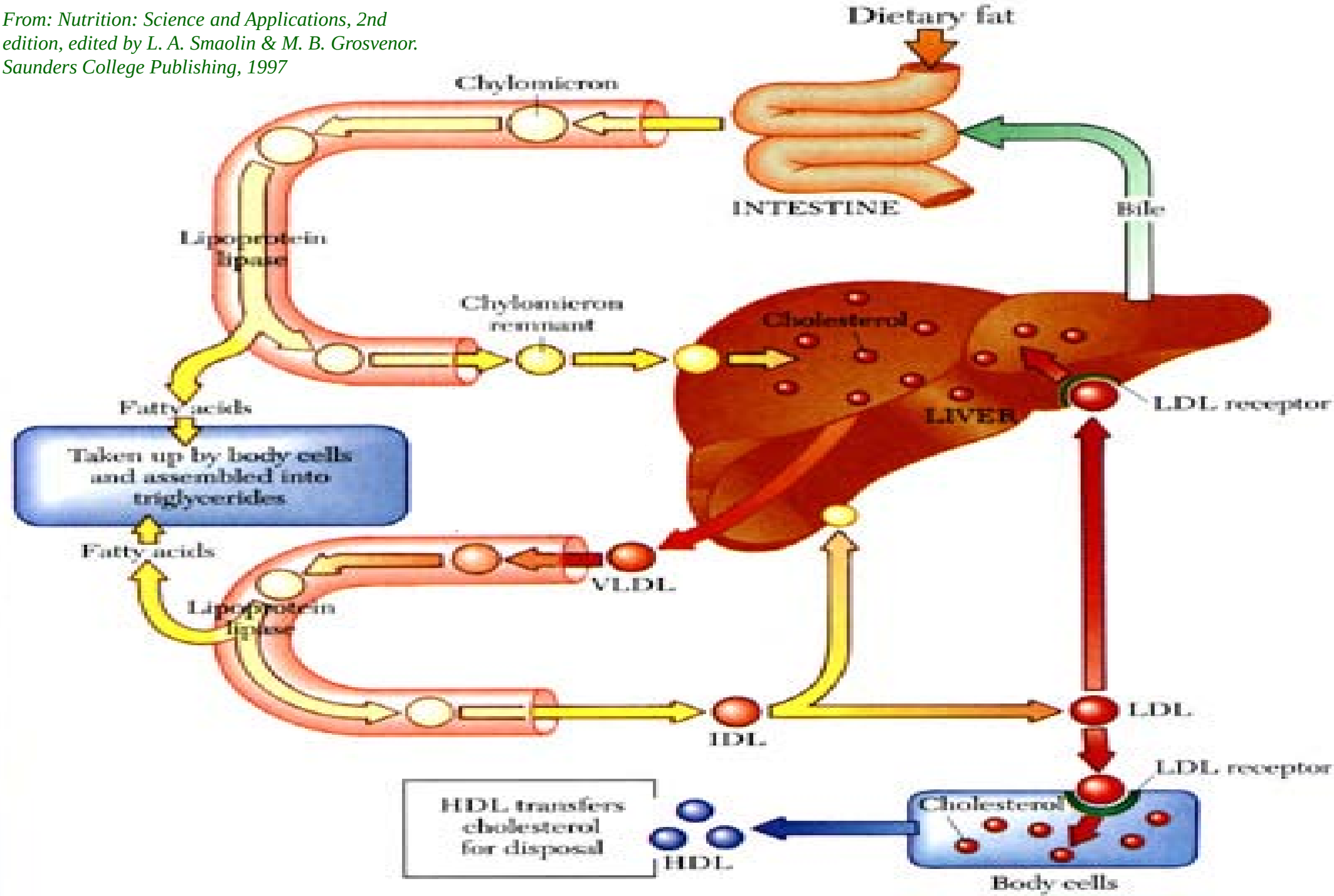
* cell differentiation (especially promielocytes and epithelial tissues)
* embryonic pattern formation,
* visual function,



• Vitamin A deficiency is ranked along with protein caloric malnutrition and iron deficiency anaemia as one of the three top priority nutritional diseases.

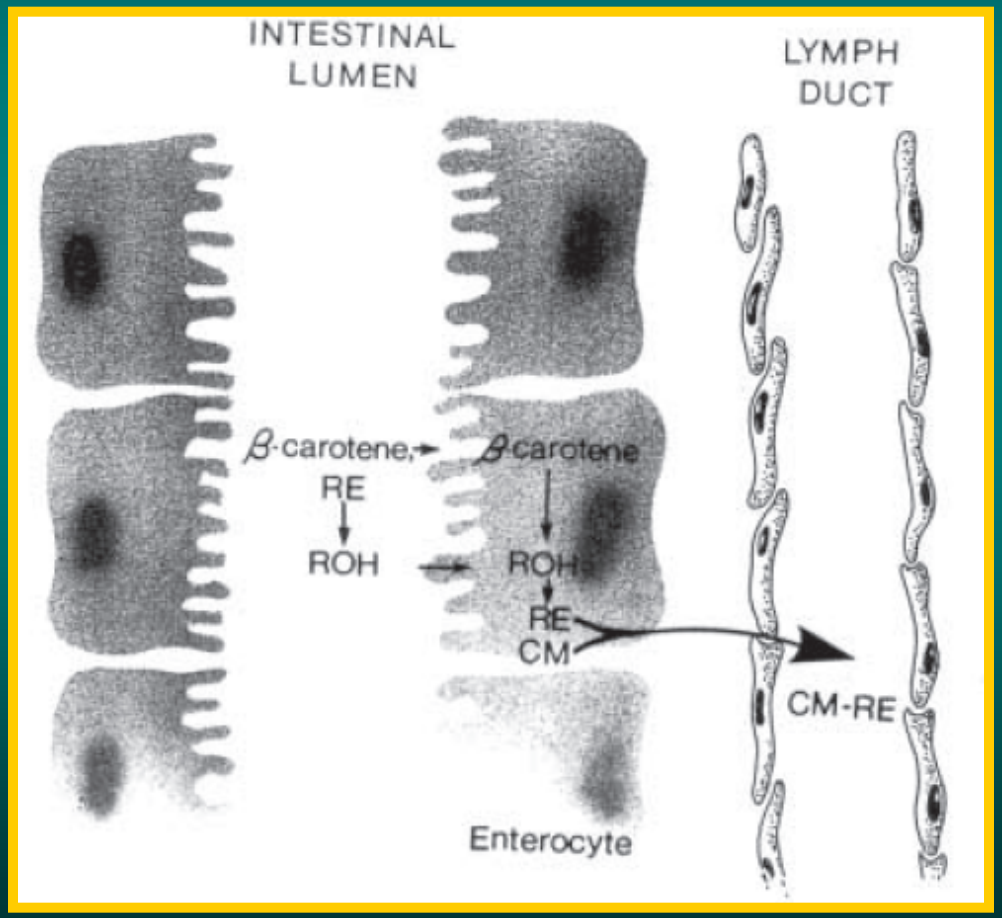
• Vitamin A deficiency is probably the foremost cause of preventable blindness in the world.

From: Nutrition: Science and Applications, 2nd edition, edited by L. A. Smaolin & M. B. Grosvenor. Saunders College Publishing, 1997



• **Intestinal lumen:** dietary retinyl esters are converted to retinol by pancreatic enzymes.

• **Small Intestinal Absorptive Cell:** retinol diffuses across the luminal membrane and binds CRBP for transport to the serosal surface. Then it is re-esterified, incorporated into chylomicrons and transporeted with lymph to the liver.



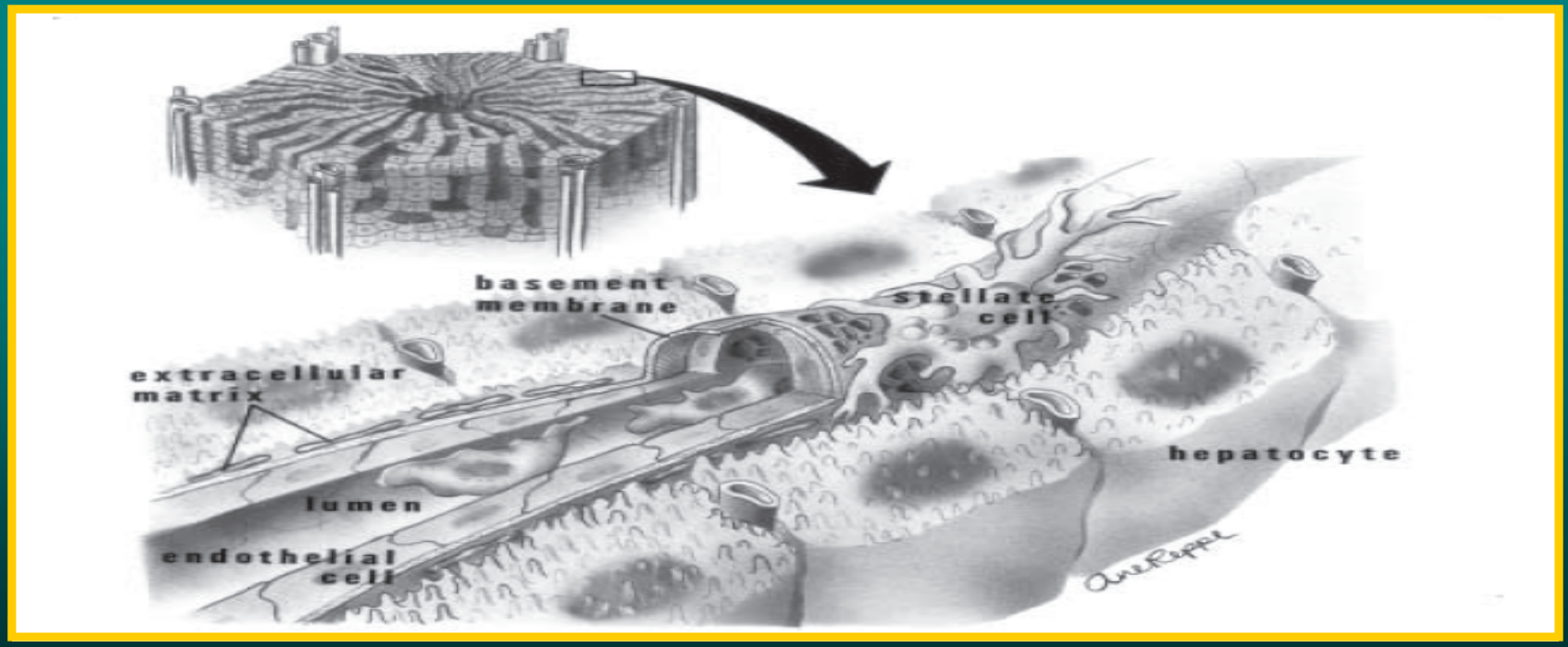
(Blomhoff, 1994).

Absorption of preformed vitamin A and provitamin A from the small intestine.

RE, retinyl ester; ROH, retinol; CM, chylomicron

• **Hepatocyte:** retinol is delivered via endocytosis of chylomicrons. Here it is stored as retinyl ester or de-estrified for release of retinol back into blood when free RBP exists. Retinoids readily accumulate in the liver, which is the major storage organ. Liver also synthesizes most of the circulatory RBP.

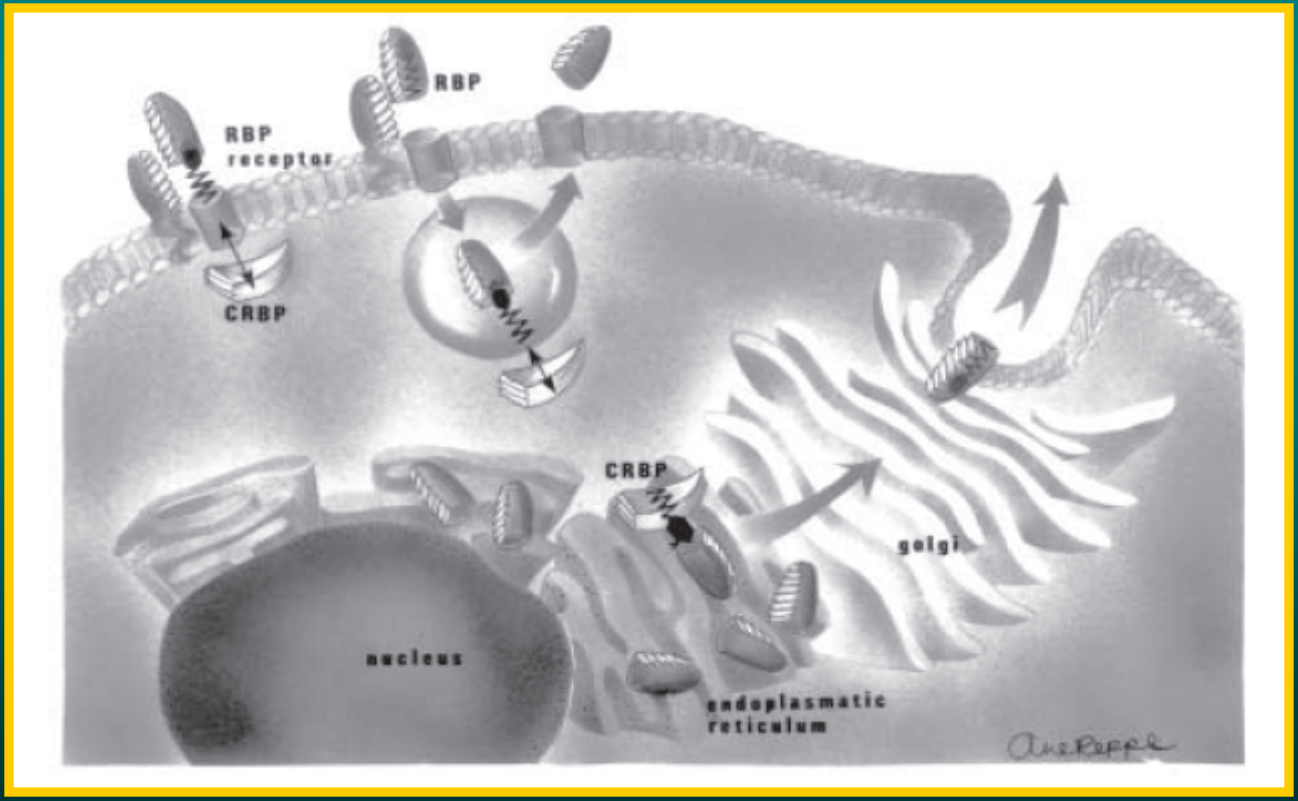
•**Blood vessels:** retinol in the blood binds retinoid-binding proteins (RBP). There are also cellular retinoid-binding proteins (CRBP). When RBP is deficient, or Vitamin A is in excess, retinyl esters bind plasma lipoproteins forming surfactants which can damage cell membranes. Retinoic acid does not bind RBP but does bind albumin.



(Blomhoff, 1994).

• **Target epithelial/mesenchymal cells:** Retinol enters the cell and binds to CRBP. In the cytoplasm or microsomes it is oxidized to retinaldehyde and then to retinoic acids (RA) for binding to nuclear receptors (RAR and RXRs).

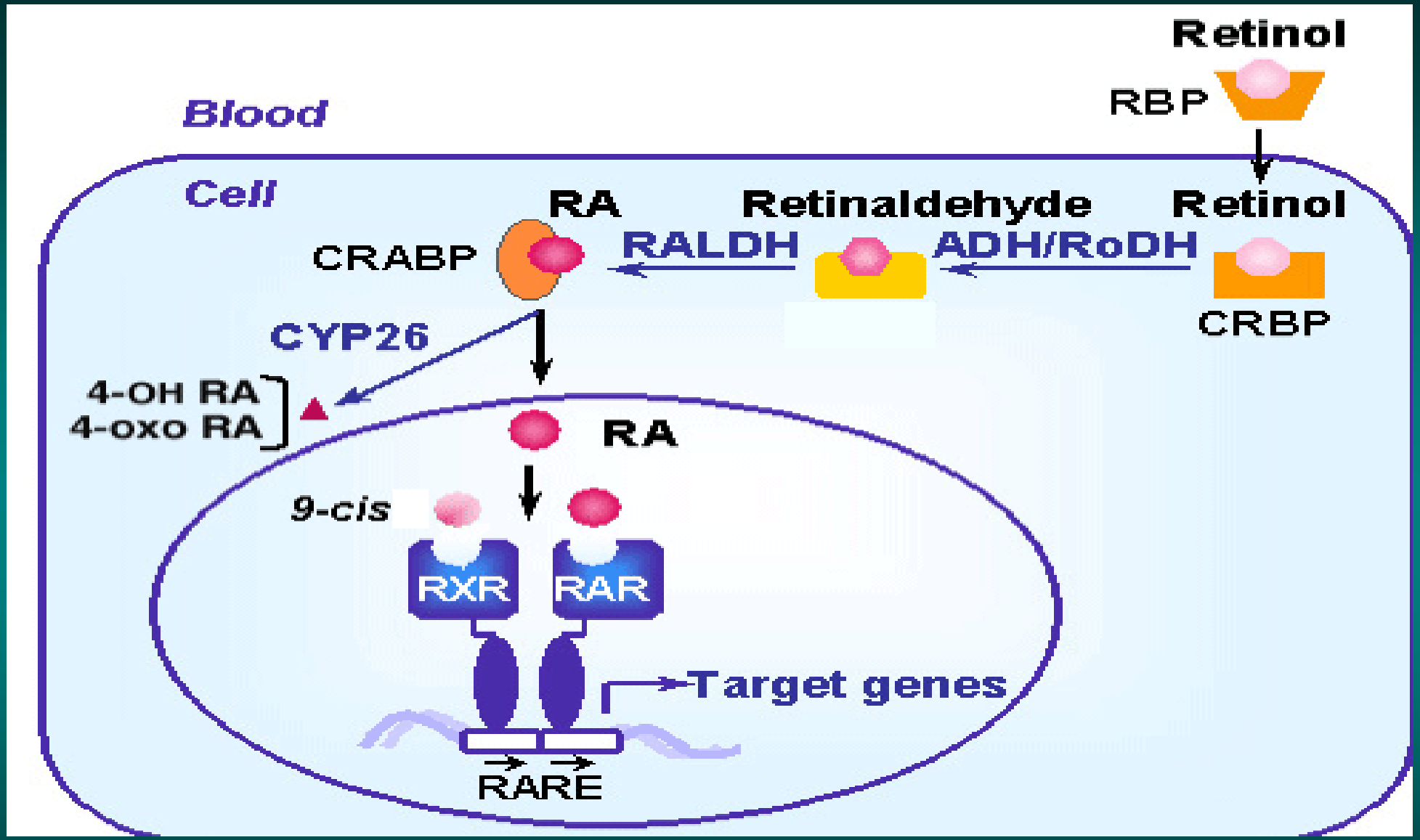
• Each cell produces its own pool of retinoids that remain intracellular to function as mediators rather than as hormones circulating in the blood stream. Intracellular isomerases may further convert ATRA to 9-cisRA, 11-cisRA, or 13-cisRA.



• RBP-retinol may be recognized by a cell surface receptor.

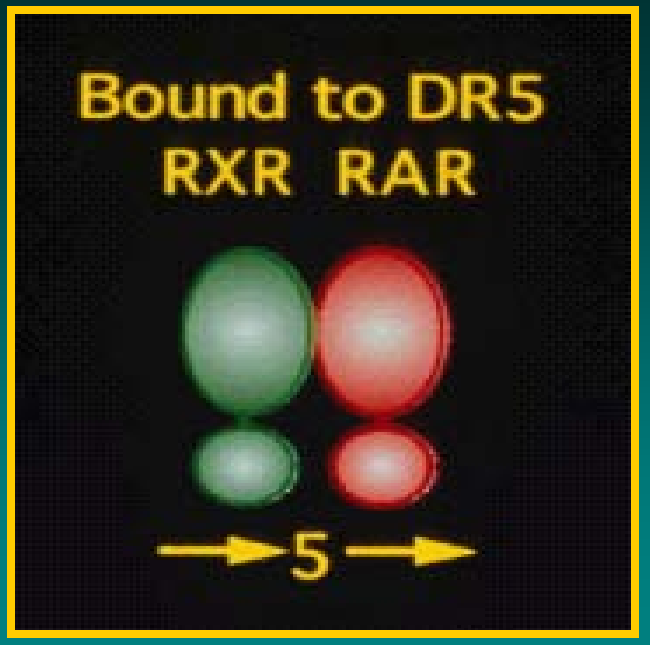
• Retinol may be transferred to CRBPs either at the cell surface or after internalization into endosomes.

(Blomhoff, 1994).



RBP – retinol binding protein; CRBP – cellular retinol binding protein; ADH– alcohol dehydrogenase; RoDH - retinol dehydrogenase; RALDH - retinal dehydrogenase ; CRABP – cellular retinoic acid binding protein; Cyp26 – hydroxylase of P450 cytochrome family.

RARα–RXRα


Bound to DR5
RXR RAR
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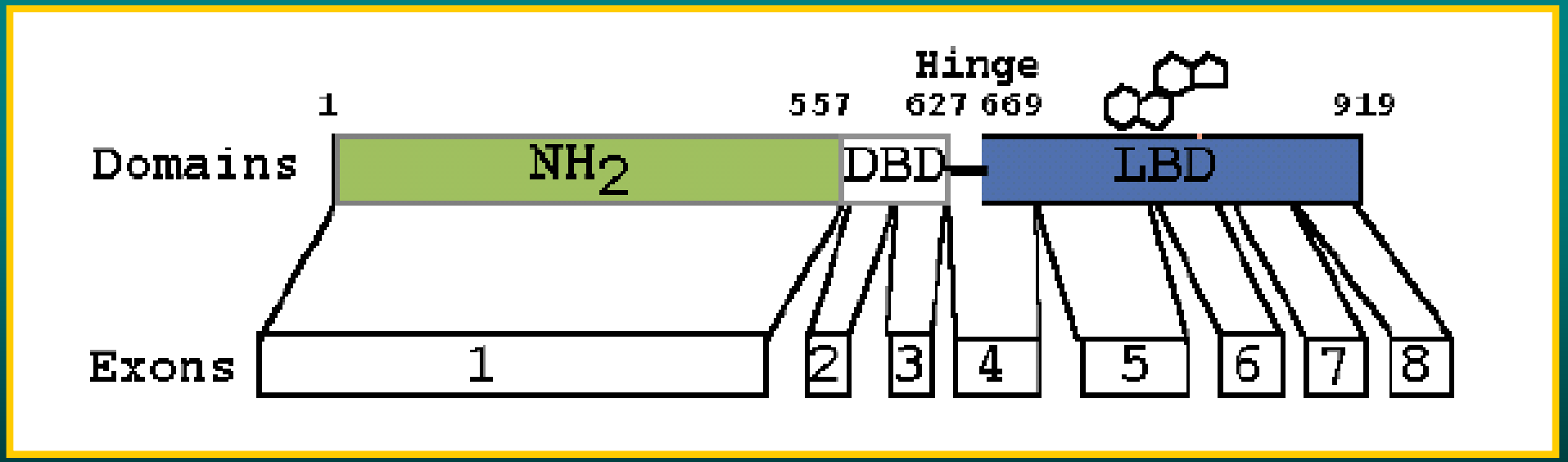
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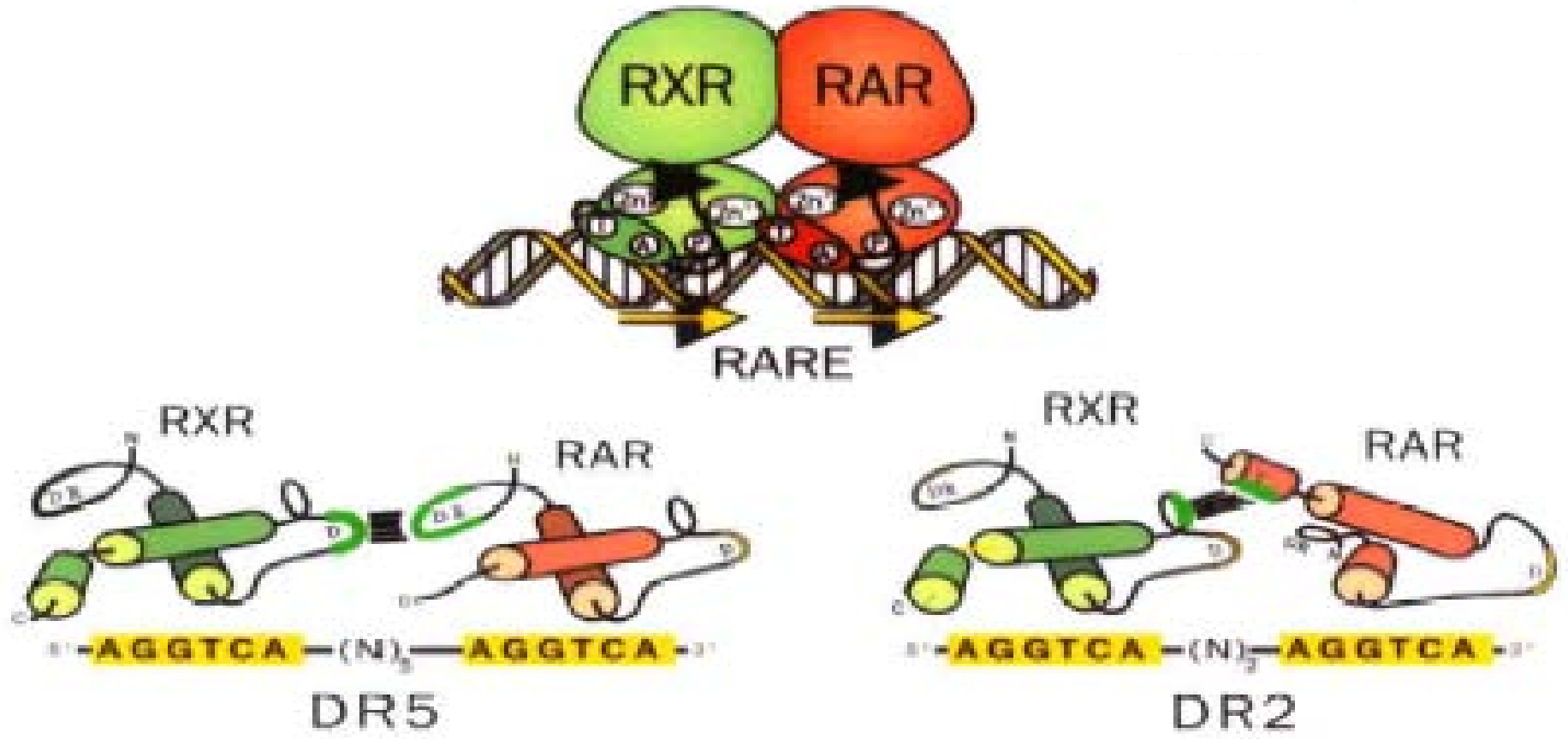
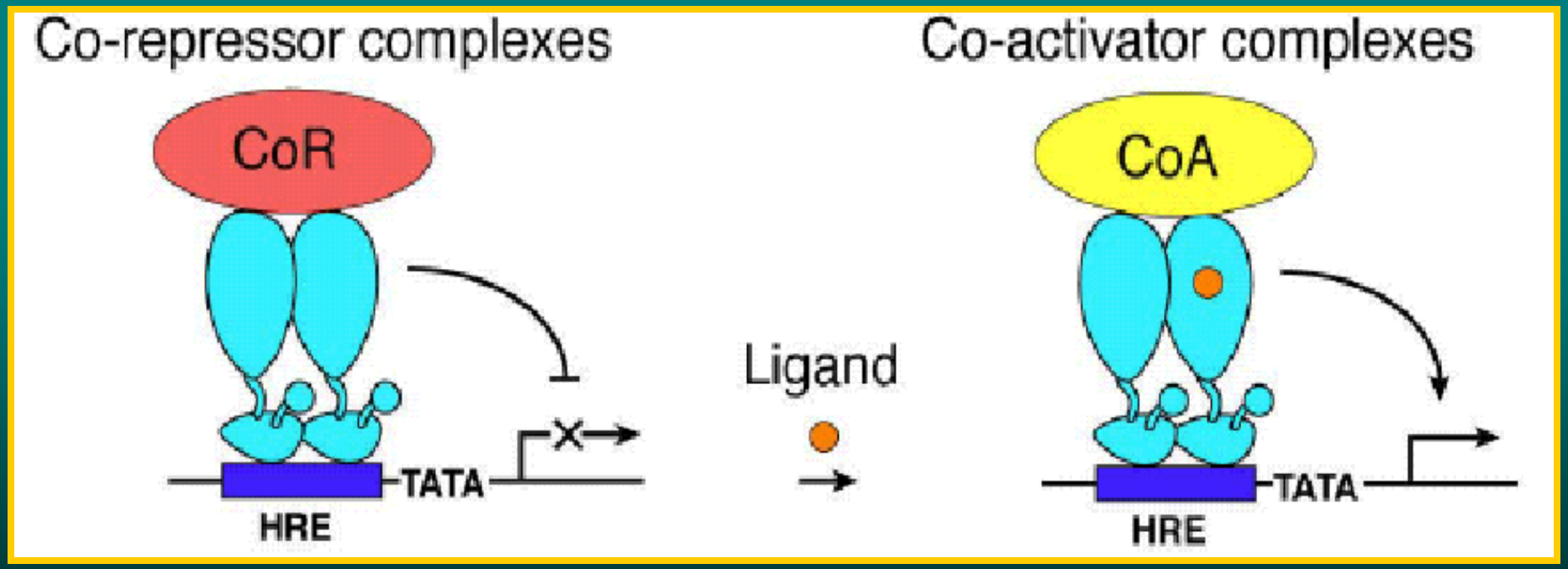
Structural features of RXR:RAR heterodimers

- In RXR:RAR heterodimers RXR is the 5'-bound receptor.
- DBDs of heterodimeric partner may bind to DR5 or (rarely) to DR2 RAREs.



RAR-RXR heterodimer

- RAR-RXR bound to a DR5 responsive element,
- corepressor masks the transcriptional activation function of the heterodimer
- binding of ligand to the RAR moiety then evokes a conformational change that releases the corepressor and allows binding of the coactivator, which then results in transcriptional activation.

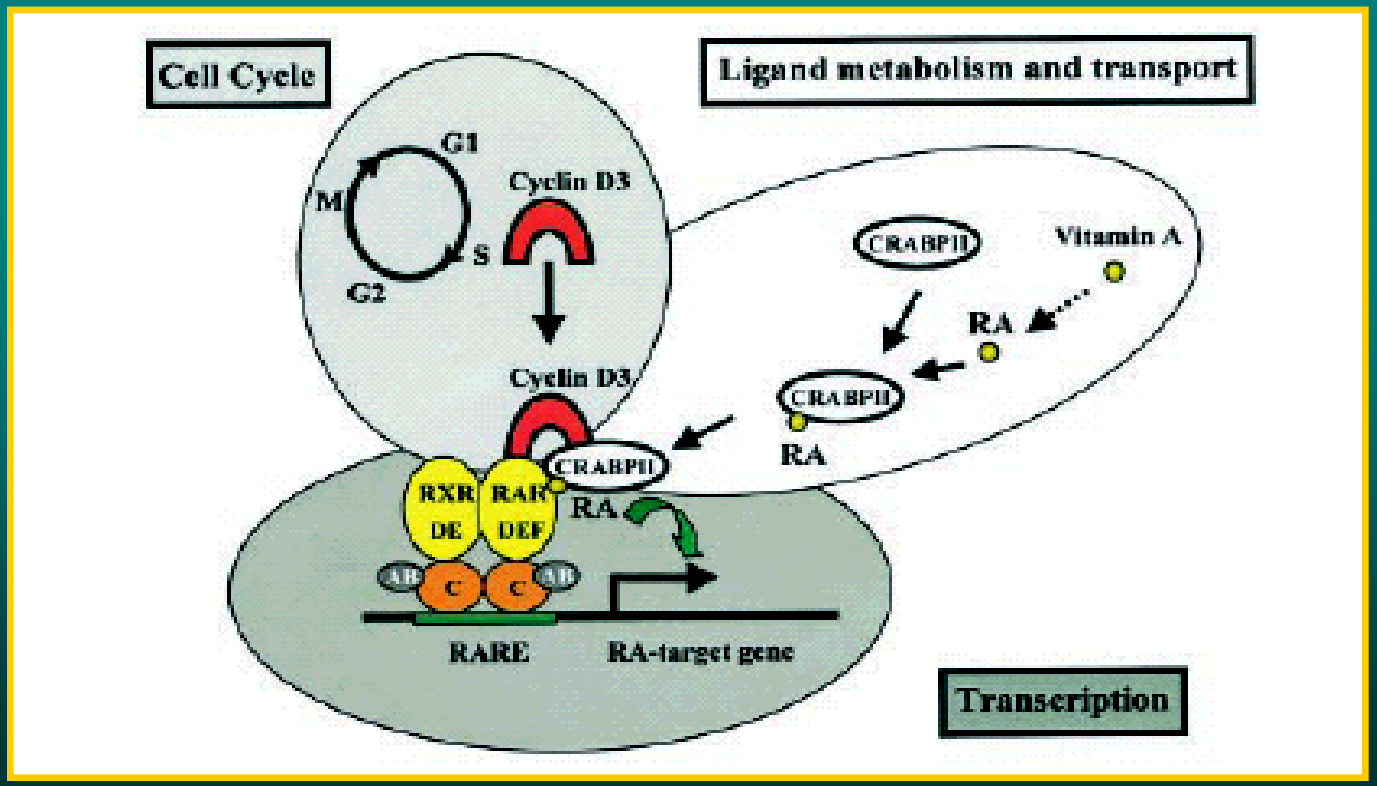


• RAR and RXR apart from cooperation with typical coactivators use also as a coactivator the CRABPII (cellular retinoic acid binding protein II).

• CRABPII forms a complex with RAR-RXR heterodimer and increases its transcriptional activity through:

* release of retinoic acids and its transfer to the receptor
* increased stability of interaction of RAR-RXR to the promoter of target gene

• CRBPII may bind cyclin D3, which additionally stabilizes the complex.



RXR, RAR, ROR and their ligands

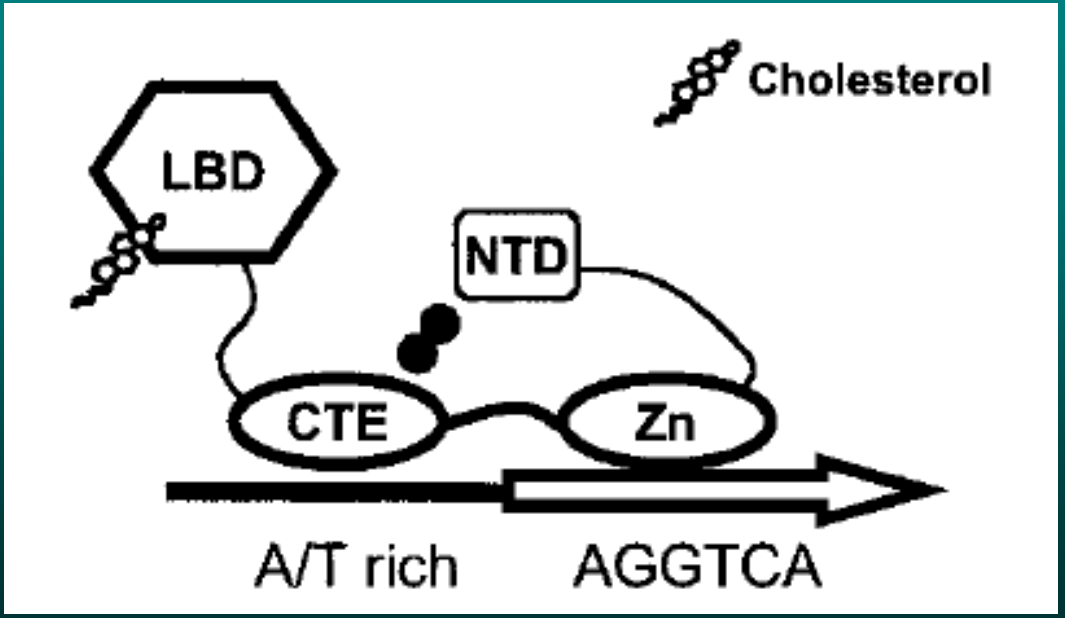
- **Retinoic acid receptors and retinoid receptor related receptors:**

*** RAR (RARα, RARβ, RARγ) – all-trans retinoic acid (ATRA), 13-cis RA**
(retinoic acid receptor)

*** RXR (RXRα, RXRβ, RXRγ) - 9-cis RA, high doses of ATRA**
(retinoic X receptor)

*** ROR (RORα, RORβ, RORγ) – cholesterol**
(retinoic acid receptor-related orphan receptor)

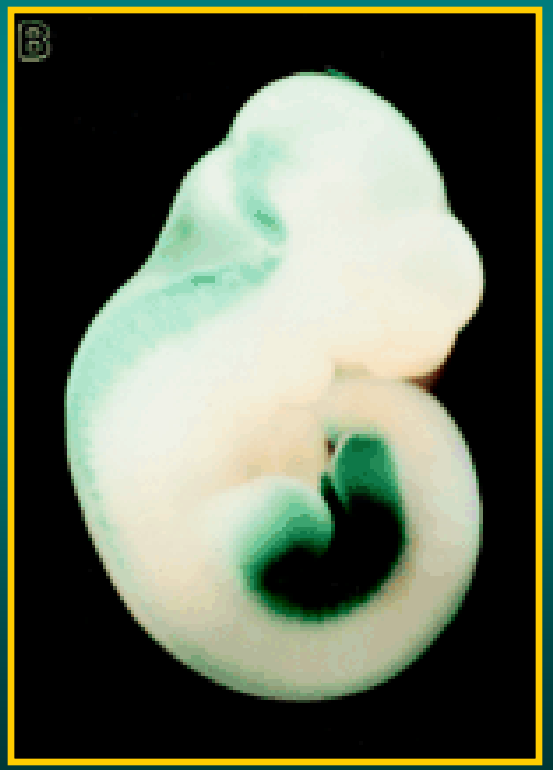
RORα

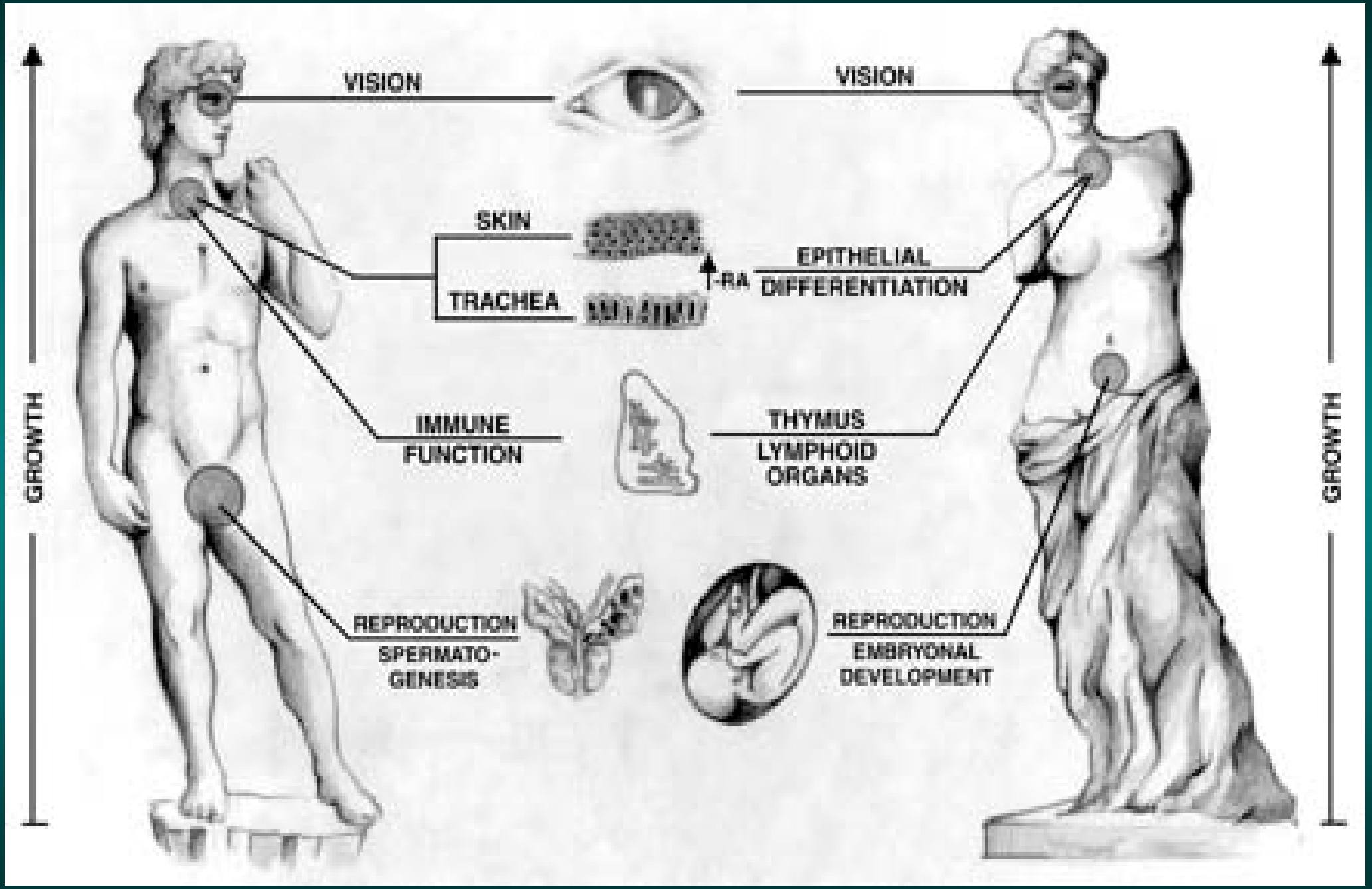


Expression of RAR isoforms in rodent embryo

- RARα transcripts are widely distributed with the highest expression in skeletal muscle, pituitary gland and various epithelia.
- RARβ is expressed in foregut endoderm, olfactory and periocular mesenchyme, urogenital region, proximal limb mesenchyme and within interdigital regions, heart outflow tract mesenchyme, intervertebral disks, and umbilical vessel walls.
- RARγ is expressed at high levels in the nervous system.

- No close relationship exists between the expression patterns of RARs and RXRs in rodent embryos:
  - * RXRα is expressed abundantly in liver, kidney, spleen, visceral tissues and skin.
  - * RXRβ mainly in the central nervous system,
  - * RXRγ is found in the peripheral nervous system and in muscle.



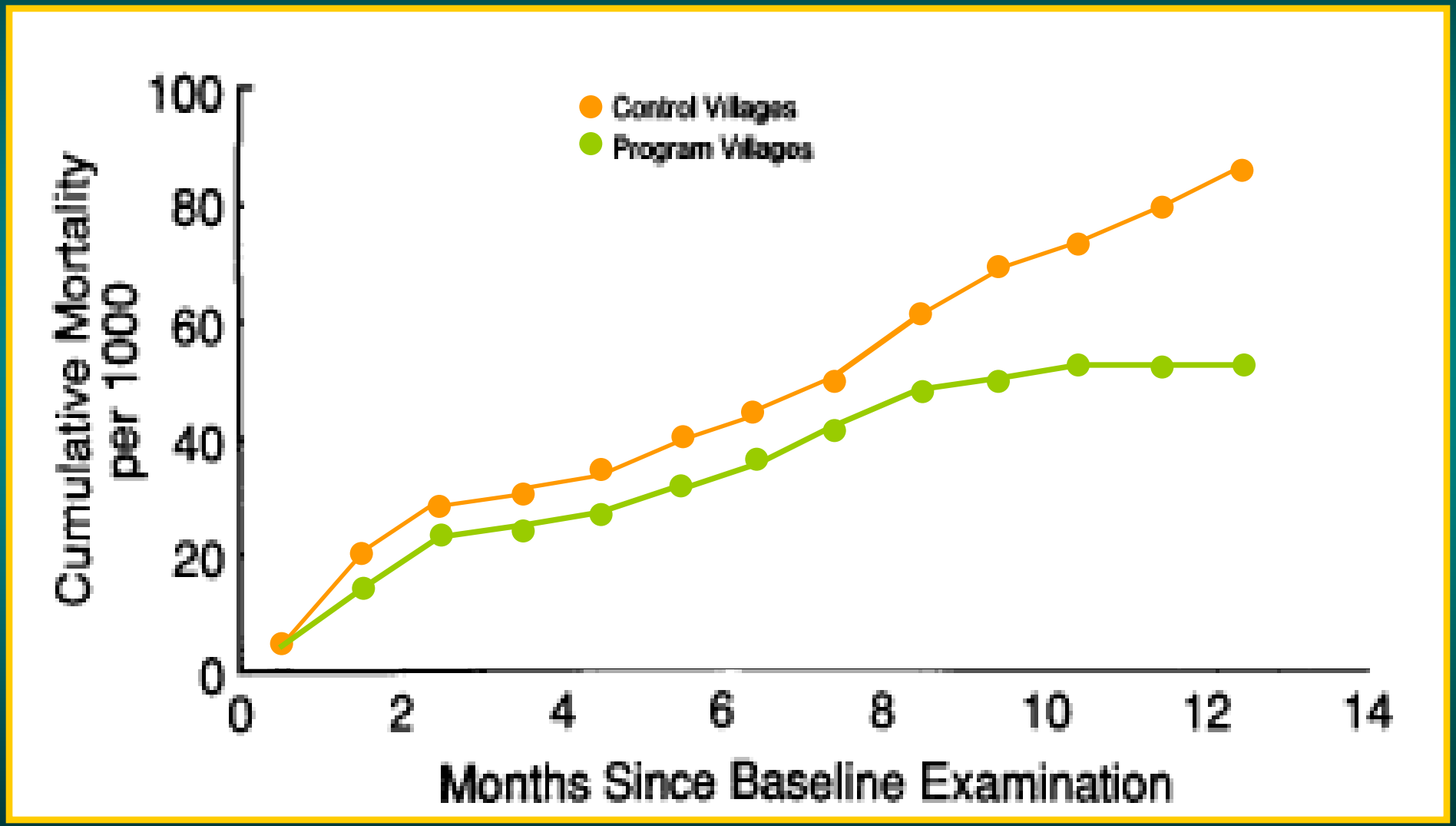


Ross et al. 2000

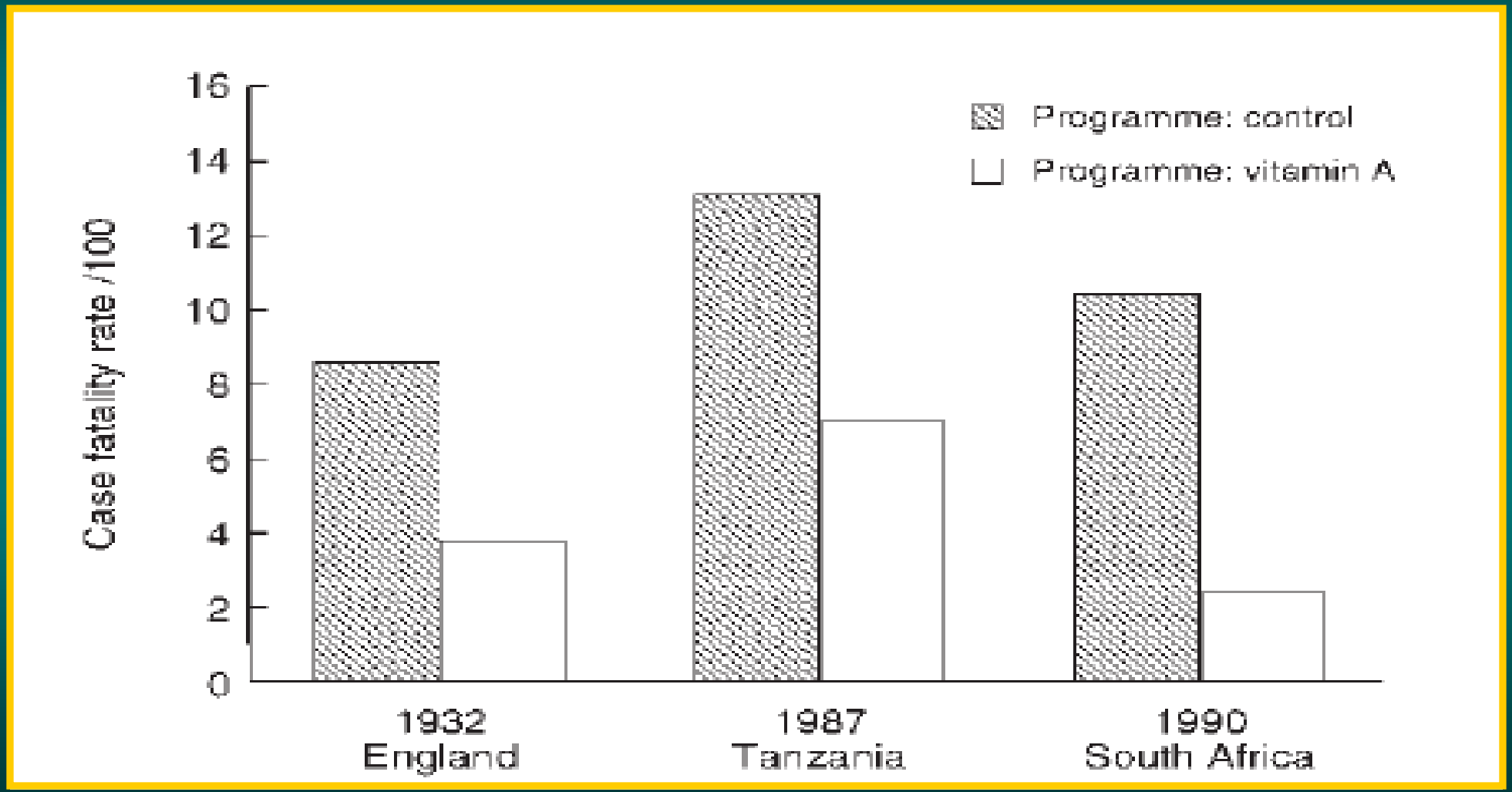
Major Community Mortality Prevention Trials

Study	*Country*	*Vitamin A Supplement*	*Reported Mortality Reduction*[a]
Aceh	Indonesia	Large dose every 6 mo	34%[b]
Bogor	Indonesia	Vitamin A	45%
NNIPS	Nepal	Large-dose every 4 mo	30%
Jumla	Nepal	One large dose follow-up at 5 mo	29%
Tamil Nadu	India	Weekly	54%
Hyderabad	India	Large dose every 6 mo	6%
Khartoum	Sudan	Large dose every 6 mo	6%
VAST	Ghana	Large dose every 4 mo	19%

Cumulative mortality of preschool children (Indonesia). Vitamin A capsules were distributed by local government twice, with interval of 6-8 months.



Measles case-fatality rates among hospitalized patients randomized to receive high-dose vitamin A (cod liver oil in the London trial) compared with those of their controls. Vitamin A supplementation reduced mortality by ~50% in all three trials.



(Sommer, West, 1996).

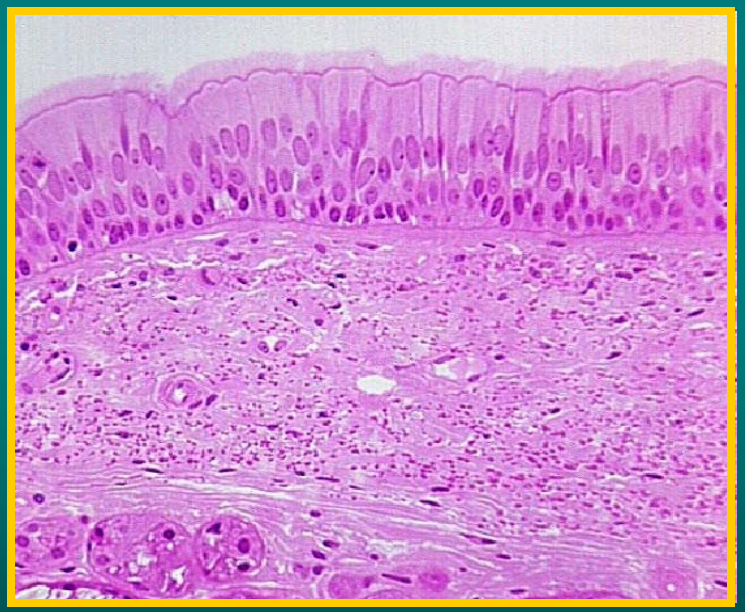
Role of Vitamin A

Epithelial cell integrity:

- Many epithelial cells appear to require vitamin A for proper differentiation and maintenance.

- Lack of vitamin A leads to dysfunction of many epithelia - the skin becomes keratinized and scaly (hyperkeratosis), and mucus secretion is suppressed (hyperplastic and metaplastic changes in the epithelia of mucous membranes).

- It seems likely that many of these effects are due to impaired transcriptional regulation caused by deficits in retinoic acid signaling.



Role of Vitamin A

Hyperkeratosis:

- Is a disease of abnormal keratinization that appears in childhood.
- The skin lesion presents with grouped follicular papules occluded by a projecting keratinous spine. The commonest sites involved are the extensor surface of the extremities, elbows, knees, and abdomen.
- Possible causes are defects in retinoic acid signaling.

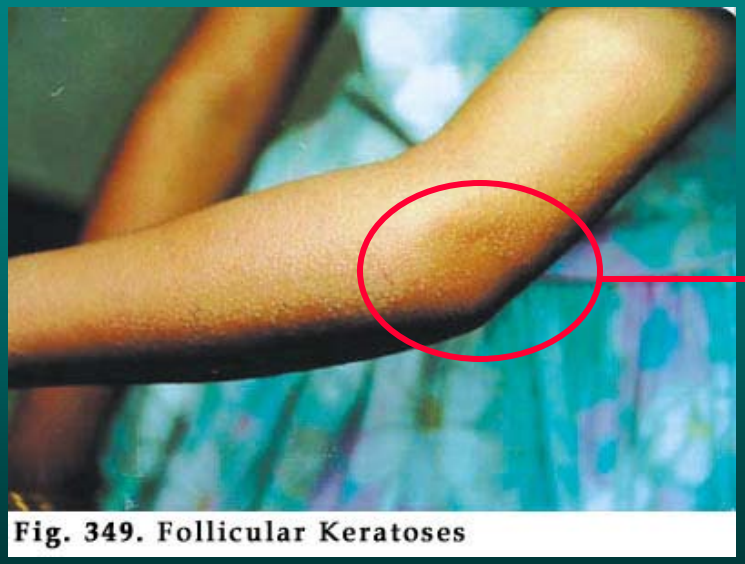
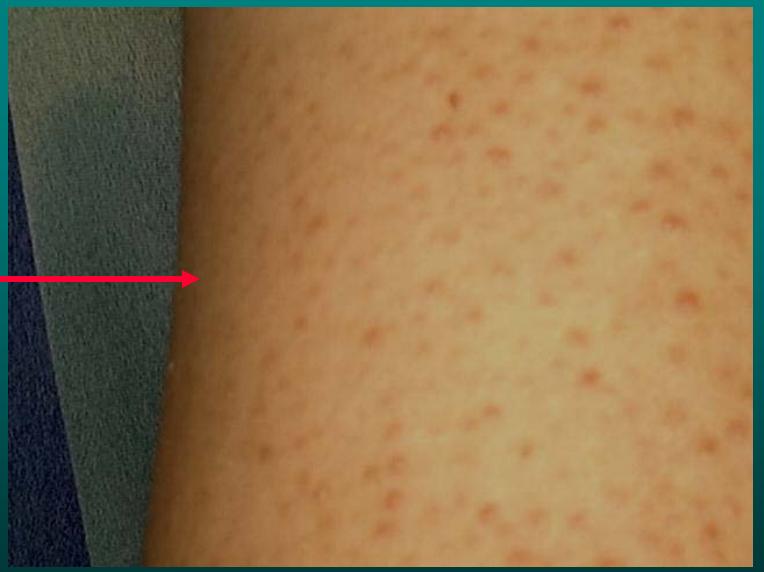


Fig. 349. Follicular Keratoses



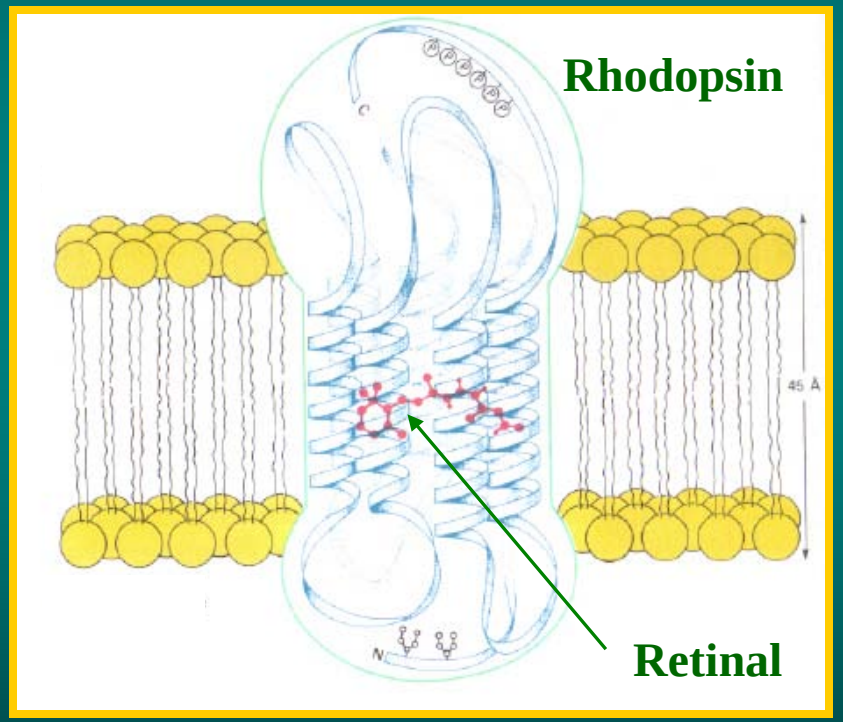
Role of Vitamin A

Vision:

- Retinal is a necessary structural component of rhodopsin, the light sensitive pigment within rod and cone cells of the retina. If inadequate quantities of vitamin A are present, vision is impaired.

- Its long-term deficiency leads initially to reversible, and ultimately to irreversible night blindness.

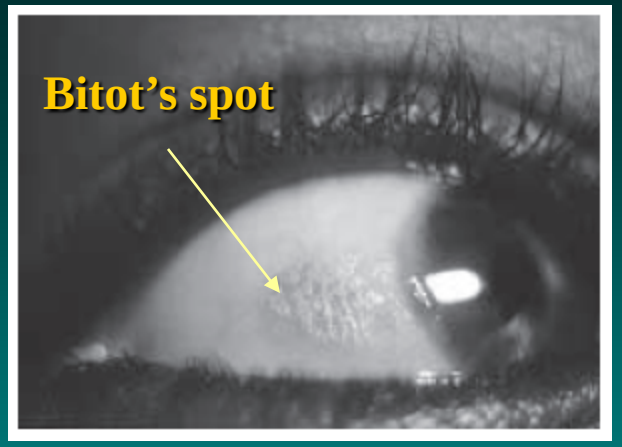
- If the first signs of vitamin A deficiency — xerophthalmia (dry eyes) – are not treated with vitamin A, the condition progresses, ultimately leading to complete blindness.



Of the estimated 5 million children who will develop xerophthalmia each year, approximately 250,000 will eventually become blind.

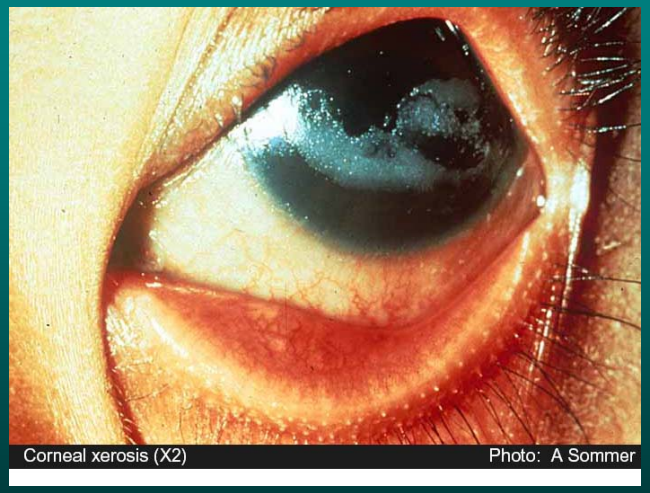
• **Bitot's spot is marker for vitamin A deficiency. The typical Bitot's spot occurs in the exposed part of the conjunctiva.**

• **A Bitot's spot consists of a heaping up of desquamated, keratinized epithelial cells which form a slightly raised area that may be readily wiped away (winking, rubbing).**

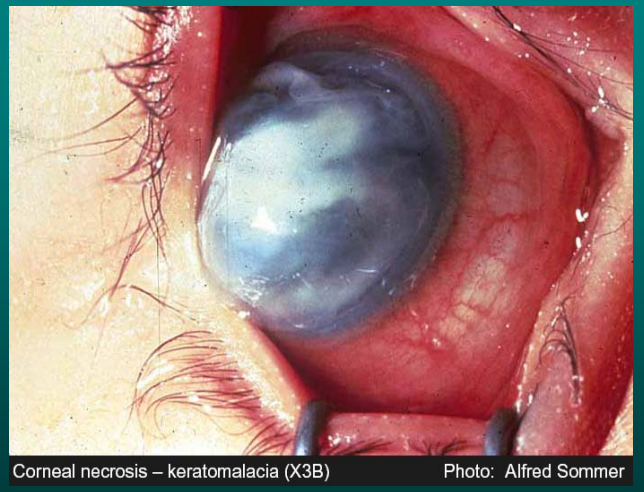


Long-term effects of vitamin A deficiency:

corneal xerosis


Corneal xerosis (X2) Photo: A Sommer

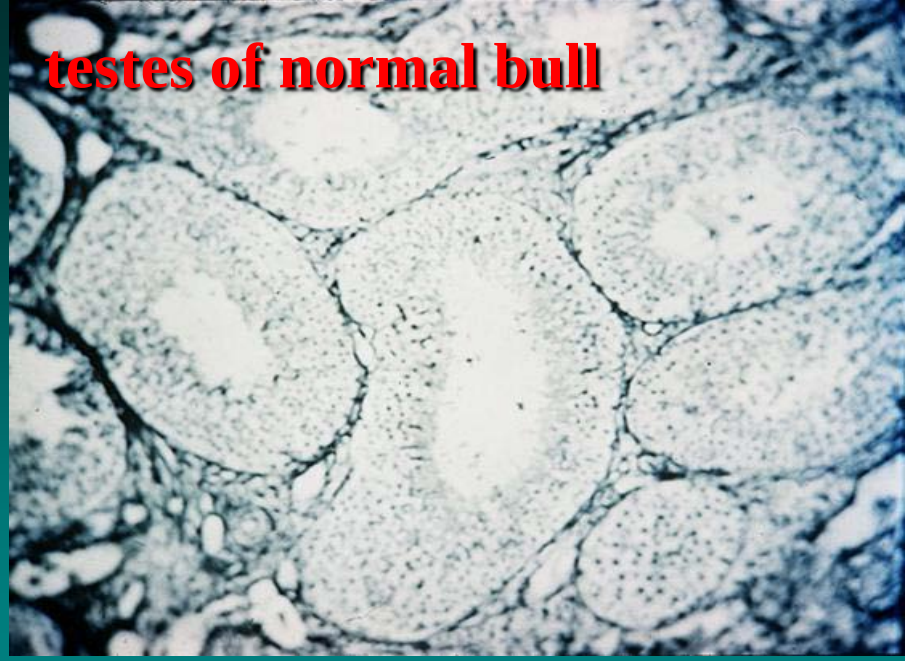
corneal necrosis

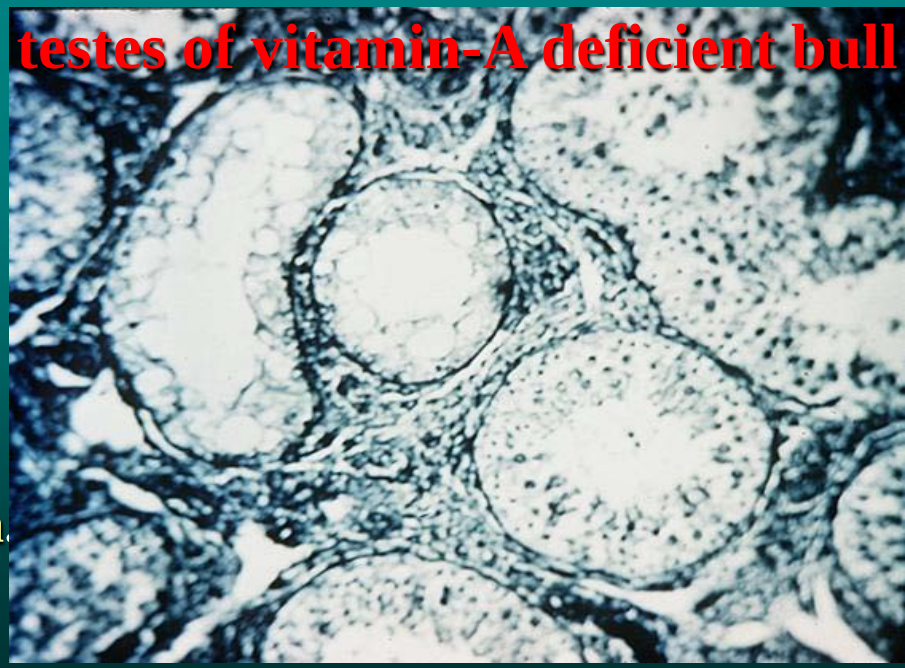

Corneal necrosis – keratomalacia (X3B) Photo: Alfred Sommer

Role of Vitamin A

Reproduction:

- High levels of CRBP have been found in the testes and high doses of RA injected peritoneally support spermatogenesis.
- Normal levels of vitamin A are required for sperm production, reflecting a requirement for vitamin A by spermatogenic epithelial (Sertoli) cells.
- In addition, dietary RA appears to be taken up by testicular interstitial Leydig cells, as it supports testosterone production.
- Similarly, normal reproductive cycles in females require adequate availability of vitamin A for prevention of placental necrosis and fetal resorption.

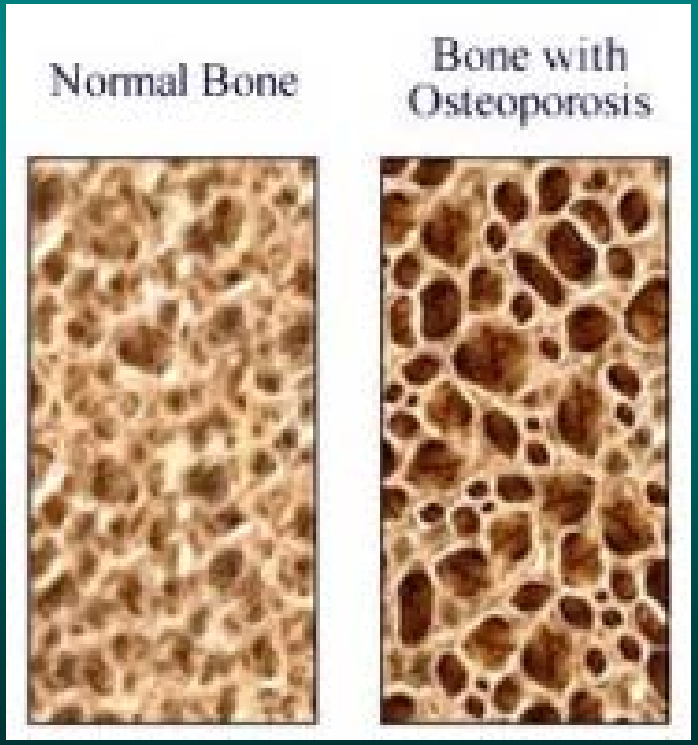




Role of Vitamin A

Bone remodeling:

- A U.S. study of more than 72,000 postmenopausal nurses found that the risk of hip fracture increased with the amount of retinol consumed and that those who were taking a vitamin A supplement had a 40 percent greater risk of hip fracture than those who did not.
- In Sweden, a country with high rates of hip fractures and diets rich in vitamin A, studies also have found a strong correlation between retinol intakes and the risk of hip fracture.
- This negative skeletal impact of vitamin A may be due to the potential of high concentrations of this vitamin to trigger an increase in the number and activity of osteoclasts (cells that cause bone breakdown). It has also been suggested that excessive vitamin A levels interfere with vitamin D, which plays an important role in preserving bone.

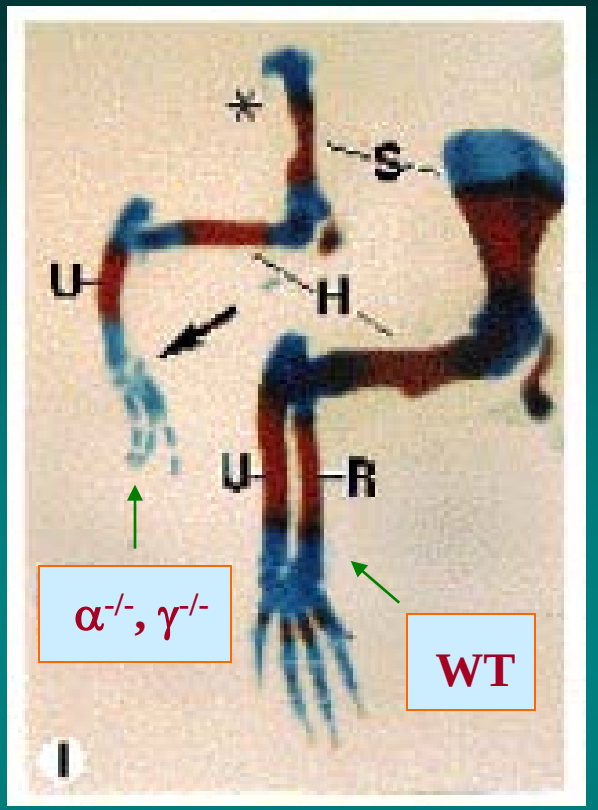


Role of Vitamin A

Bone remodeling:

- Normal functioning of osteoblasts and osteoclasts is dependent upon vitamin A.

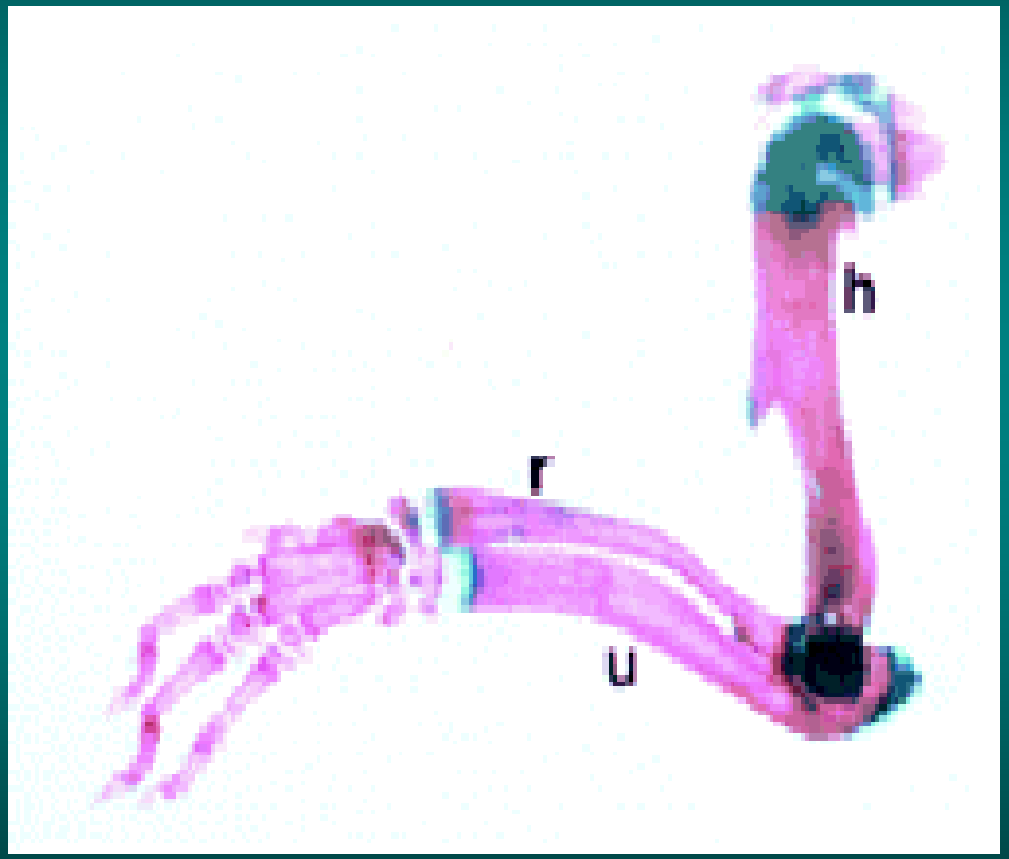
- Retinoic acid receptor may regulate bone resorption by suppressing in bone cells:
  - * alkaline phosphatase activity,
  - * osteocalcin production,
  - * interleukin-6 synthesis



- Its importance in limb development was demonstrated by the observation that compound homozygotes of null alleles of RAR exhibited a range of limb abnormalities from reductions to duplications.

- Retinoic acid is important in normal limb ontogeny and in excess is a potent teratogen, causing characteristic perturbations of normal limb development.

RAR overexpression



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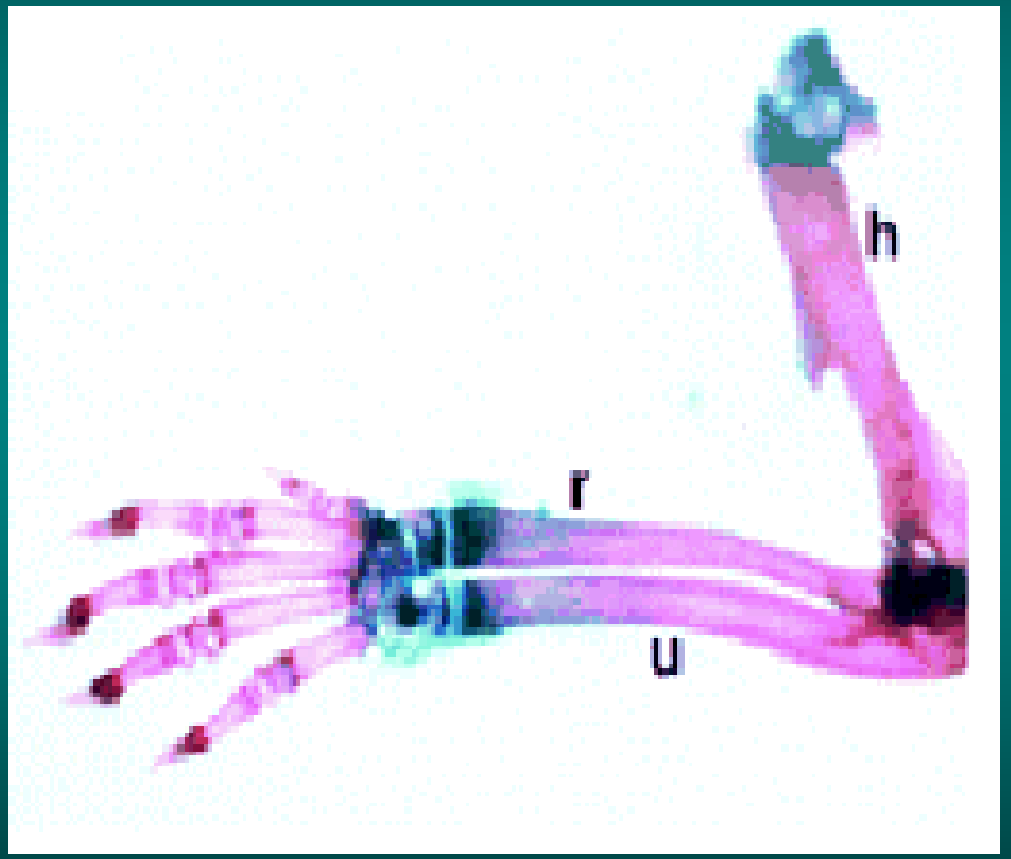

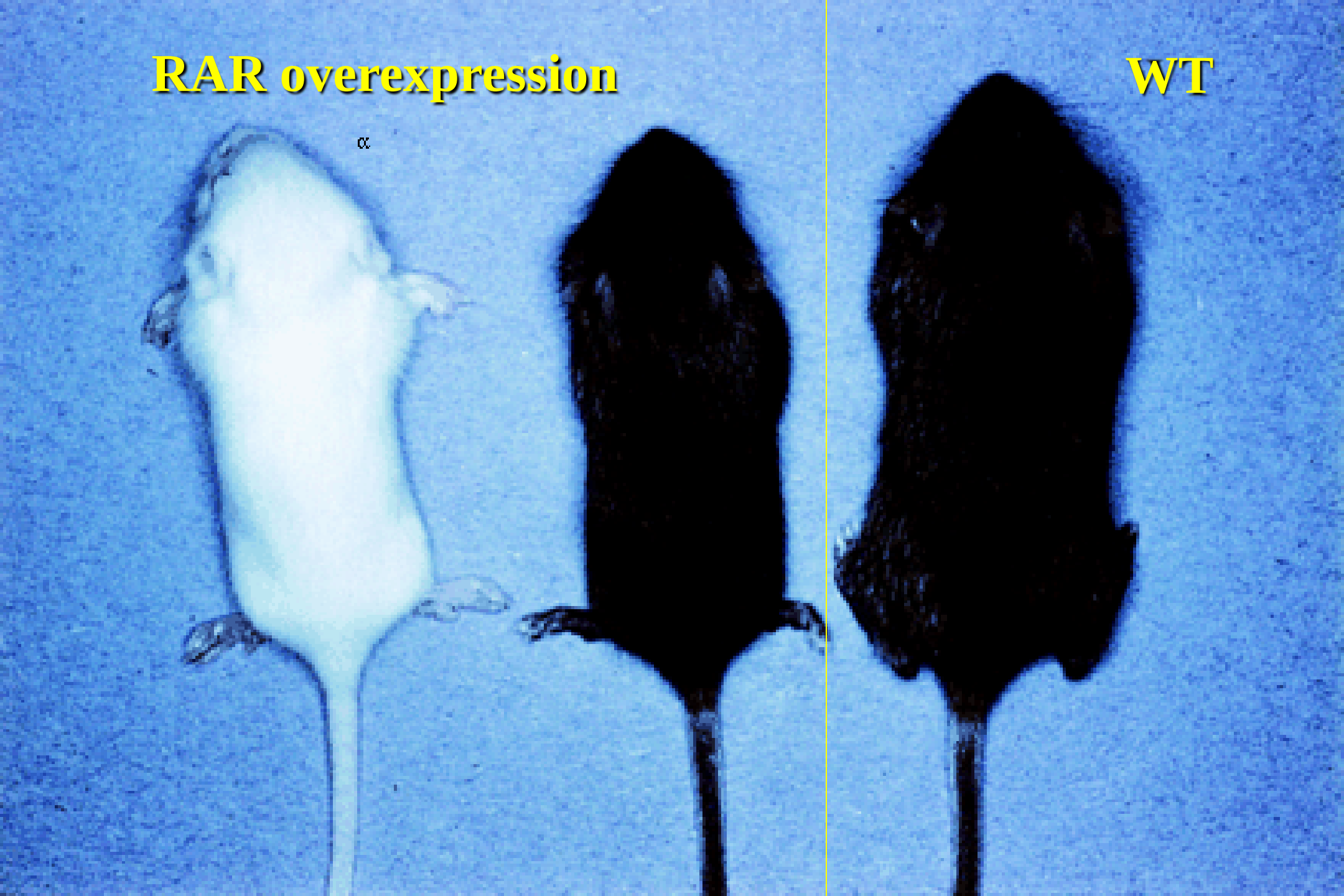
RAR overexpression
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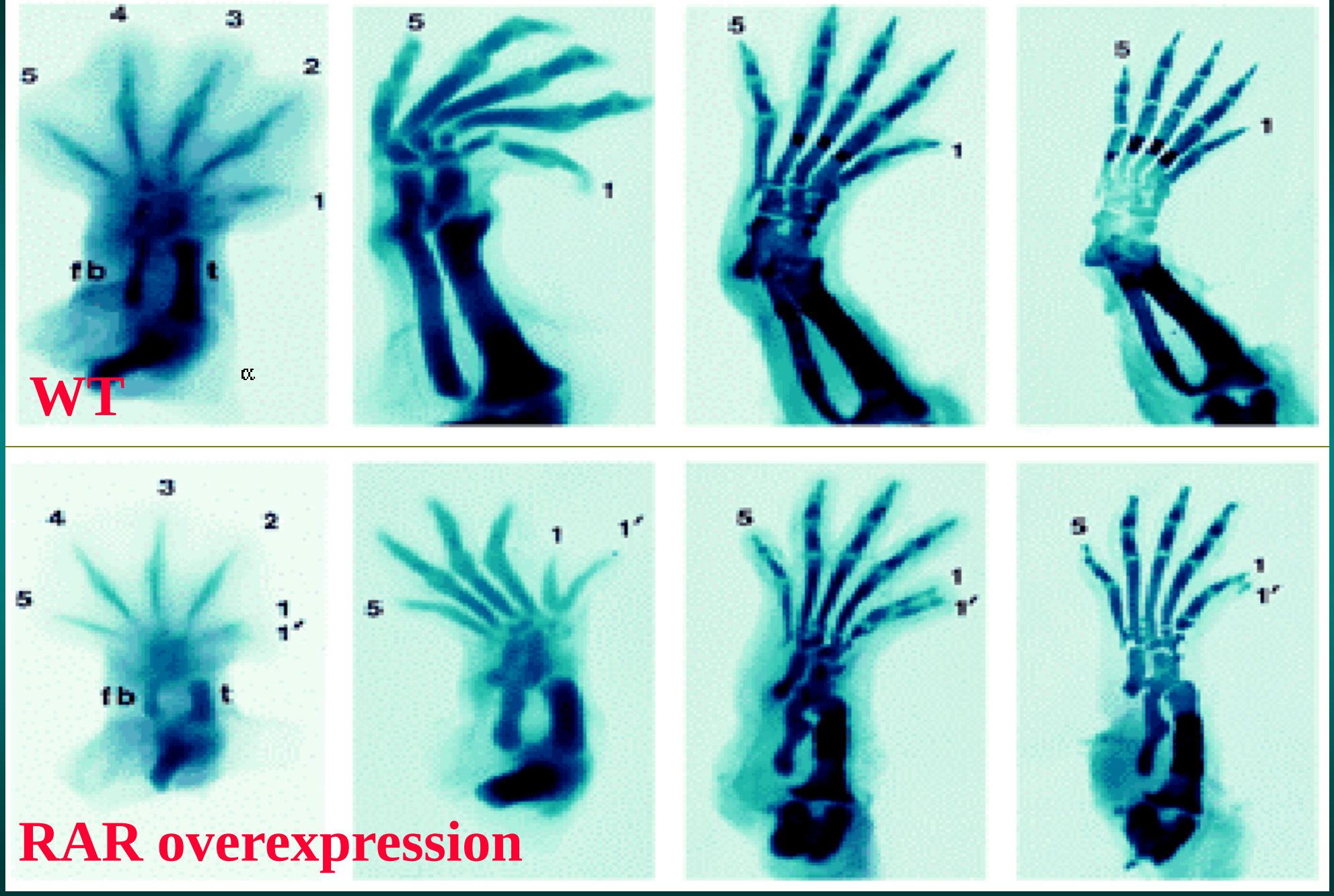
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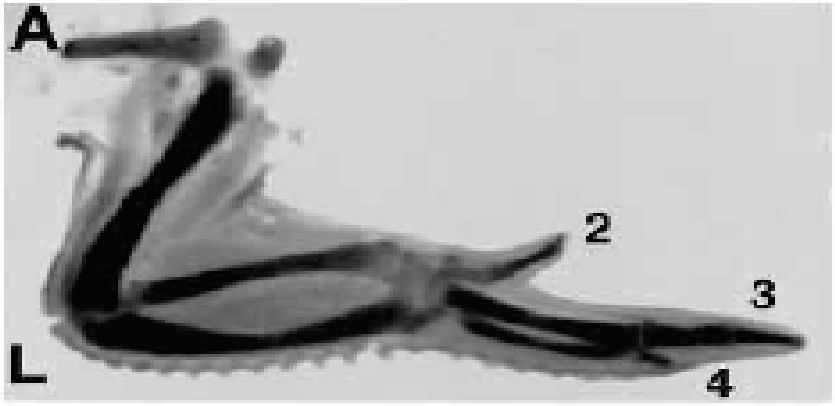
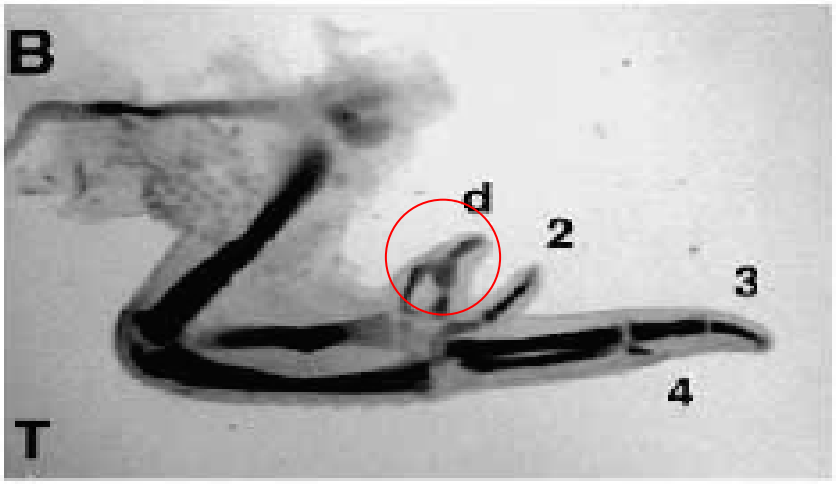
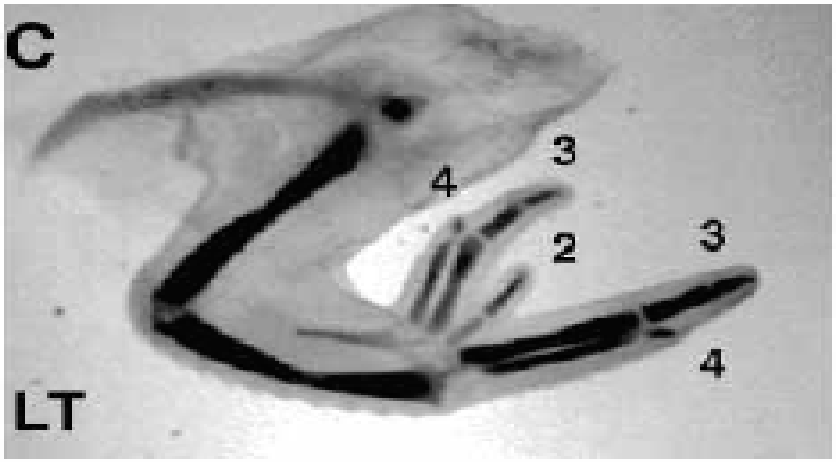
RAR overexpression



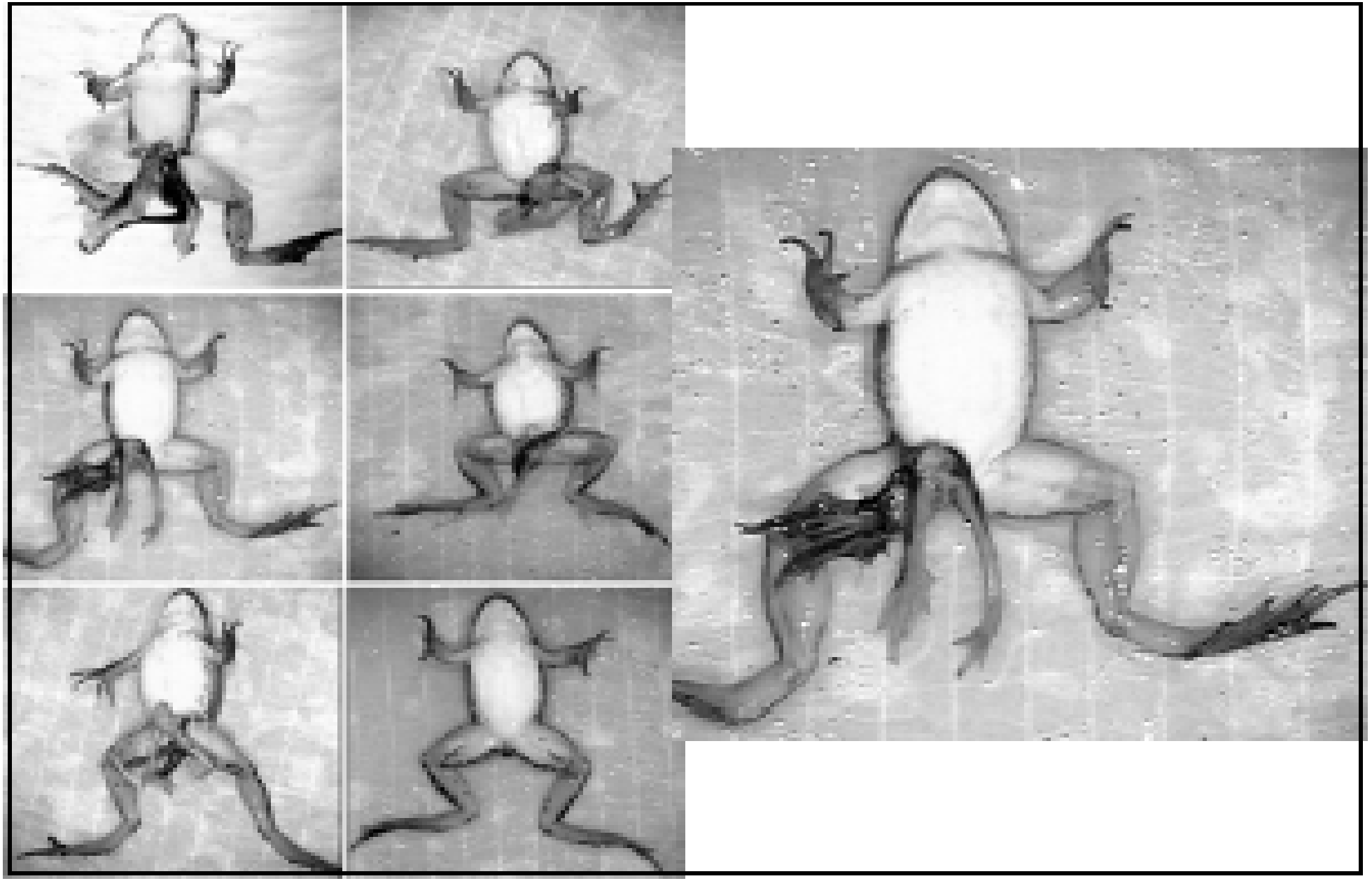
control



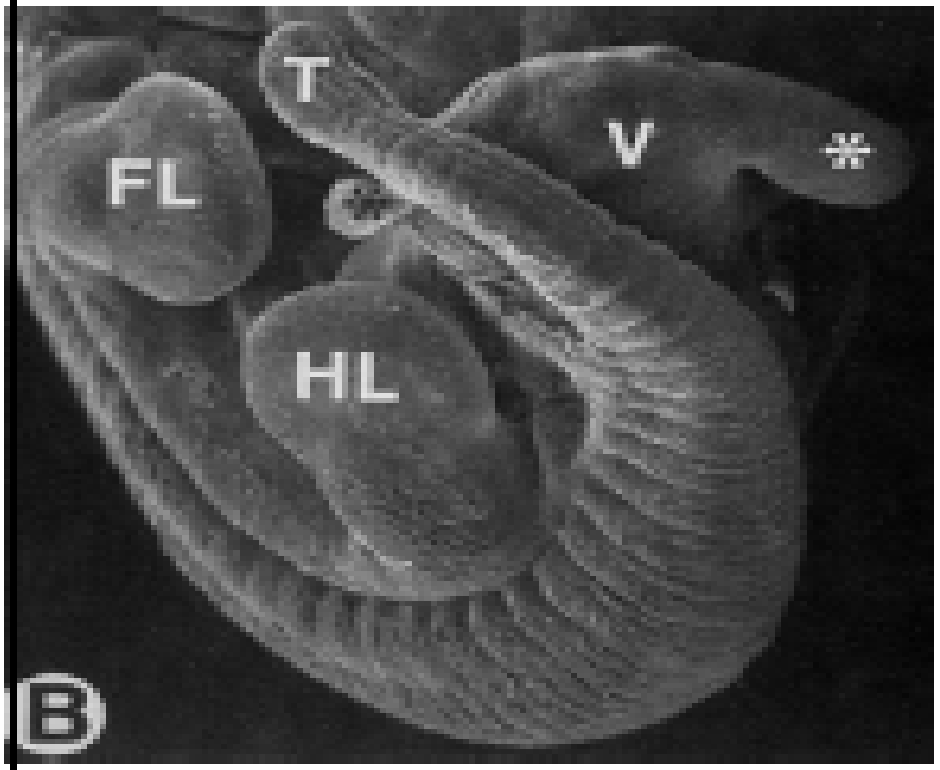
TTNTB (RAR specific agonist)

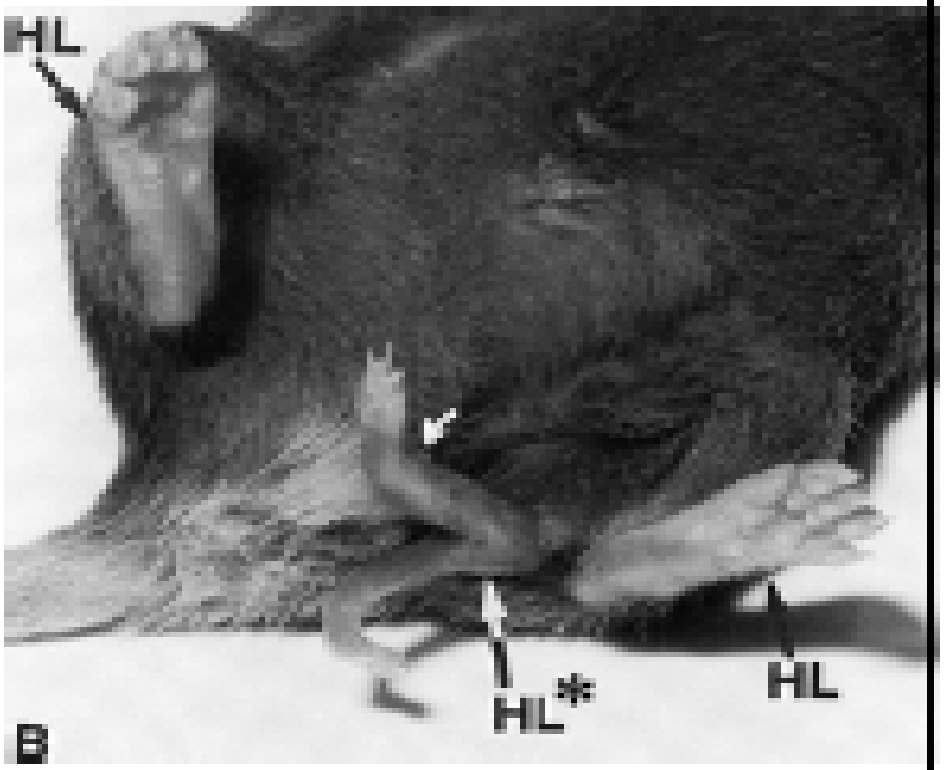


TTNTB (RAR specific agonist)
+ LG69 (RXR specific agonist)



RA Induces Extra Limbs



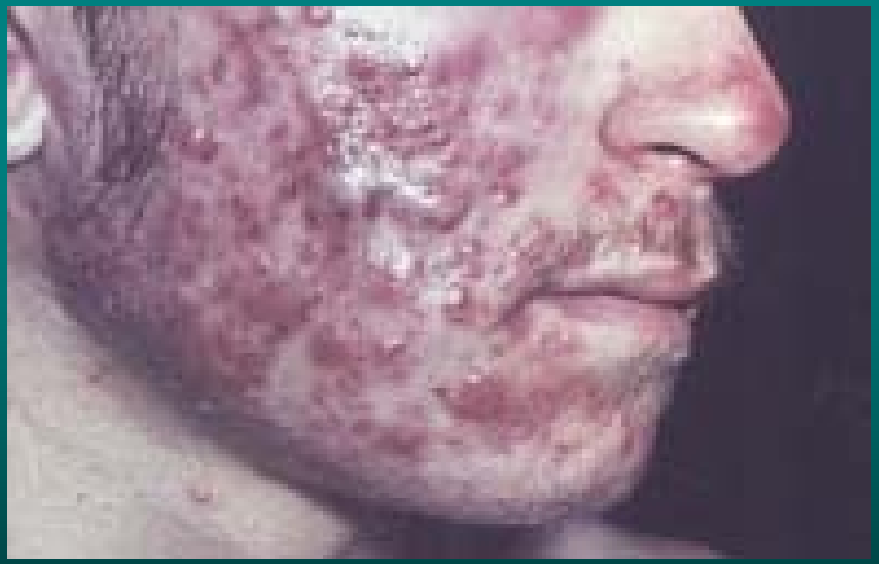


13-cis-retinoic (Isotretinoin) acid or Accutane

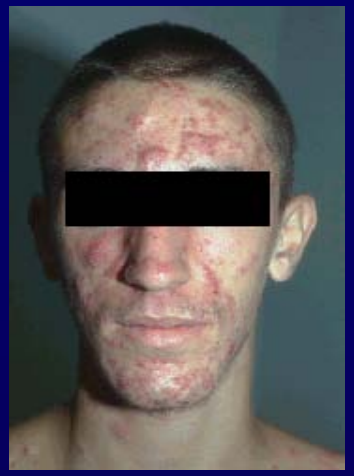
- Derivatives of Vitamin A and are used to treat skin disorders such as acne.

- Their effect in acne treatment is based on a reduction in the secretion and size of the sebaceous glands, with a corresponding reduction in microbial colonisation and resulting inflammation. The formation of comedones (black accumulations of sebum in the secretory ducts of a sebaceous gland) is also decreased.

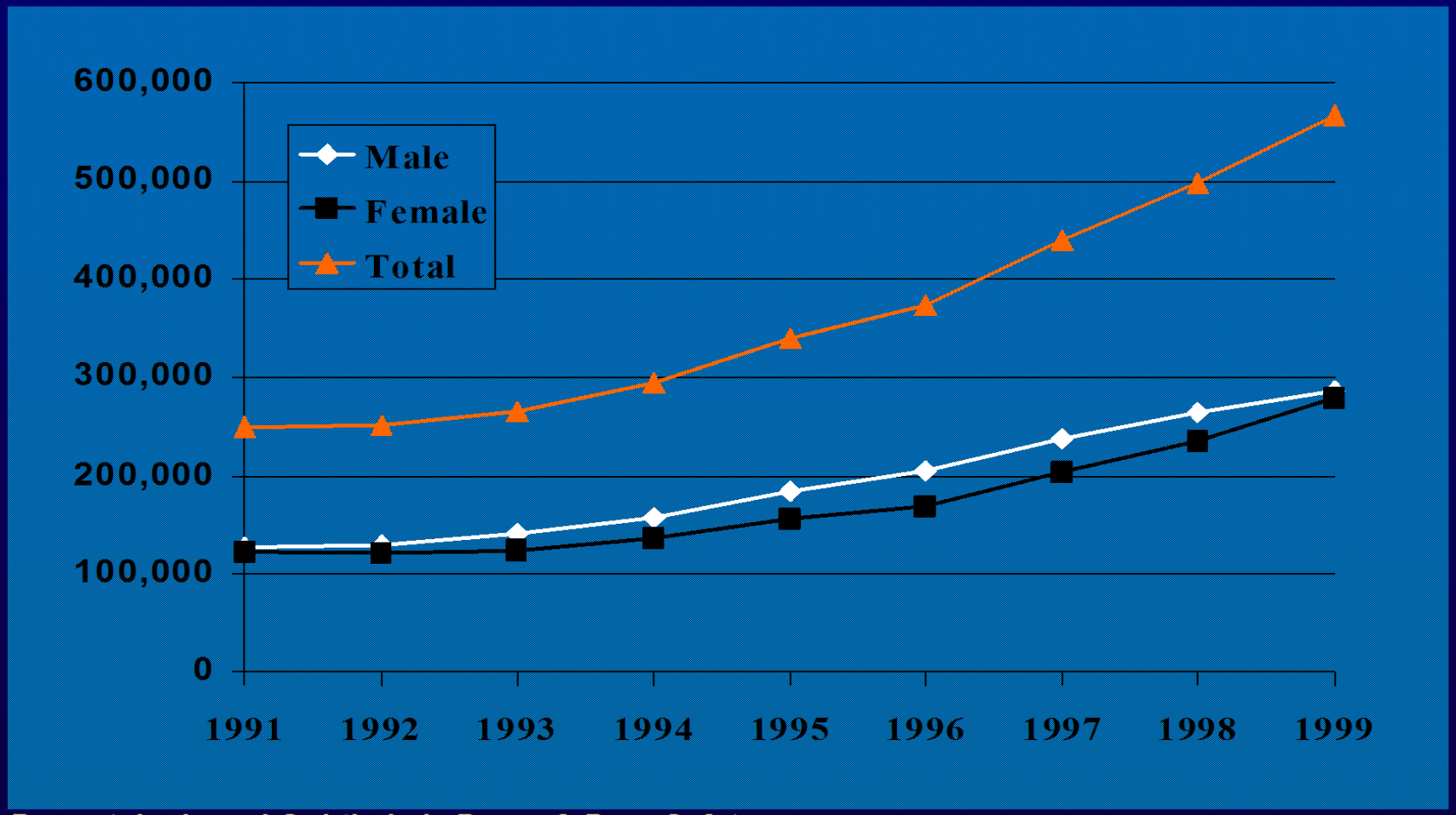
- Isotretinoin is indicated for the treatment of severe recalcitrant nodular acne. Nodules are inflammatory lesions with a diameter of 5 mm or greater.



Estimated Number of Patients Using Accutane



Number of Patients

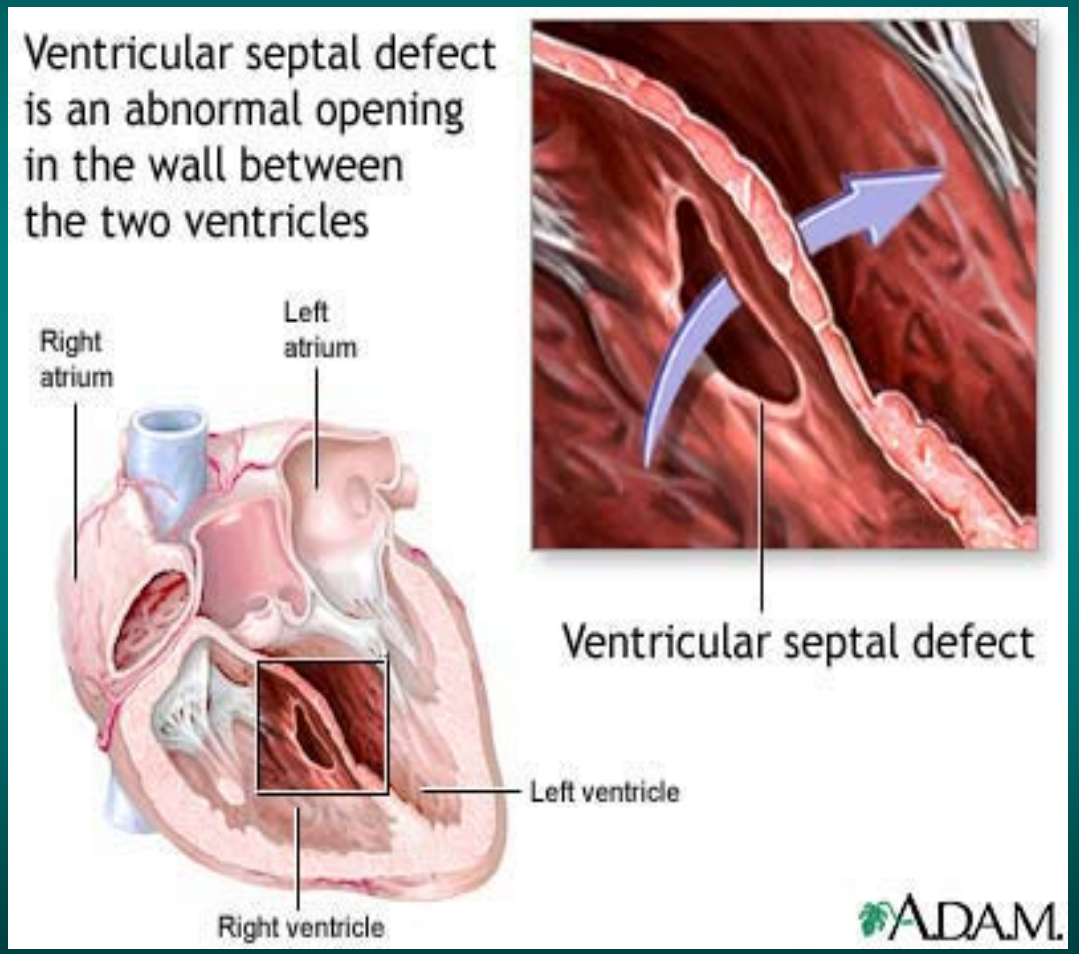


Isotretinoin--Teratogen

- Increased risk of spontaneous abortion and premature birth
- Malformations: craniofacial, cardiac, thymus, CNS, functional
- 28% of exposed pregnancies affected at birth
- Indicated only for use in females who are not pregnant and males

13-cis-retinoic (Isotretinoin) acid or Accutane

- Women who used Accutane while pregnant may give birth to babies with birth defects:
- hydrocephaly,
- microcephaly,
- mental retardation,
- ear and eye abnormalities,
- cleft lip and palate, and other facial abnormalities
- heart defects.



- A study sponsored by the National Institute of Child Health and Human Development (NICHD) compared vitamin A intake during pregnancy in women who had given birth to infants who were healthy, and who had a neural tube defect or cranial-neural-crest defect. No differences were found in consumption of vitamin A in these groups.

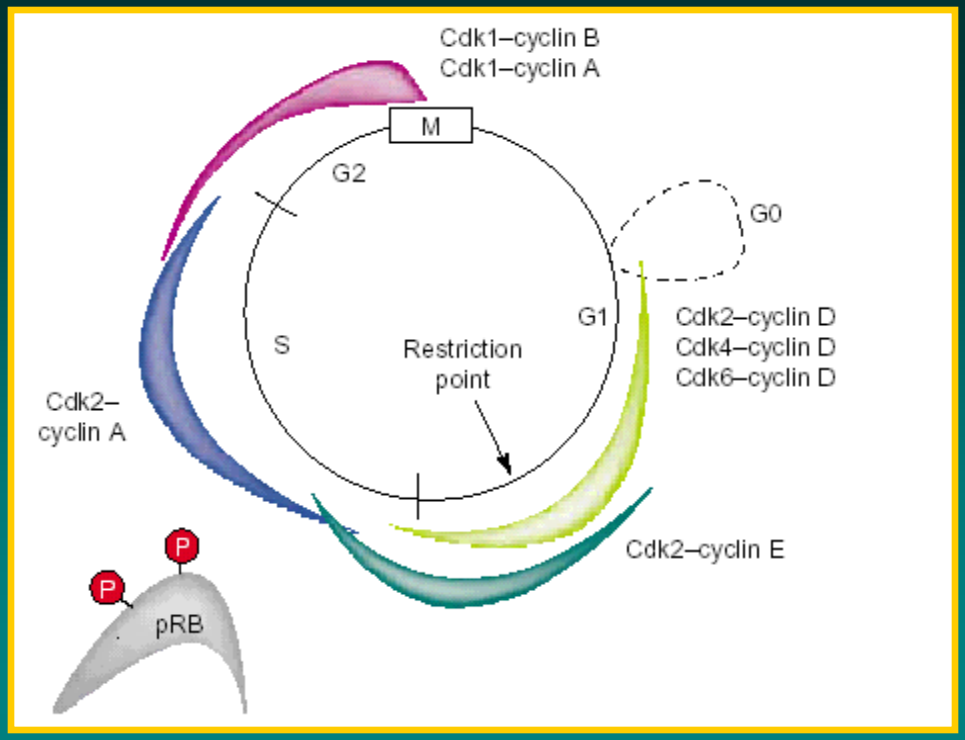
- A similar study conducted at the Boston University School of Medicine compared vitamin A intake during pregnancy in women who had given birth to healthy infants or who had defects. The study found that women who took about 10,000 IU or more vitamin A during pregnancy were more likely to give birth to a child with a cranial-neural-crest defect. It was estimated that intakes of greater than 10,000 IU of vitamin A by pregnant women could result in a defect in one of every 57 infants.

Vitamin A – Retinol and its derivatives

They are essential for the embryo and the adult

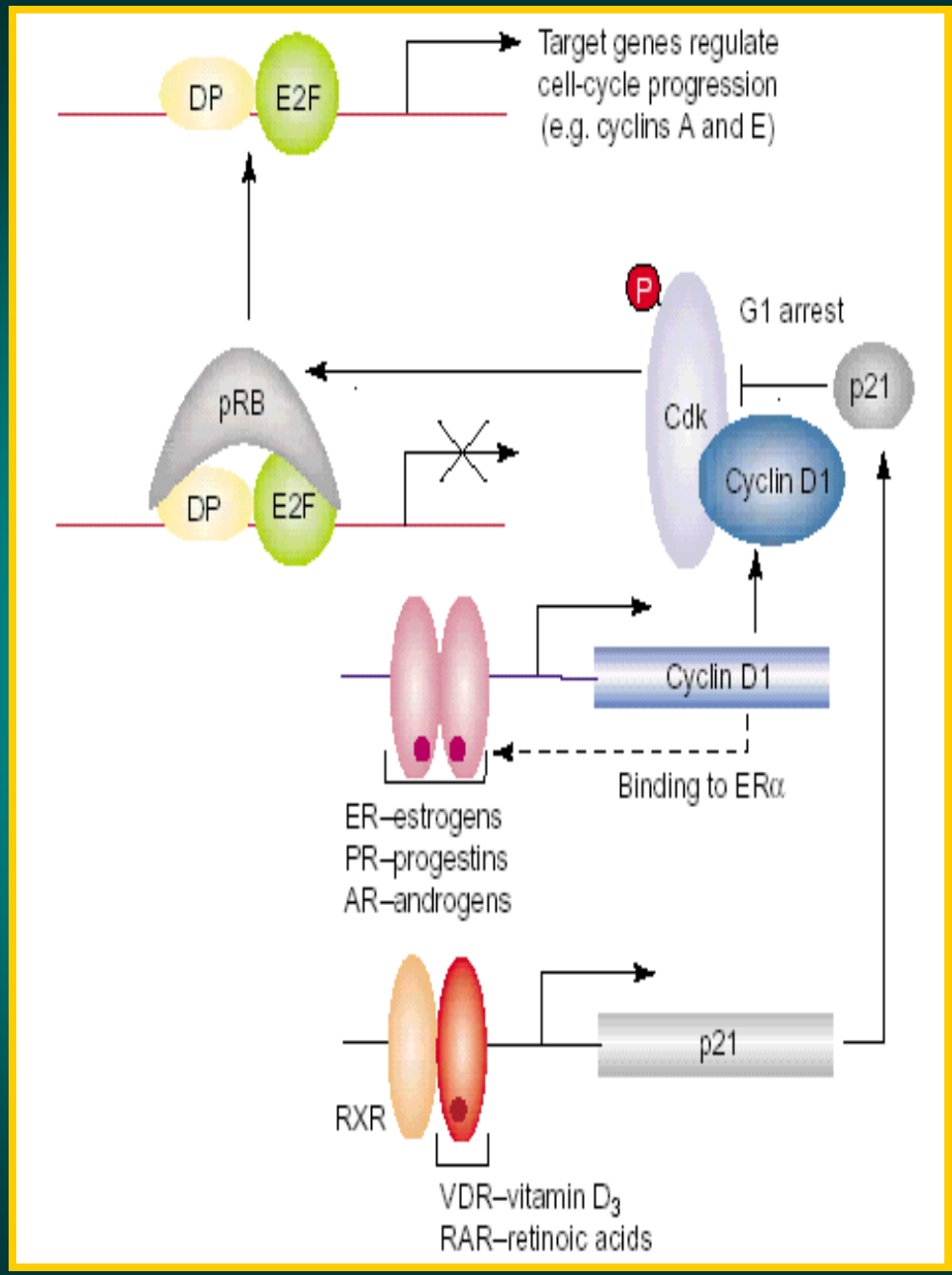
Too little – abortions

Too much - malformations



Some nuclear receptors (ER, AR, PR) stimulate expression of cyclin D, which activates Cdk4. It leads to phosphorylation of pRB, and increases transcription of genes increasing proliferation.

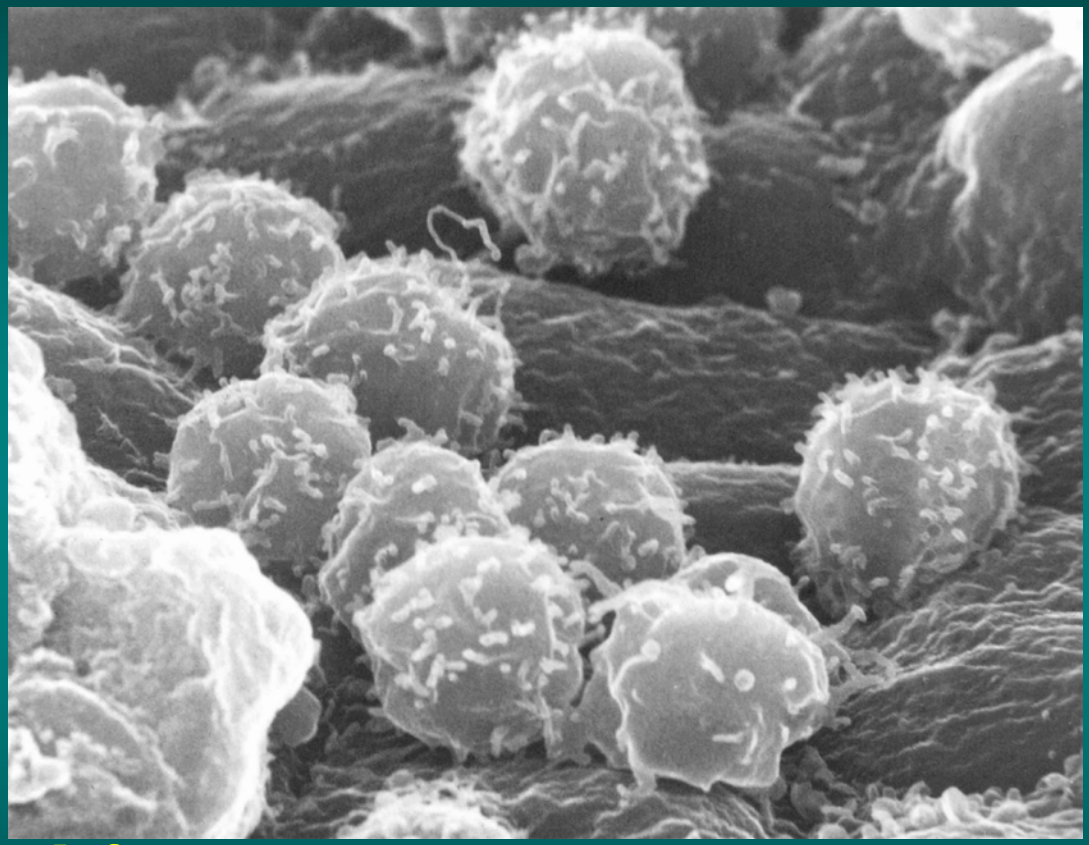
Others receptors (VDR, RAR) increase p21 expression, thus block Cdk activity, which keeps cells at G1 phase.

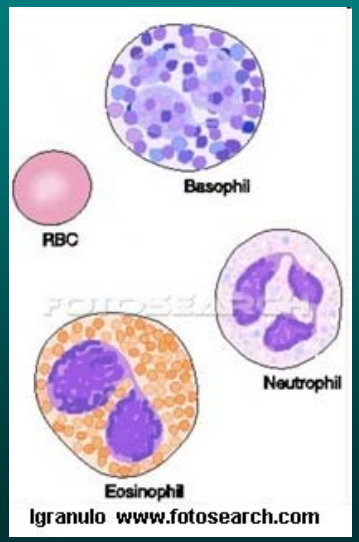


RAR-RXR heterodimer influences cell cycle

- **Retinoids and RAR/RXR were shown to cause:**

  * downregulation of NF-κB
  * downregulation of c-myc
  * downregulation of c-myb
  * downregulation of cyclin A, E and cdks 2,4,6.
  * downregulation of telomerase activity
  * **upregulation of p21**
  * upregulation of SUMO-1
  * upregulation of cEBPε
    (crucial for maturition of granulocytes)
  * upregulation of TRAIL





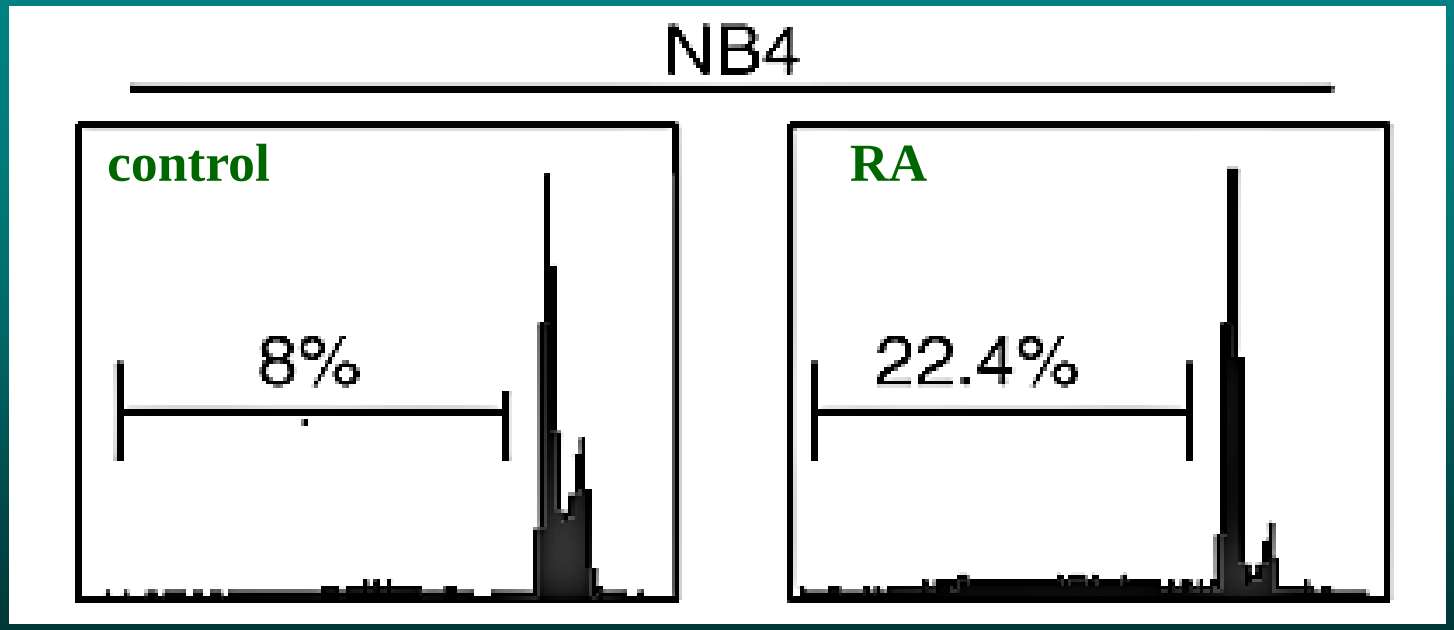
This leads to:

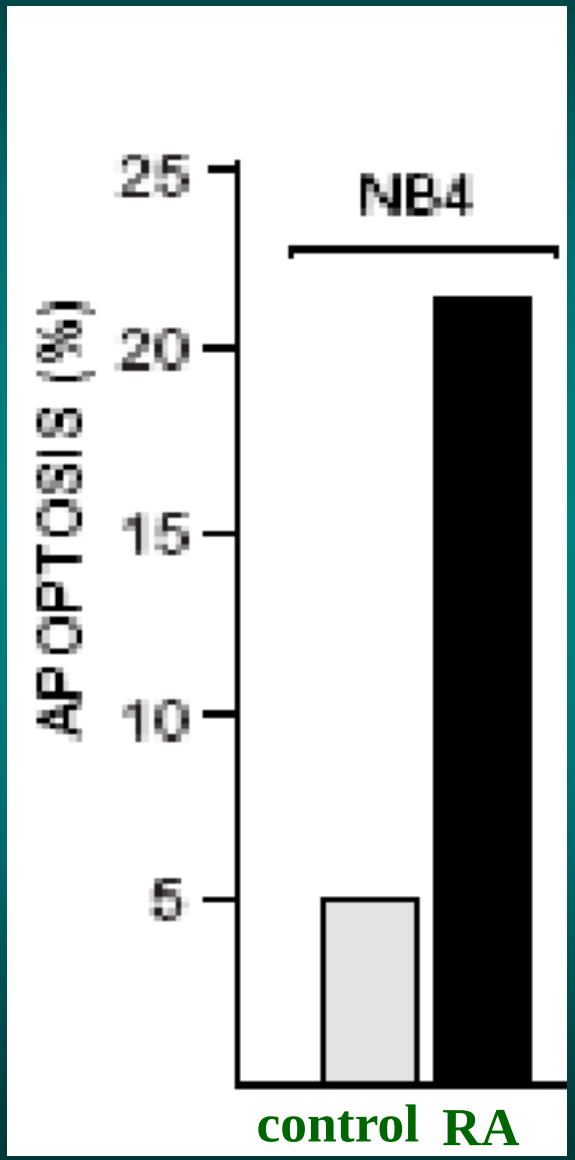
* decreased proliferation,
* **differentiation of granulocytes**,
* apoptosis of blasts

Apoptosis of promyelocyte afer treatment with RAR agonist

Promyelocytic NB4 cells,

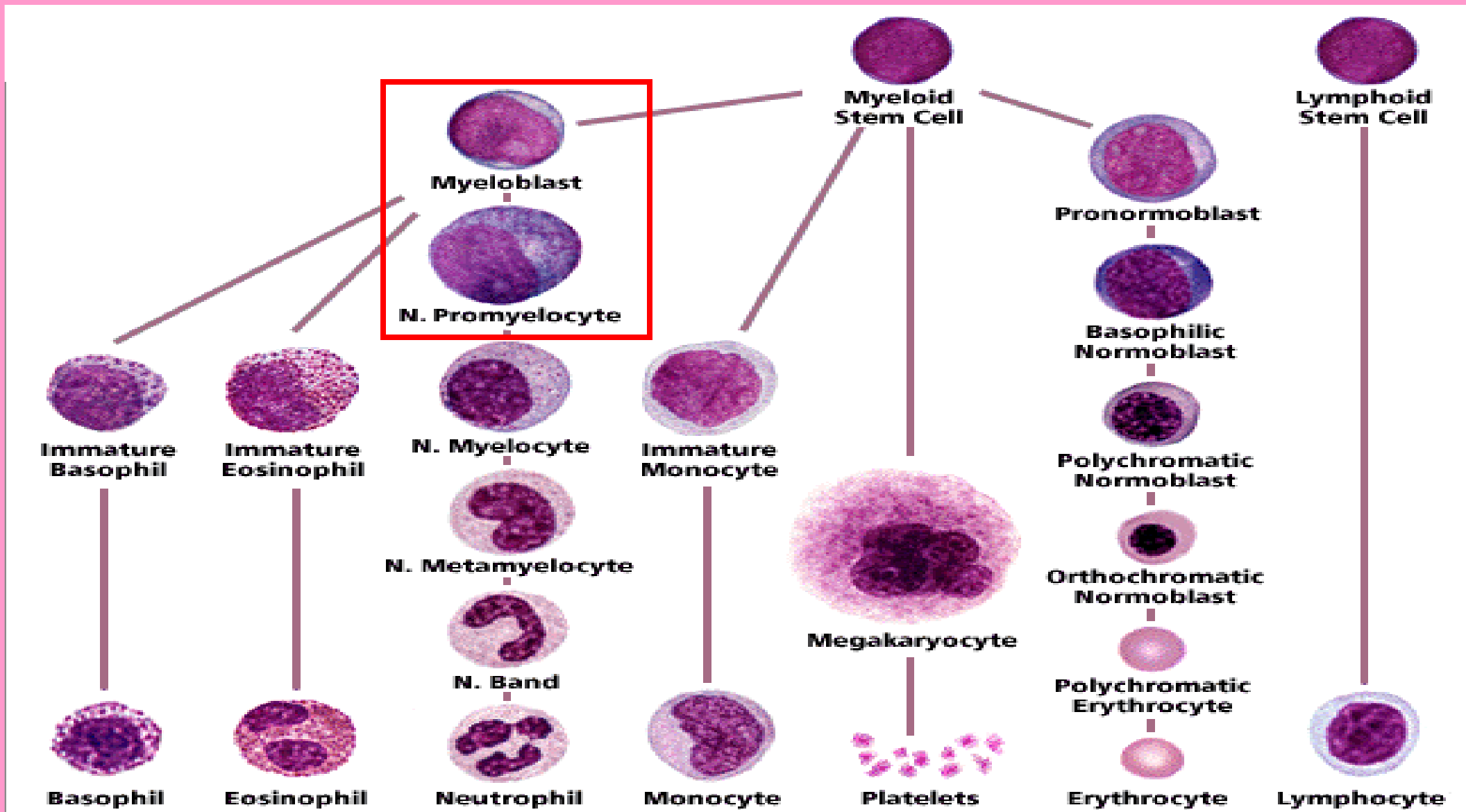
FACS analysis of propidium iodide labelled cells.



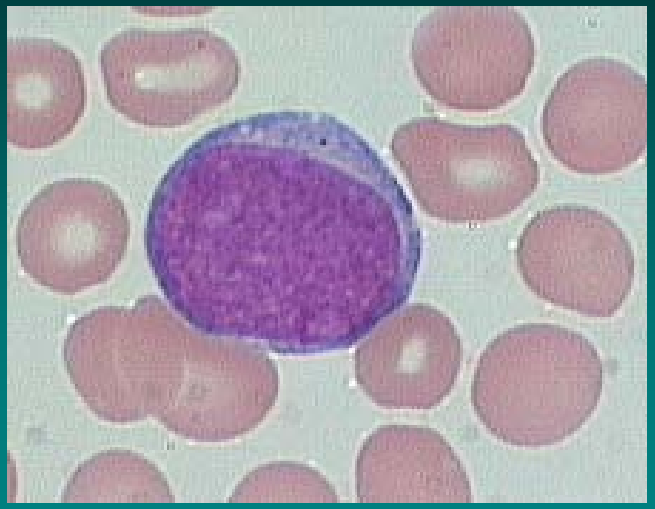


Altucci et al. Nature, 2001.

Hematopoietic Differentiation



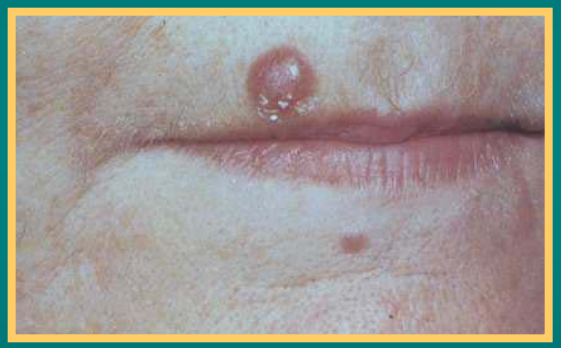
Acute Promyelocitic Leukaemia



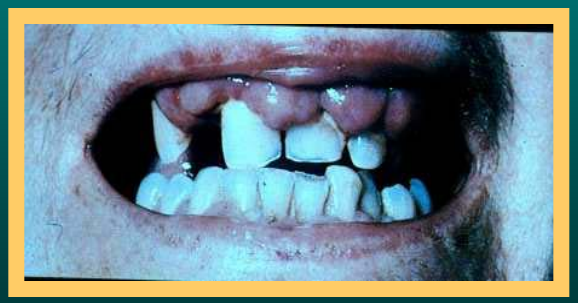
- Acute promyelocitic leukaemia (M3) can affect adults of all ages.
- The signs and symptoms of APL are nonspecific and include fatigue, minor infections, or a tendency to bleed (hemorrhagic diathesis).
- It represents 10% of all acute myelocytic leukaemias in adults (~3 000 cases yearly in USA).
- It is a rapidly progressing type of leukaemia that affects promyelocite, which continue to divide, but do not mature.
- These immature dividing cells fill up the bone marrow and prevent it from making blood cells properly.
- As the leukaemia cells do not mature, they cannot do the work of normal white cells, leading to an increased risk of infection and haemorrhages. And as the bone marrow cannot make the right numbers and quality of red blood cells and platelets, symptoms such as anaemia and bruises also occur.

Leukemic infiltrations

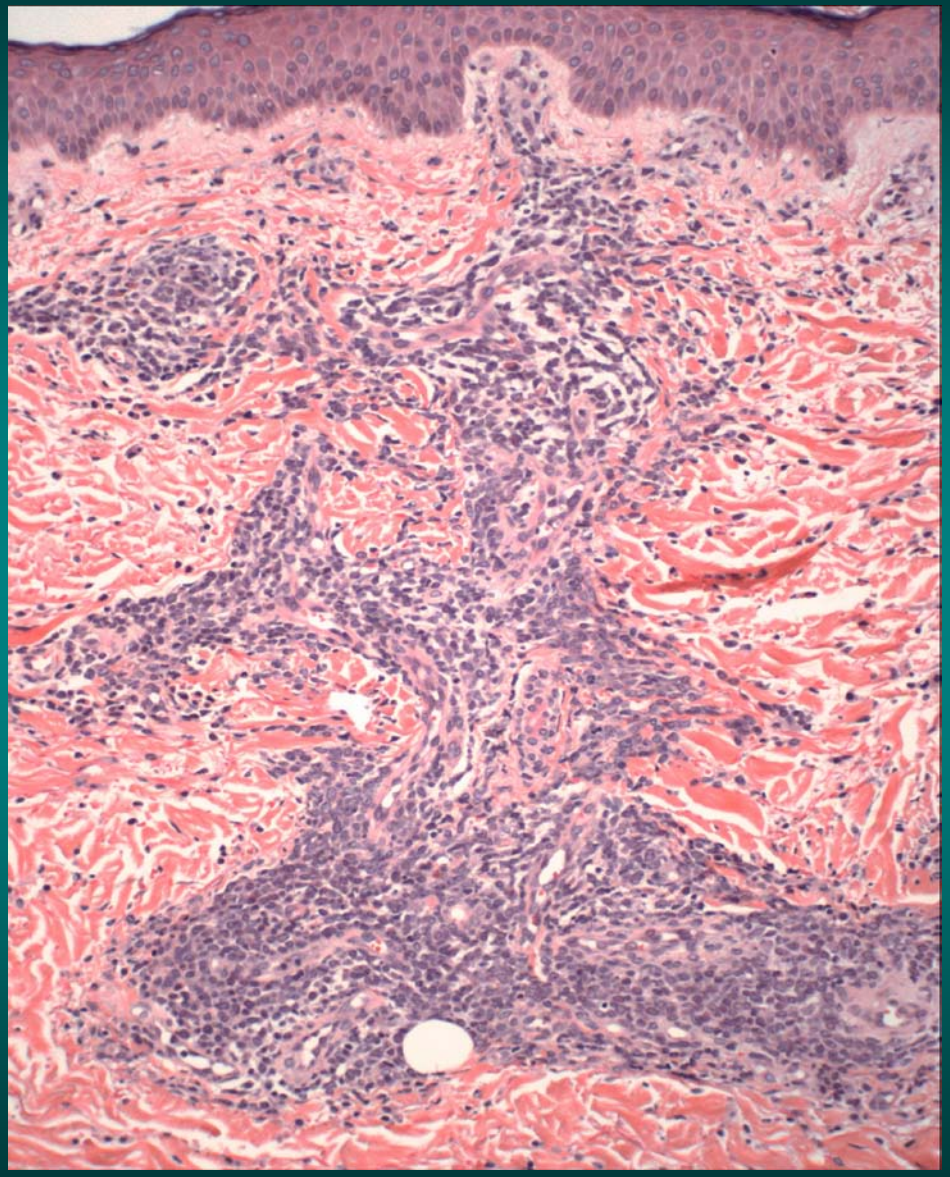
Petechiae



Leukemic infiltrate of skin



Gum infiltration in leukemia



PML-RAR

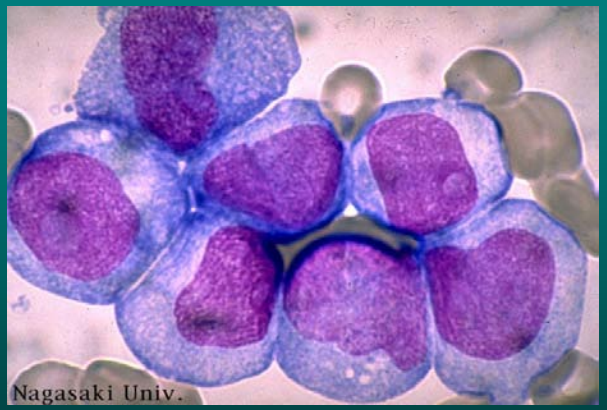
• In 99% of patients there is an exchange of fragment of chromosome 15 and 17, which is a marker of this disease. Chronologically there was a second example (after Philadelphia chromosome) of understanding the genetical background of cancerogenesis.

• Translocation involves RARα on chromosome 17 and PML (promyelocytic leukemia gene) on chromosome 15 creating a chimeric protein PML-RAR.

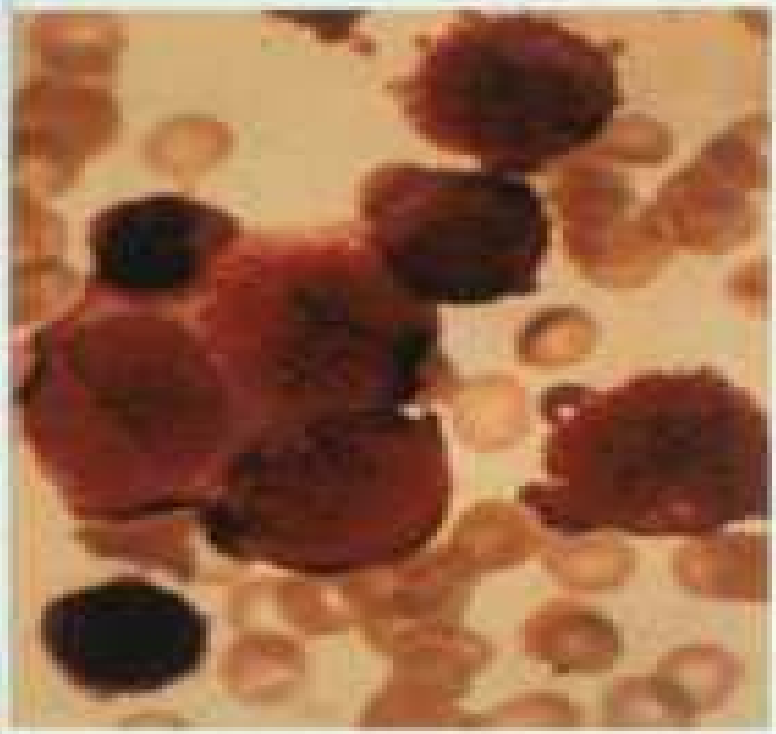
• Therapeutical benefits:

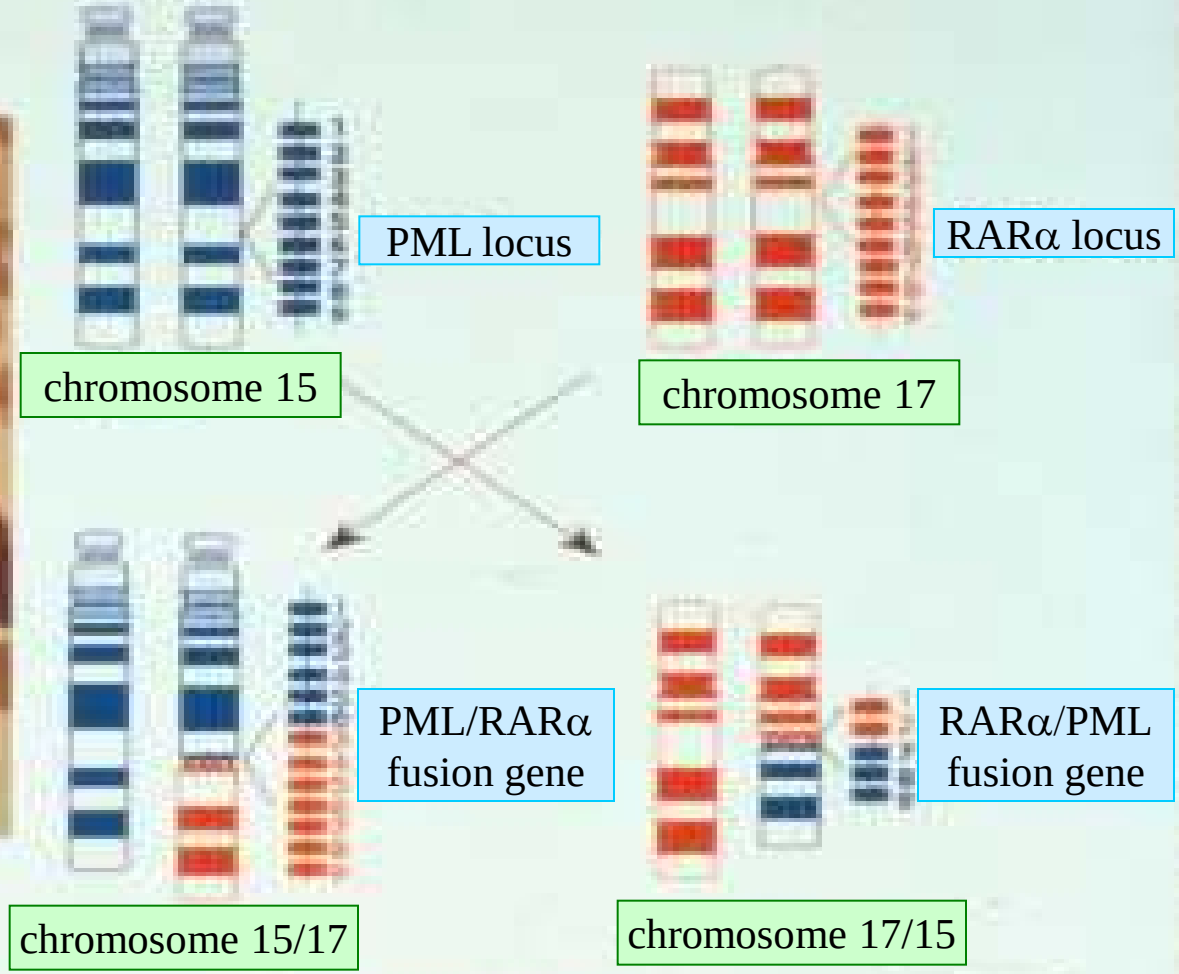
* in 1960s time from diagnosis APL to death of patients ranged from 1 to 2 weeks, depending on the quality of care (!).

* currently ~80% of cases are fully curable.



APL with t(15;17) PML-RARα



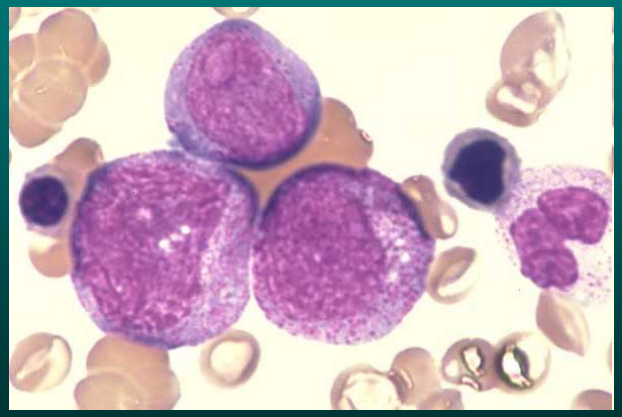


PML-RAR

• Normal protein PML is present in nucleus (Kremer bodies, 10-30/nucleus), where colocalizes with SUMO-1, Sp-100, Sp140, CBP, DAXX, RB and p53 proteins.

• PML-RARα interacts with PML, leading to destabilization of Kremer bodies. Proteins normally included into the bodies are located in different places of nucleus. Thus, they cannot function normally.

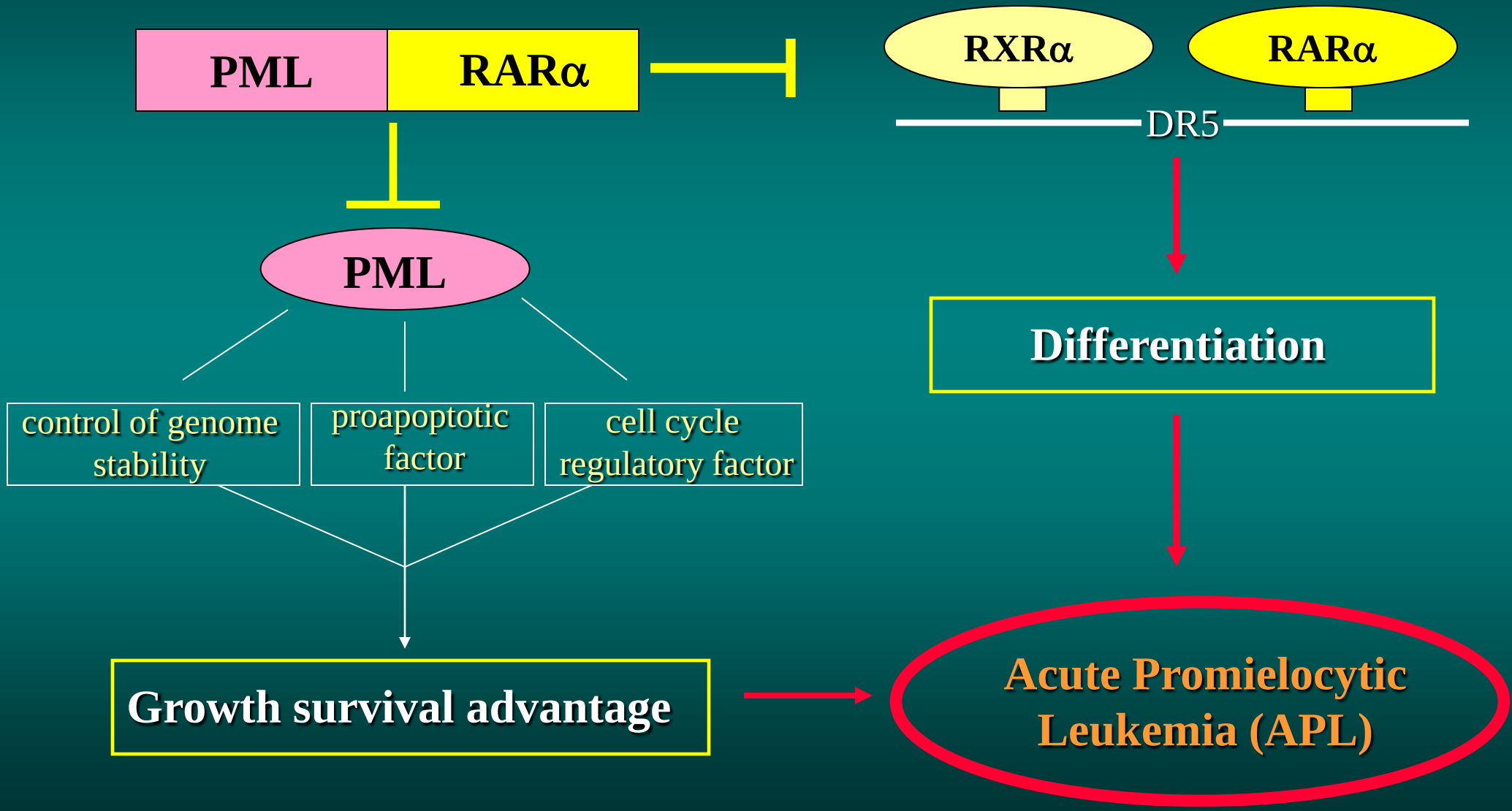
• PML is crucial for maintaining the correct structure of Kremer bodies, so PML-RARα acting through PML disturbs activity of all proteins located in the Kremer bodies.



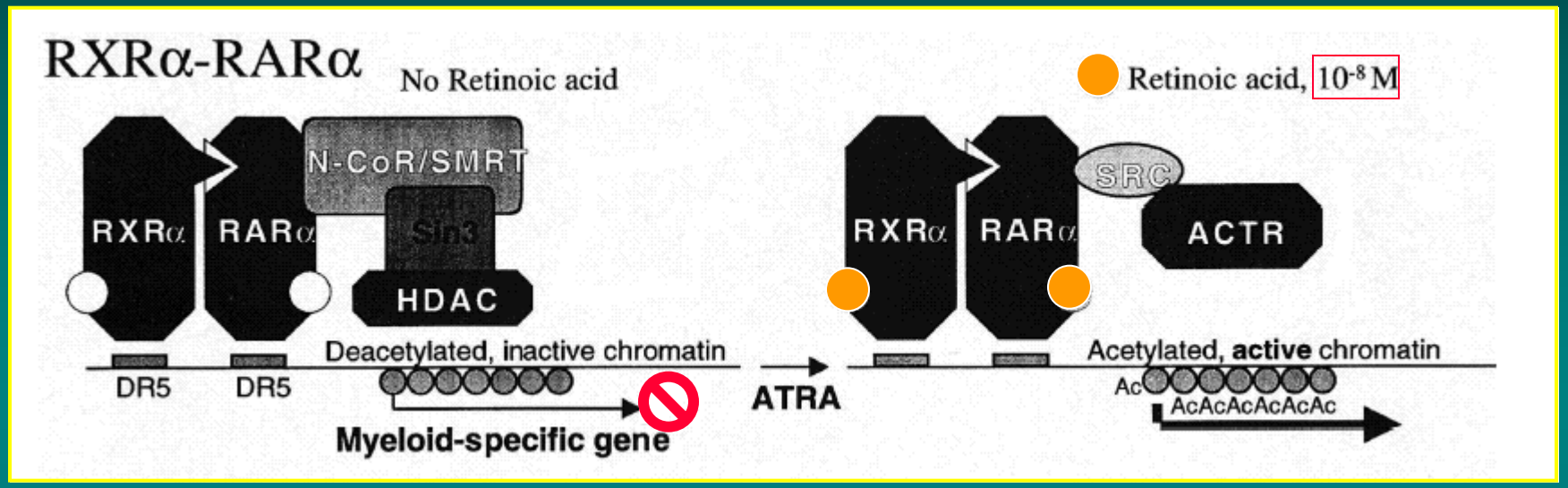
It causes

* inhibition of promyelocyte differentiation
* augmenting the cell proliferation
* increased instability of genome

Dual leukemic function of PML-RARα



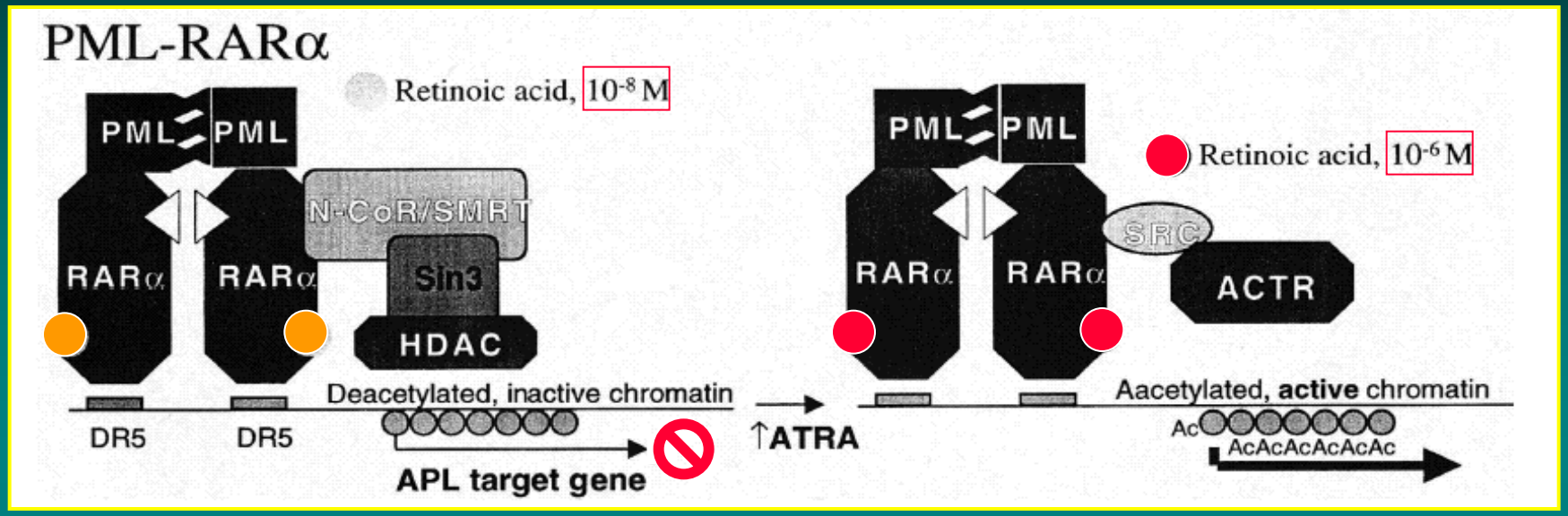
PML-RAR: inhibition of differentiation



- In absence of retinoic acid RAR-RXR heterodimers inhibit transcription of target genes through recruiting repressors and histone deacetylases.

- Physiological doses of retinoic acids induce release of repressors, assembly of coactivators and expression of target genes, which leads to inhibition of proliferation and increase in differentiation of many cell types, including promyelocytes.

PML-RAR: inhibition of differentiation

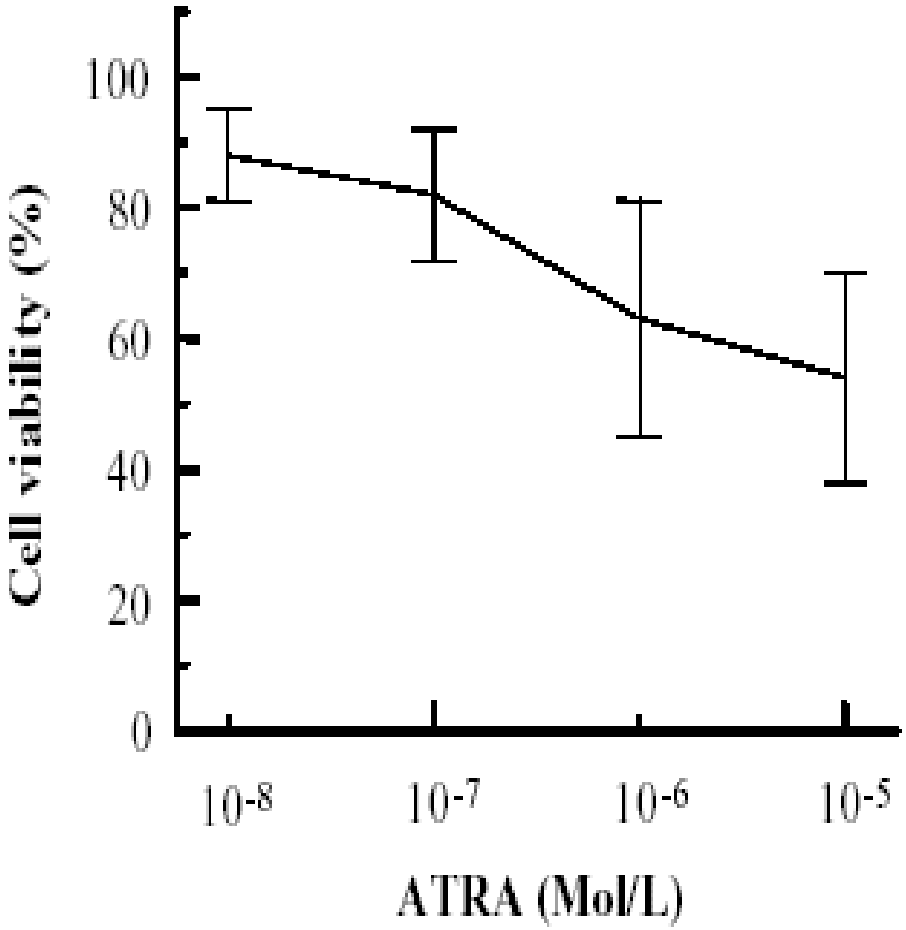


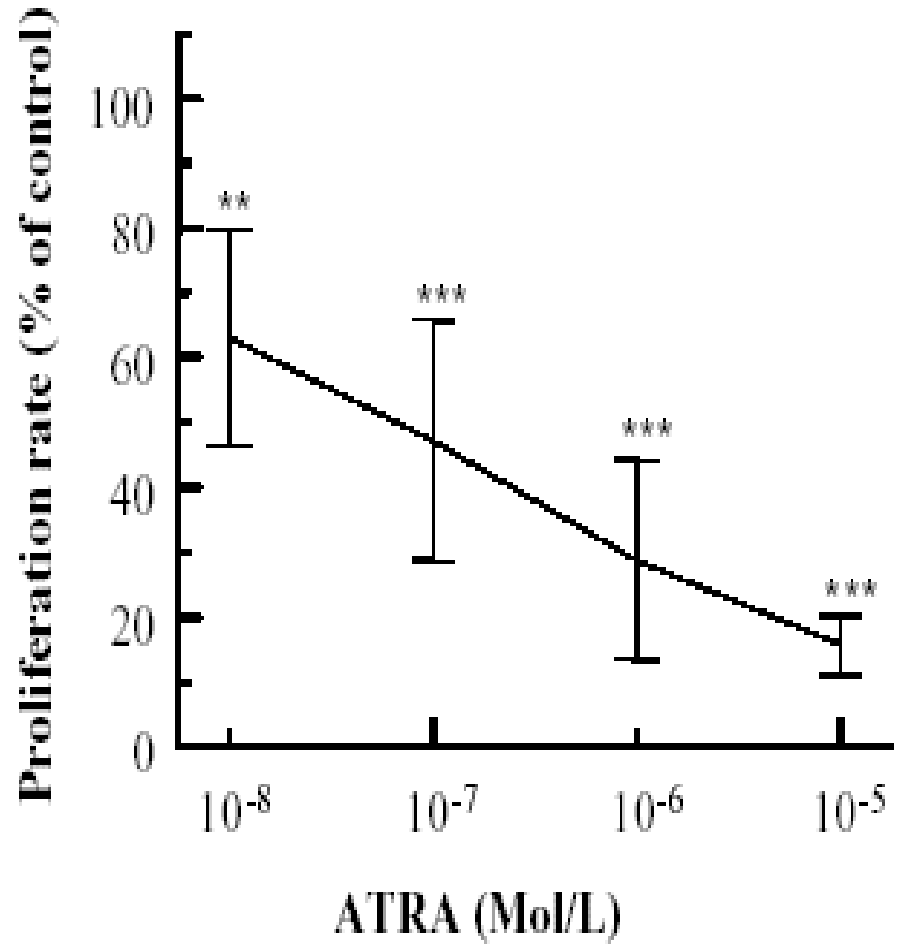
- PML-RARα may bind to RARE as a dimer or may dimerize with RXRα. Moreover, PML-RARα bind retinoic acid with affinity the same as RARα.

- At physiological doses of retinoic acids, PML-RARα acts as a strong repressor of transcription, because of increased affinity to corepressors and histone deacetylases.

- This effect is overcome with pharmacological doses of retinoic acids, when repressory complex dissociate away and coactivators are recruited, leading to transcriptional activation of target genes.

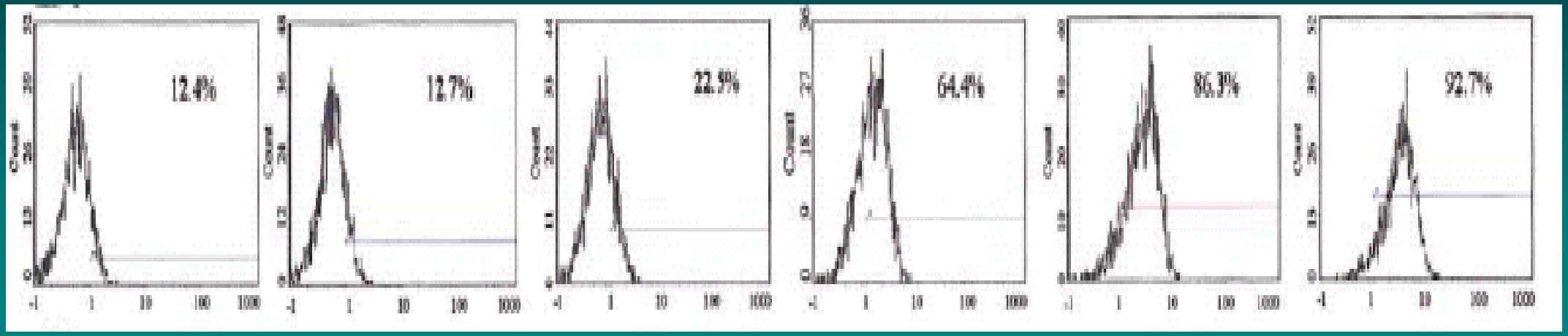
Viability (A) and proliferation rate (B) of cells exposed to ATRA at different concentrations in suspension culture for 72 hours. The results anre mean values (±SD) of eight OU-AML cell lines in two independent experiments.



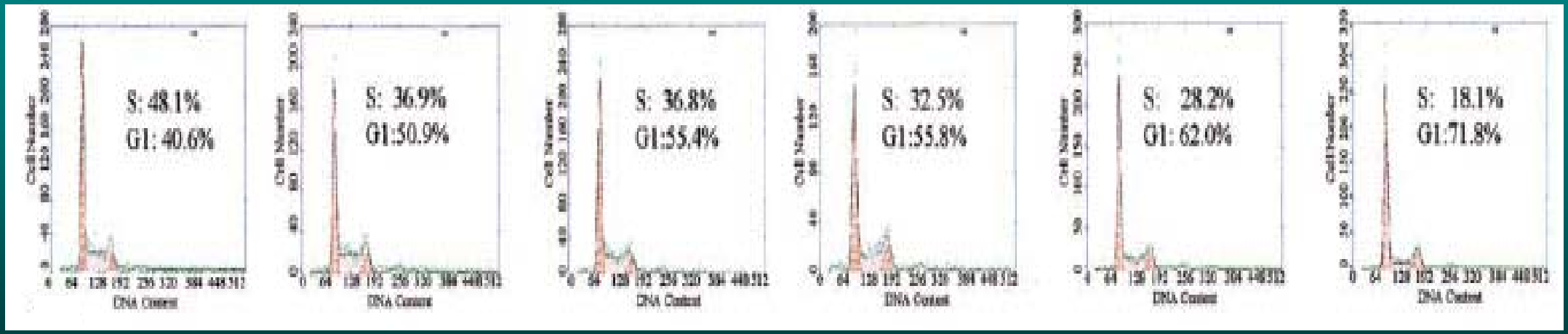


Effect of ATRA treatment on APL cells

CD11b expression (FACS analysis)



proliferation (FACS analysis, PI staining)

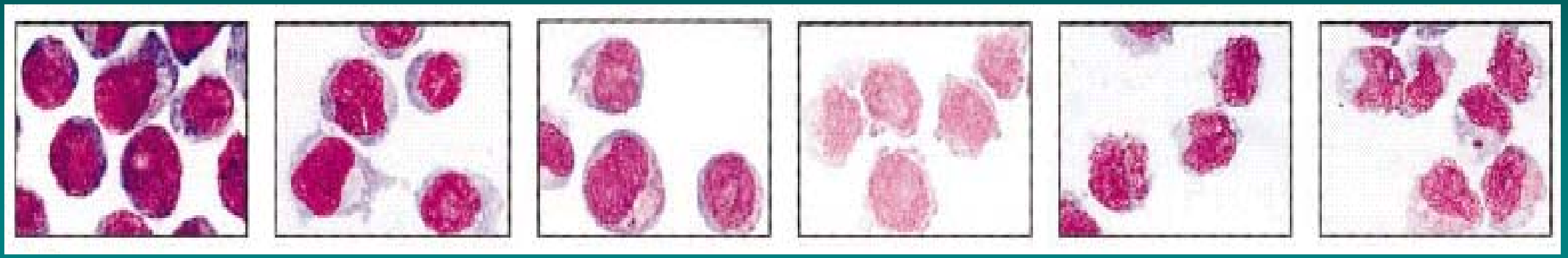


control

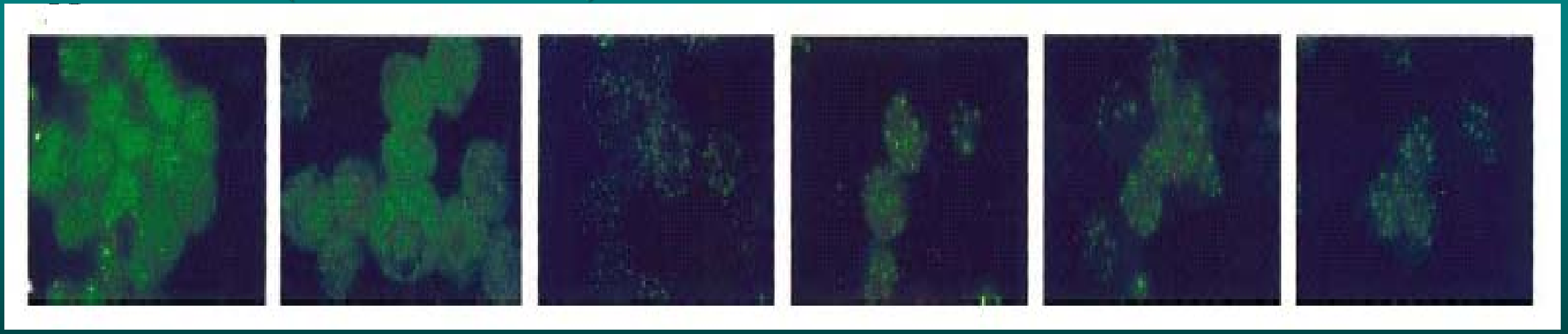
ATRA (time)

Effect of ATRA treatment on APL cells

morphology



PML localization (immunofluorescence)



control

ATRA (time)

all trans retinoic acid (ATRA) and APL treatment

- In patients suffering from APML treatment with high doses of ATRA can induce differentiation of promyelocytes into granulocytes.
- The results of clinical trials suggest that combined ATRA/chemotherapy (with anthracycline) is superior than treatment with ATRA alone: relapse at 2 years was reduced from 16% to 6%, and event-free survival at 2 years was increased from 77% to 84% in patients who received combined ATRA/chemotherapy compared with those who received ATRA alone.
- This relatively small difference, however, indicates that ATRA alone remains an excellent choice for patients who are poor candidates for aggressive chemotherapy.
- Despite the effectiveness of ATRA (+chemotherapy), a small percentage of patients continue to die of complications experienced during this phase of therapy.
- Catastrophic bleeding accounts for many of these deaths and is due to a hemorrhagic disorder that results from activation of both the fibrinolytic system and the coagulation cascade.

Thank you and see you next week...

What would be profitable to remember in June:

- pathogenesis of APL – role of retinoic acid in treatment of APL

- characteristic of PML-RAR proteins

- effects of vitamine A deficiency and overloading

- Accutane – therapeutic effects and side-effects

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

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