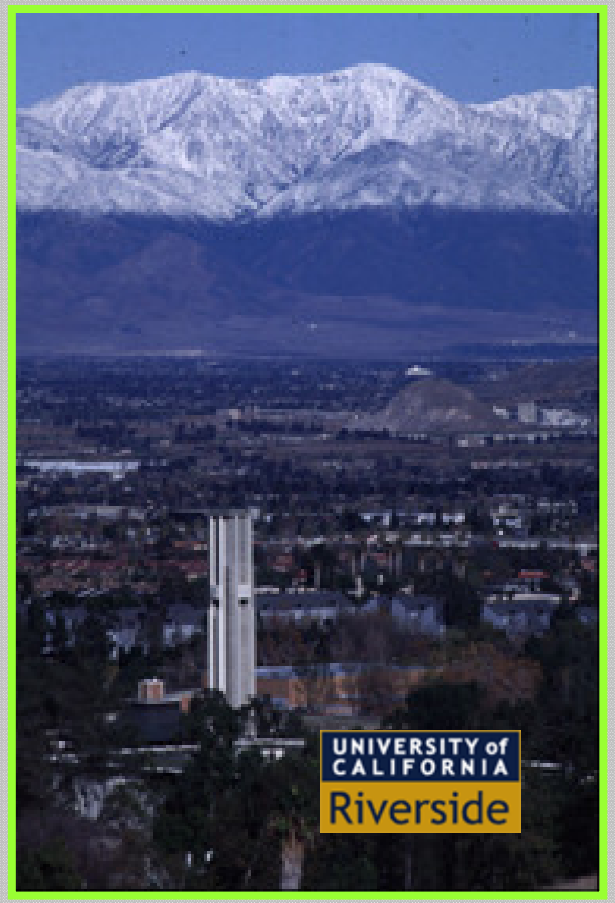
0mics GROUP CONFERENCES
2nd International Conference on ENDOCRINOLOGY
FROM VITAMIN D TO HORMONE D: FUNDAMENTALS OF THE VITAMIN D ENDOCRINE SYSTEM



[October 20th, 2014]

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Ph.D.
Department of Biochemistry &
Division of Biomedical Sciences
University of California
Riverside, CA

OUTLINE

CONCEPT OF A VITAMIN

CONCEPT OF AN ENDOCRINE SYSTEM

INTRODUCTION TO VITAMIN D

VITAMIN D ENDOCRINE SYSTEM

VITAMIN D RECEPTOR

MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY

OUTLINE

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SUMMARY

WHAT IS A VITAMIN ??

An organic molecule *essential* to metabolic processes, but which is *not* biosynthesized by the organism

AND.....

Therefore, to sustain life, there is a regular (~daily) dietary requirement !!

OUTLINE

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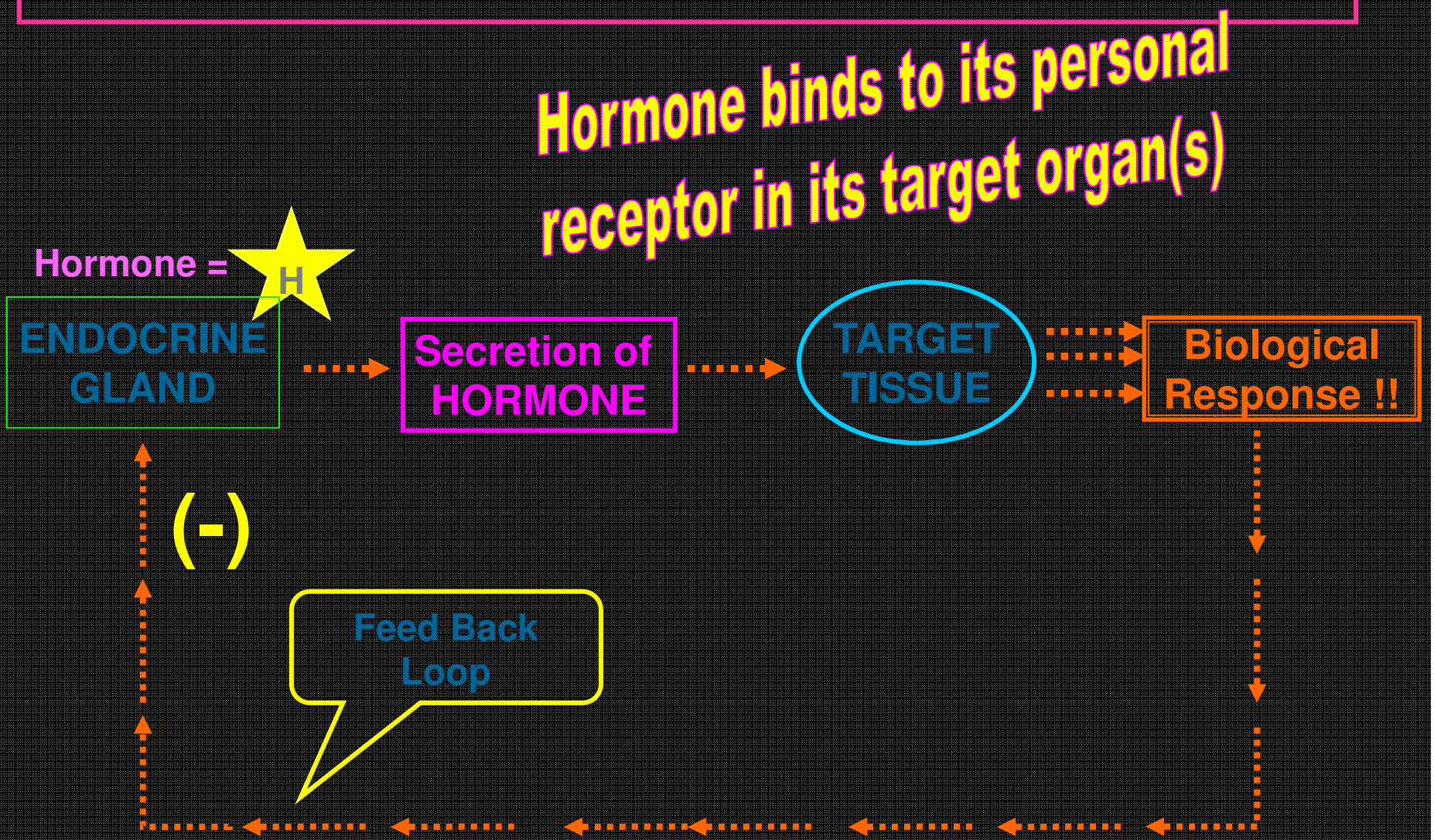
VITAMIN D ENDOCRINE SYSTEM

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SUMMARY

GENERAL MODEL OF HORMONE ACTION



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SUMMARY

WHAT IS THE HISTORICAL FUNCTION OF VITAMIN D ? ?

WITHOUT VITAMIN D

1. POOR CALCIUM ABSORPTION BY THE INTESTINE (from food)

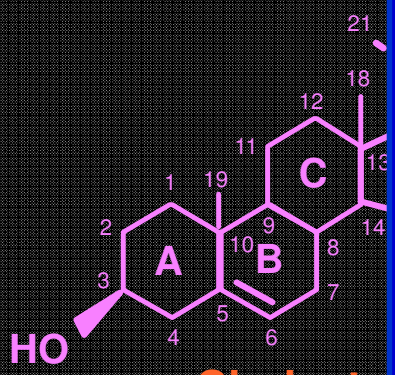
2. POOR INCORPORATION OF CALCIUM INTO THE BONES

BONE DISEASE OF RICKETS



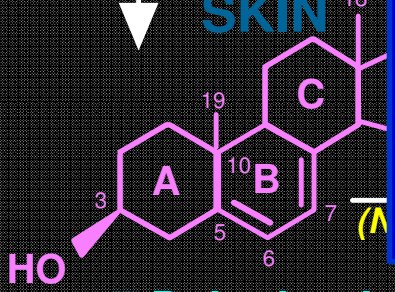
SO DOES VITAMIN D3

FUNCTION AS A
VITAMIN ? ?
or as a
STEROID HORMONE ??

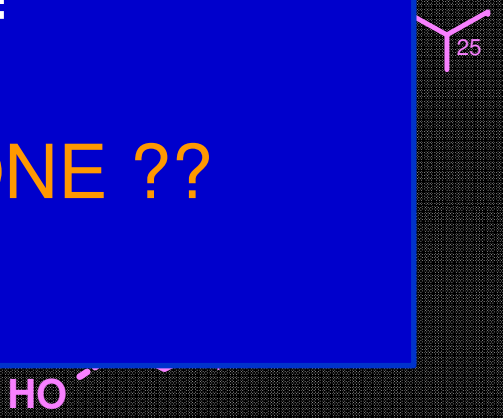


Choleste

In the SKIN



7-Dehydrocholesterol
(Pro-Vitamin D_3)



Vitamin D_3 or
Cholecalciferol

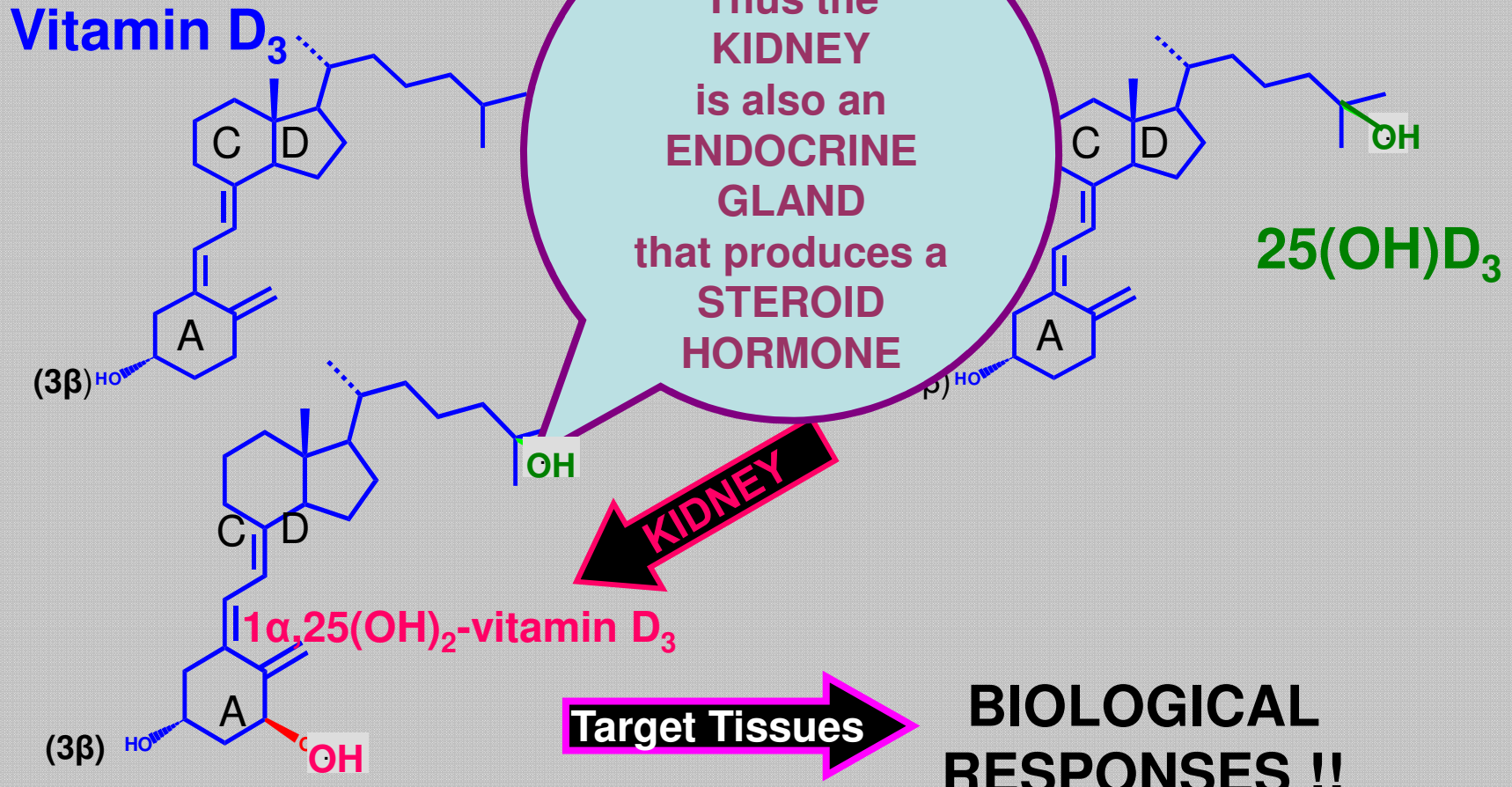
An Important Question for VITAMIN D

IN THE ABSENCE OF SUNLIGHT, DIETARY SUPPLEMENTATION WITH VITAMIN D_3/ CHOLECALCIFEROL PROMOTES IT TO A VITAMIN STATUS

E ANSWER

Vitamin D is inert.

Thus the KIDNEY is also an ENDOCRINE GLAND that produces a STEROID HORMONE



A $1\alpha,25(OH)_2D_3$ is a STEROID HORMONE !!

THE TISSUE DISTRIBUTION OF THE NUCLEAR $1\alpha,25(OH)_2D_3$ RECEPTOR (VDR) DEFINES THE SCOPE OF THE VITAMIN D ENDOCRINE SYSTEM

Adipose Hair Follicle Pituitary

Intestine Placenta

(1969 – 2012
Many Labs

The orignal three
VDR containing
target organs where
$1,25(OH)_2D_3$
was known in 1971 to produce
biological effects were

Intestine, Bone, Kidney

Some surprising new VDR target organs !!

KEY DISCOVERIES ALL AT UCR

JBC **243:**4055-4064 (1968)

The association of a metabolite of vitamin D_3 with intestinal mucosa chromatin *in vivo.*

Mark Haussler, Jim Myrtle, and Anthony W. Norman

Proc Natl Acad Sci U S A. 1969 January; **62(1):** 155–162.

CHROMOSOMAL RECEPTOR (VDR) FOR A VITAMIN D METABOLITE*

Mark R. Haussler and Anthony W. Norman

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA (RIVERSIDE)

"A Nutrition Classic": Nutrition Reviews **43:** 181-183 (1985

Science. 1971 Jul 2;173(991):51-4.

1,25-dihydroxycholecalciferol: Identification of the proposed active form of vitamin D3 in the intestine.

Norman AW, Myrtle JF, Midgett RJ, Nowicki HG, Williams V, Popjak G.

Science AAAS

FIRST CLINICAL USE OF 1α,25(OH)$_2$-VITAMIN D$_3$

New England J. Medicine **287:891** -895 (1972)

Action of 1,25-dihydroxy cholecalciferol, a potent kidney-produced metabolite of vitamin D$_3$ in uremic man.

A. S. Brickman, J. W. Coburn and A. W. Norman



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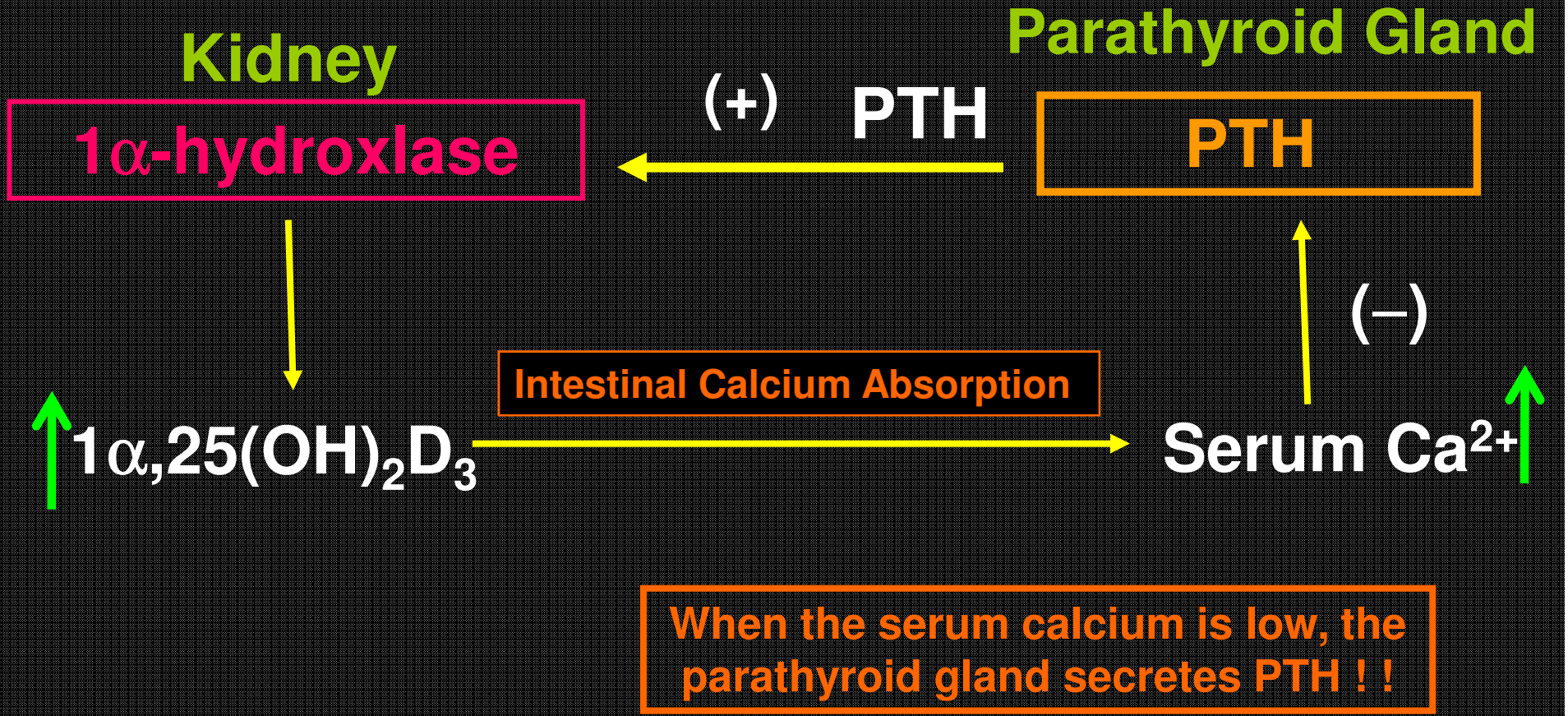
VITAMIN D ENDOCRINE SYSTEM

VITAMIN D RECEPTOR

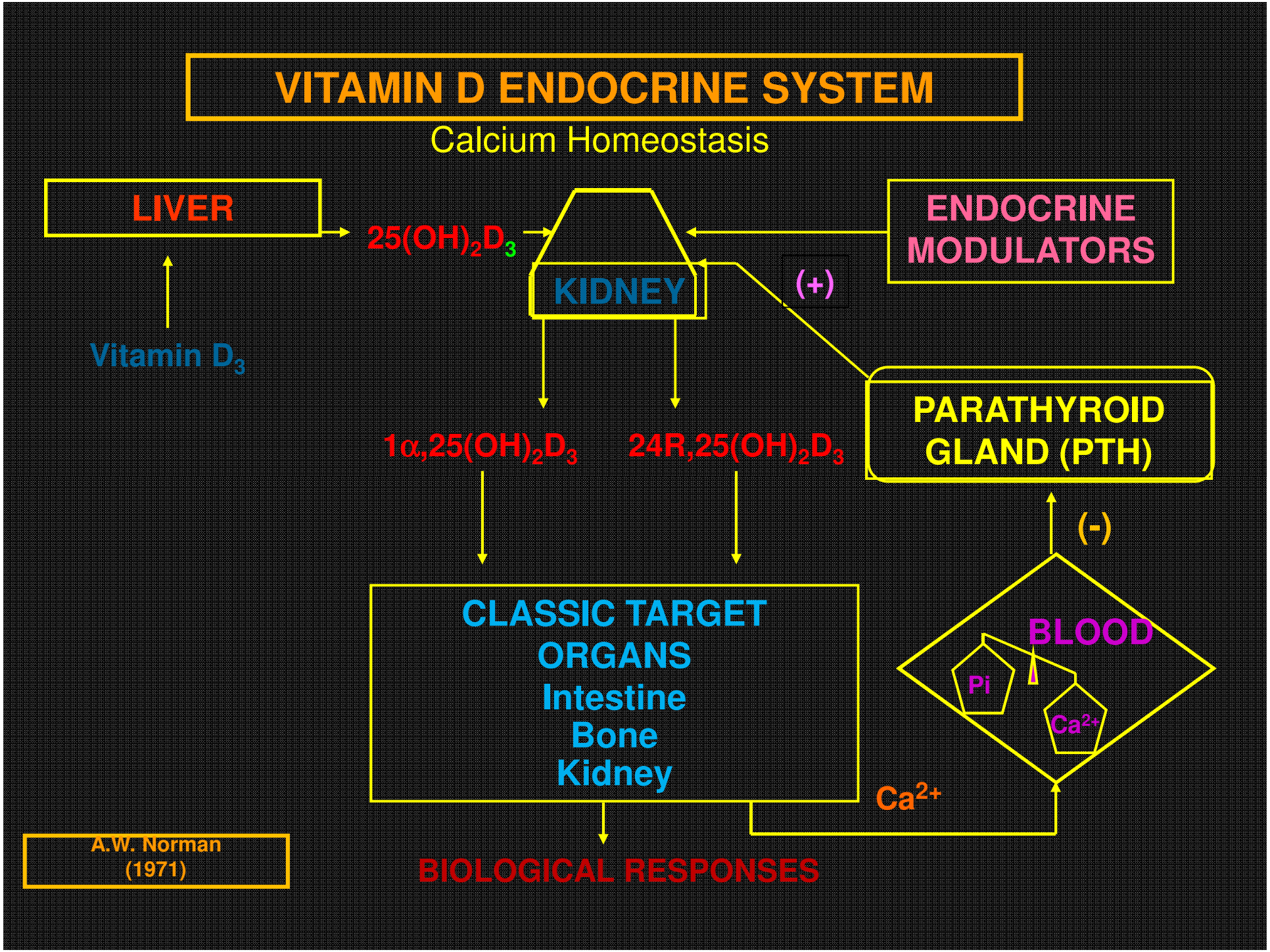
MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY

KIDNEY 25(OH)D-1α-Hydroxylase PRODUCTION OF $1\alpha,25(OH)_2D_3$ IS REGULATED VIA A FEEDBACK LOOP



When the serum calcium is low, the parathyroid gland secretes PTH ! !


VITAMIN D ENDOCRINE SYSTEM
Calcium Homeostasis
LIVER
25(OH)2D3
KIDNEY
ENDOCRINE MODULATORS
(+)
Vitamin D3
1α,25(OH)2D3
24R,25(OH)2D3
PARATHYROID GLAND (PTH)
(-)
CLASSIC TARGET ORGANS
Intestine
Bone
Kidney
BLOOD
Pi
Ca2+
Ca2+
A.W. Norman (1971)
BIOLOGICAL RESPONSES

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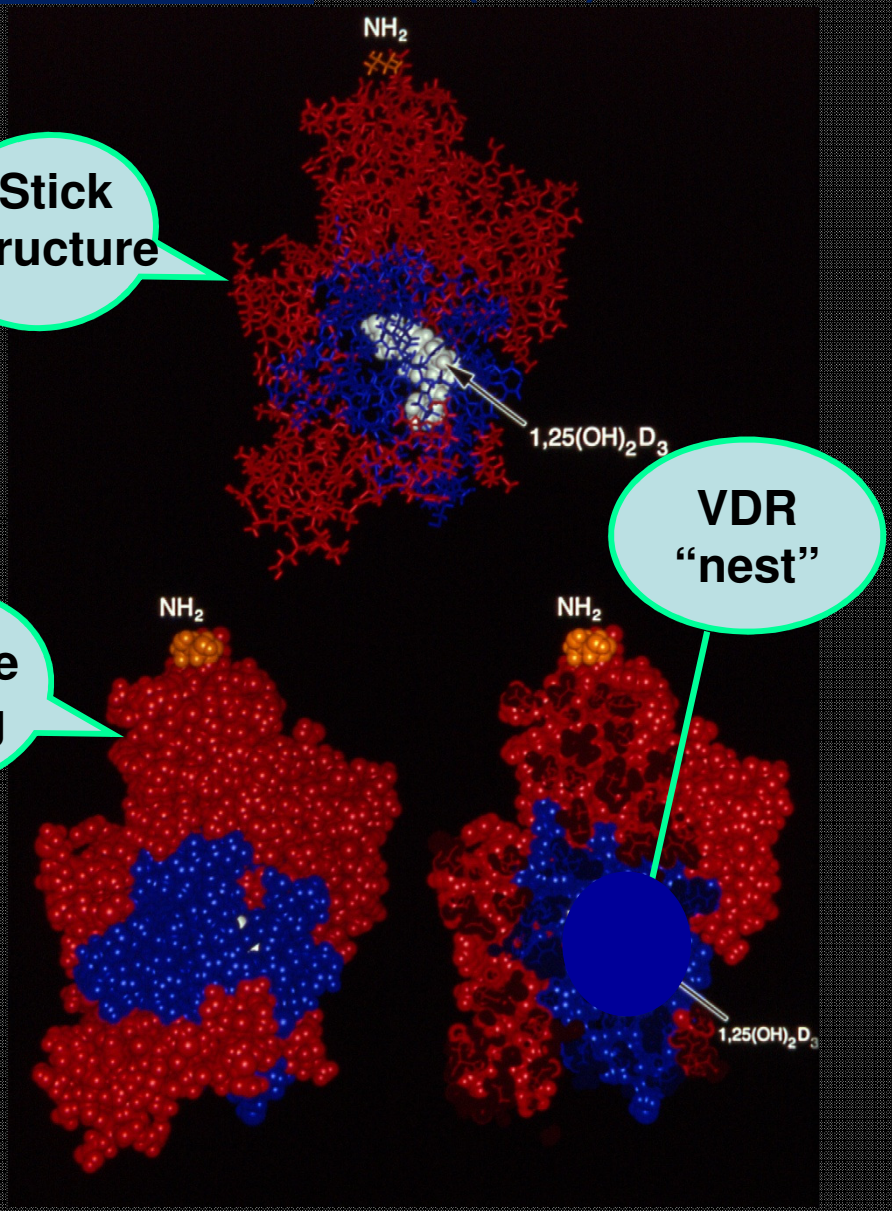
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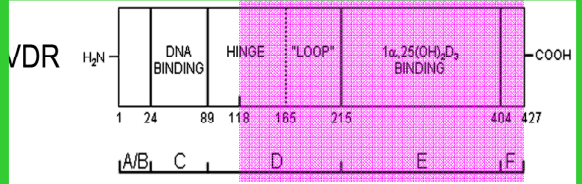
SUMMARY

X-RAY STRUCTURE OF VITAMIN D RECEPTOR

Rochel *et al.* Molecular Cell 5:173 (2000)

VDR LBD X-ray structure has 259 amino acids with approx. 5100 atoms.





VDR Crystal Structure (aa 118-427; Δ165-215)

LOSS OF THE VDR RECEPTOR RESULTS IN ALOPECIA



Loss of hair ! !

What is alopecia ???

The VDR is in the hair follicle.



Hereditary Vitamin D-resistant Rickets, type 2 [K45E]

X-RAY STRCTURE OF THE VITAMIN D BINDING PROTEIN (DBP)

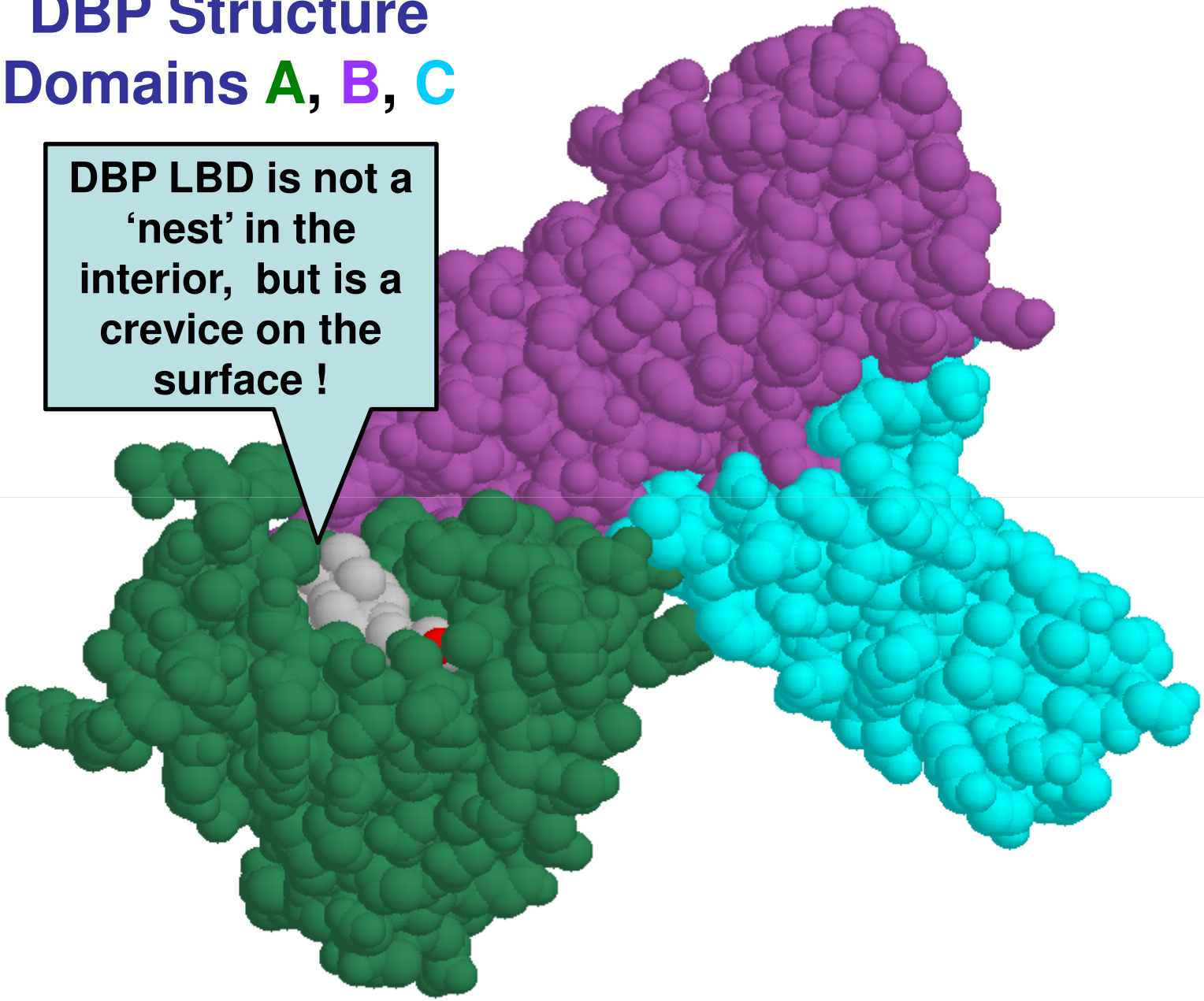
A structural basis for the unique binding features of the human vitamin D-binding protein

Christel Verboven[1], Anja Rabijns[1], Marc De Maeyer[2], Hugo Van Baelen[3], Roger Bouillon[3] and Camiel De Ranter[1]

[1]Laboratorium voor Analytische Chemie en Medicinale Fysicochemie, Faculteit Farmaceutische Wetenschappen, Katholieke Universiteit Leuven, E. Van Evenstraat 4, B-3000 Leuven, Belgium. [2]Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology (VIB), Herestraat 49, B-3000 Leuven, Belgium. [3]Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium.

nature structural biology • volume 9 number 2 • 131-136 • february 2002

DBP Structure
Domains A, B, C



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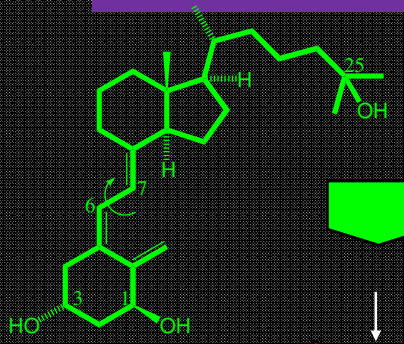
VITAMIN D ENDOCRINE SYSTEM

VITAMIN D RECEPTOR

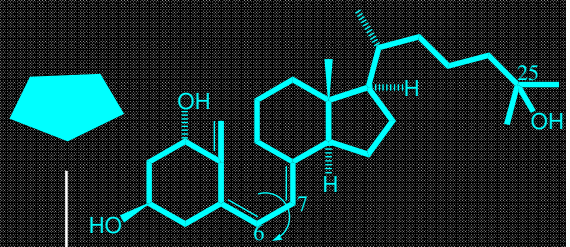
MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY

Modes of Action of $1\alpha,25(OH)_2D_3$

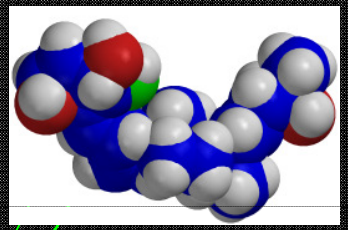


6-s-*trans* $1\alpha,25(OH)_2D_3$ 6-s-*cis*

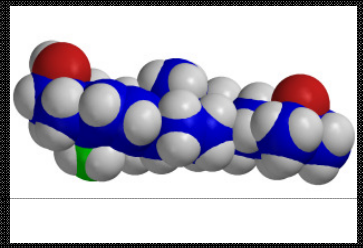


VDR_{Nuc}

VDR_{CavMem}



Ligand- nVDR complex



Ligand mVDR Complex

DNA

Up regulation

Down regulation

Genomic responses

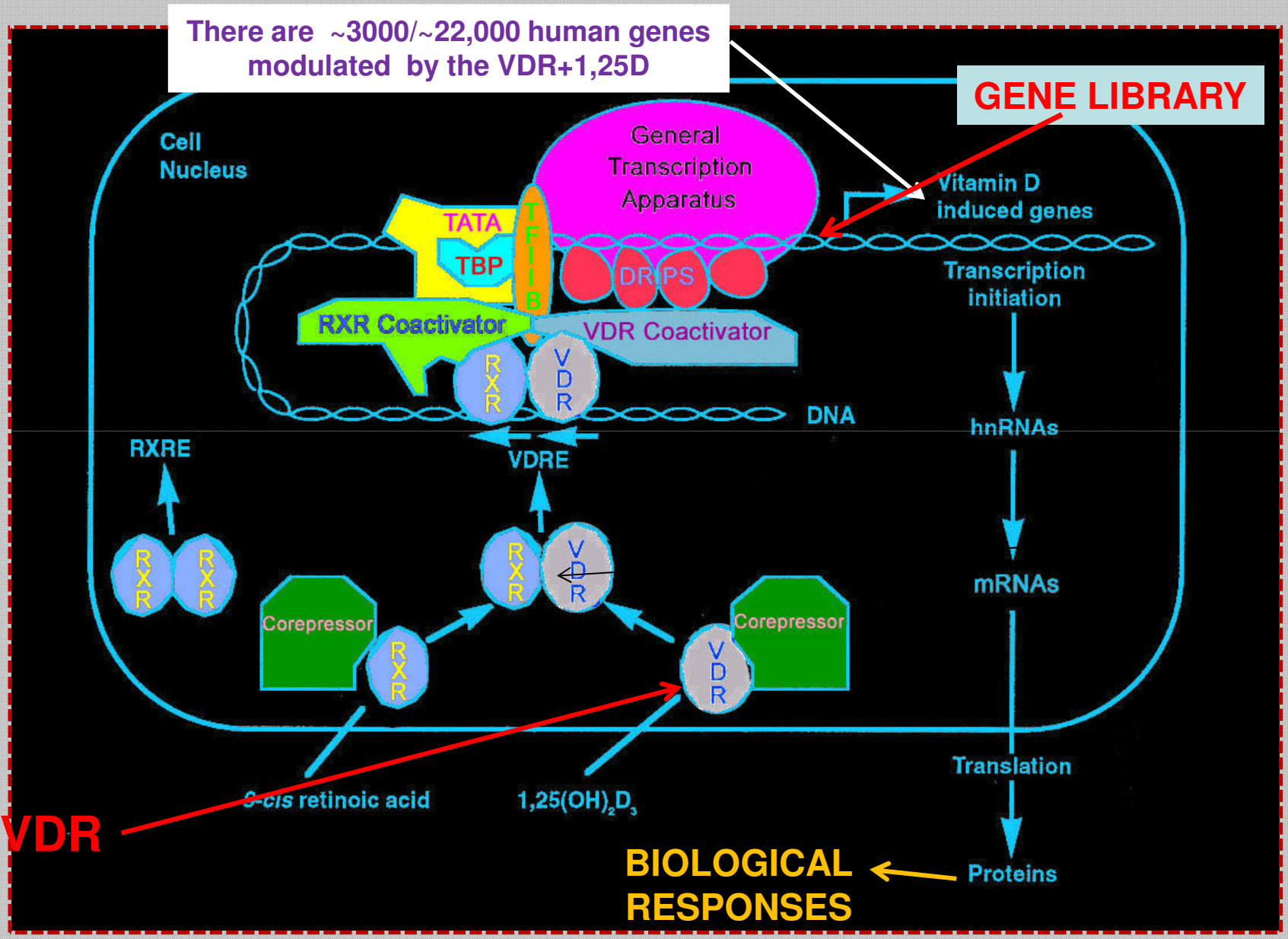
Rapid responses

Hours to Days

Biological Responses

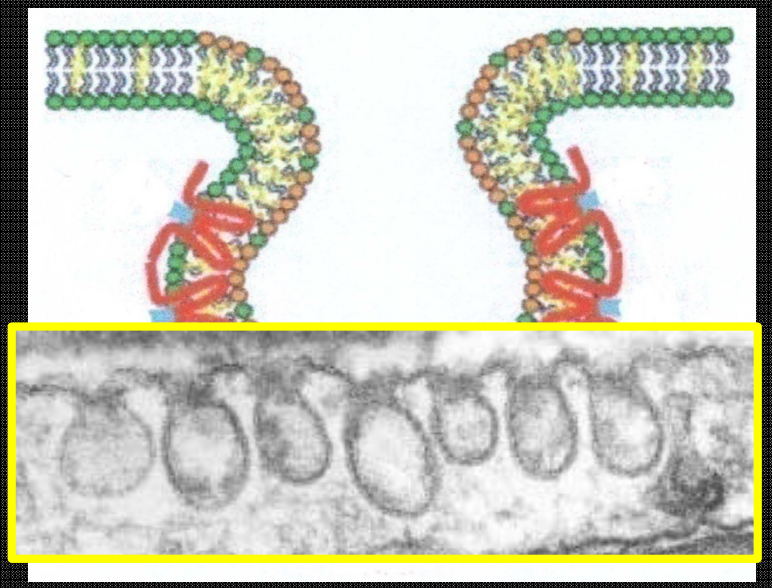
Seconds To Minutes

MODEL OF 1α,25(OH)$_2$D$_3$ TRANSCRIPTIONAL ACTIVATION OF THE GENE LIBRARY



Isolation of Caveolae Membrane Fractions Is there a VDR_{mem} present?

- **Approach:**
  - Differential Centrifugation
  - Absence of Detergents
- **Lipid raft-Caveolae (LR-C)**
  - Platforms
  - Unique buoyant density
  - Detergent-resistant

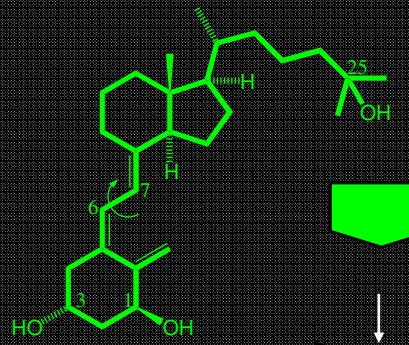


100 nm flask-shape invaginations or vesicles of the plasma membrane

http://www.rzuser.uni-heidelberg.de/~t73/plasmal/endoct.html

Smart *et al.* PNAS 92:10104 (1995)

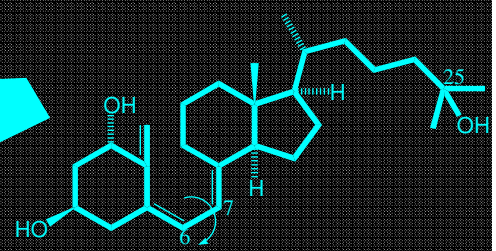
Modes of Action of $1\alpha,25(OH)_2D_3$



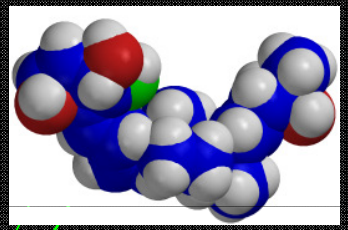
6-s-trans

$1\alpha,25(OH)_2D_3$

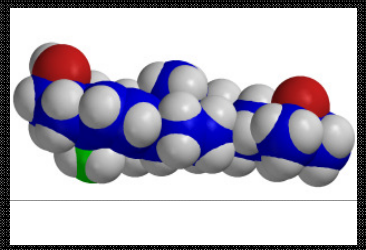
6-s-cis



VDR_{Nuc}



Ligand- nVDR complex



Ligand mVDR Complex

VDR_{CavMem}

DNA

Up regulation

Down regulation

Genomic responses

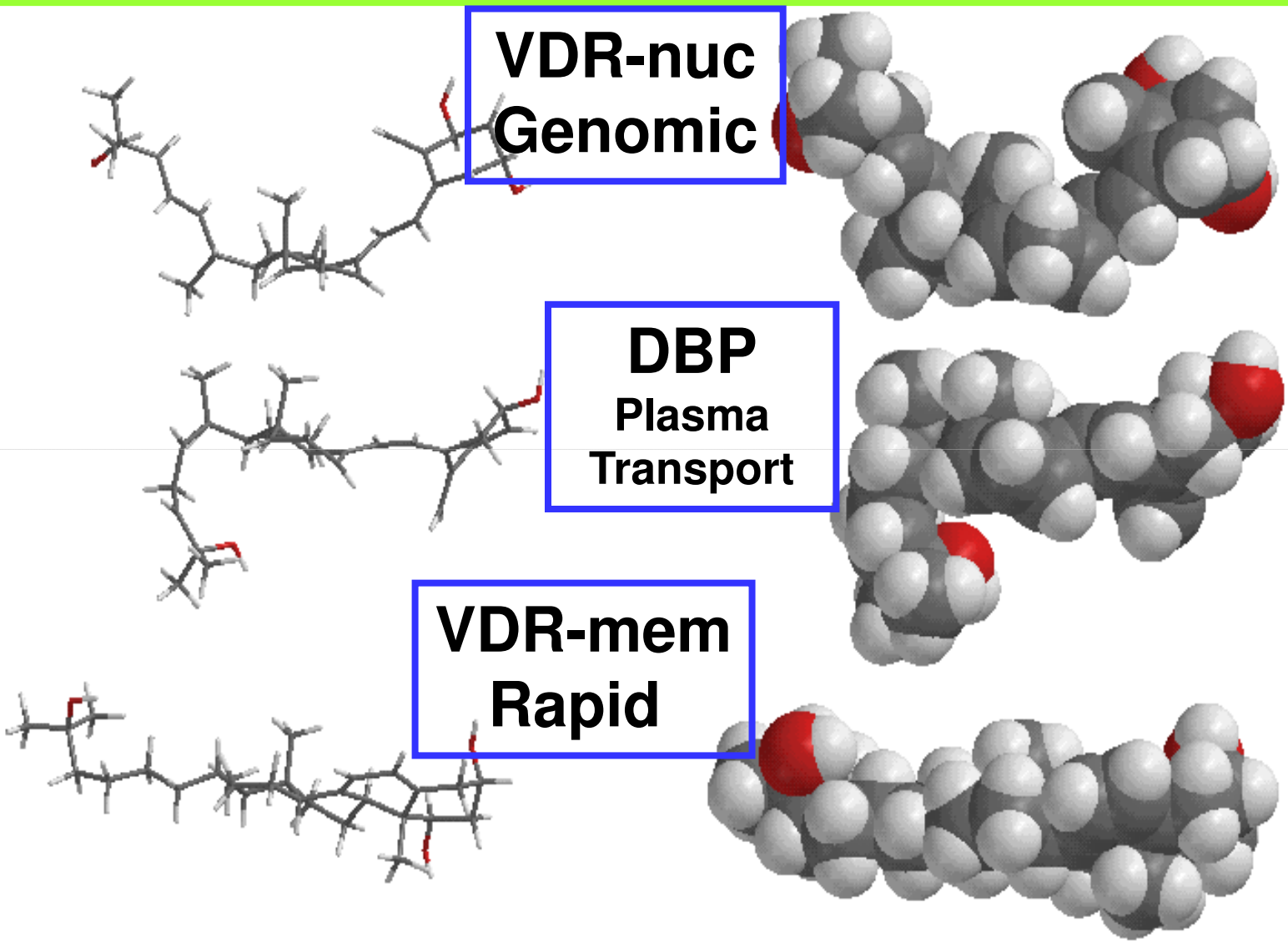
Rapid responses

Hours to Days

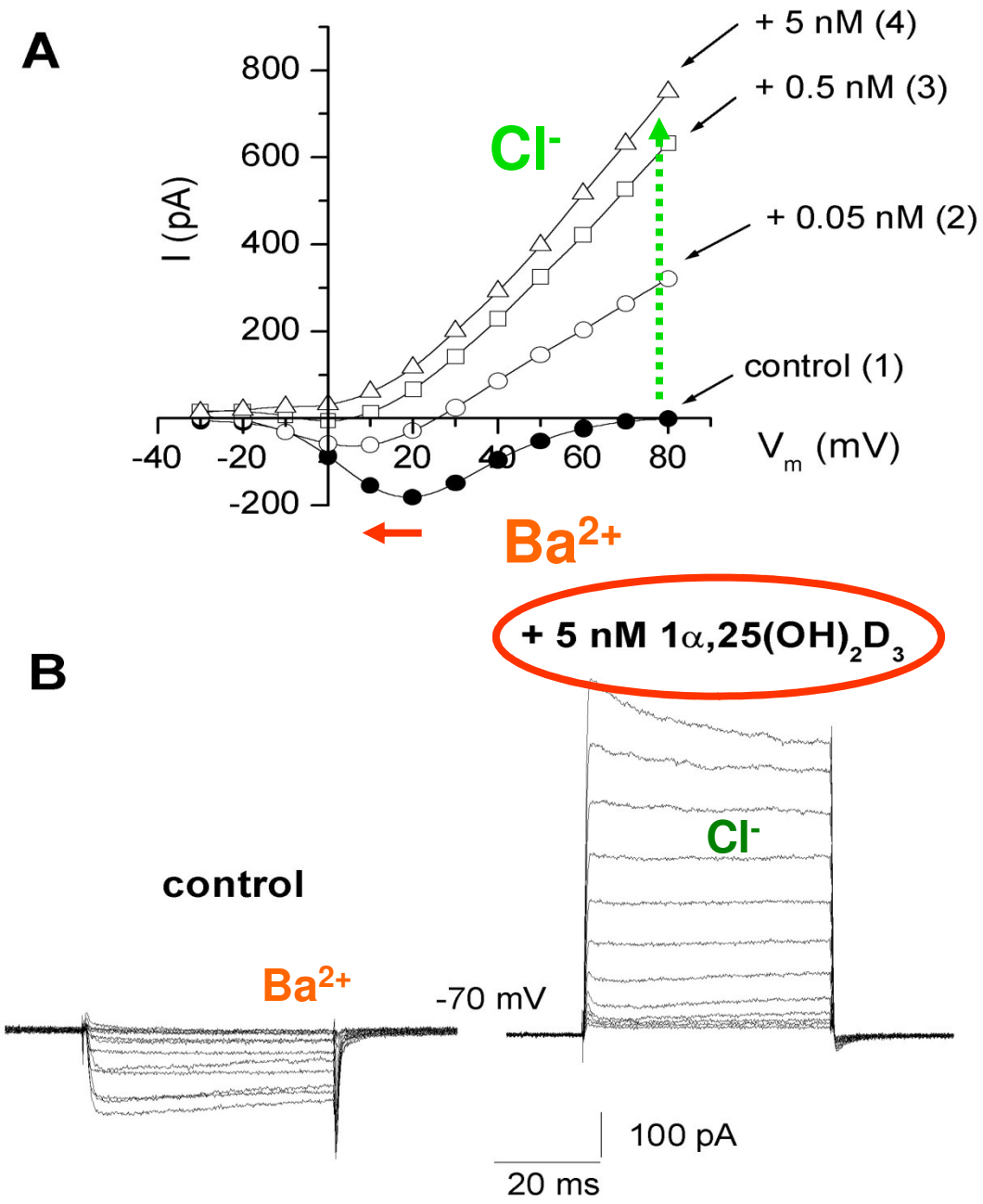
Biological Responses

Seconds To Minutes

SUMMARY OF OPTIMAL LIGAND SHAPES

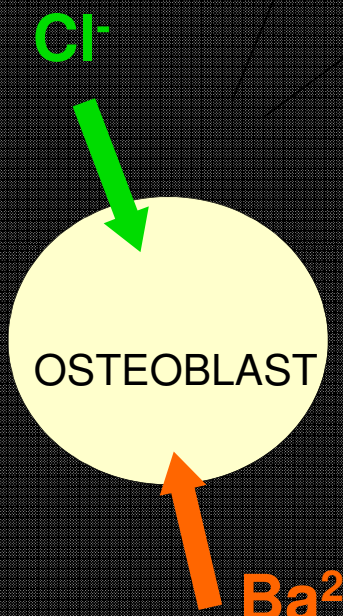


POTENTIATION OF ION CURRENTS BY 1,25D_3 IN OSTEOBLASTS



Whole-cell patch-clamp recordings

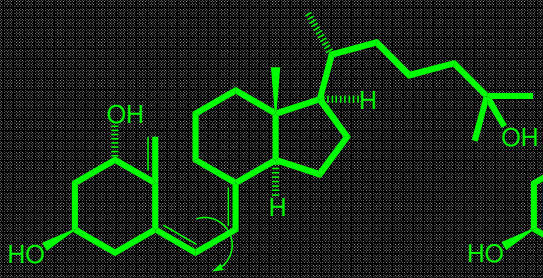
RAPID RESPONSES



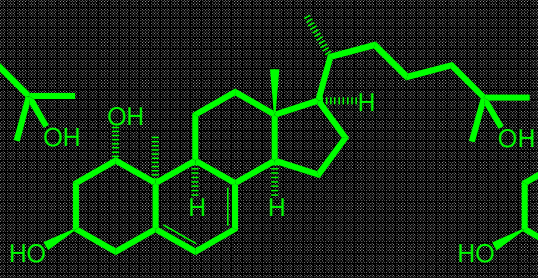
Zanello and Norman, JBC, 199

MOLECULAR TOOLS

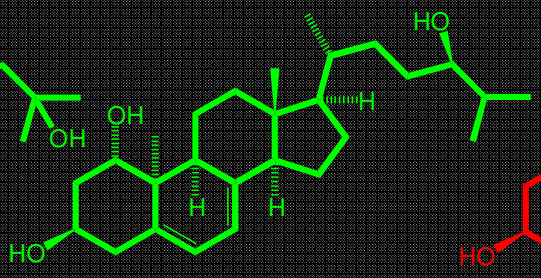
RAPID RESPONSES VDR_{Mem}



$1\alpha,25(OH)_2D_3$

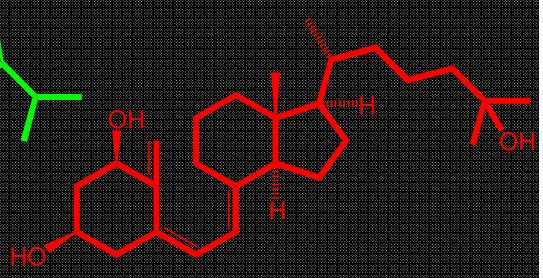


$1\alpha,25(OH)_2$-Lumisterol$_3$
JN



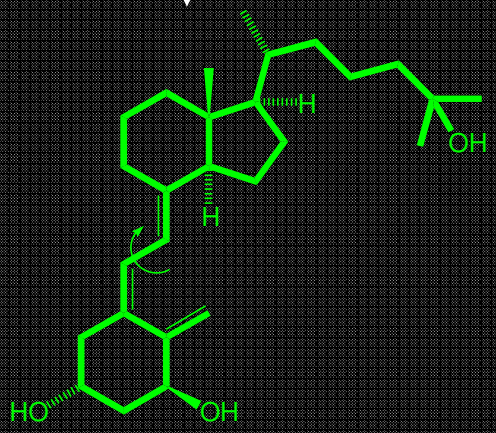
$1\alpha,24R(OH)_2$-Lumisterol$_3$
MZ

ANTAGONIST

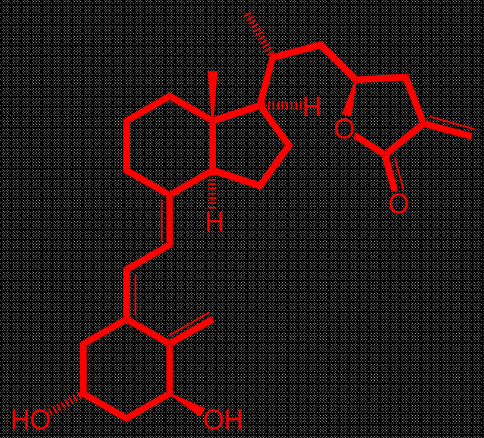


$1\beta,25(OH)_2D_3$
HL

GENOMIC RESPONSES VDR_{Nuc}

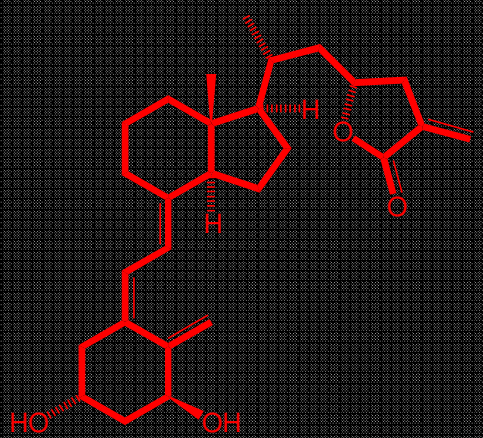


$1\alpha,25(OH)_2D_3$



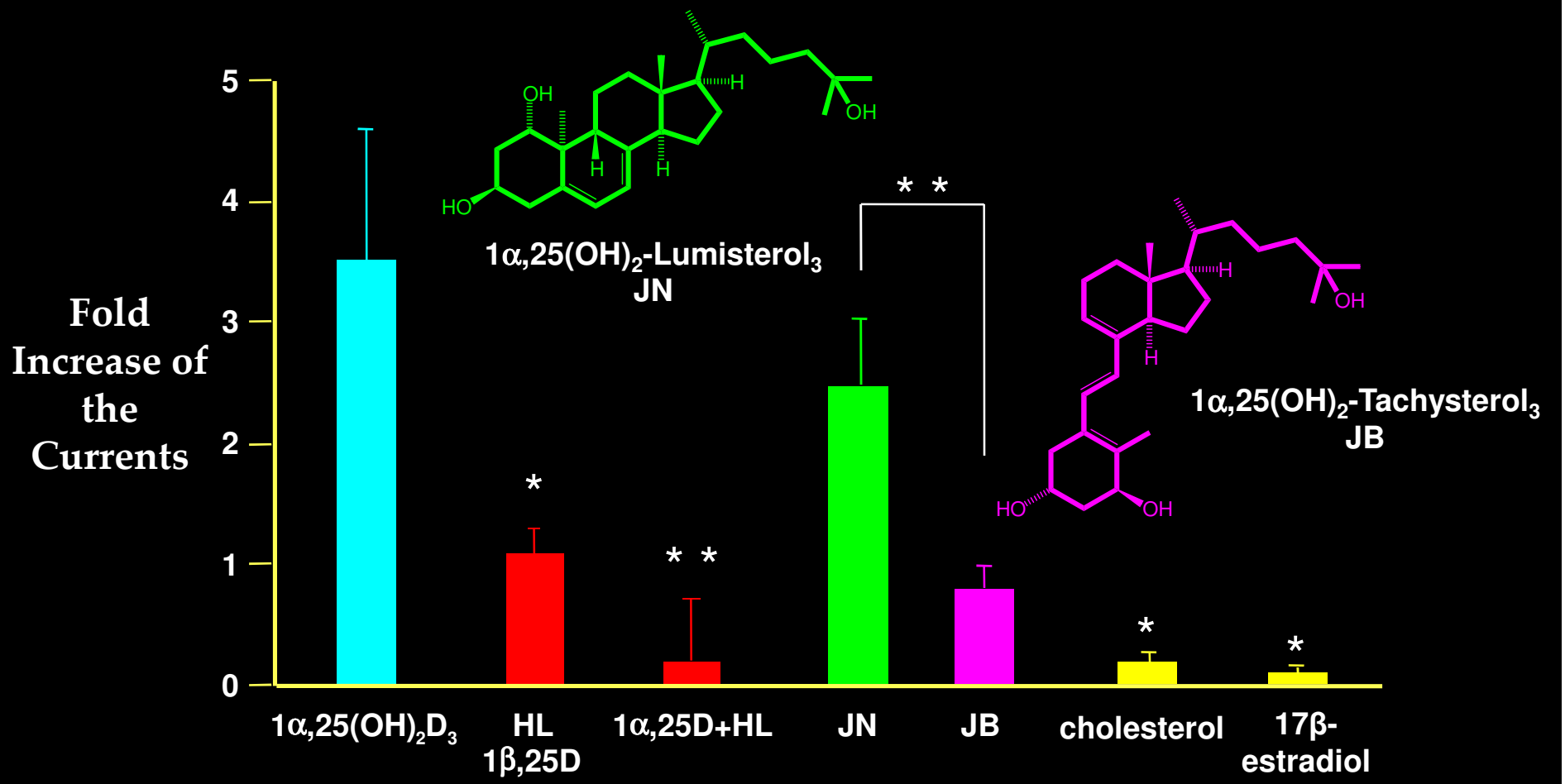
TEI-9647
MK

ANTAGONISTS



TEI-9648
ML

STRUCTURE FUNCTION STUDIES CONCERNING $1\alpha,25(OH)_2D_3$ STIMULATED CHLORIDE CHANNELS



$1\alpha,25(OH)_2D_3$ STRUCTURE-FUNCTION ANALYSIS OF *RAPID RESPONSES*

Response → / Agonist ↓	Cell/Organ: Chick Intestine	Pancreatic β-cells	Endothelial cells	Osteoblasts
	Transcaltachia (rapid Ca^{2+} absorption)	Insulin secretion	Smooth muscle cell migration	Opening Cl^- channels & secretion
$1\alpha,25(OH)_2D_3$ Conformationally Flexible	Yes	Yes	Yes	Yes
(JN) 6-s-*cis* locked	Yes	Yes	Yes	Yes
(JB) 6-s-*trans* restricted	No	...	...	No
(HL) $1\beta,25(OH)_2D_3$	Antagonism	Antagonism	Antagonism	Antagonism
Reference	Norman, Bouillon, Farach-Carson Mol. Endocrin. 11:1518 (1997)	Ishida, Norman Endocrin. 140: 4706 (1999)	Liao, Norman Circ. Res. 91:17 (2002)	Zanello, Norman JBC 272:22617 (1997, 2004)

OUTLINE

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CONCEPT OF AN ENDOCRINE SYSTEM

INTRODUCTION TO VITAMIN D

VITAMIN D ENDOCRINE SYSTEM

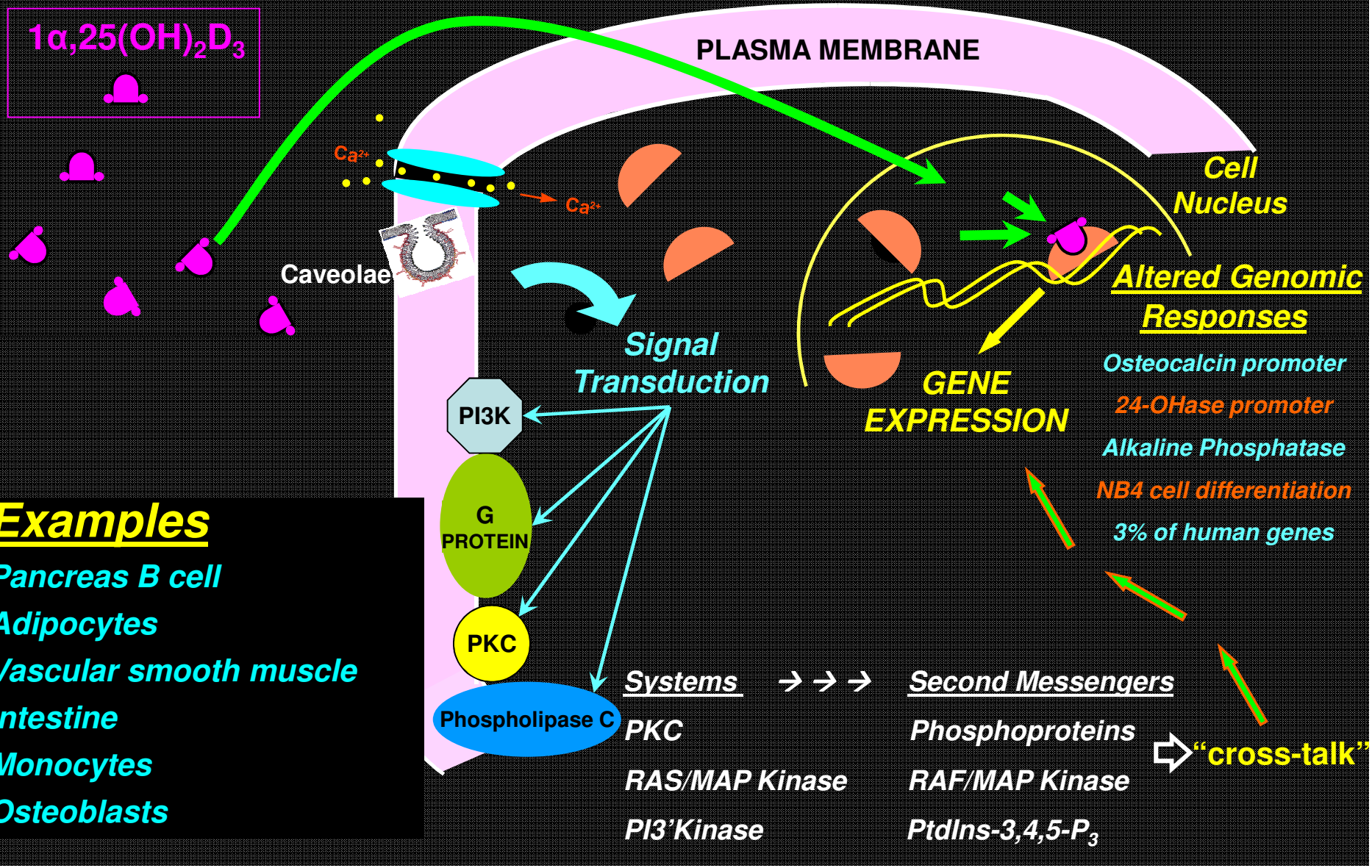
VITAMIN D RECEPTOR

MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY

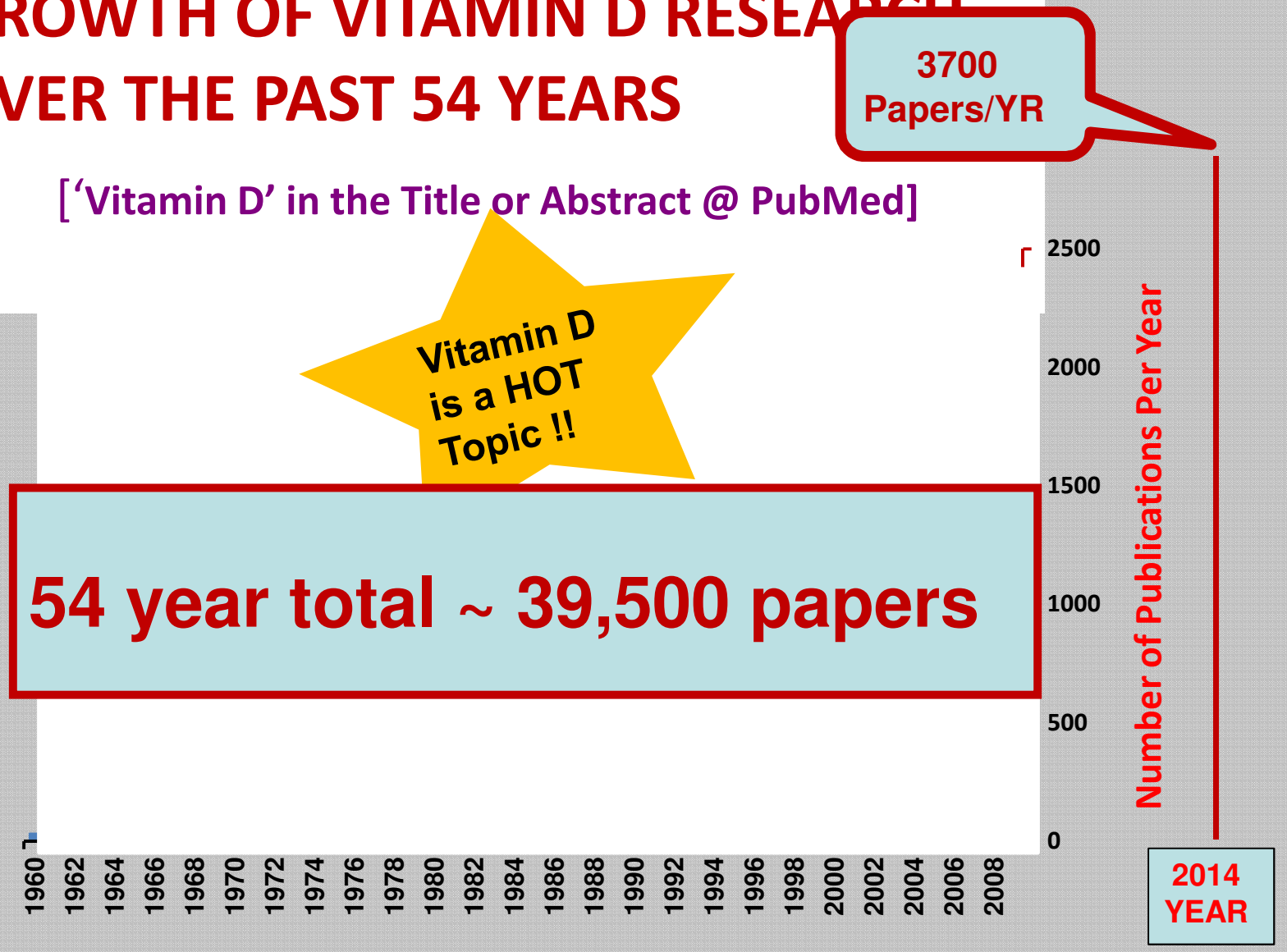
$1\alpha,25(OH)_2D_3$ + VDR = RAPID RESPONSES

Ligand Shape Matters **Affects Target Cell Biology**



GROWTH OF VITAMIN D RESEARCH OVER THE PAST 54 YEARS

['Vitamin D' in the Title or Abstract @ PubMed]



$1\alpha,25(OH)_2D_3$ ACTIVATES THE INNATE IMMUNE SYSTEM

Cutting Edge: 1,25-Dihydroxyvitamin D_3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression[1]

Tian-Tian Wang, Frederick P. Nestel,* Véronique Bourdeau,*¶ Yoshihiko Nagai,*‡ Qiuyu Wang,* Jie Liao,* Luz Tavera-Mendoza,† Roberto Lin,* John H. Hanrahan,* Sylvie Mader,¶ and John H. White[2]*†*

J. IMMUNOLOGY 173: 2909 (2004)

"The innate immune system provides front-line protection against…..
..infectious agents such as tuberculosis and antibiotic-resistant pathogens such as *Pseudomonas aeruginosa, the agent responsible for long term* infection in many cystic fibrosis patients".

Toll-Like Receptor Triggering of a Vitamin D–Mediated Human Antimicrobial Response

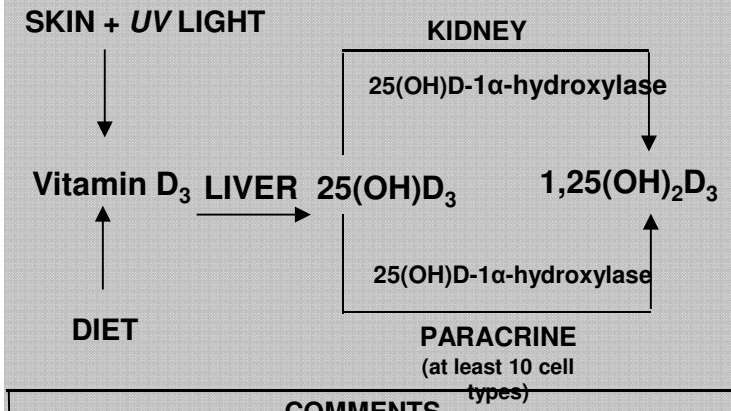
Philip T. Liu,[1,2]* Steffen Stenger,[4]* Huiying Li,[3] Linda Wenzel,[4] Belinda H. Tan,[1,2] Stephan R. Krutzik,[2] Maria Teresa Ochoa,[2] Jürgen Schauber,[5] Kent Wu,[1] Christoph Meinken,[4] Diane L. Kamen,[6] Manfred Wagner,[7] Robert Bals,[8] Andreas Steinmeyer,[9] Ulrich Zügel,[10] Richard L. Gallo,[5] David Eisenberg,[3] Martin Hewison,[11] Bruce W. Hollis,[12] John S. Adams,[11] Barry R. Bloom,[13] Robert L. Modlin[1,2]†

SCIENCE 311: 1770 (2006)

Norman, A.W. History of the discovery of vitamin D and its daughter steroid hormone. See Annals Nutrition & Metabolism 61:199 (2012).

A. W. Norman 2014

CONTRIBUTIONS OF VITAMIN D TO GOOD HEALTH



COMMENTS		
Vitamin D itself is biologically inactive. It is a precursor of 1α,25(OH)$_2D_3$.	Serum 25(OH)D_3 is a marker of vitamin D nutritional status. Its serum level should be 40 – 80 ng/ml	1α,25(OH)$_2D_3$ is a steroid hormone that produces biological responses via binding to its receptor (VDR) in at least 37 tissues.

Serum 25(OH)D		Nutritional Descriptor
ng/ml	nmoles/L	
< 5	<12	Severe D deficiency
< 10	< 25	Vit. D deficiency
10 - 20	25 – 50	Vit D. insufficiency
> 40	>100	Vit D. sufficiency
>100	>250	Risk for toxicity

PHYSIOLOGICAL SYSTEMS	BIOLOGICAL RESPONSES	VITAMIN D DEFICIENCY RELATED DISEASES
ALL CELLS	Cell cycle regulation Cell proliferation inhibition	Cancer Prostate, breast, colon cancer (prevention) Leukemia(s) (treatment)
CALCIUM HOMEOSTASIS	Intestinal calcium absorption & Bone remodeling	Rickets, Osteomalacia, Osteoporosis
IMMUNE SYSTEM INNATE ADAPTIVE	Stimulate synthesis of antimicrobial peptides Dendritic and T-cell function	Increased prevalence of infection e.g. tuberculosis Increased autoimmune diseases; e.g. type 1 diabetes, multiple sclerosis, inflammatory bowel disease, psoriasis
PANCREAS β Cells	Facilitate insulin secretion	Impaired glucose tolerance and type-II diabetes
HEART & CARDIOVASCULAR	Renin-angiotensin regulation, Coagulation, fibrinolysis, heart muscle function	High renin hypertension, increased cardiovascular risk factor, increased thrombogenesis
MUSCLE	Promote normal skeletal muscle development; improve muscle strength	Muscle myopathy; increased falls
BRAIN	*In progress* Brain has VDR & 1α-Hydroxylase	Vitamin D deficiency *in utero* alters adult behavior

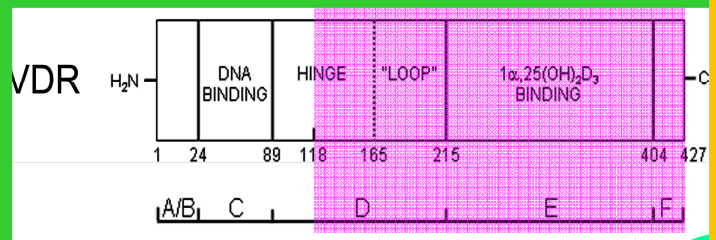
New evidence indicates that vitamin D3 intake should be about 2000-5000 IU/day. Everyone should have their physician order a serum 25(OH)D level determined at least once yearly. Vitamin D3 soft gel capsules (1000 or 5000 IU) .can be ordered from Bio-Tech Pharmacal on-line; their URL is http://www.biotechpharrmacal on-line. More health information is available at Grassroots Health More health information is available at their home page URL = **http://www.grassrootshealth.org/index.php [Email >** anthony.norman@ucr.edu]


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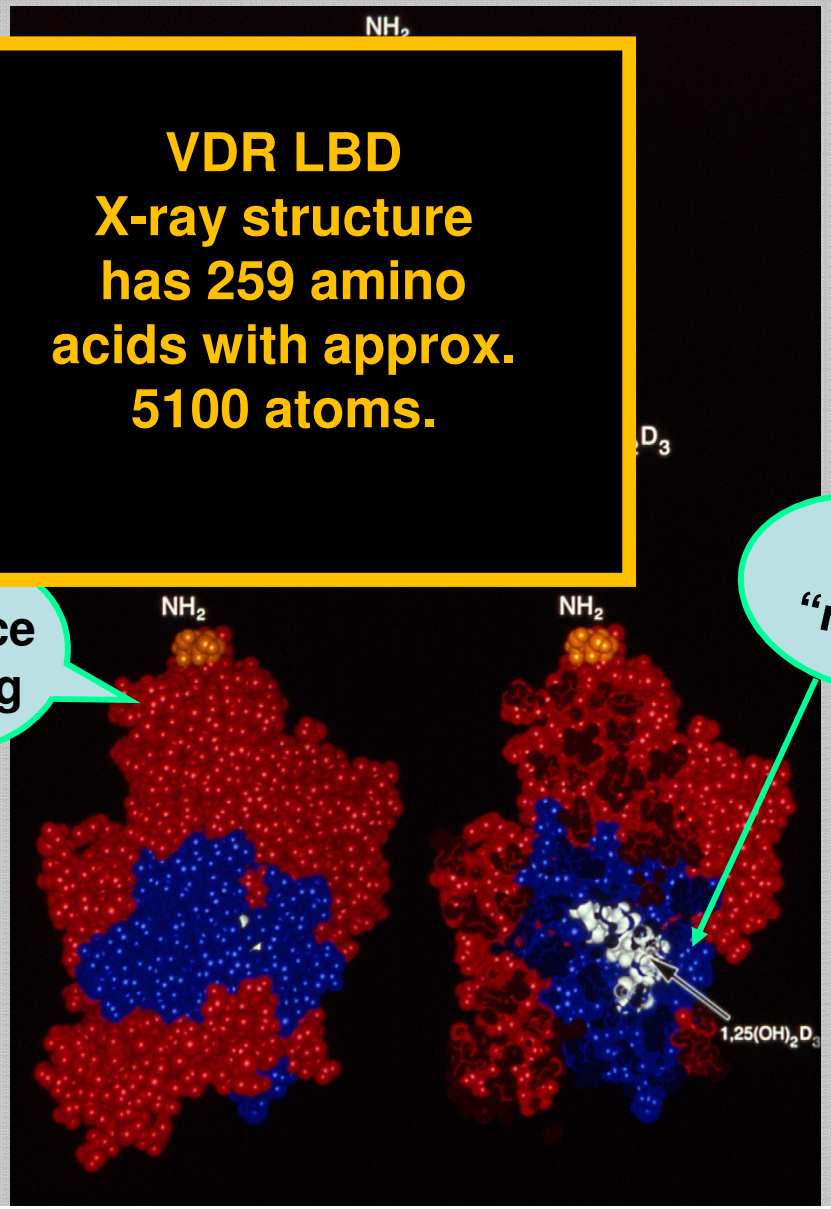
X-RAY STRUCTURE OF VITAMIN D RECEPTOR

Rochel *et al.* Molecular Cell 5:173 (2000)

VDR LBD
X-ray structure has 259 amino acids with approx. 5100 atoms.



VDR Crystal Structur
(aa 118-427; Δ165-215)



Space filling

VDR "nest"

OUTLINE

CONCEPT OF A VITAMIN

CONCEPT OF AN ENDOCRINE SYSTEM

INTRODUCTION TO VITAMIN D

VITAMIN D ENDOCRINE SYSTEM

VITAMIN D RECEPTOR

MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY

OUTLINE

WHAT IS A ‘VITAMIN’ ?

VITAMIN D ENDOCRINE SYSTEM

GENERAL MODEL OF HORMONE ACTION

DISCOVERY OF VITAMIN D RECEPTOR & DBP

MODES OF ACTION OF $1\alpha,25(OH)_2D_3$

SUMMARY