Aluminofluoride complexes (AlF_x) – the most dangerous combination of fluoride and aluminum

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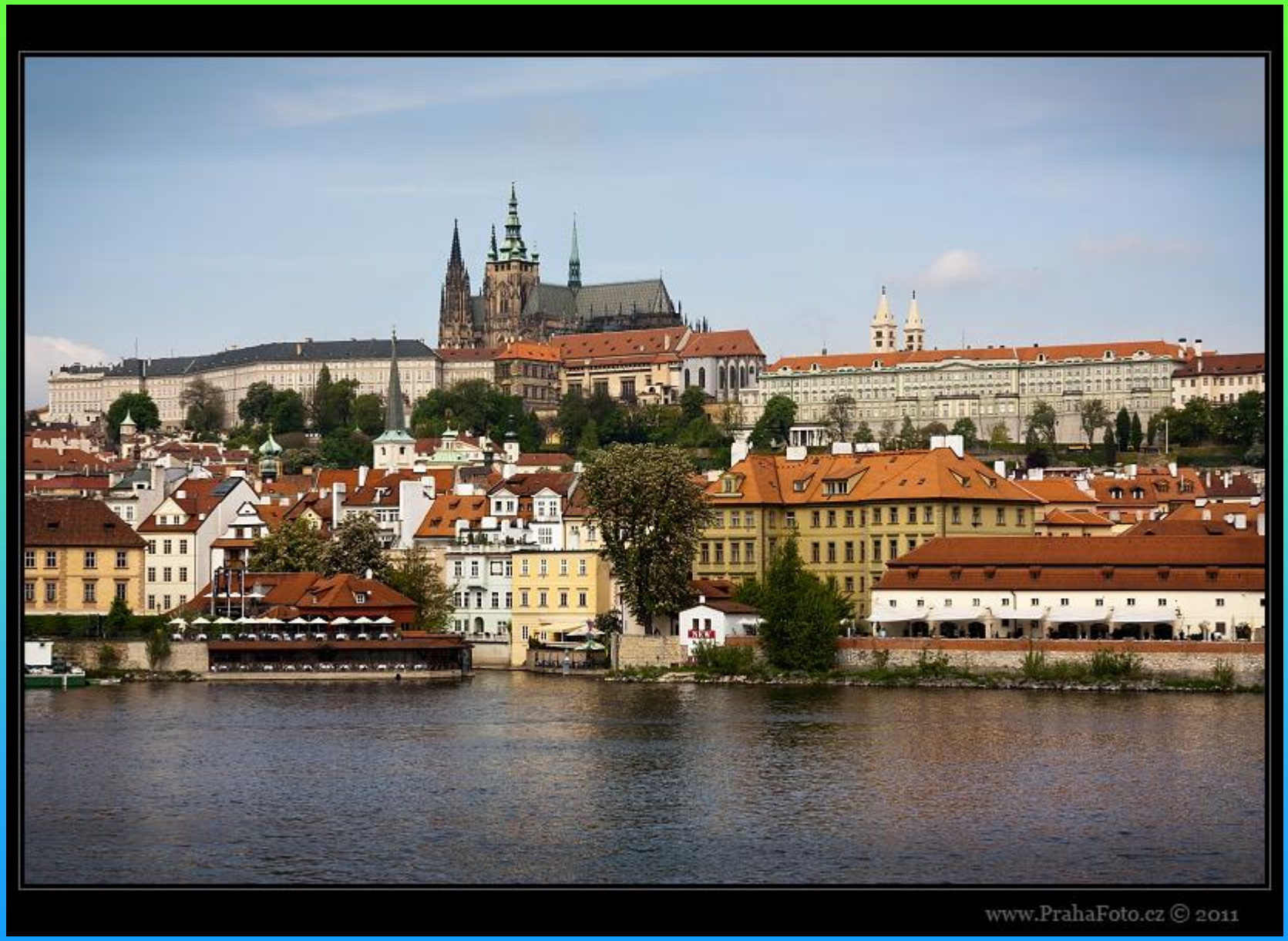
- **Introduction**
- **Interactions of AlF_x with G proteins**
- **Chemistry of AlF_x and phosphoryl-transfer reactions**
- **The role of G proteins in cell signaling: G protein coupled receptors**
- **Danger of fluoride and aluminum overload for the human health**

- References are available on CD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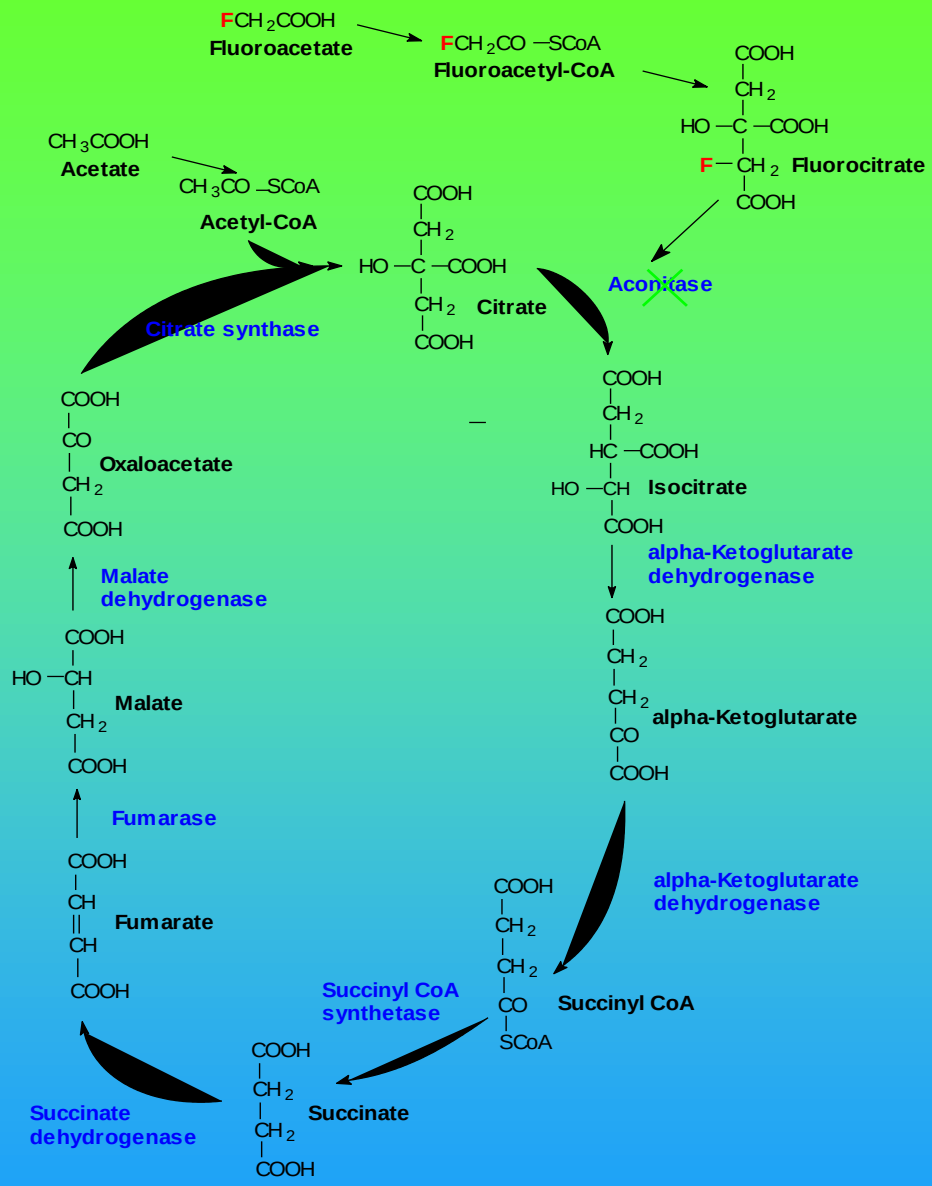


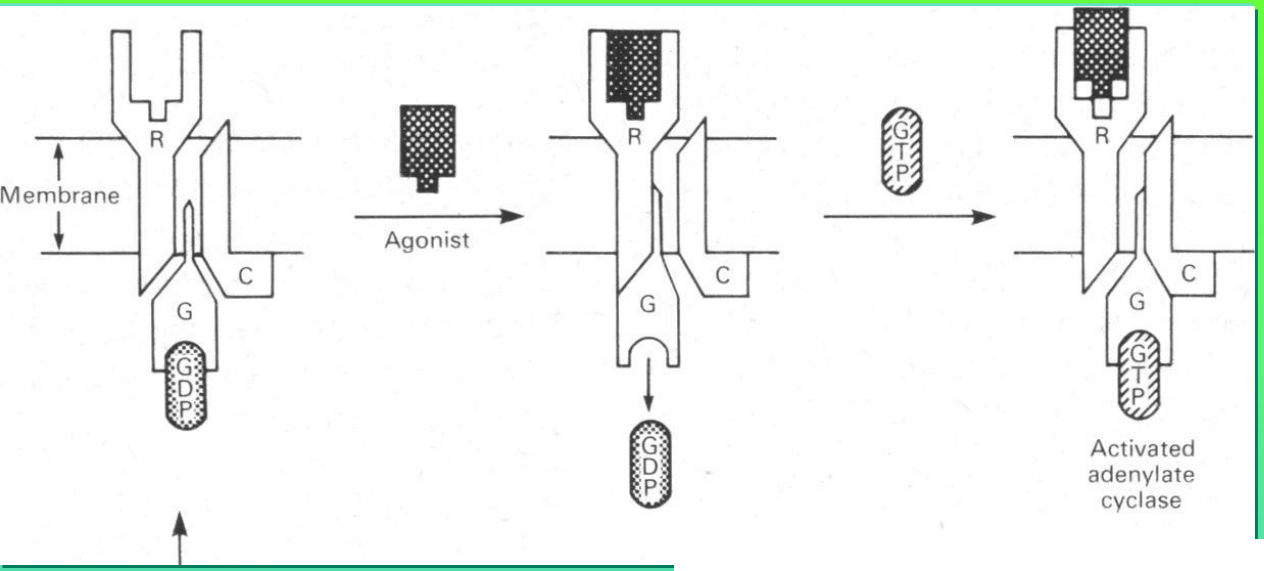
The use of NaF in laboratory helped in the discovery of glycolytic and Krebs-cycle pathways

INHIBITION

ENZYME	SOURCE	NaF
acid phosphatase	ram semen osteoblasts osteoclasts bone marrow, kidney	20-200 µM mM mM <0.5 mM
aconitase	liver	mM
adenylyl cyclase	liver fibroblasts	up to 10 mM 5 mM
AChE	red blood cell brain	0.01-10 mM 5-50 mM
arginase	liver, kidney	>4 mM
BuChe	blood plasma	50 µM
enolase	red blood cell hepatocytes embryonic cells oral bacteria	1-50mM 3 mM 1mM, 50µM 16-54 µM
F-ATPase	mitochondria	mM
glucose-6-phosphatase	liver	µM
glycogen synthase	hepatocytes	2-15 mM
IMPase	fibroblasts brain	mM 20 mM
lactate dehydrogenase	ram semen fetal osteoblast bone marrow	20-200 µM 6 – 60 µM <0.5 mM
lipase	pankreas, liver	10 mM
L-Ca^{2+}channels	heart	10 mM
Na^+/K^+ ATPase	plasma membrane kidney	1-10 mM 1-10 mM 5mM
PKC	retina	mM
PLD	liver, brain, lymphocyte	mM
protein phosphatase	liver bone	10 – 50 mM µM
pyrophosphatase	yeast	5 mM
pyruvate kinase	red blood cell	10-50 mM
succinate dehydrogenase	heart, liver, kidney	mM
urease	animal	mM

STIMULATION

ENZYME	SOURCE	NaF
adenylyl cyclase	heart, liver, brain lymphoma cell smooth muscle heart turkey RBC brain kidney	10 mM 10 mM 10 mM 1-10 mM 10 mM 10 mM 10 mM
alkaline phosphatase	bone cells	10-100 µM
aspartate transaminase	ram semen	20-200 µM NaF
Ca^{2+}-ATPase	heart, muscle SR	1-10 mM
c PIPsynthase	Liver	10mM
cytidylate cyclase	rat brain	mM
ERK	bone	1 – 10 mM
glu S-transferase	ram semen	20-200 µM
$K^+[ACh]_M$ channel	heart	>1 mM
K^+ATP channel	heart	mM
lactate dehydrogenase	hepatocytes ram semen	1-30 mM 20-200 µM NaF
L-type Ca^{2+} channel	rabbit femoral artery	10 mM
MAP kinases	lung	5-7.5 mM
glycogen phosphorylase	hepatocytes	1-50 mM
PI-3 kinase	human HepG2 cells	30 mM



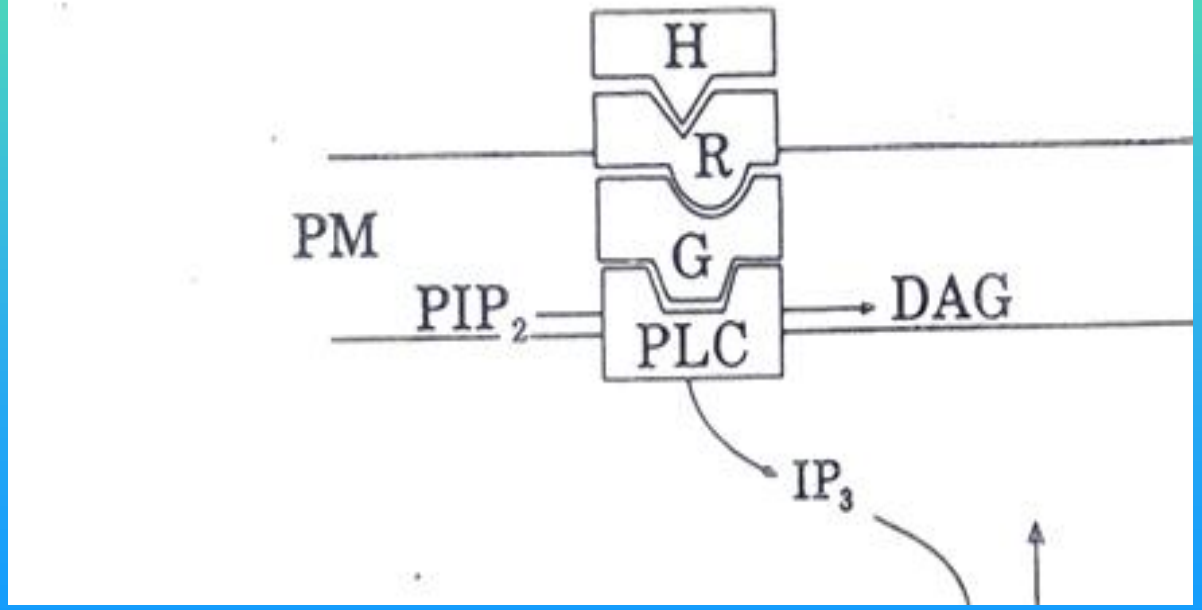
Adenylate cyclase

ATP → **cAMP**

R – receptor

G – G protein

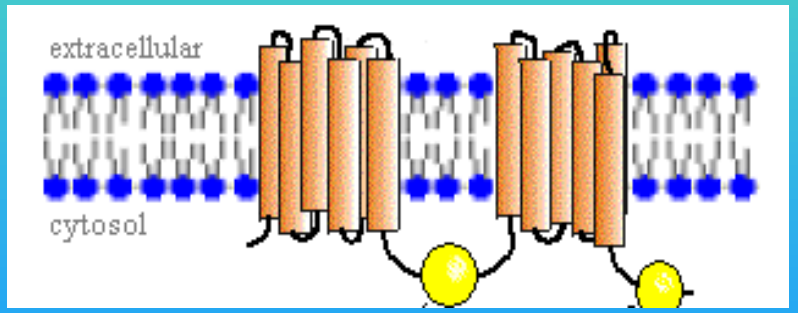
C – adenylate cyclase



Martin Rodbell

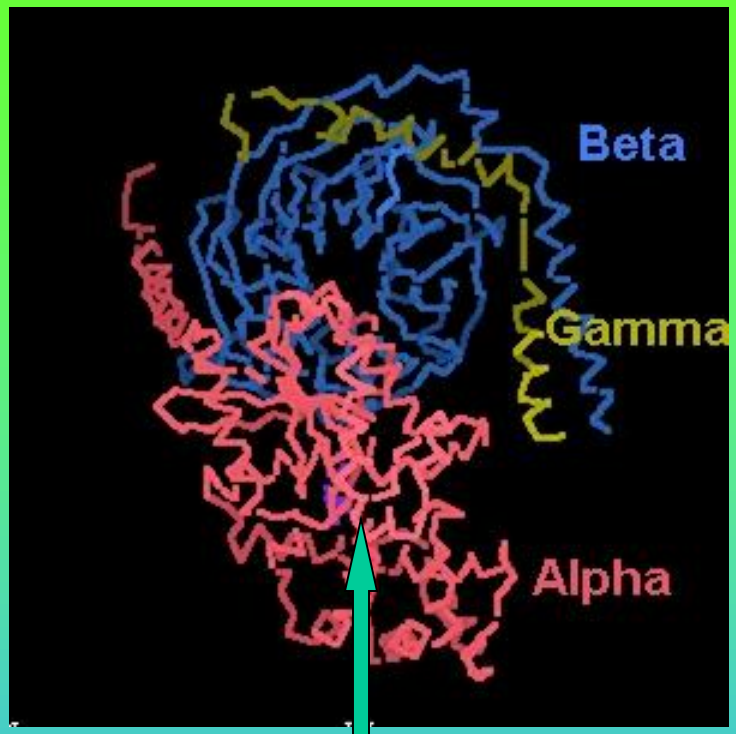
Alfred G. Gilman

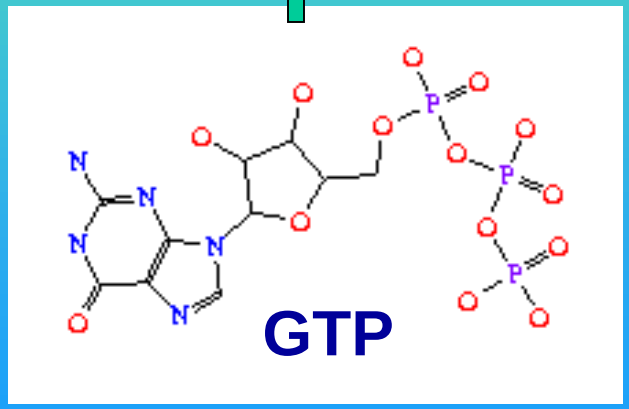
Fluoride was an important tool for the discovery of the **role of G proteins in cell signaling (Nobel Prize 1994).**



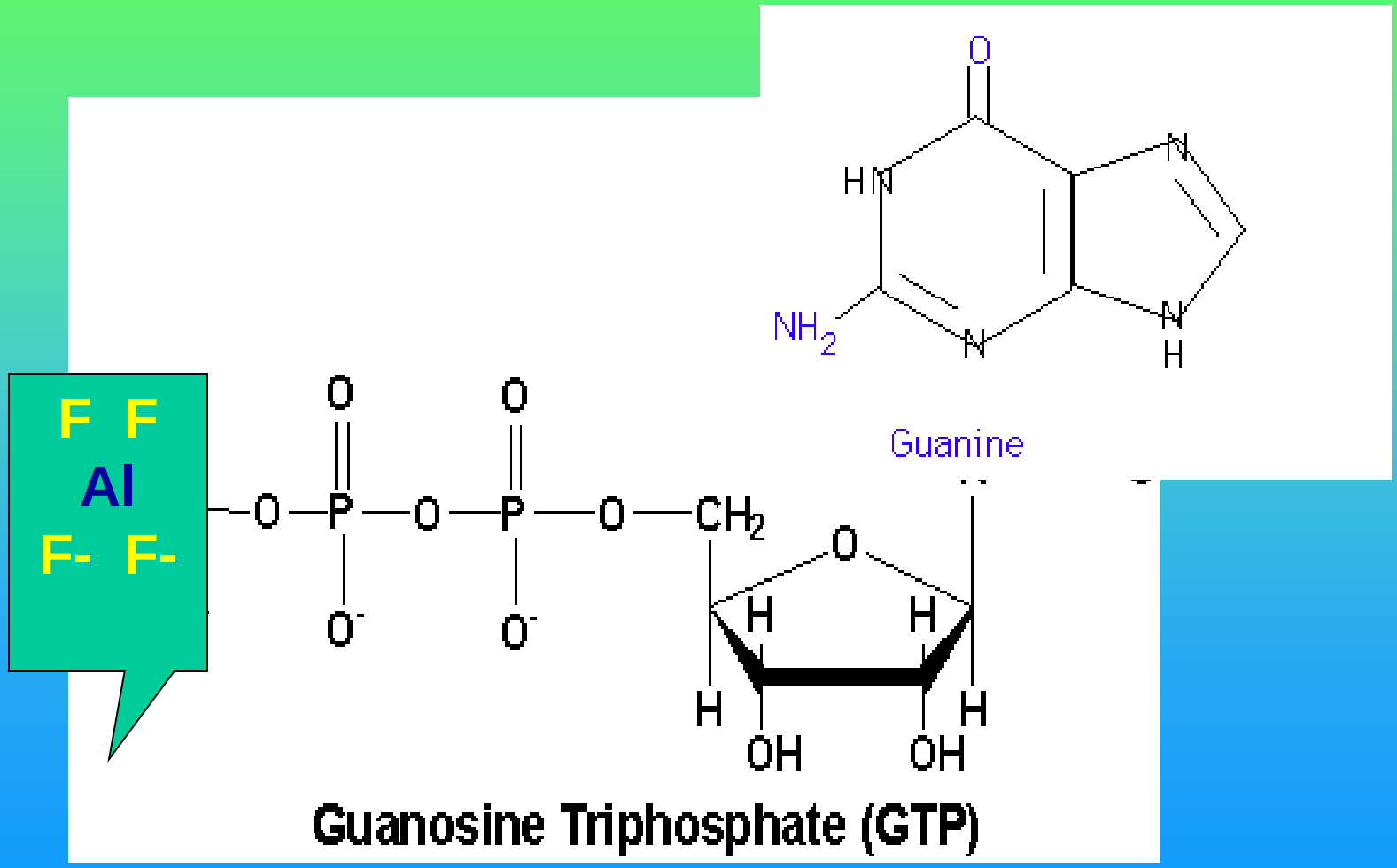
Rodbell 1982: Aluminum is a requirement for activation of adenylate cyclase by fluoride

Heterotrimeric G protein





AlF_4^-



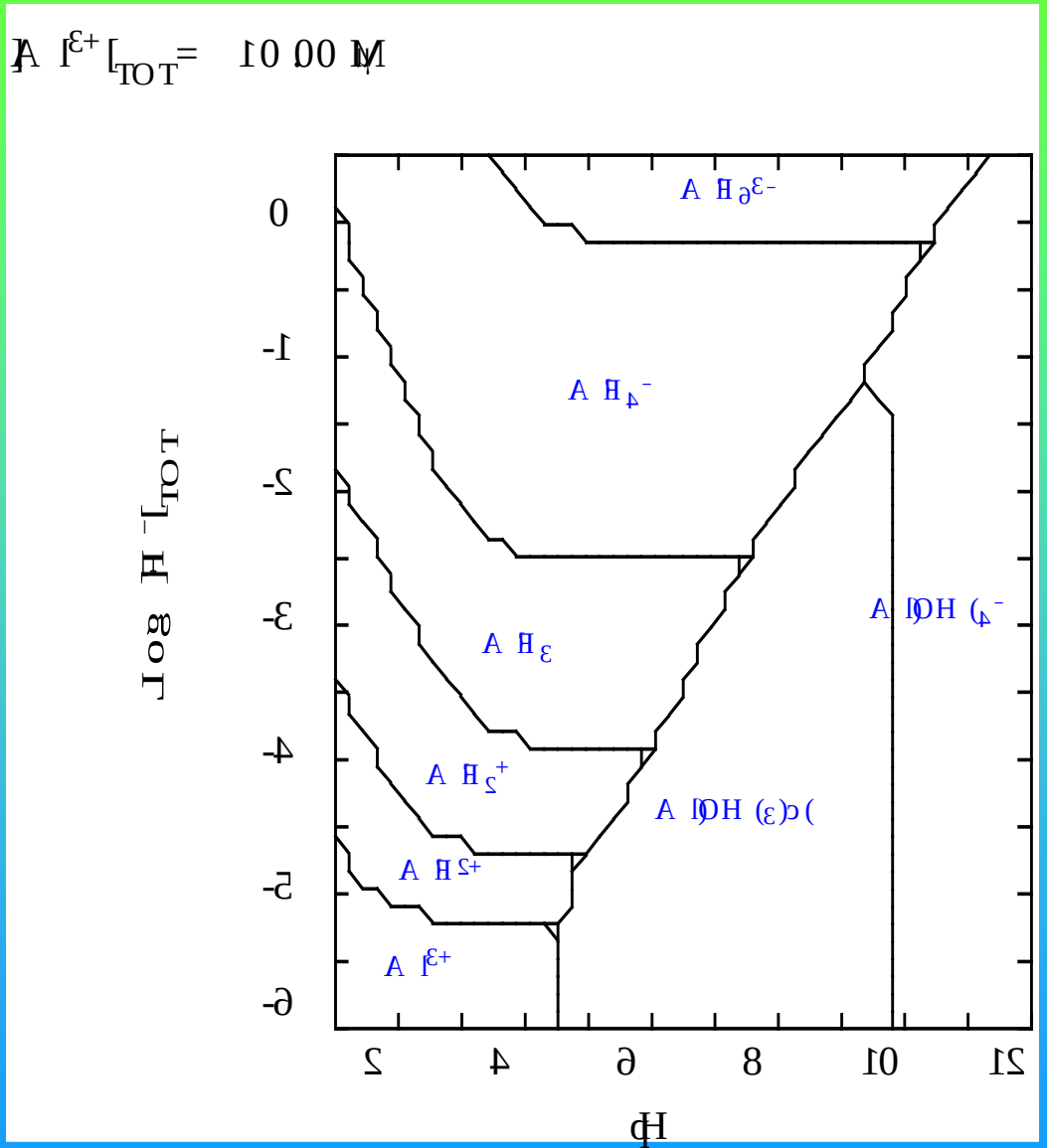
Guanosine Triphosphate (GTP)

Some effects of NaF plus Al^{3+} observed in laboratory investigations

CELL TISSUE	BIOCHEMICAL RESPONSE	FUNCTIONAL RESPONSE
Liver - hepatocytes	Ca^{2+}↑ IP3↑ cAMP↓	Activation of glycolysis, Fatty acid oxidation Activation of catabolic processes
Kidney	Ca^{2+}↑ cAMP↑ ion channels affected	Glomerular hypercellularity and distortions Renal mesangial proliferation
Platelets	Ca^{2+}↑↓ IP3↑↓	Agreggation
Red blood cells	IP3↑	Shape changes Disorganization of cytoskeleton
Fibroblasts	Ca^{2+}↑ IP3↑ cAMP↓	Growth, movement Production of extracel. matrix
Osteoblasts	PG synthesis ↑ Tyrosine phosphorylation ↑ Phosphate transport↑	Mitogenic effect proliferation↑ Life span ↑ Anabolic action ↑
Osteoclasts	cAMP Ca^{2+}↑	Inhibition of bone resorption Cellular retraction
Neurons	IP3↑ Ca^{2+}↑	Spike amplitude
Brain	IP3↑ Ca^{2+}↑	Enhancement of synaptic transmission and spike amplitude
Pars tuberalis	Ca^{2+}↑ Inositol phosphates↑	Binding of iodomelatonin ↓

ALUMINUM – FLUORIDE PREDOMINANCE DIAGRAM

This diagram demonstrates HOW solubility of Al hydroxide is changing with pH or fluoride concentration.



PO_4^{3-}

AlF_4^-

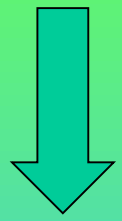
AlF_x – molecule of the year 1997

Recent three-dimensional structures of phosphoryl transfer enzymes in their aluminum fluoride bound state and corresponding biochemical data have shown how diverse biological problems can be investigated using this small inorganic molecule.

Alfred Wittinghofer
Current Biology 1997, 7:R682–R685

LIFE IS truly BUILT around PHOSPHATE

The phosphate analogue model of AlF_x has been extended to many enzymes that bind phosphate groups



We can predict hundreds of reactions, which might be influenced by AlF_x

Fluoride Interactions: From Molecules to Disease
Strunecka A., Patocka J., Blaylock R.L., Chinoy N.J.*Current Signal Transduction Therapy, 2007, 2, 190.*

Inhibitory effects of fluoride + Al^{3+} on enzymatic activities. Yes means that Al^{3+} is required

ENZYME	SOURCE	NaF	Al^{3+}
adenylyl cyclase	liver fibroblasts	up to 10 mM 5 mM	Yes Yes
AChE	red blood cell	0.01-10 mM	Yes
F-ATPase	mitochondria	mM	Yes
glucose-6-phosphatase	liver	μM	Yes
glycogen synthase	hepatocytes	2-15 mM	Yes
IMPase	fibroblasts	mM	Yes
Na^+/K^+ ATPase	plasma membrane	1-10 mM	Yes
PKC	retina	mM	Yes
PLD	liver, brain, lymphocyte	mM	Yes

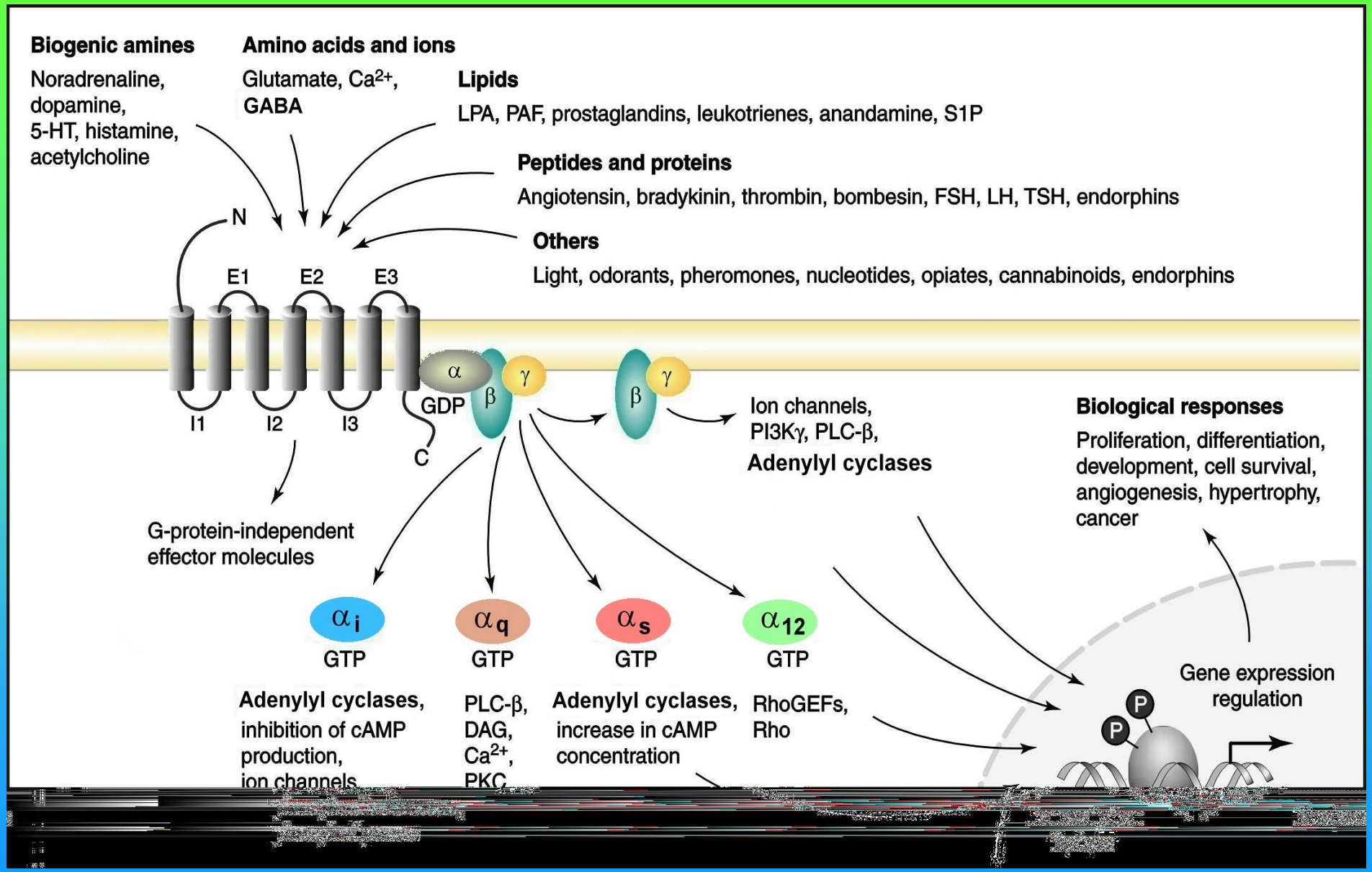
Stimulatory effects of fluoride on enzymatic activities. Yes means that Al^{3+} is required

ENZYME	SOURCE	NaF	Al^{3+}
PKC	lung	5-7.5 mM	Yes
PLA2	platelets	5 - 10mM	Yes
	macrophages	5 – 10 mM	Yes
	endothelial cells	5 – 20 mM	Yes
PLC	hepatocytes	1-50 mM	Yes
	RBC	1 mM	Yes
	rabbit femoral artery	10 mM AlFx	Yes
	astrocytes	intracellulary	Yes
PLD	platelets	5-10 mM	Yes
	rat atria	10 mM	Yes
	canine cer. cortex	AlFx only	Yes
tyrosine kinase	osteoblasts	1 – 10 mM	Yes
		10 – 100 µM	Yes

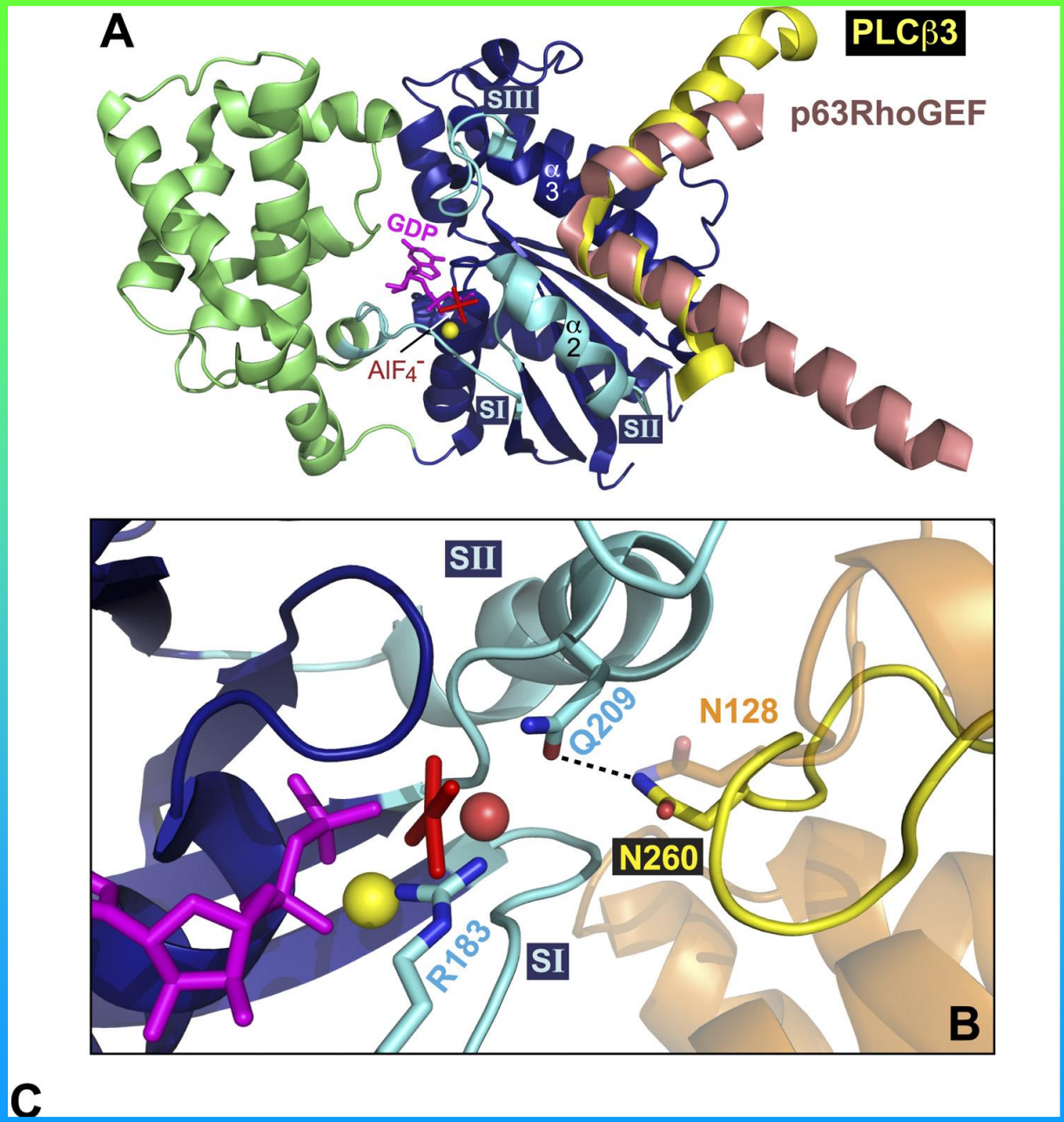
Stimulatory effects of fluoride on enzymatic activities. Yes means that Al^{3+} is required

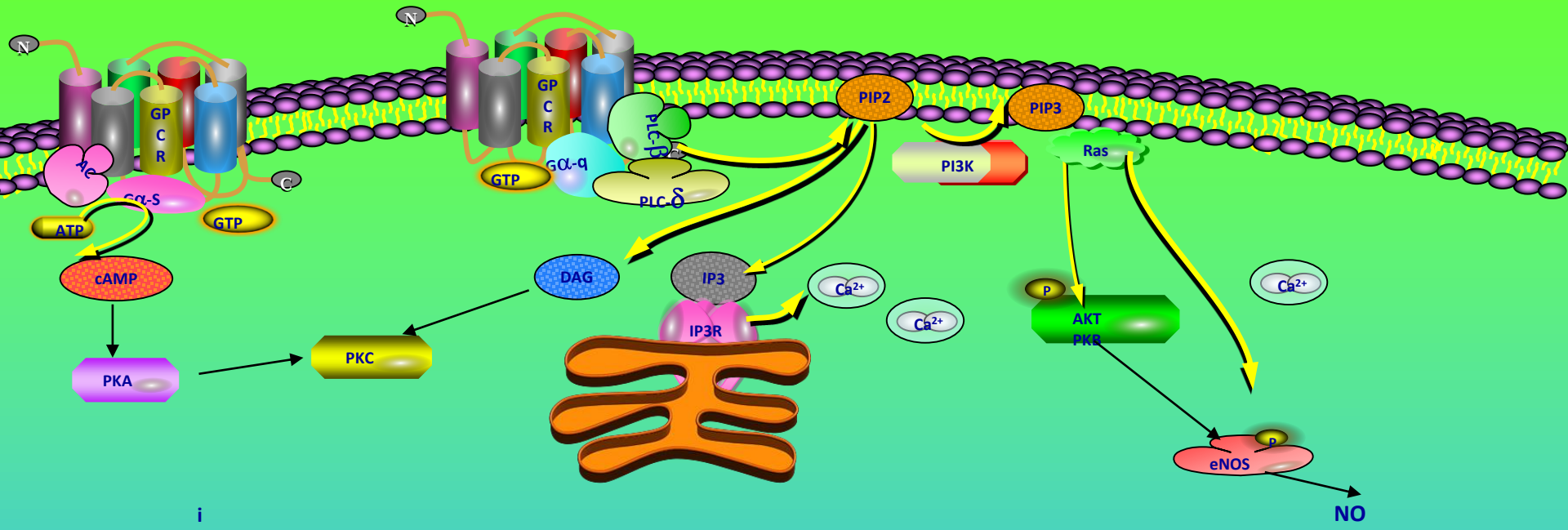
ENZYME	SOURCE	NaF	Al^{3+}
adenylyl cyclase	heart, liver, brain lymphoma cell smooth muscle heart turkey RBC brain	10 mM 10 mM 10 mM 1–10 mM 10 mM 10 mM	Yes Yes Yes Yes Yes Yes
alkaline phosphatase	bone cells	10–100 μM	Yes
cytidylate cyclase	rat brain	mM	Yes
ERK	bone	1–10 mM	Yes
K^+ATP channel	heart	mM	Yes
MAP kinases	lung	5 mM	Yes
Glycogen phosphoryl	hepatocytes	1–50 mM	Yes
PI 3-kinase	HeLa cells	30 mM	Yes

General scheme of G protein-mediated transmembrane signalling



Marinissen and Gutkind, 2001

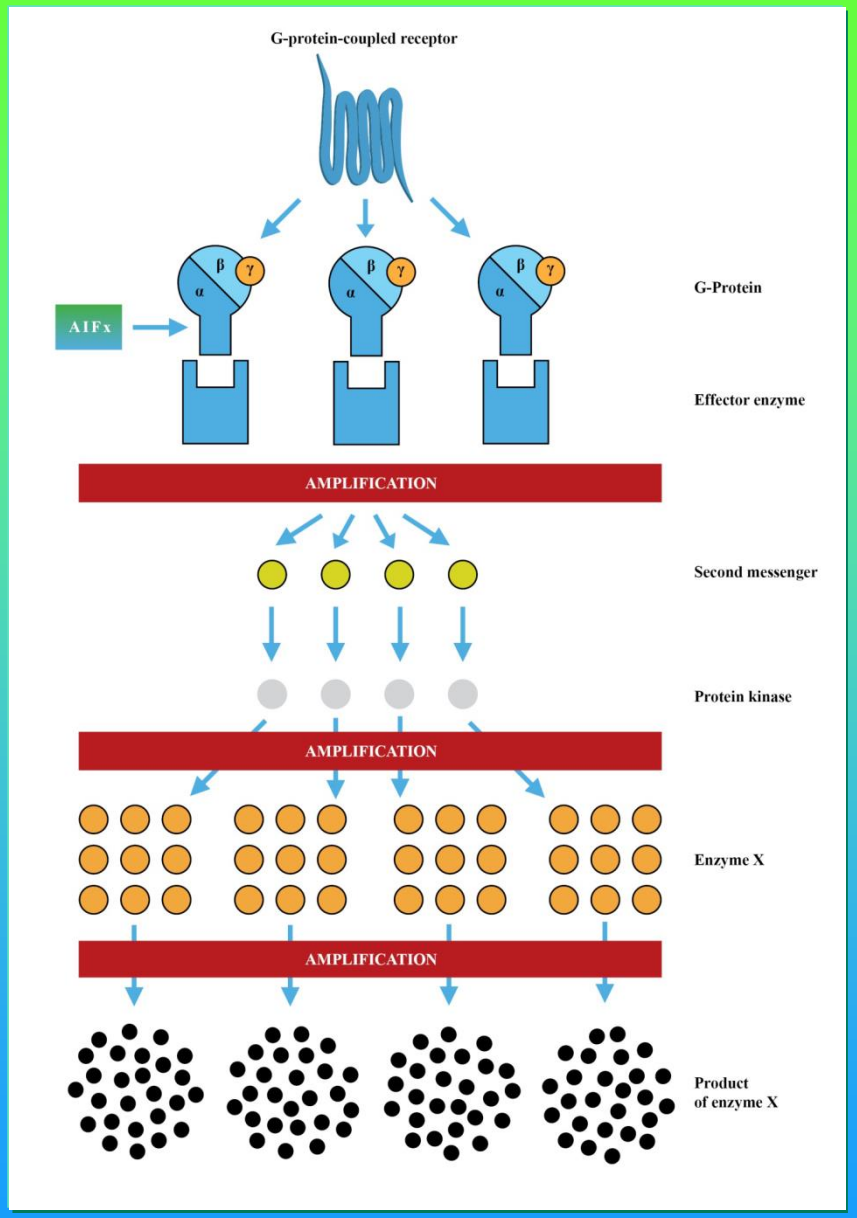

A
PLCβ3
p63RhoGEF
SIII
α3
GDP
α2
AlF4-
SI
SII
SII
Q209
N128
N260
R183
SI
B
C



GPCRs
G-protein coupled receptors

AlF_x MESSENGER of FALSE INFORMATION

Its message is greatly amplified



European Fluoride and Aluminum Network of Excellence

May 2002 – February 2003

37 participants – 17 countries
354 researchers

EU Member States, Bulgaria,
Turkey, India, China, Chile

Fluoride Interactions: From Molecules to Disease

Anna Strunecka, Jiri Patocka , Russell L. Blaylock, and Niloufer J. Chinoy†

Current Signal Transduction Therapy, 2007, 2, 190-213

ALUMINUM

CONCLUSIONS

AlF_x is a molecule giving false information, which is amplified by processes of signal transmission. Biological signaling pathways interact with one another to form complex networks. Yet, it seems that we shall not probably find any physiological process, which is not potentially influenced by AlF_x. The synergistic action of fluoride and aluminum in the environment, water, and food chains, can evoke various and multiple pathological symptoms. AlF_x might induce the alterations of homeostasis, metabolism, growth, and differentiation of the living organism.

Russell Blaylock USA

Jiri Patocka Czech Republic