Analysis of central nerve system development and differentiation

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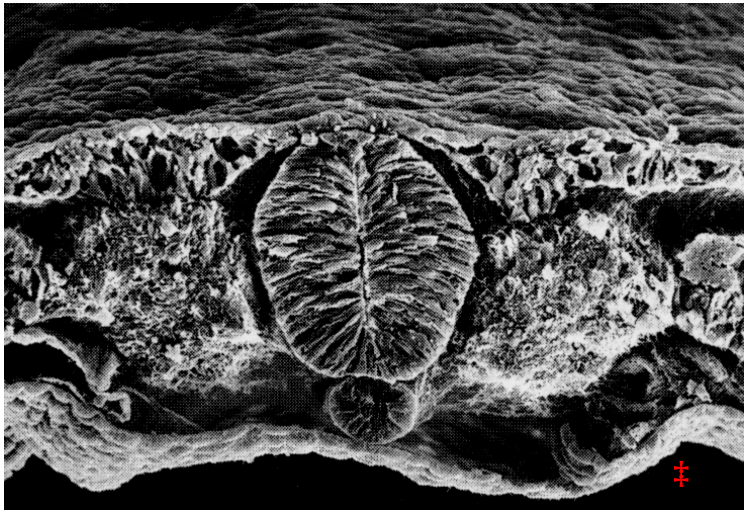
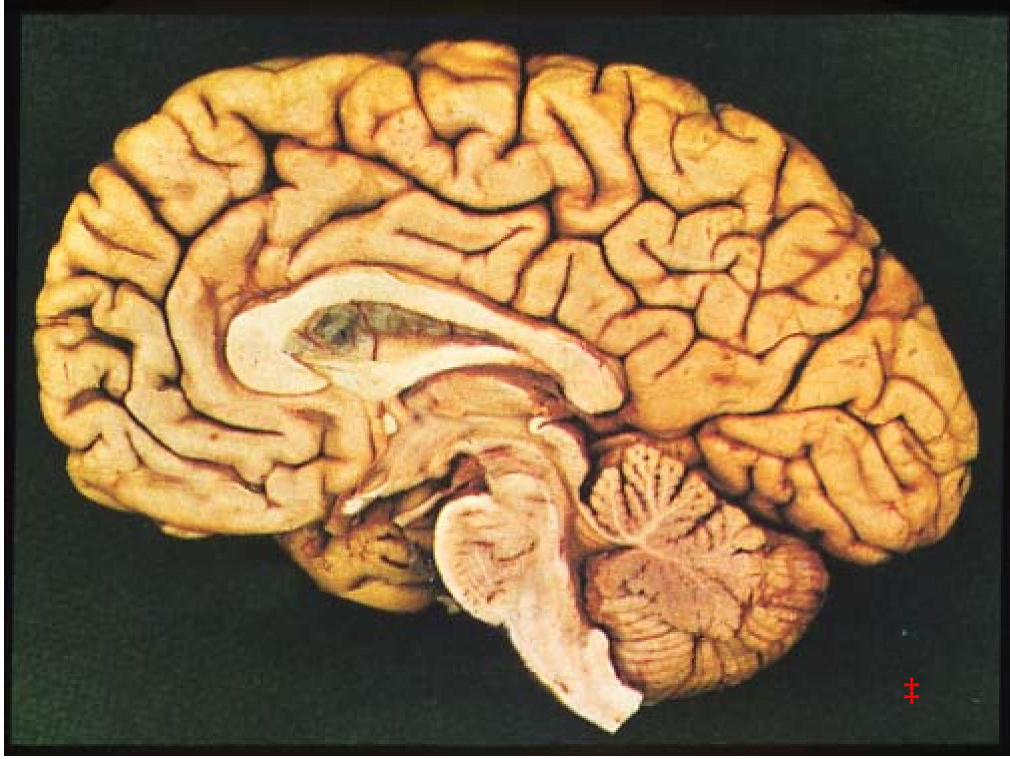
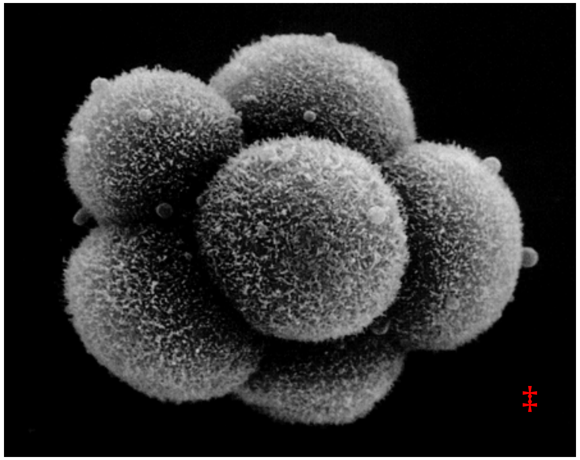
Understanding the molecular basis of neural development







Molecular biology,
Biochemistry,
Biophysics,
Structure biology

NORMAL

↕

ABNORMAL

- ***Normal —Abnormal***

- ***Health — Disease***

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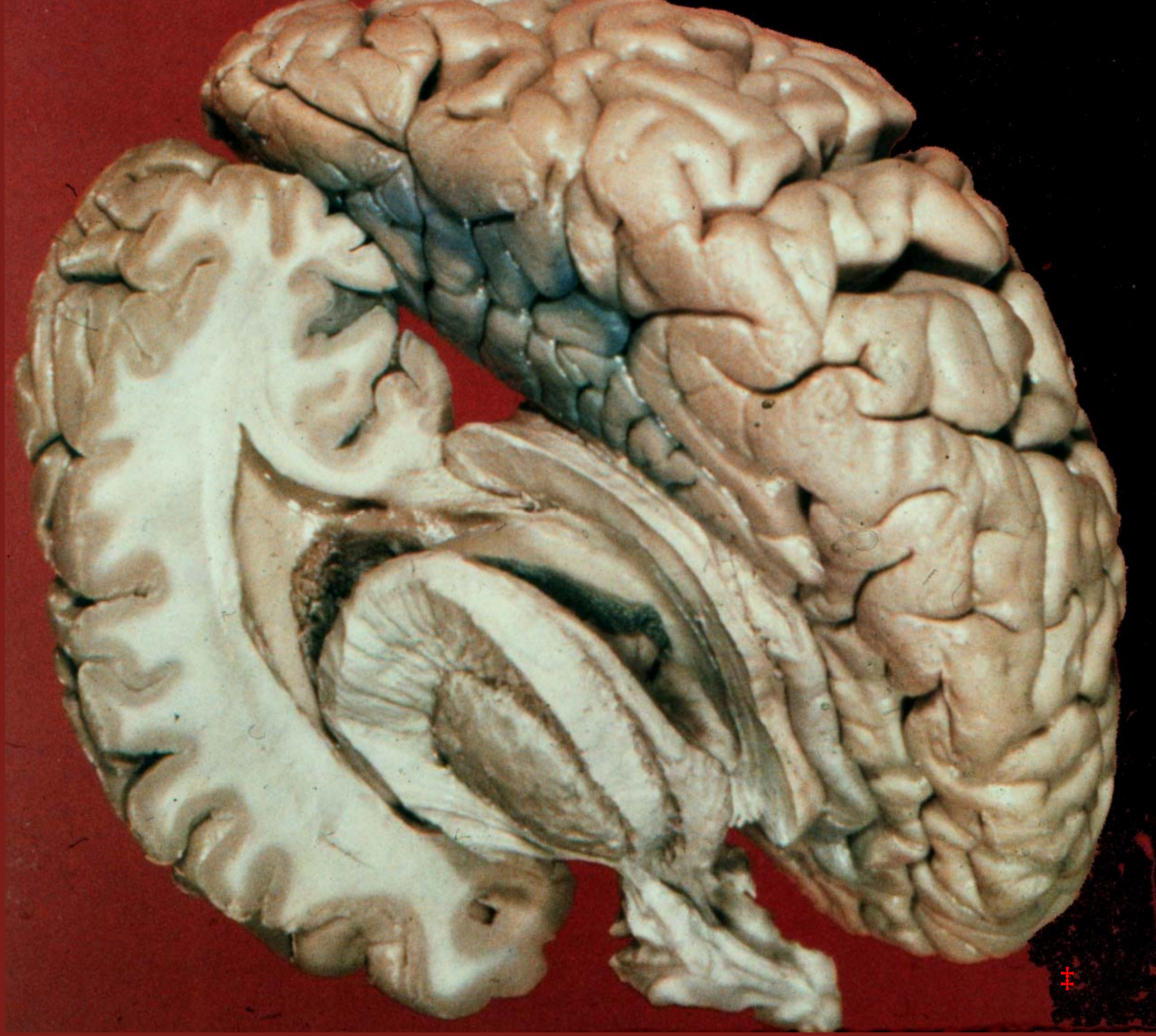
cerebellar ataxia mutant mouse

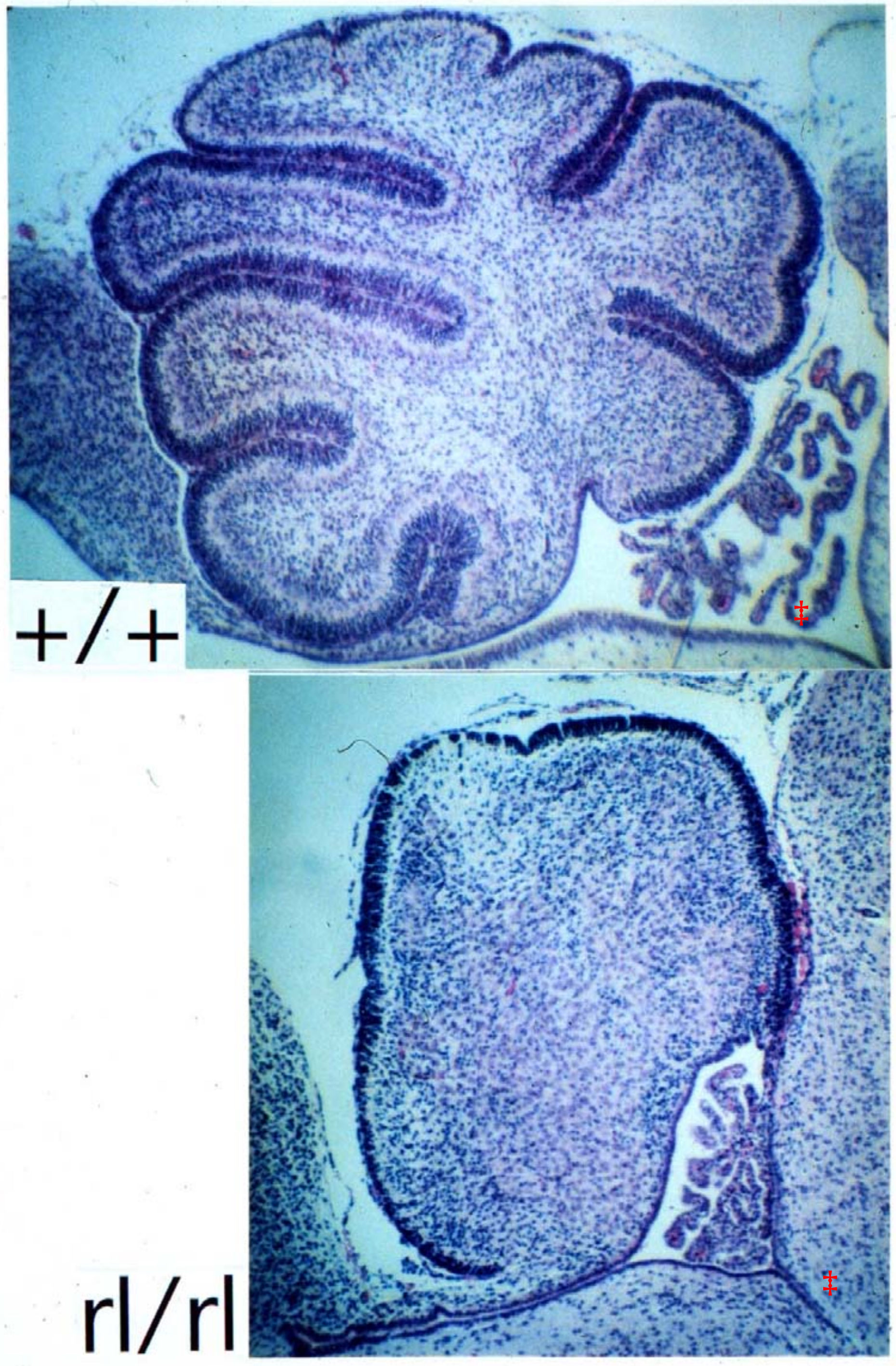
Possible to link molecular disorder—morphologic disorder—functional disorder

1. Positioning of nerve cells and formation of wrinkles in the brain
2. Discovery & analysis of Zic gene
3. Discovery & analysis of IP_3 receptor



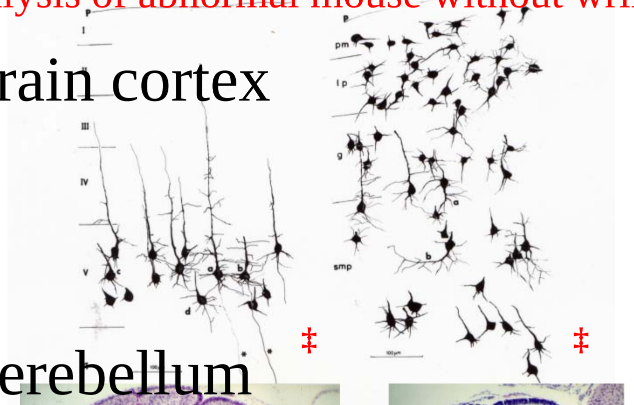
How are wrinkles in brain formed？
How are positions of nerve cells determined?

<u>Lissencephaly</u>
（human,mouse）
reeler mouse
yotari mouse

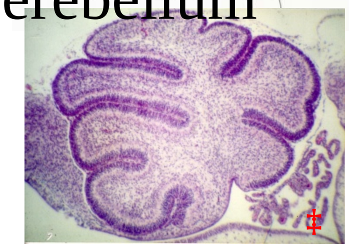


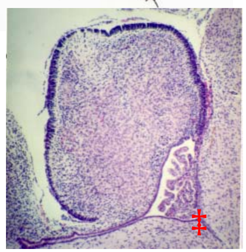
Analysis of abnormal mouse without wrinkles in brain and with wrong nerve cell positions

brain cortex



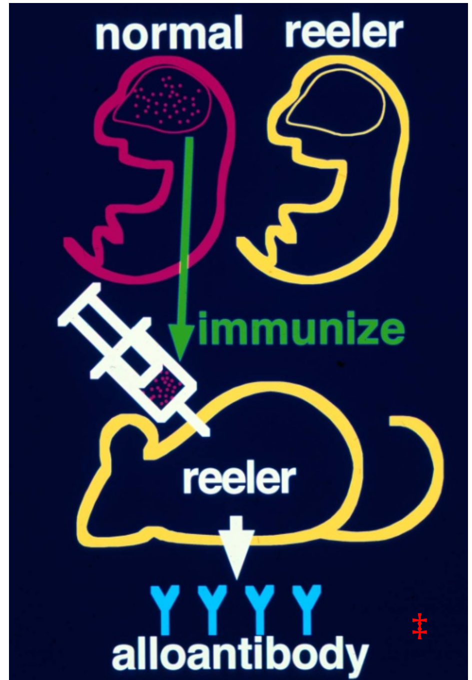
cerebellum





normal mouse

reeler
（without brain wrinkle）
（wrong nerve cell positions）

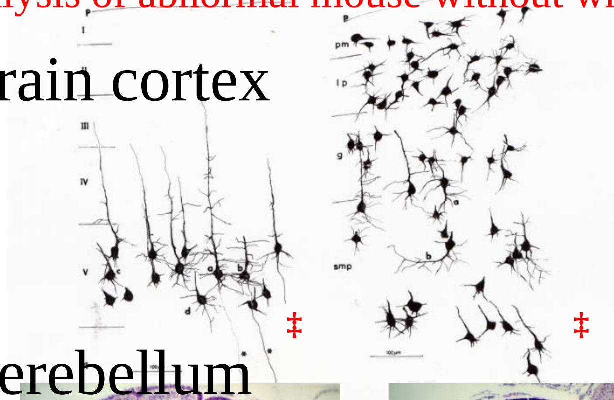


Breakthrough in molecular biology researches by immunizing brain of normal mouse to reeler mouse, making antibodies against product of gene(reelin) and identifying the gene

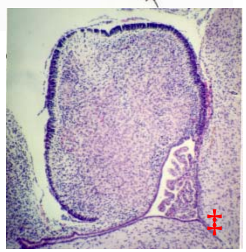
(Neuron 1995)

Analysis of abnormal mouse without wrinkles in brain and with wrong nerve cell positions

brain cortex

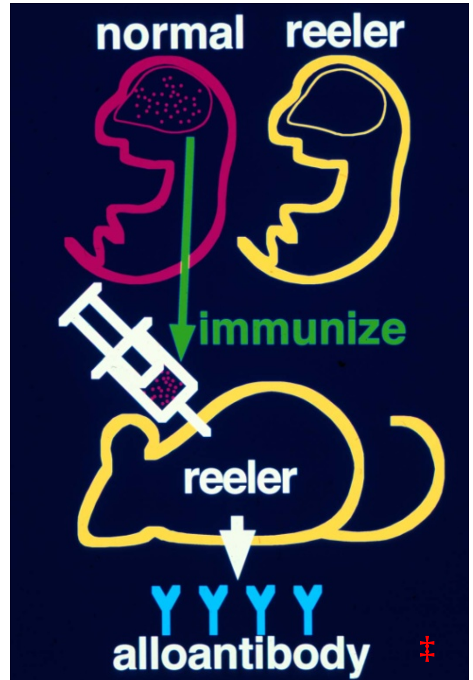


cerebellum



normal mouse

reeler
(without brain wrinkle)
(wrong nerve cell positions)



Breakthrough in molecular biology researches by immunizing brain of normal mouse to reeler mouse, making antibodies against product of gene(reelin) and identifying the gene

(Neuron 1995)

Yotari discovery and naming of mutant mouse

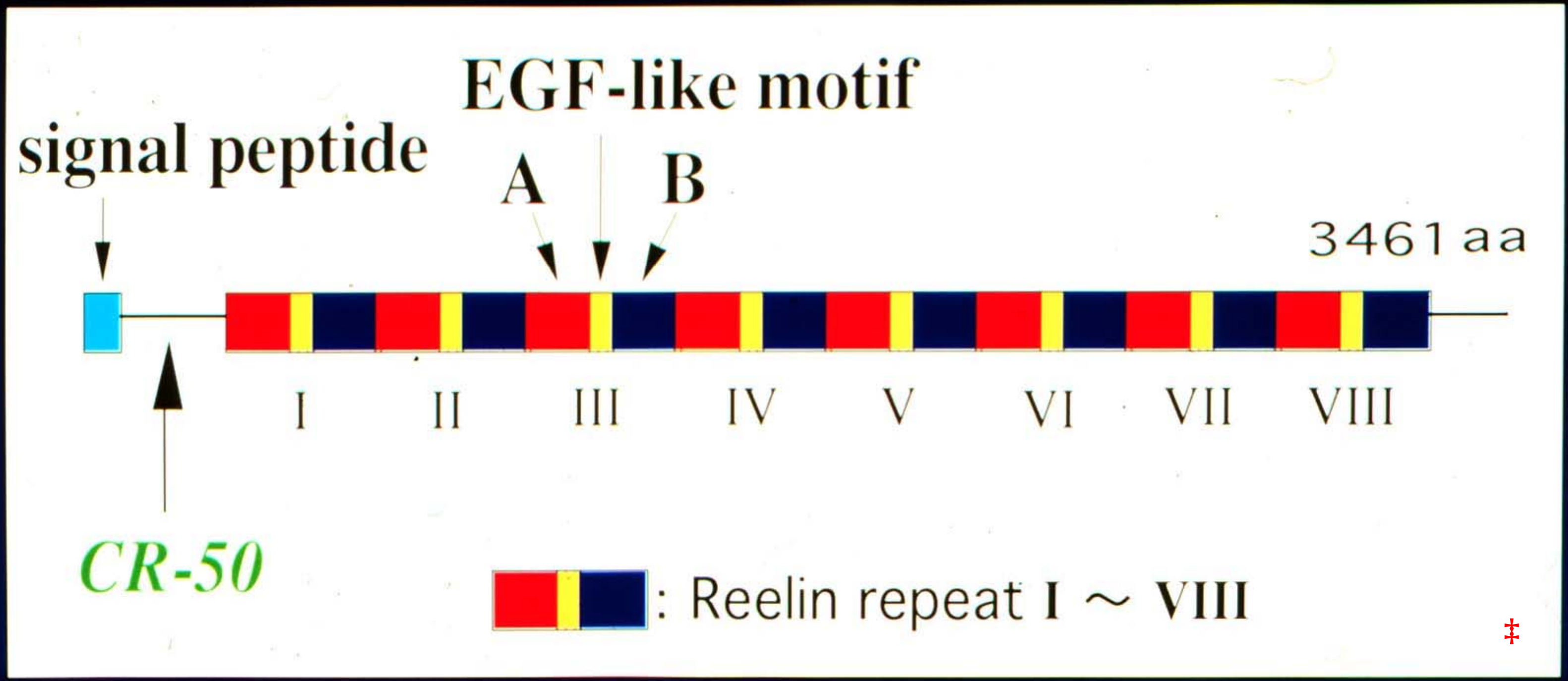
1) without formation of brain wrinkle

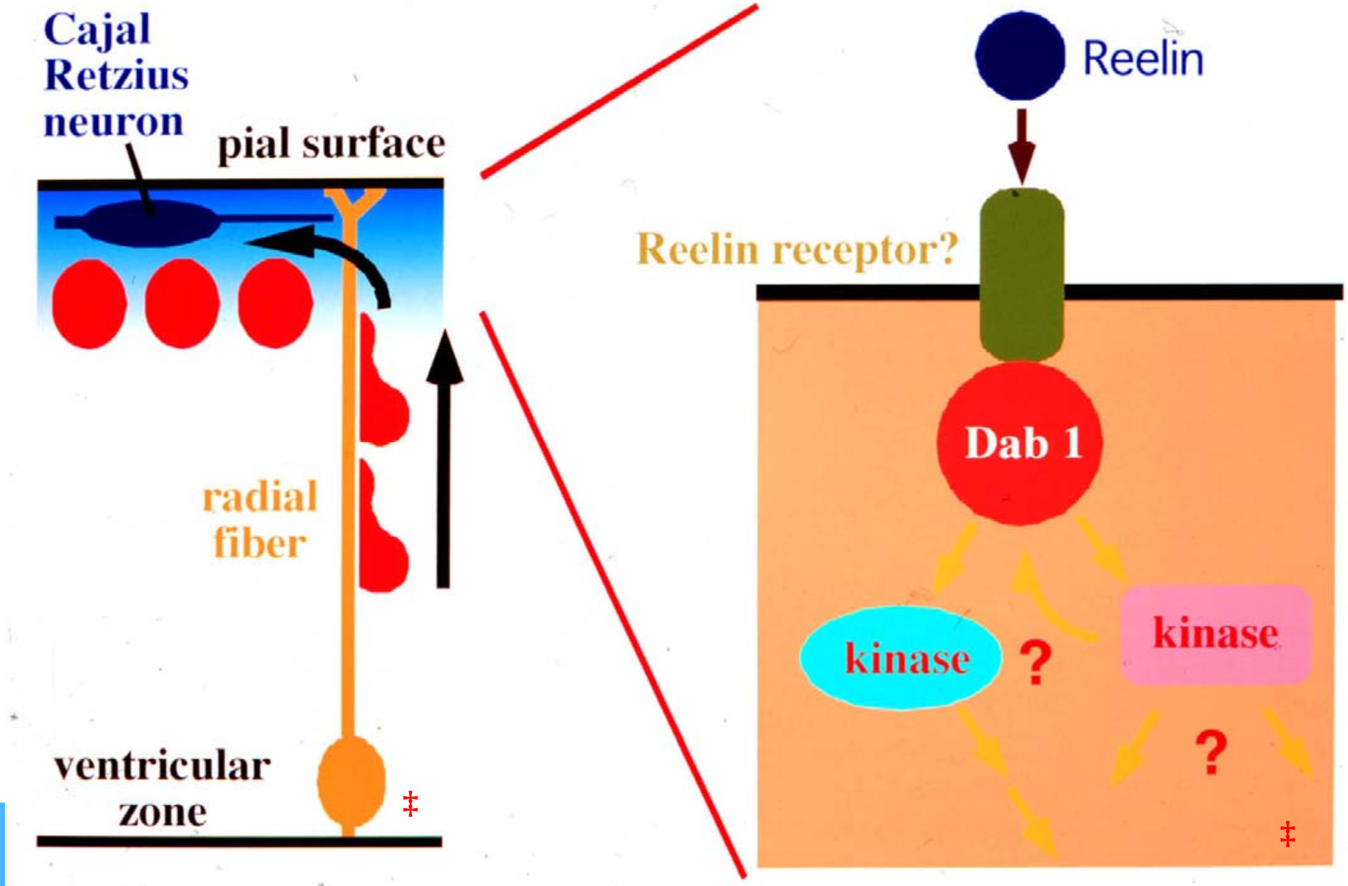
2) downstream of reelin

3) *disabled-1 (*Src,Abl and Fyn) adaptor of mutated tyrosine kinase protein

(Nature 389 730-733 1997)

Reelin structure




Cajal
Retzius
neuron
pial surface
radial
fiber
ventricular
zone
Reelin
Reelin receptor?
Dab 1
kinase
?
kinase
?

1. Positioning of nerve cells and formation of wrinkles in the brain
2. Discovery & analysis of Zic gene
3. Discovery & analysis of IP_3 receptor

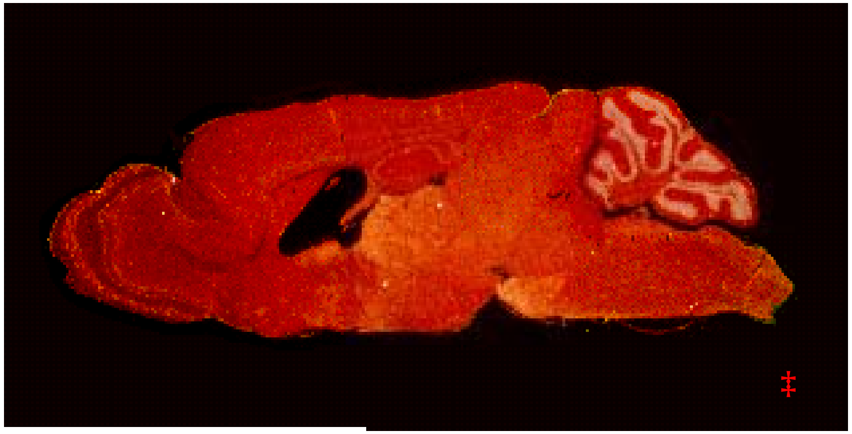
Zic (Zinc finger protein enriched in the cerebellum)

Odd-paired (*Drosophila* homologue, single gene) regulates *wingless* and *engrailed*

Wingless----***Wnt*** (vertebrate)

Engrailed----***En*** (vertebrate)

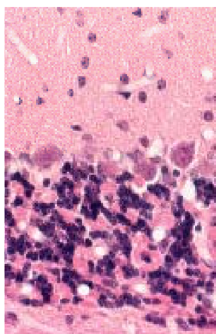
Zic (*Zinc finger protein of the cerebellum*)

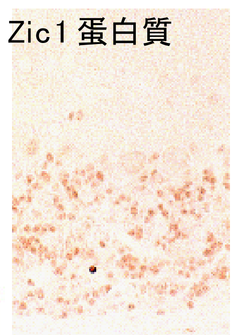


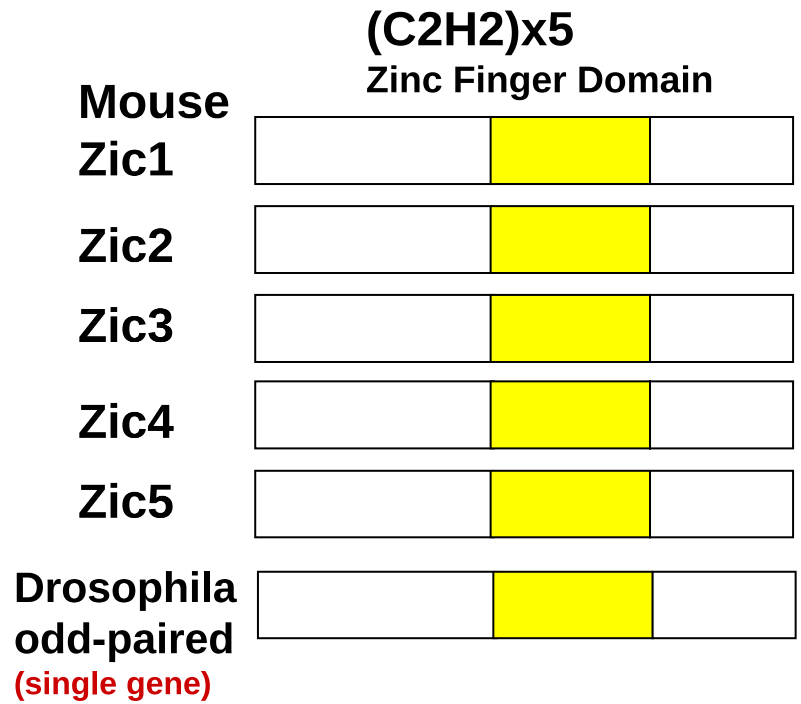
Molecular layer

Purkinje cell layer

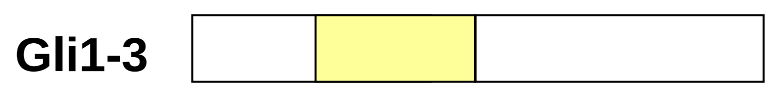
Granule cell layer







Odd-paired modulates gene expression of wingless and engrailed.



Gli mediates hedgehog signal as a transcriptional regulator.

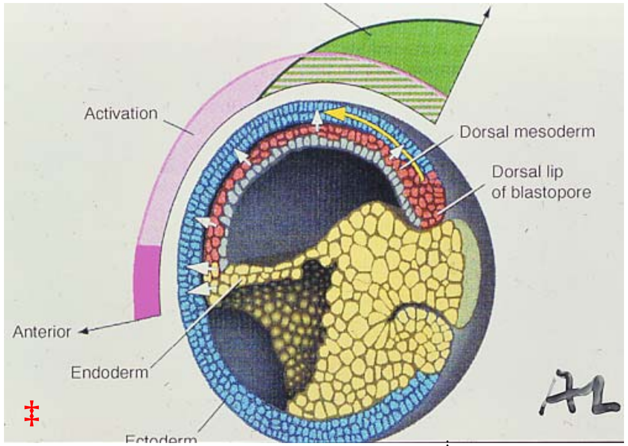
Morphogenetic anomaly of brain caused by Zic defect

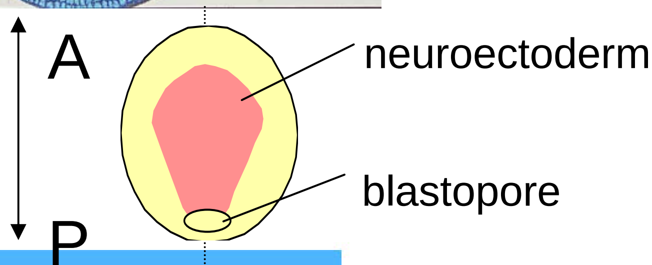
- Zic 1 responsible for patterning of cerebellum

- Zic 2 which regulates development of whole central nerve system

- Zic 3 which determines left-right axis

Neuroectodermal differentiation (neural induction)





neural inducer
Noggin
Chordin
Follistatin

↓

ectoderm **Zic**

↓

neuroectoderm
(neural plate)

↓

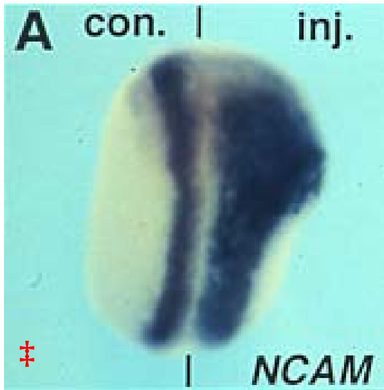
"proneural gene"
NeuroD
Neurogenin
Ash etc

↓

Neuronal differentiation

Zic promotes differentiation of neural ectoderm

Zic1 was overexpressed in the right side



neuroectodermal marker

epidermal marker

(dorsal view of Xenopus neurula)

neural inducer

Noggin

Chordin

Follistatin

↓

ectoderm **Zic**

↓

neuroectoderm (neural plate)

↓

“proneural gene”

NeuroD

Neurogenin

Ash etc

↓

Neuronal differentiation

Morphogenetic anomaly of brain caused by Zic defect

- Zic 1 responsible for patterning of cerebellum
- Zic 2 which regulates development of whole central nerve system
- Zic 3 which determines left-right axis

morphologic aberration caused by Zic

PNAS 97 1618-1623 2000

gene defected mouse

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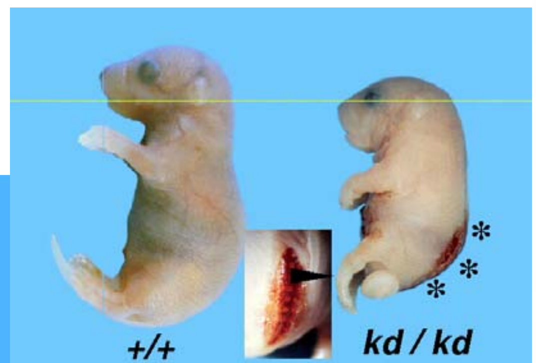
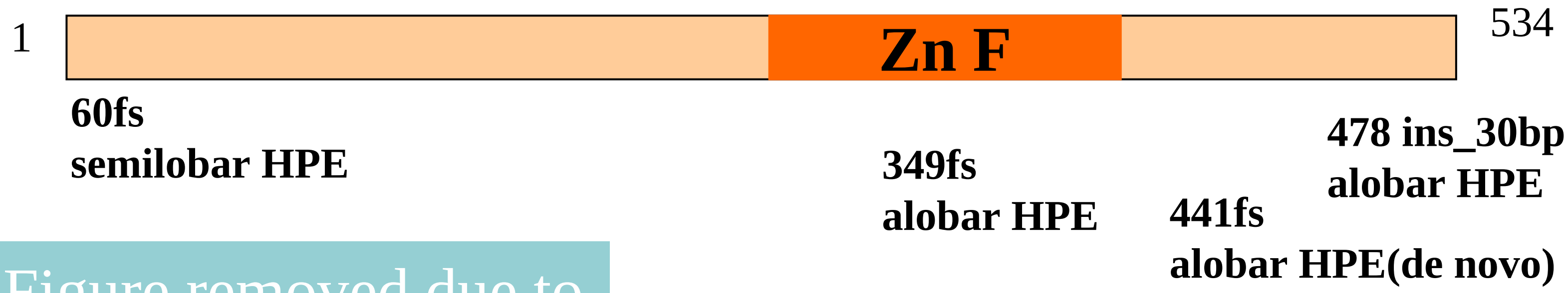
13q deletion syndrome



(From Brown, 1993, Courtesy of S. Brown)

Human ZIC2 is a causal gene of holoprosencephaly



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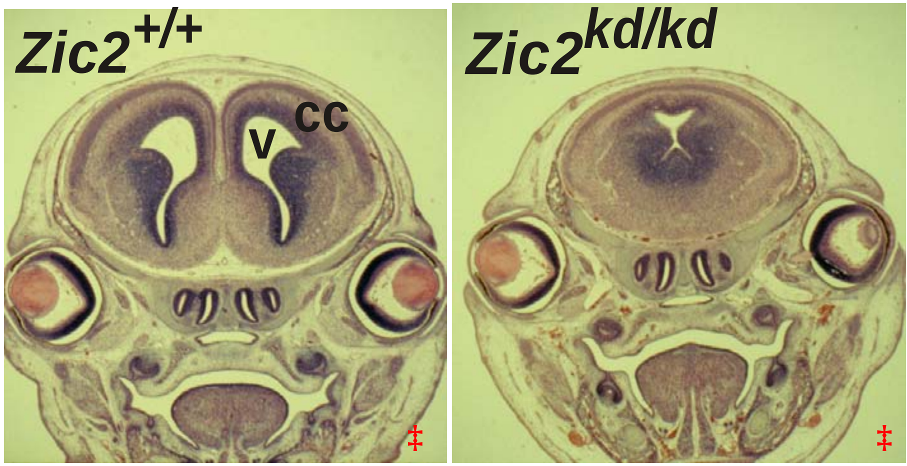
Brown et al., Nat.Genet,20,180-183 (1998)



Zic2 mutant mice show a similar phenotype to holoprosencephaly.

Nagai et al., **PNAS, 97, 1618 (2000)**

Morphogenetic anomaly of brain caused by Zic defect

- Zic 1 responsible for patterning of cerebellum
- Zic 2 which regulates development of whole central nerve system
- Zic 3 which determines left-right axis

Zic 3 is responsible for determining left/right
(Kitaguchi et al. Development 127 4787-4795 2000)

Zic 3 defect： Situs inversus
Situs ambiguus
X-linked situs abnormality results from mutations in Zic 3 (Nature Genetics 17, 305-308 1997)

Figure removed due to copyright restrictions

Zic (Zinc finger protein enriched in the cerebellum)

discovered and named as a protein rich in brain granule cells

Zic1: abnormal patterning of cerebellum

Zic3:situs inversus

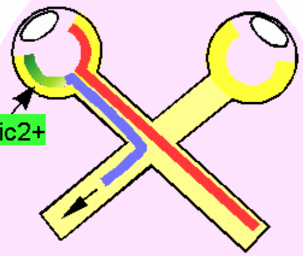
(Development 2000)

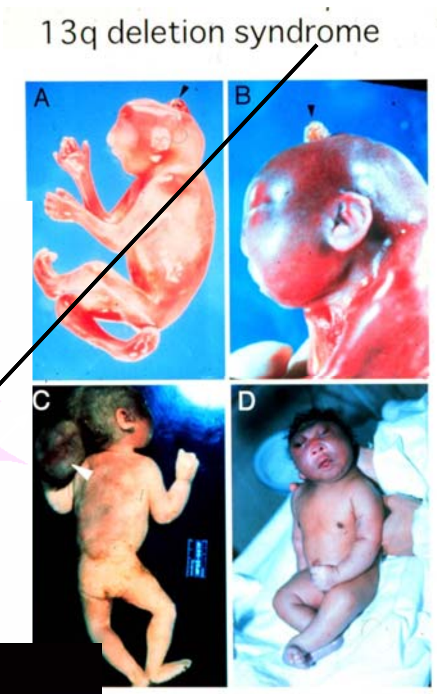
Zic2:

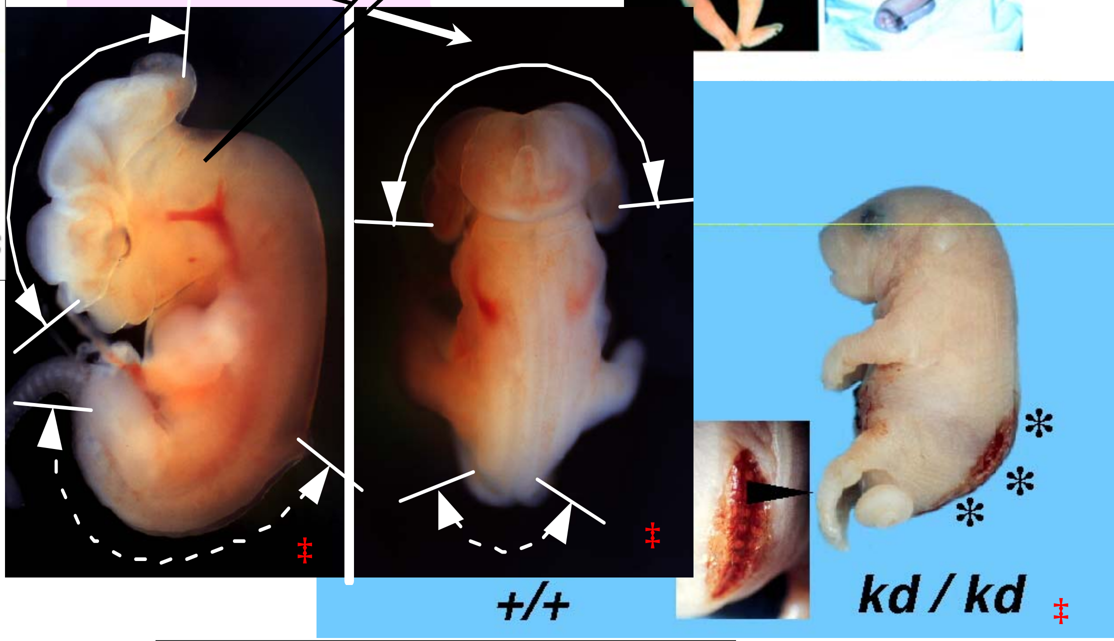
a)stereovision defect*(Cell 2003)*

b)exencephalia *(PNAS 2000)*

c) holoprosencephaly(septal defect)*(PNAS 2000)*







Zic2 knockout mouse

1. Positioning of nerve cells and formation of wrinkles in the brain
2. Discovery & analysis of Zic gene
3. Discovery & analysis of IP_3 receptor

Cerebellar ataxia mouse by mutation

—behavioral anomaly—

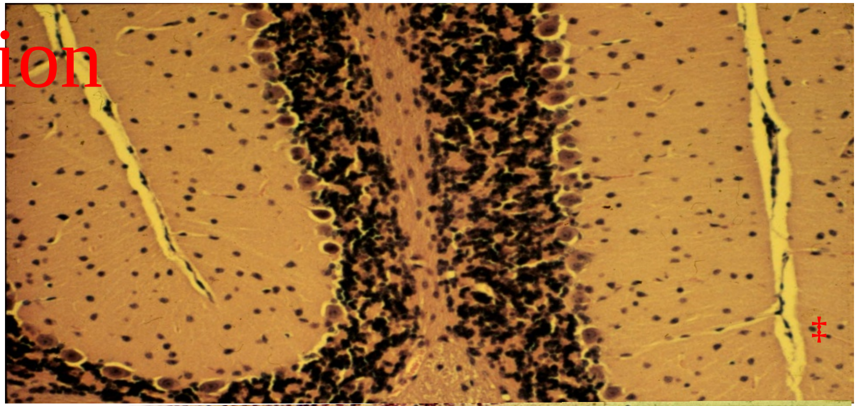
—morphogenesis anomaly—

—abnormal protein—

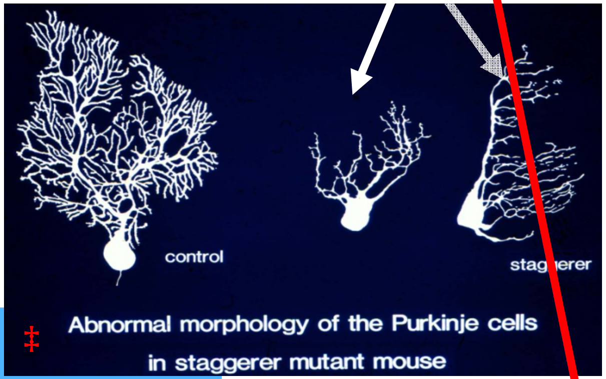
(search for a defected protein)

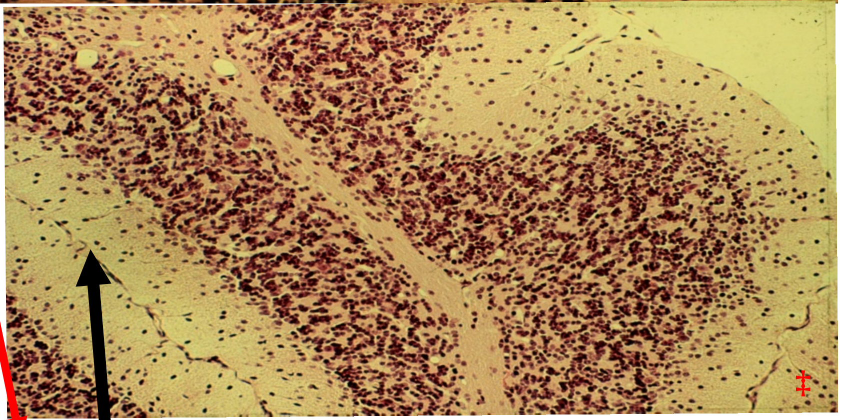
normal

P400 deletion



Synapse defected(dendrite dysgenesis)mouse

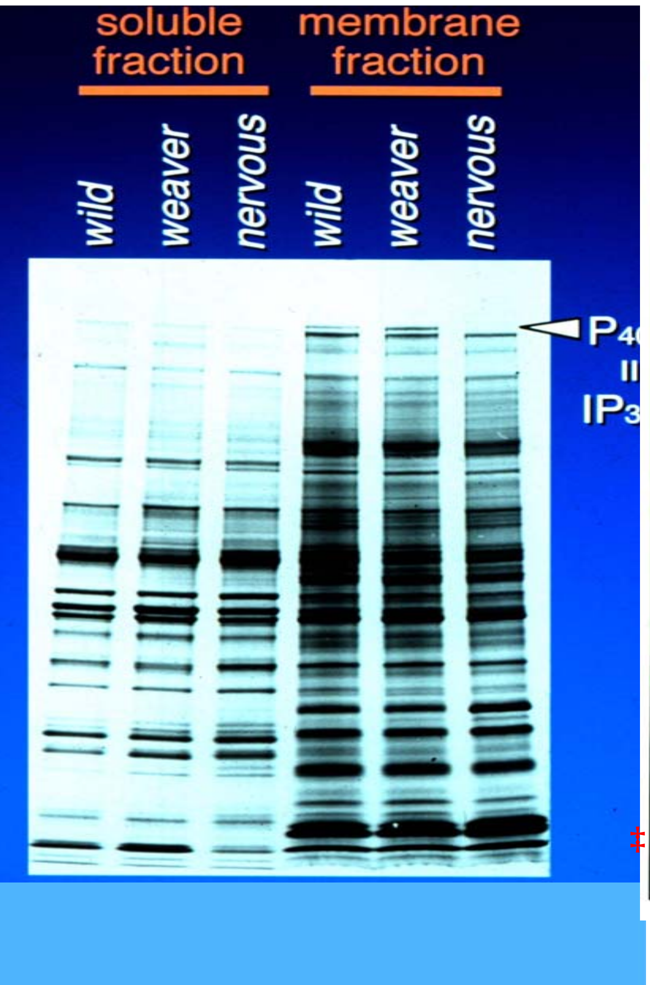




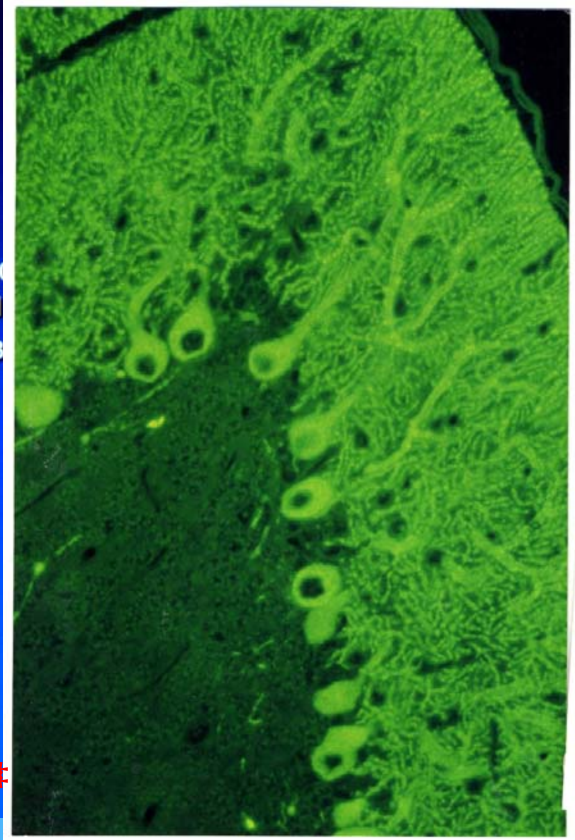
Purkinje cell defected mouse(nervous, pcd)

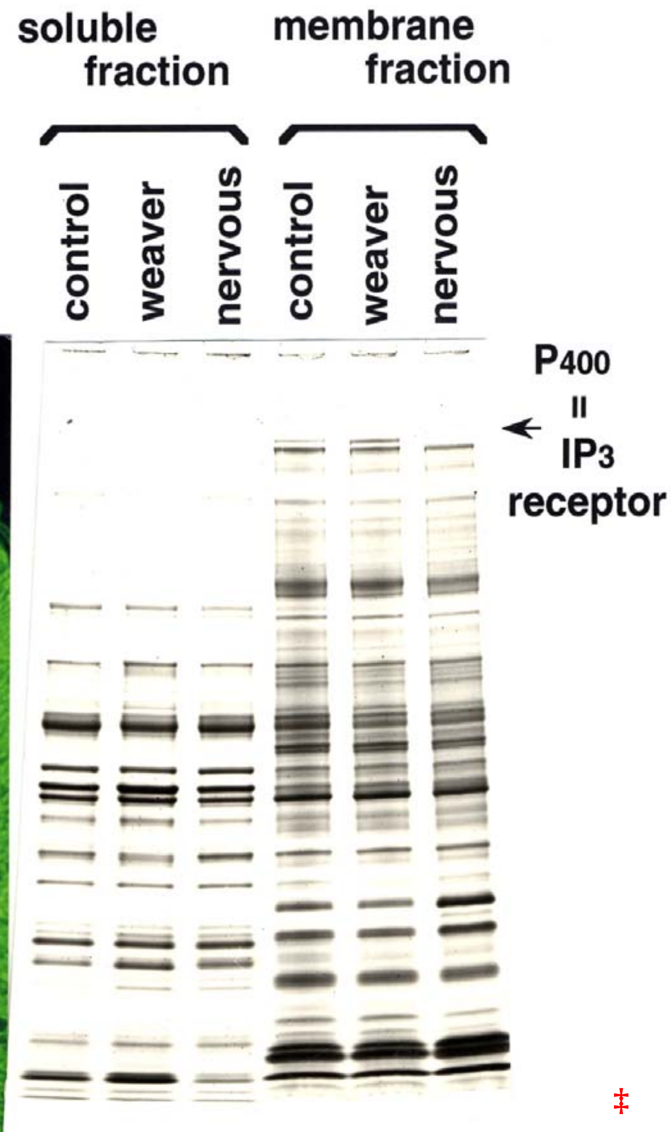
P400 and IP_3 Receptor are same molecule

(Nature 342 32-38 1989, EMBO J. 61-67 1990)

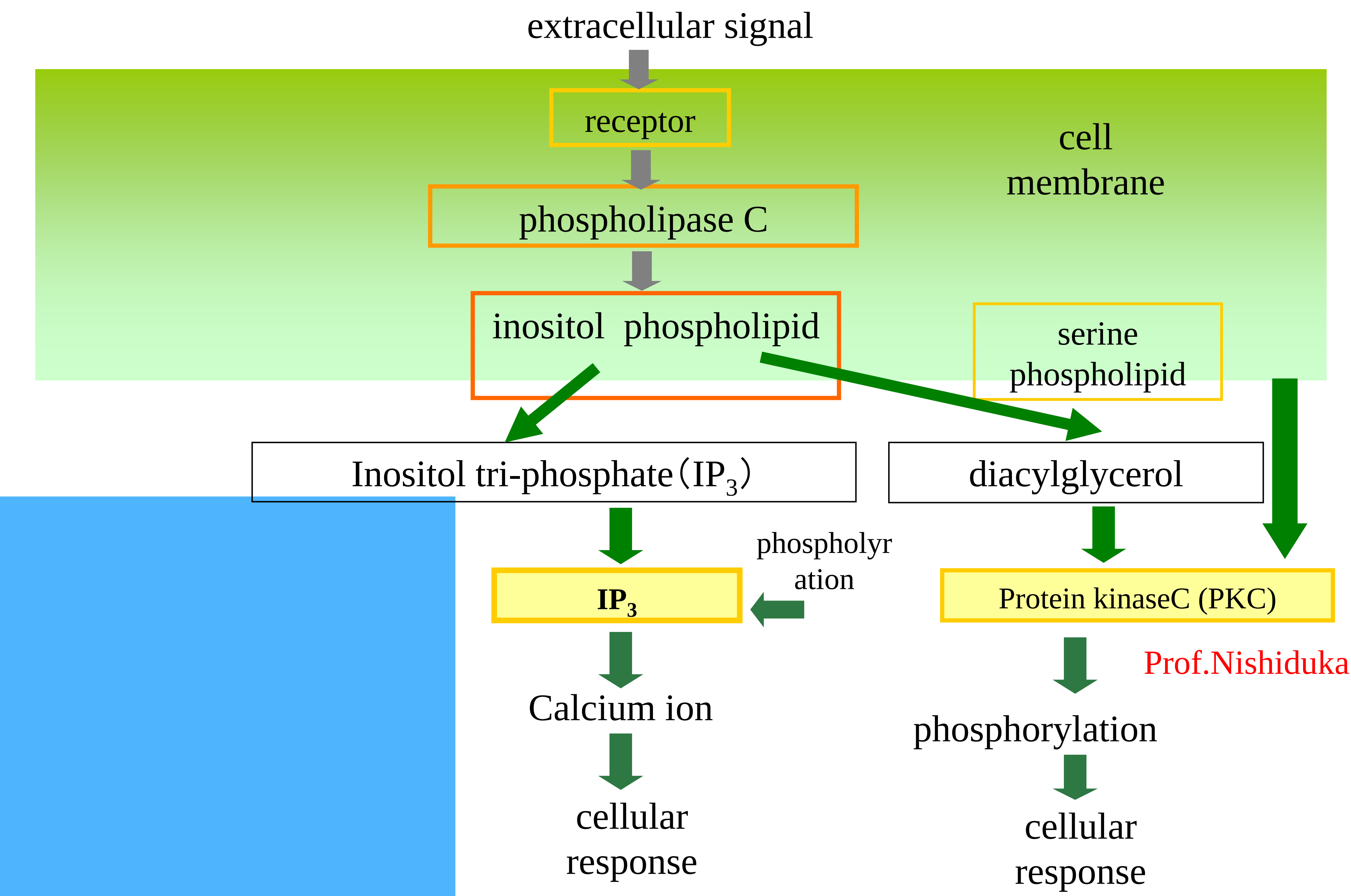


immunostaining of P_{400}/IP_3 receptor





Activation mechanism of IP_3 receptor and protein kinase C



progress toward IP_3 RECEPTOR discovery & analysis

P400 **(IP3 receptor): analysis as a glycoprotein phosphorylated specifically to developmental stages**

Moshiba et al. 1977

calcium release by IP_3 from sites other than mitochondria

Behridge et al. 1983

GP-A : glycoprotein in synapse **Kelly 1984**

PCPP-260 : phosphorylated protein **Greenguard 1986**

P400 was discovered to be an IP_3 receptor. Whole length was determined by cDNA cloning（1989(Nature)), and it was proved to be a calcium channel（1991(J. Biol. Chem.)90(Neuron)）


EXIT
PLENARY
NH
June 20-25, 1993
OLE
PETERSEN
UNIVERSITY OF LIVERPOOL


LONG EARS BECAUSE...
HEY, HE'S A RABBIT
WHITE GLOVES ASSIST
CONCERTO PIANO
EXPERT MASONRY,
ROBIN
IRVINE
CAMBRIDGE UNIVERSITY
KATSUHIKO
MIKOSHIBA

How P400 and IP3 receptor were connected

１）Purkinje cell of a synapse-defected（dendrite dysgenesis） mouse (staggerer mouse)having no calcium spike was reported from the electrophysiology lab. (Crepel et al. J. Physiol 1984) ーーーRelationship between P400 and calcium was assumed

Many calcium-binding proteins were reported to be high in cerebellum. However, most of them were soluble and low molecular. ーーThey may not be the ones.

２）IP3 was reported to release calcium as a second messenger (Behridge et al.,1983）

IP3 binding activity is highest in cerebellum.
Concentration of P400 is also highest in cerebellum.

The relationship between P400 and IP3 receptor was assumed from these data.

＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝

Experiments were conducted (in 1983-1989) to see the relationship between P400 and IP3 binding protein.

Experiment ２

Biochemical explanation for equivalence of **P400 and IP3**

１）Specific monoclonal antibody was successfully made. （To make a specific antibody all anyhow,2 years and a half were devoted to acquire monoclonal antibody.）

（Commonly, researchers used to cut out gel from a band and immunized rabbits.
ーーーSpecificity is disputable.）
By immunizing mouse with purified P400 ,and using homogenate of Purkinje cell defected mouse, screening of antibody by Western blotting was conducted for 2 years and a half in my lab.（at protein lab, the U. of Osaka）

２）「Because **P400** was purified under tough conditions, **there were no activity to bind to P3**」So, there were no clue to find out the relationship betweenIP3 receptor.

３）IP3 binding protein was purified, and the research using antibody was conducted to consider whether it is homologous to P400.（IP3 was labeled by isotope, and we devoted a year to purify the protein, using **IP3 conjugation as an indicator**）

４）At each procedures of purification, **IP3 binding activity** and P400 monoclonal antibody reacted.

５）Final product of purification reacted to **P400 monoclonal** antibody.

６）By **P400 monoclonal** antibody, IP3 binding ability lowered.
From these results, **P400 was concluded to be homologous to IP3 binding protein.**

On the other hand,
１）Whole cDNA cloning of P400 using monoclonal antibody was successfully done（second largest in the world then）（It was later found to be IP3 receptor by the experiment above.）
２）By exhaustive screening of monoclonal antibody, antibody which inhibits the function was also obtained. ーーThis finding developed into a research to inhibit fertilization.

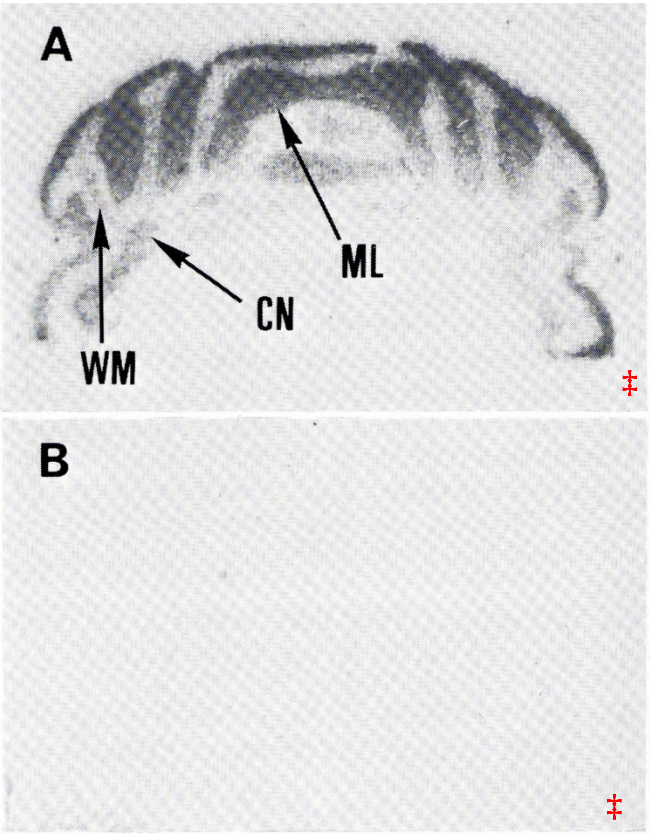
Experiment 1. Binding to IP3 was studied by using a slice of cerebellum of Purkinje cell defected mouse. （Later, published in EMBO J.1990）

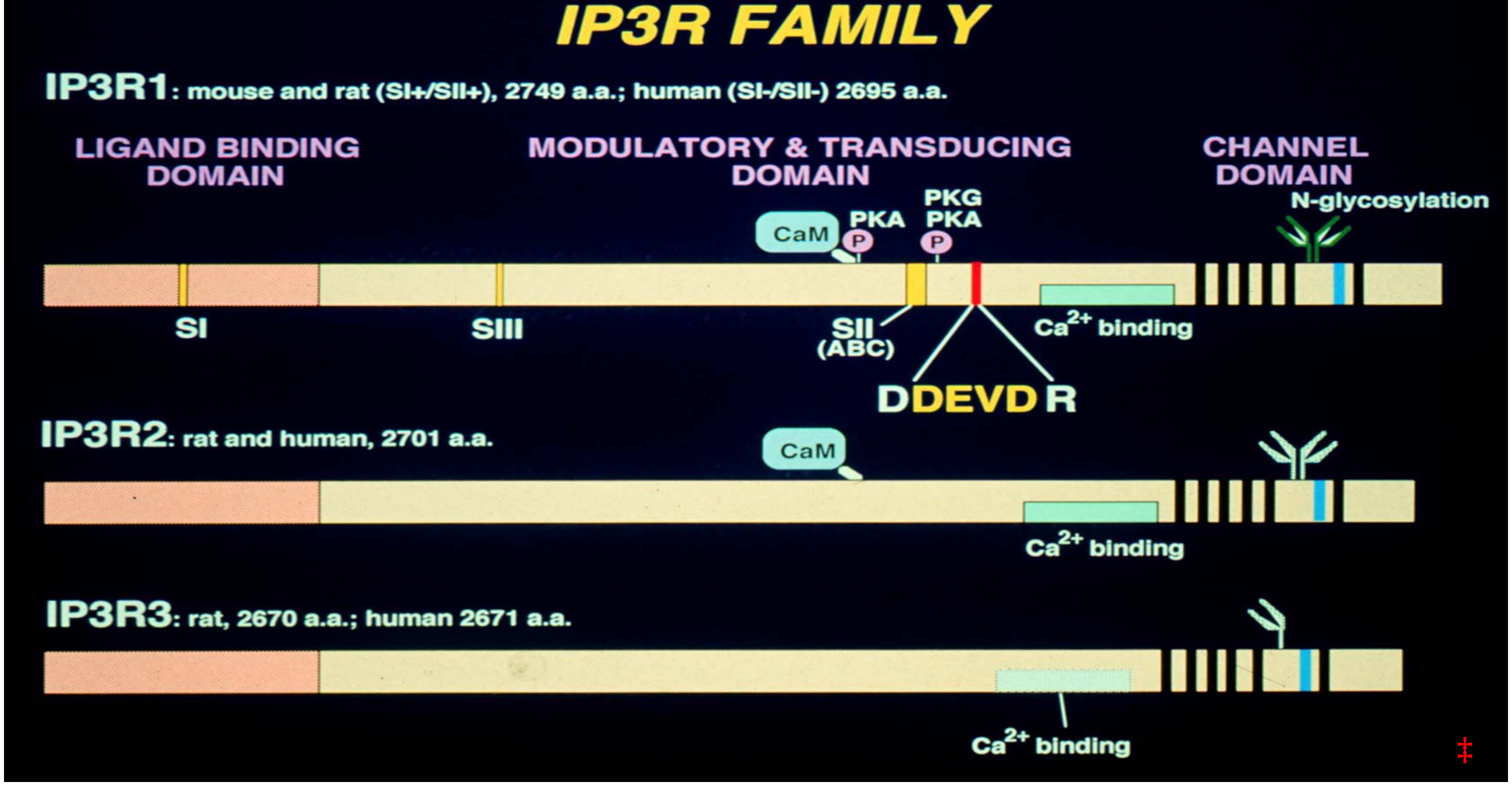
No bindings to IP3 was discovered.（Fig.B below）(1983━1989) ——

From this result, the relationship between IP3 binding protein and P400 became authentic.

Auto-radiography indicating IP3 binding to a slice of wild-type mouse cerebellum （Signals in Purkinje cells）

There is no IP3 binding in cerebellum of Purkinje cell defected mouse.





MW: 314kD

Three types

Mouse IP3 R (1,2,3)
Human IP3R (1,2,3)
***Xenopus laevis* IP3R (1)**

Single gene

***C. elegans* IP3R**
***Drosophila* IP3R**
Starfish IP3R

HISTORY OF IP_3 RECEPTOR

P400 (IP_3 R) : developmentally regulated glyco-phospho-protein

Mikoshiba et al.   1976

IP_3 releases Calcium from non-mitochondrial store

Streb, Irvine, Berridge, Schulz   1983

GP-A : synaptic junctional glycoprotein

Kelly et al.   1984

PCPP-260 : phospho-protein

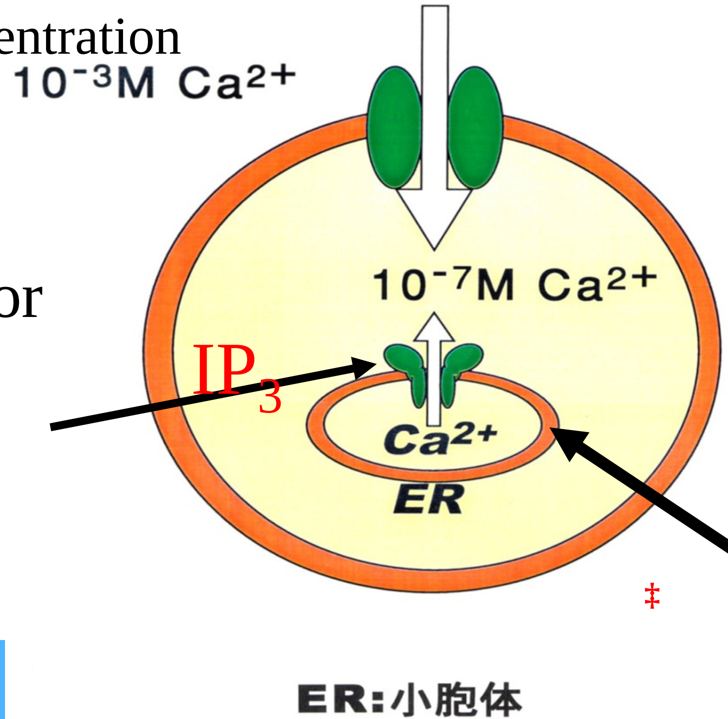
Greengard et al.   1986

P400 = IP_3 Receptor cDNA cloning,
Calcium channel

Mikoshiba Lab.   1989

Ca^{2+} concentration in/out of a cell, release of **Ca^{2+}** from internal store by **IP_3**

extracellular Ca^{2+} concentration



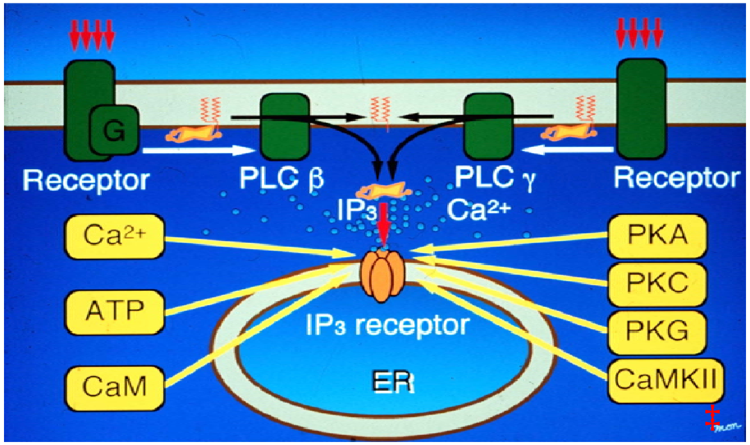
Whether IP_3 receptor is a channel or not was unknown.

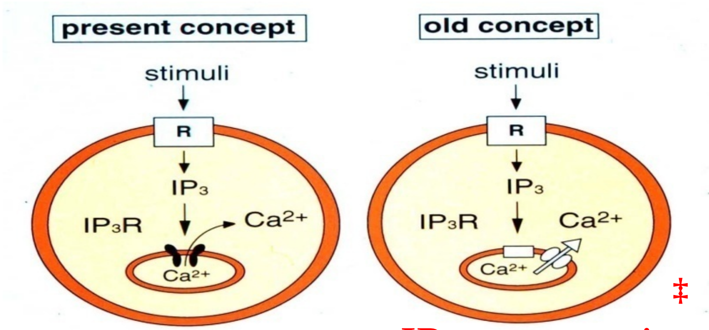
intracellular Ca^{2+} concentration

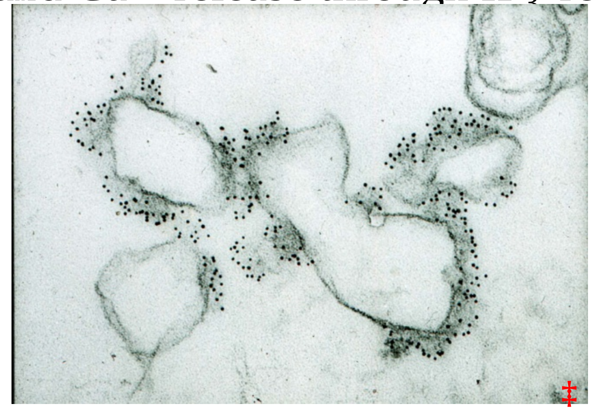
Entity of intracellular Ca^{2+} storage was unknown.

Ca^{2+} concentration out of the cell is mM concentration as in the blood, but it is 10^{-7}M inside the cell. Since Ca^{2+} triggers various physiological activities, all those activities were assumed to be caused by Ca^{2+} flux from outside of the cell. However, by development of Ca^{2+} indicators such as Fura-2,Ca^{2+} storage inside the cell was discovered.

IP_3 production by stimuli from outside of the cell and Ca^{2+} release through IP_3 receptor







Ca^{2+} storage was proved to be endoplasmic reticulum（1991）
（immuno-electric microscope image by gold colloid method）

Experiment of artificial lipid bilayer
（J.Biol.Chem. 1991）
Overexpression experiment：
Amount of Ca^{2+} release and IP_3 binding activity increased.
（Neuron 1990）(Nature 1989)

IP_3 receptor is a Ca^{2+} channel.

IP_3 receptor was thought to be IP_3 binding protein, and Ca^{2+} channel was thought to be another molecule.
However, by incorporating IP_3 binding protein into artificial lipid bilayer, IP_3 binding protein was discovered to be a good Ca^{2+} channel.（J.Biol.Chem. 1991) By overexpressing cDNA of cloned IP_3 receptor (Nature 1989) in a cell, IP_3 binding activity and Ca^{2+} channel activity increased.（Neuron 1990)。From these results, IP_3 receptor was proved to have both properties of IP_3 binding protein and Ca^{2+} channel.

calcium

metallic ion

cell poison

component of bone（vertebrates）

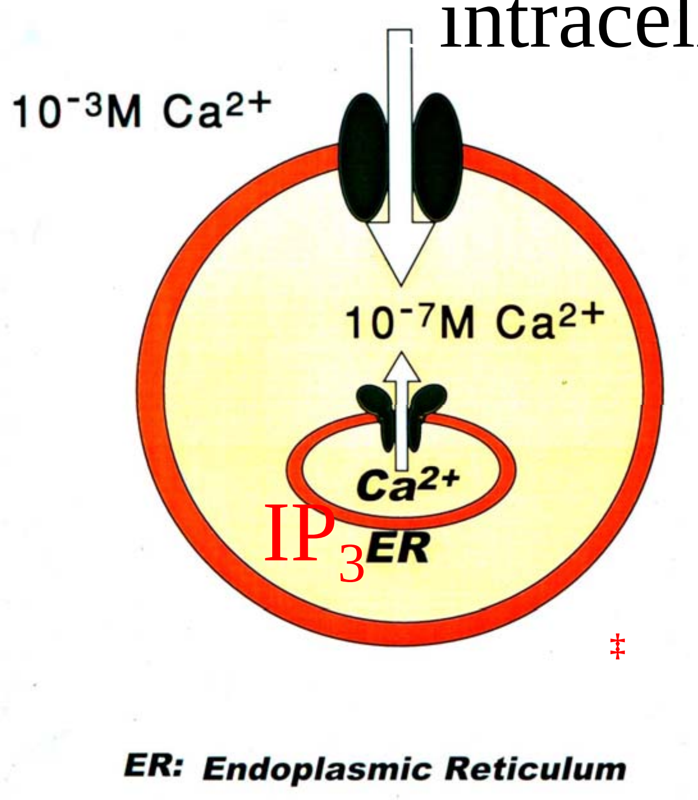
cuticle（insects）

essential second messenger（intracellular）

basic————clinical

normal————disease

intracellular calcium dynamics



1) **Calcium Influx**

(through the plasma membrane)

2) **Calcium Release**

(from the intracellular stores)

(Ca^{2+} wave and oscillation)

Ca^{2+} oscillation during fertilization

IP_3 receptor was found to be the starter of **Ca^{2+} oscillation**。*(Science 1992)*

Control

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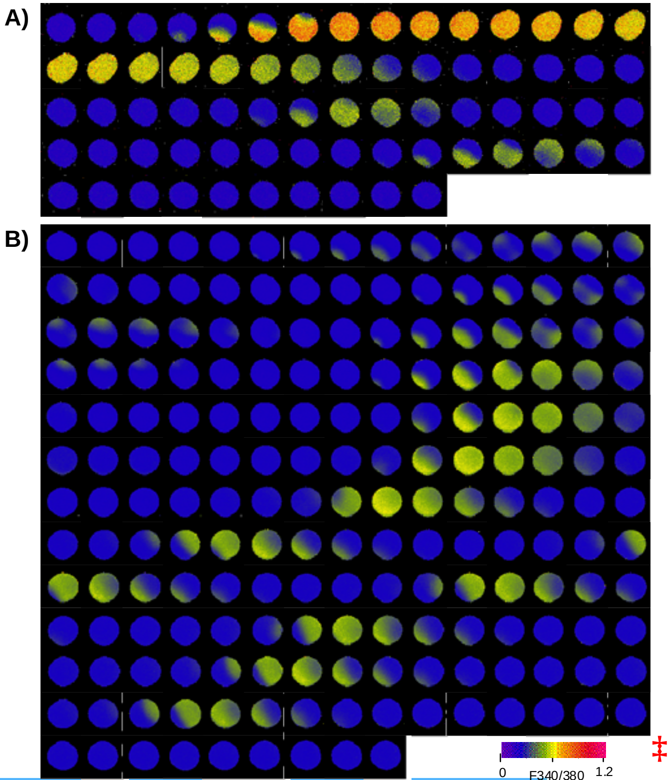
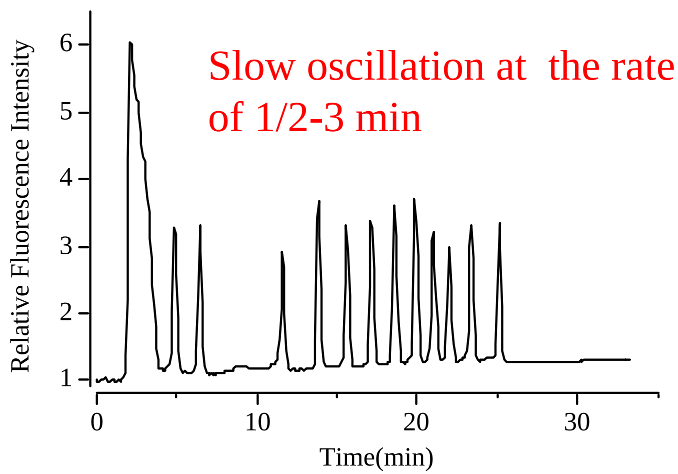
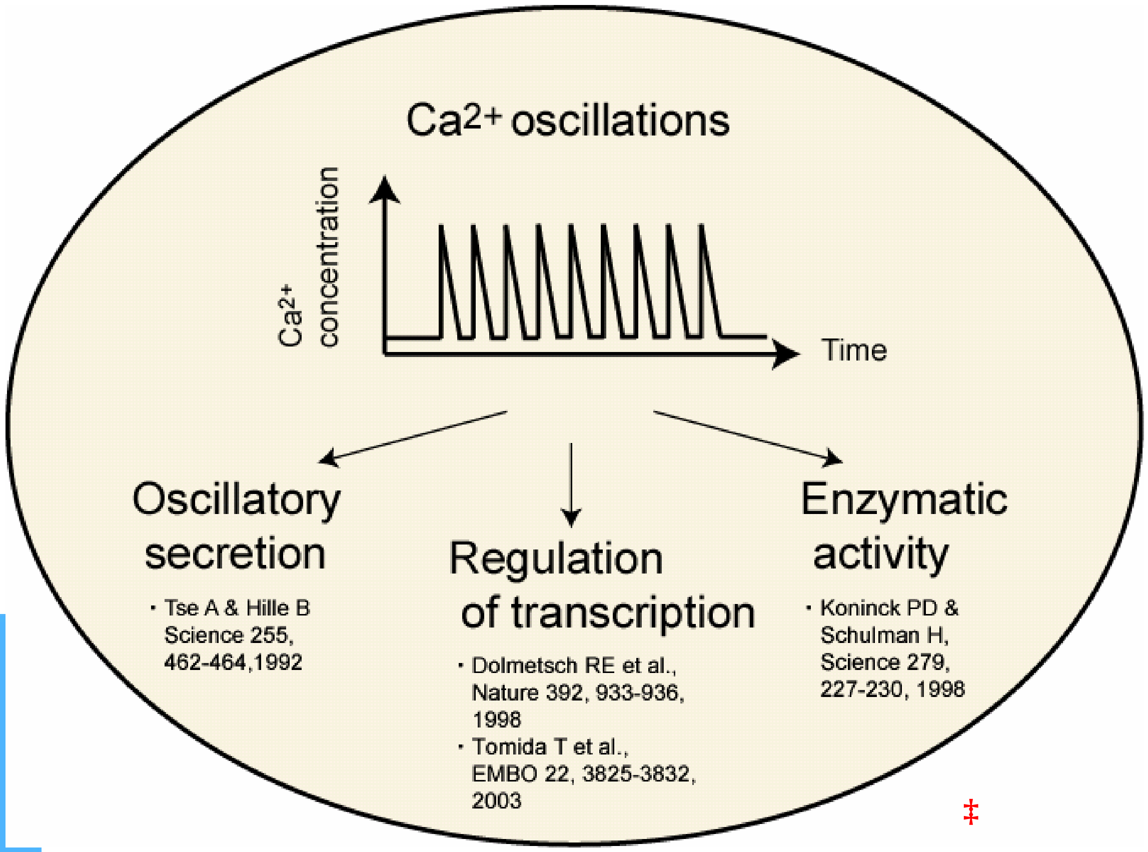




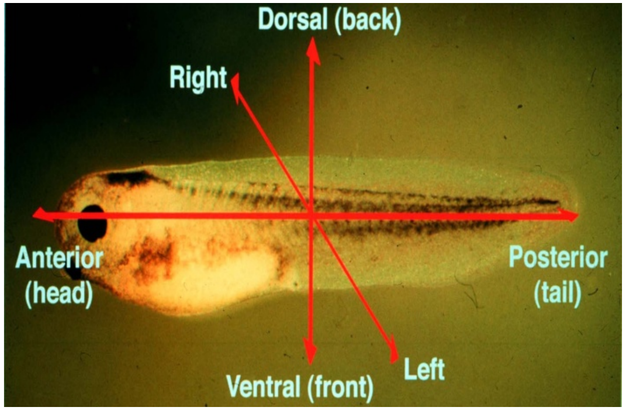
Slow oscillation at the rate of 1/2-3 min

Amplitude and frequency of Ca^{2+} oscillation determine the targets inside the cell.



Nerve/ventral-dorsal axis formation

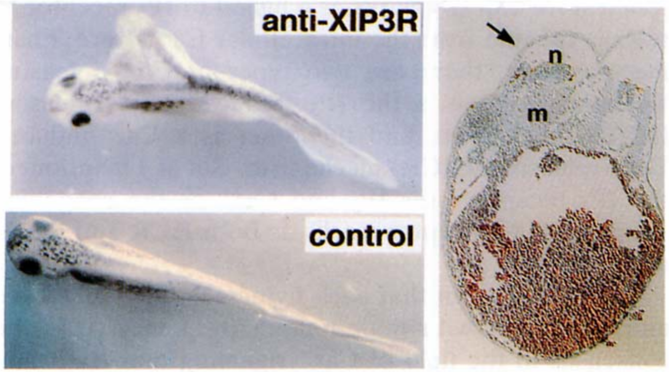
As dorsalization and neuralization means the same at developing period, ventral-dorsal axis formation is important for formation of nerve system. Ventral-dorsal axis formation is determined at 4 blastomere phase after fertilization.



‡

Effect of lithium

１）lithium formulation, remedy for manic-depressive
２）inhibition of inositol polyphosphate 1-phosphatase,
inositol monophosphate phosphatase
３）developmental anomaly（secondary axis formation）
ventral side（4 cells phase）
→dorsalization

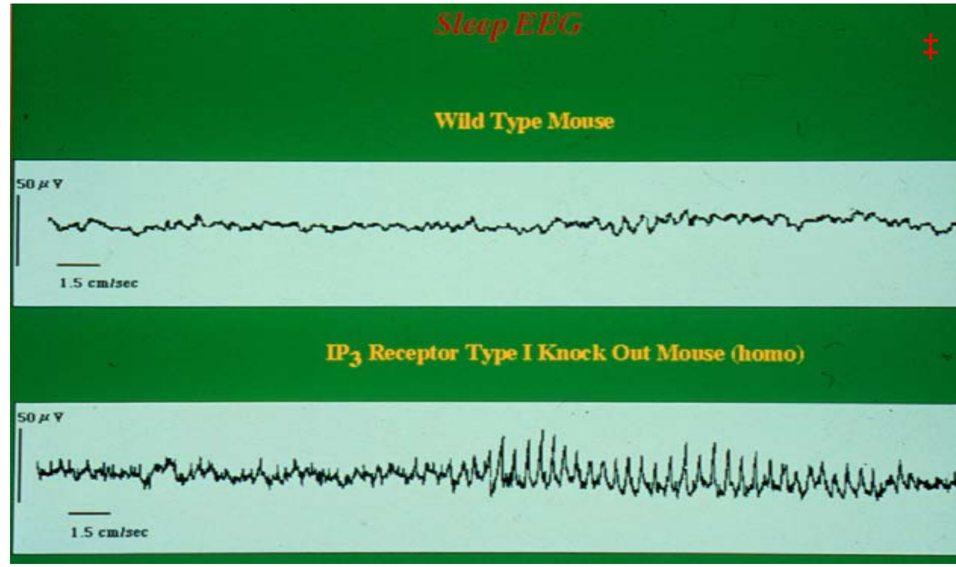


IP_3 receptor involvement in ventral-dorsal axis formation ***(Science 278, 1940-1943, 1997) (Nature 417 295-299 2002)***

Effects of lithium written above were known, and from the fact that it inhibits enzymes of inositol phosphatase system, Prof. Mikoshiba assumed that it also inhibits the functions of IP_3 receptor. He devoted 2 years to produce monoclonal antibody for inhibiting functions of Xenopus IP_3 receptor. When this was injected to ventral part of the early embryo, dorsal differentiation was induced, and secondary axis was formed. From this experiment, that Ca^{2+} release from IP_3 receptor is concerned in ventral-dorsal axis formation was proved.(Science 278, 1940-1943, 1997) NF-AT was identified to be a downstream factor（Nature 417 295-299 2002)

Mouse with IP_3 receptor gene defect has epilepsy and cerebellar ataxia(Nature 379,168-171,1996)

Figure removed due to copyright restrictions



IP_3 receptor's role in neural plasticity was discovered
a slice of cerebellum : long-term depression（LTD）disappears (J.Neurosci. 18, 5366-5373,1998)

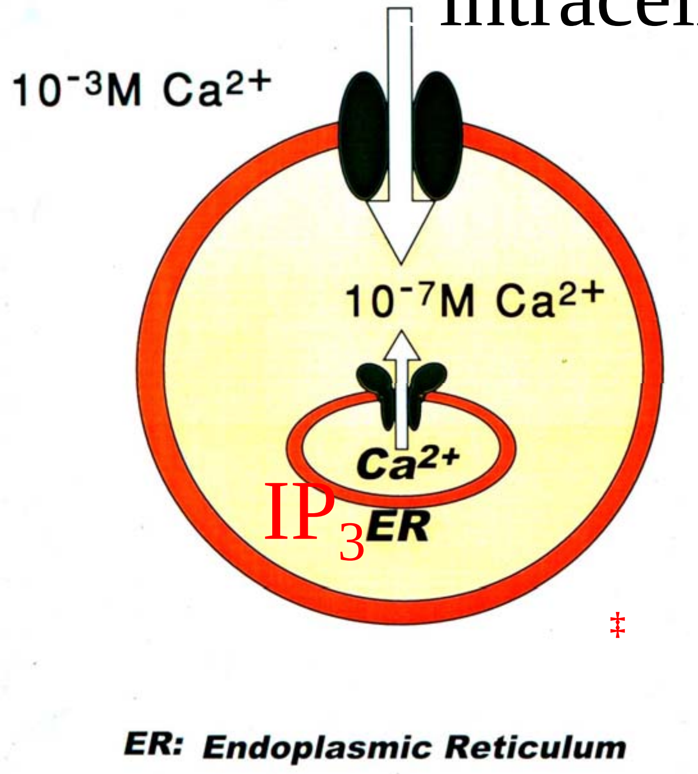
a slice of hippocampus（CA1）：long-term potentiation（LTP）is enhanced, LTP suppression disappears

De-potentiation disappears, no change in LTD

(Learning & Memory 7, 312-320,2000)

Related to specificity of synapse **(Nature 408,584-588,2000)**

intracellular calcium dynamics



1) **Calcium Influx**

(through the plasma membrane)

2) **Calcium Release**

(from the intracellular stores)

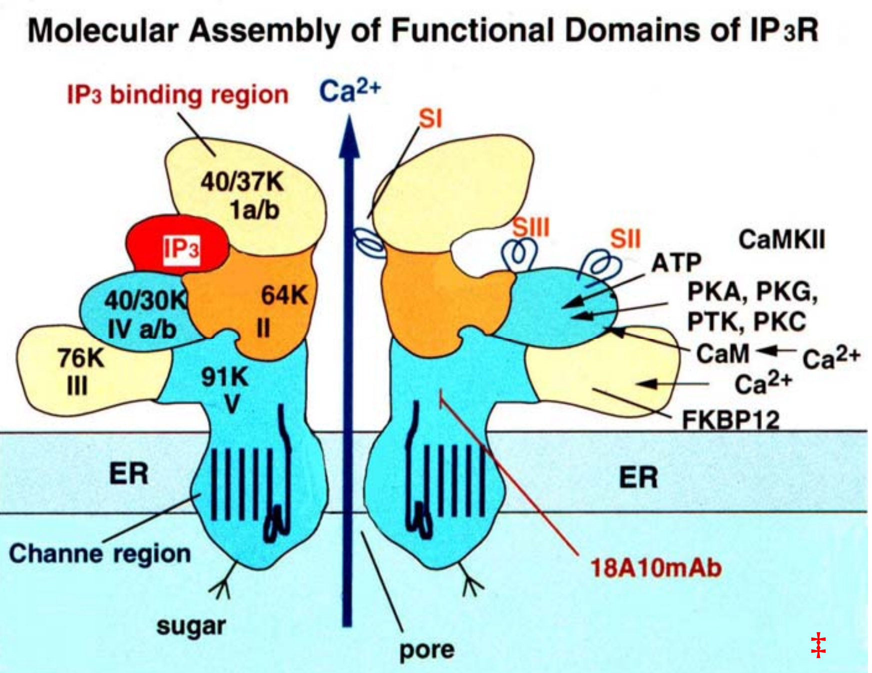
(Ca^{2+} wave and oscillation)

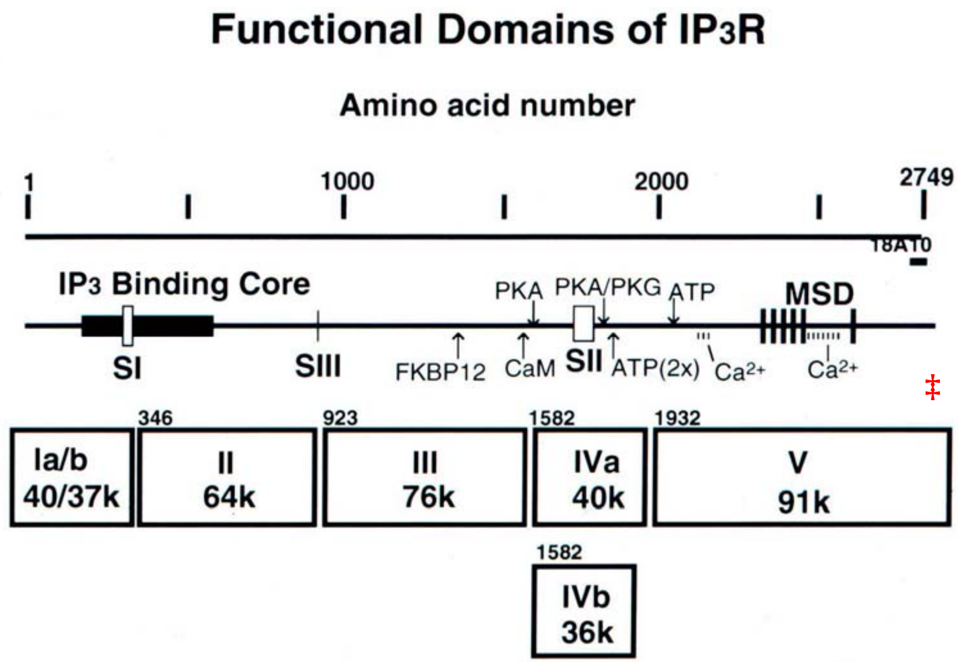
Unique biochemical properties of IP_3R

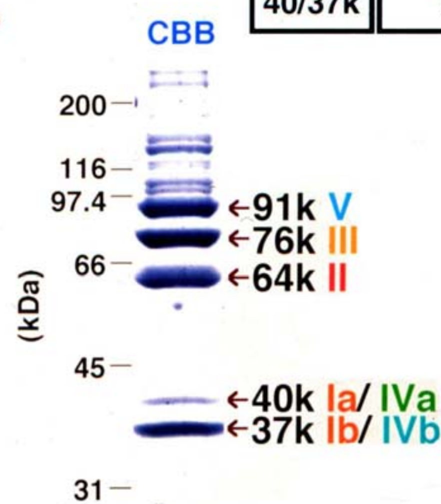
1) high affinity IP_3 binding (IP_3 sponge)
2) molecular assembly of fragmented
   functional units of IP_3 R
3) quantal Ca release
4) translocation of IP_3R to the PM
   (
5) a) reticular ER movement, b) vesicular ER movement,

c) mRNA transportation, d) IP_3R clustering
vesicular ER movement
6) porous structure with cavities
7) allosteric structural change
8) IRBIT is released from IP3R
9) Redox regulation is linked to Ca^{2+} signaling

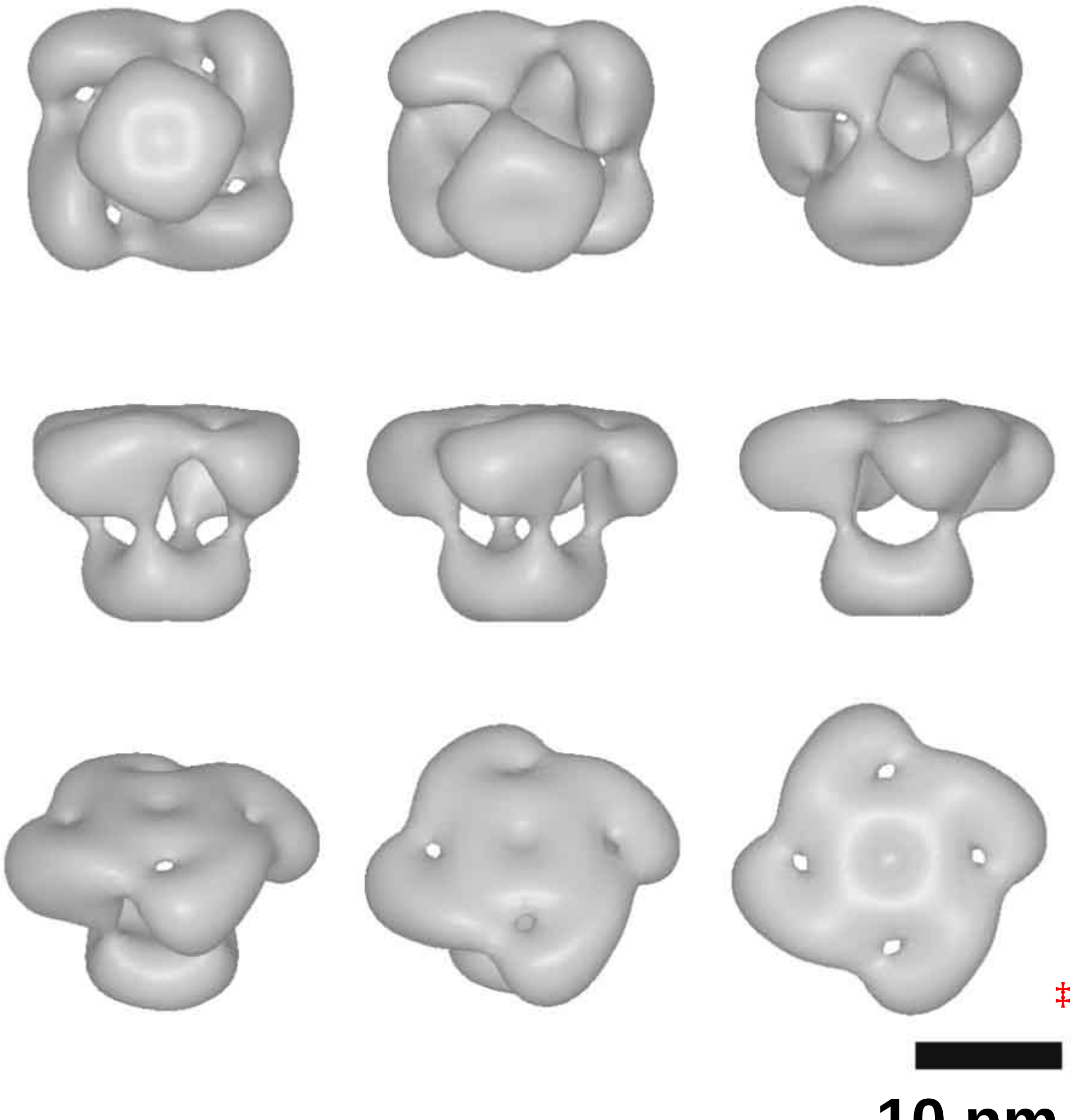
After segmentation by trypsin, IP3 receptors reassembly.
IP3 binding activity and Ca2+ release activity are same as control (J. Biol Chem. 274 316-327 1999)



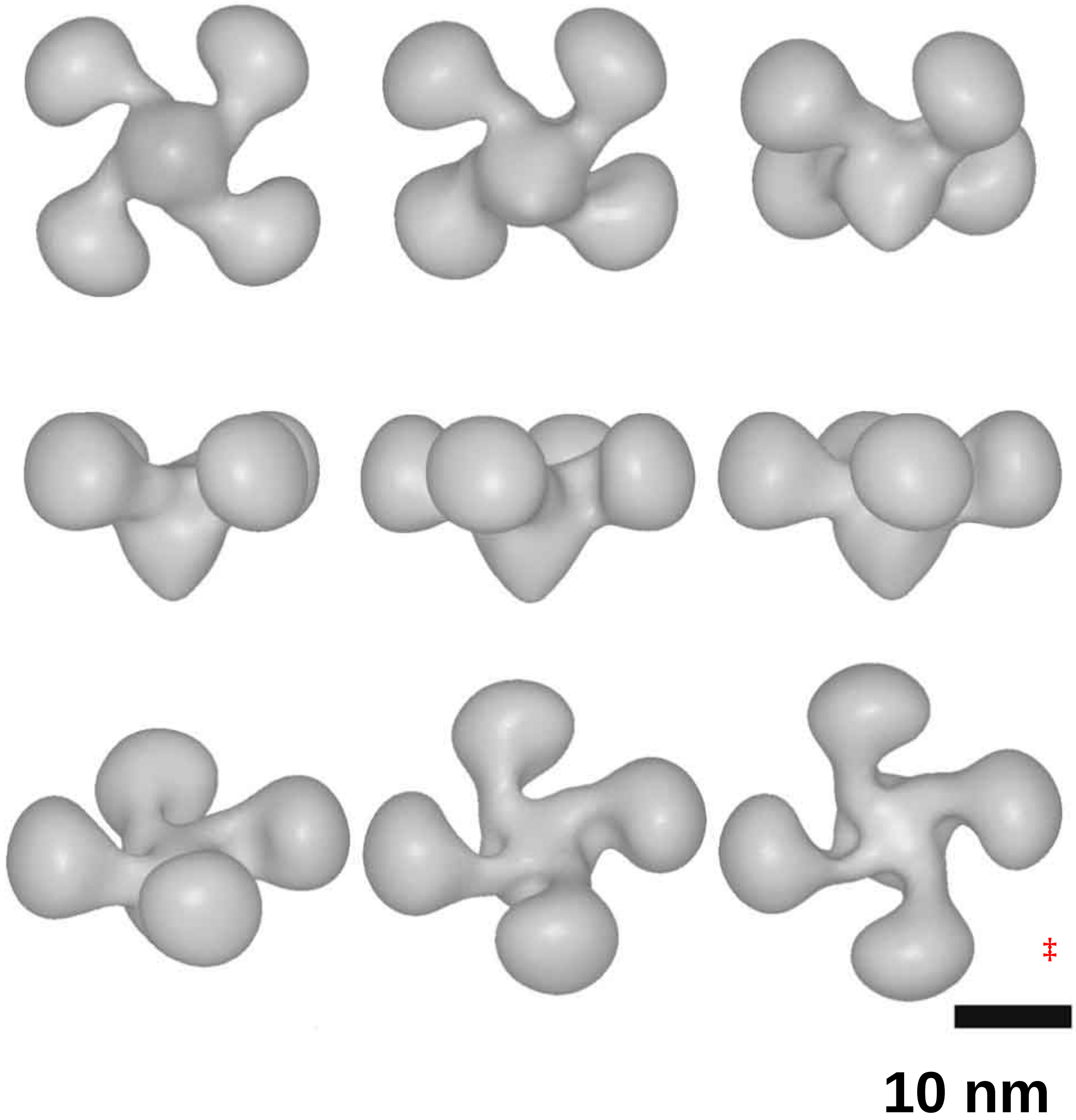




A 3D-Structure of IP_3R1 in the Ca^{2+}-unbound State



A 3D-Structure of IP_3R1 in the Ca^{2+}-bound State



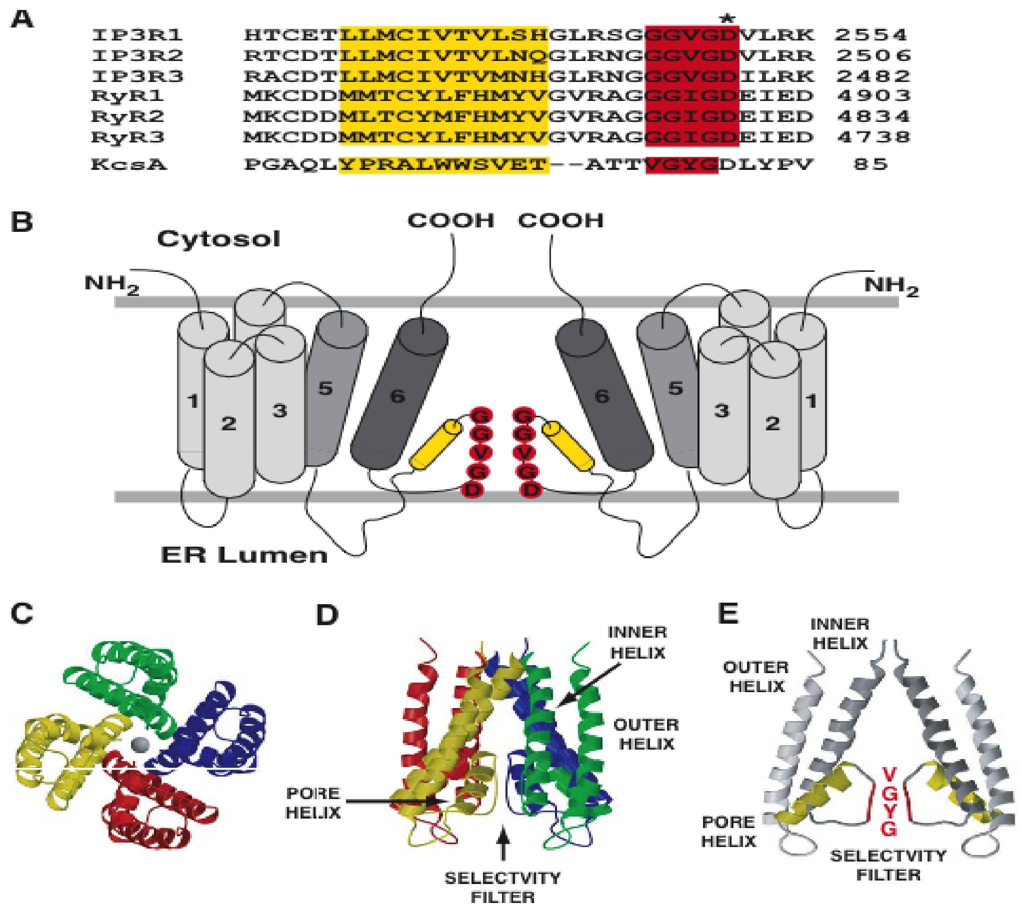
Gating mechanism of IP_3 receptor

Pore structure of IP_3 receptor/Ca^{2+} channel

Comparison of each channel's sequences

‡

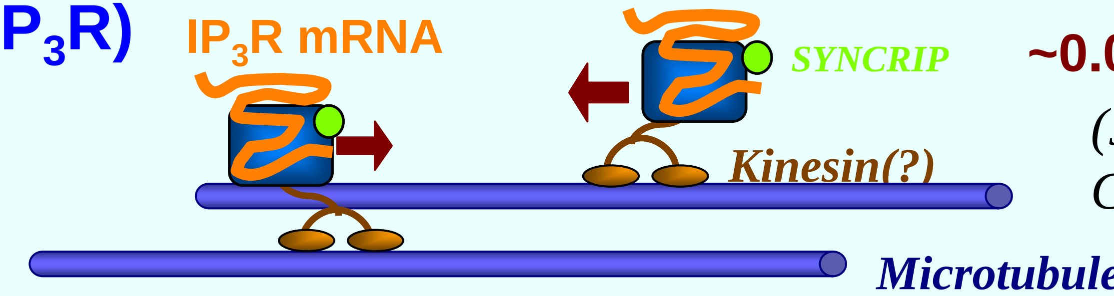
Pore structure of IP_3receptor



Structure of KCSA channel

4 ways of intercellular transportation of IP3receptor

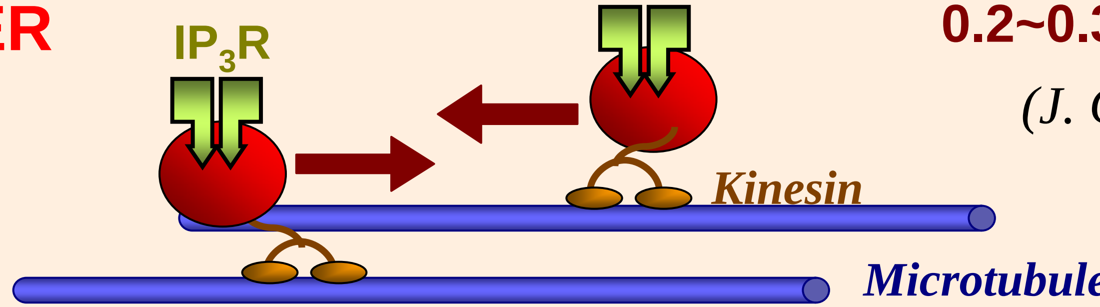
1) mRNA (IP_3R)



~0.05 μm/s

(J. Biol. Chem. 2004a)

2) granular ER

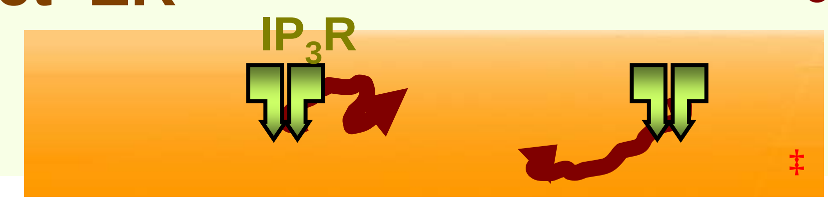


0.2~0.3 μm/s

(J. Cell Sci. 2004)

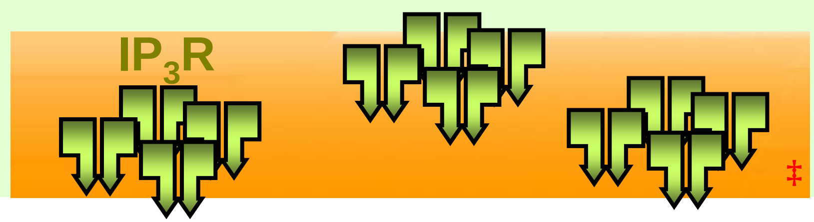
3) net ER

0.26 μm²/s



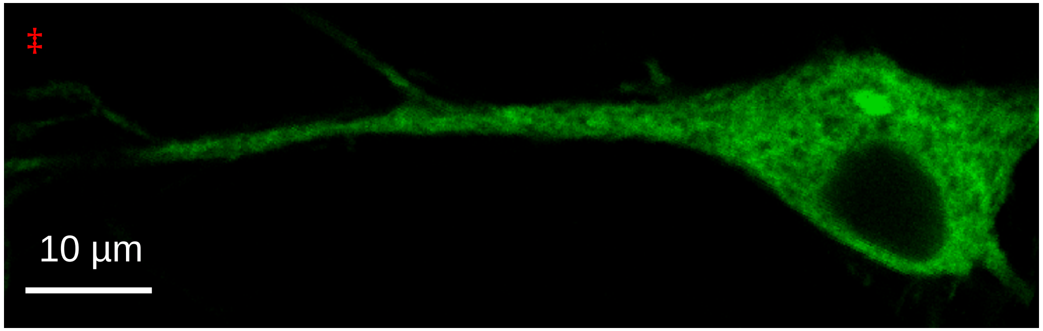
(J. Biol. Chem. 2004b)

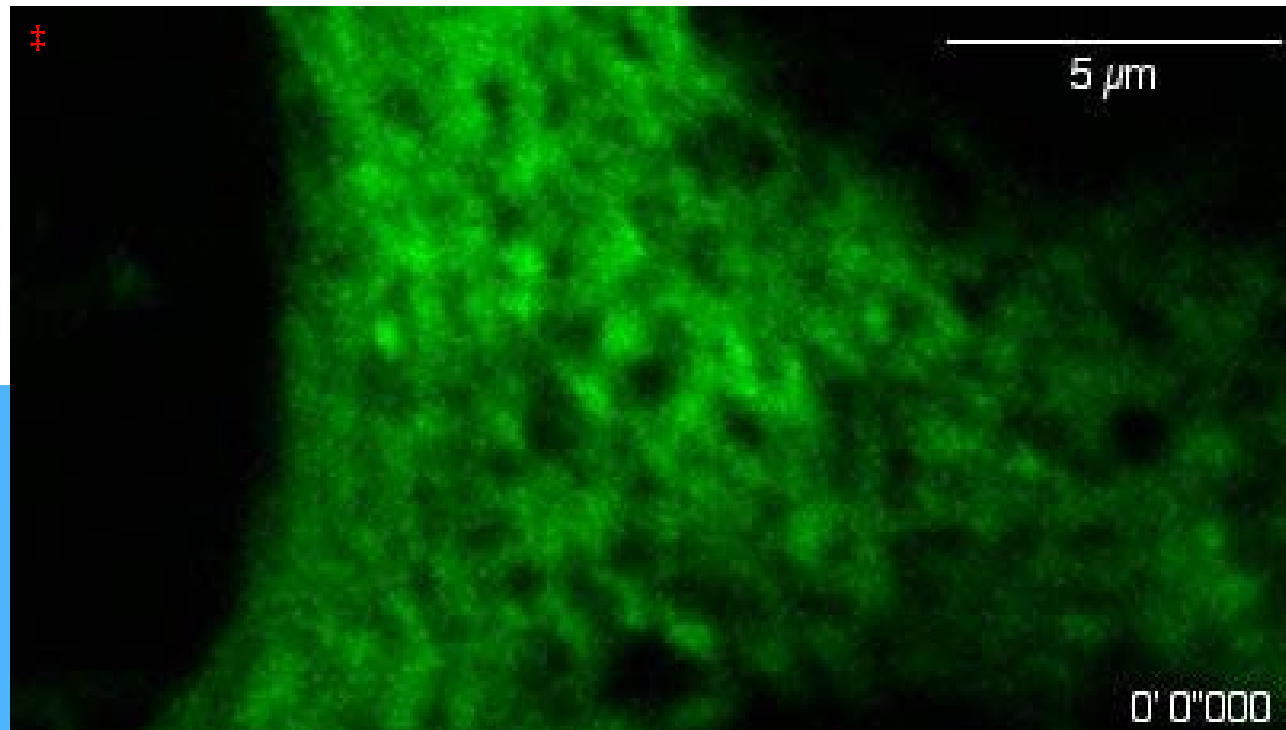
4) cluster formation



(J. Biol. Chem. 2005)

ER in Neuron Is a Meshwork, Mainly

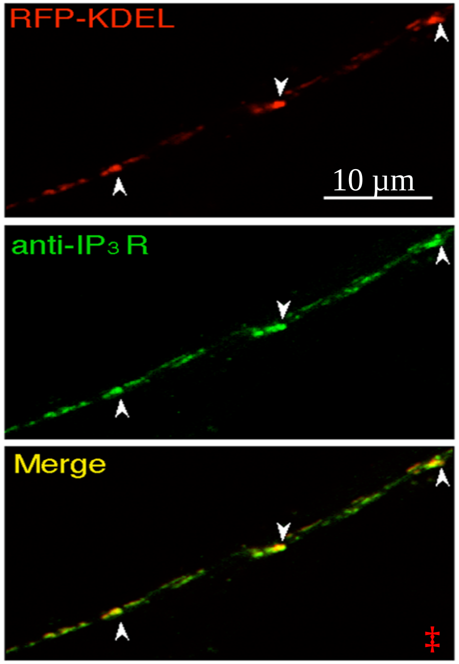




ER meshwork is <u>rather static</u>

ER marker (RFP-KDEL) co-localizes with IP_3R1 and calcium pump

ER granule moves bi-directionally in dendrites *(J. Cell Sci. 2004)*



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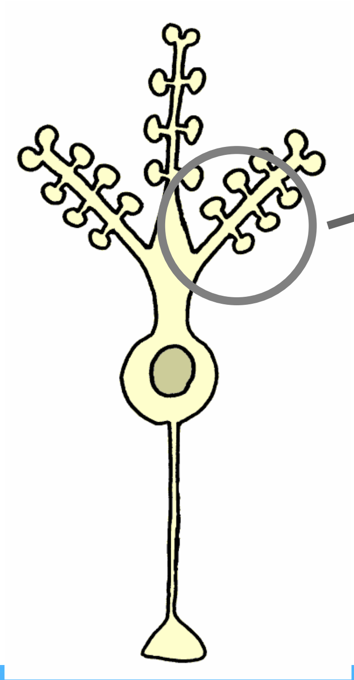
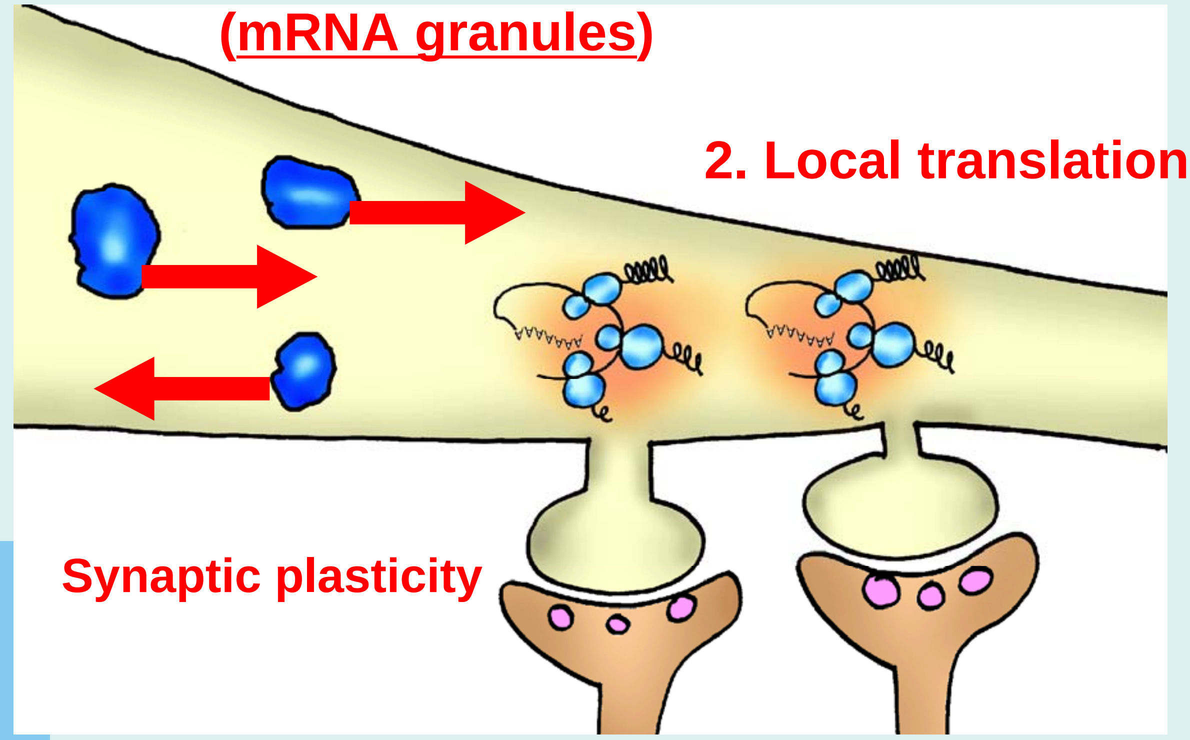
RFP-KDEL : A marker for ER

Local Protein Synthesis in the Neuronal Dendrite



Protein synthesis in the dendrite

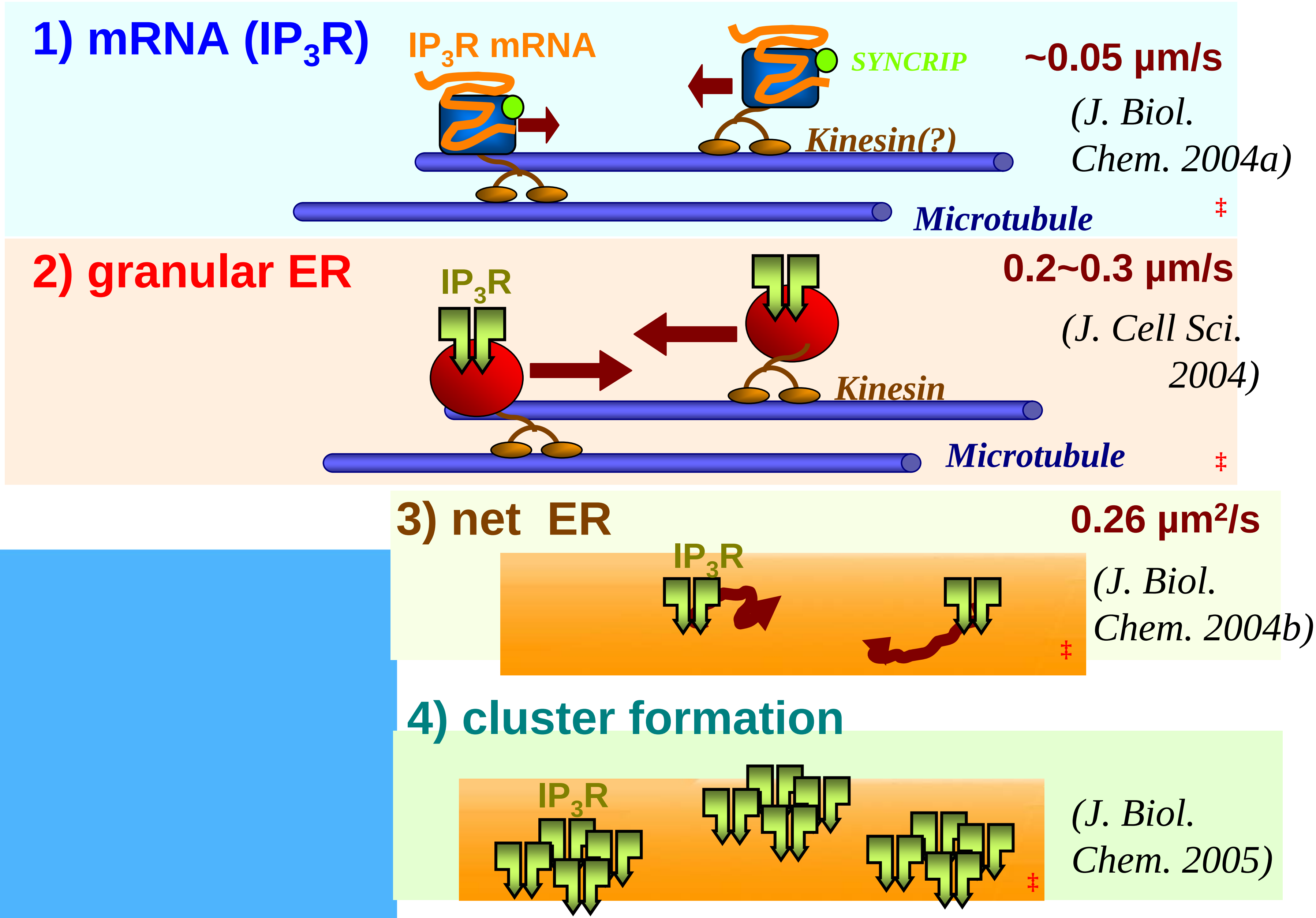
‡



Various kinds of mRNAs are targeted to the dendrite

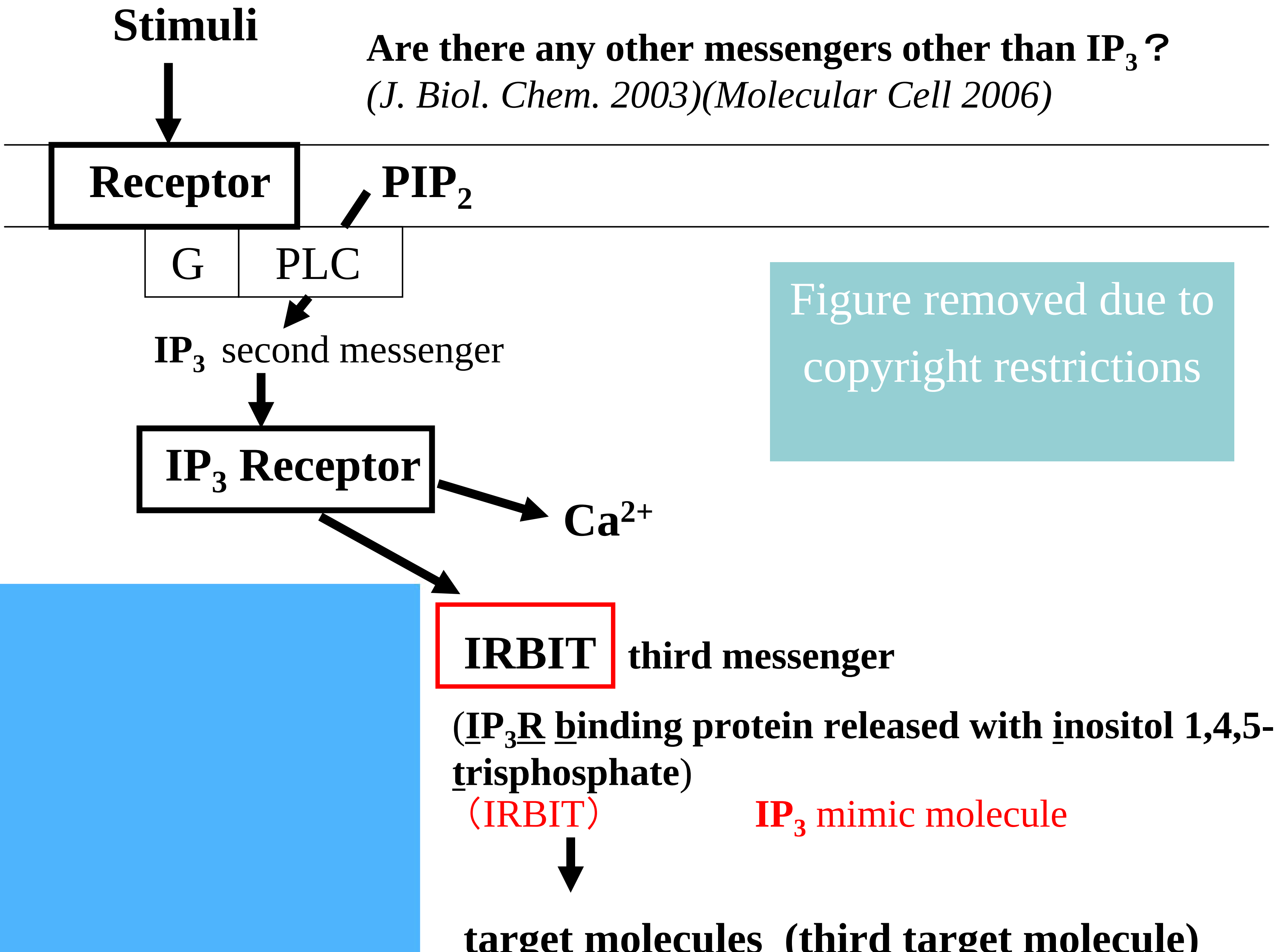
Cytoskeletal proteins (MAP2, β-actin, Arc, neurofilament protein)
Kinase (CaMKIIα)
Receptors and Channels (Glycine receptor, Glutamate receptor, IP_3R1)

4 ways of intercellular transportation of IP3receptor



Are there any other messengers other than IP_3？

(J. Biol. Chem. 2003)(Molecular Cell 2006)



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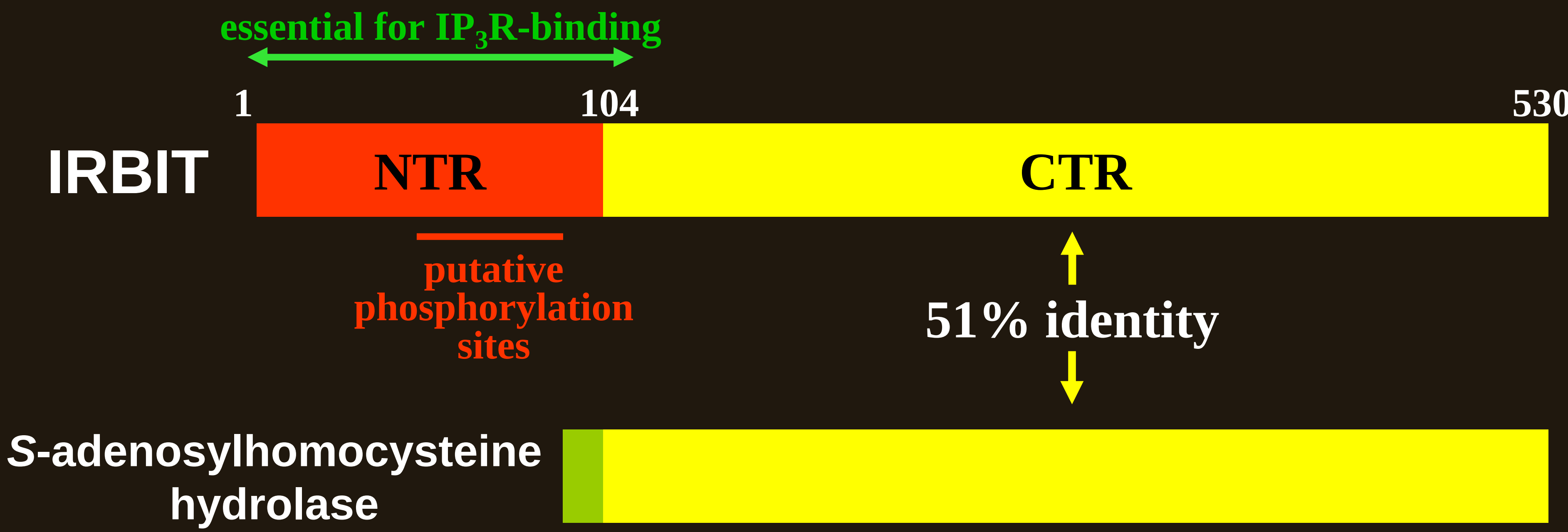
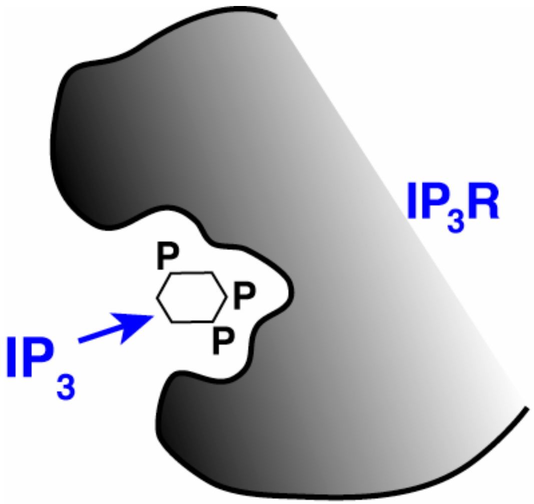
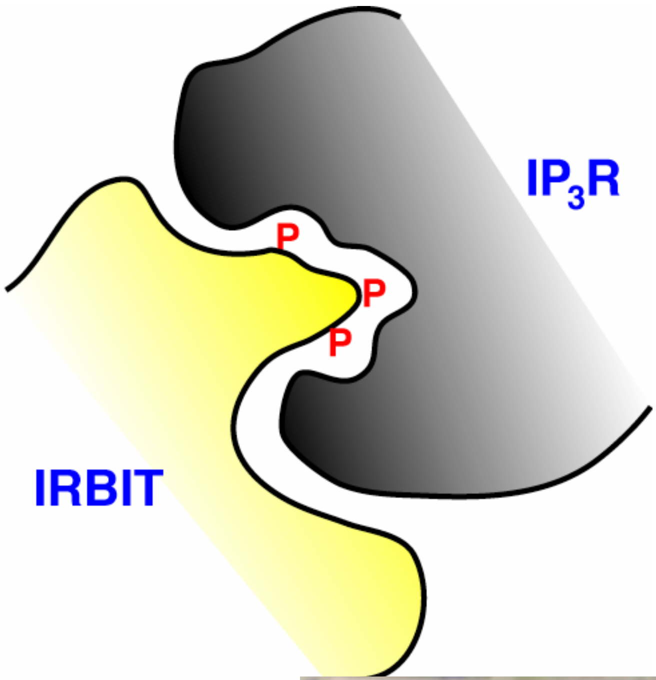
Structure of IRBIT



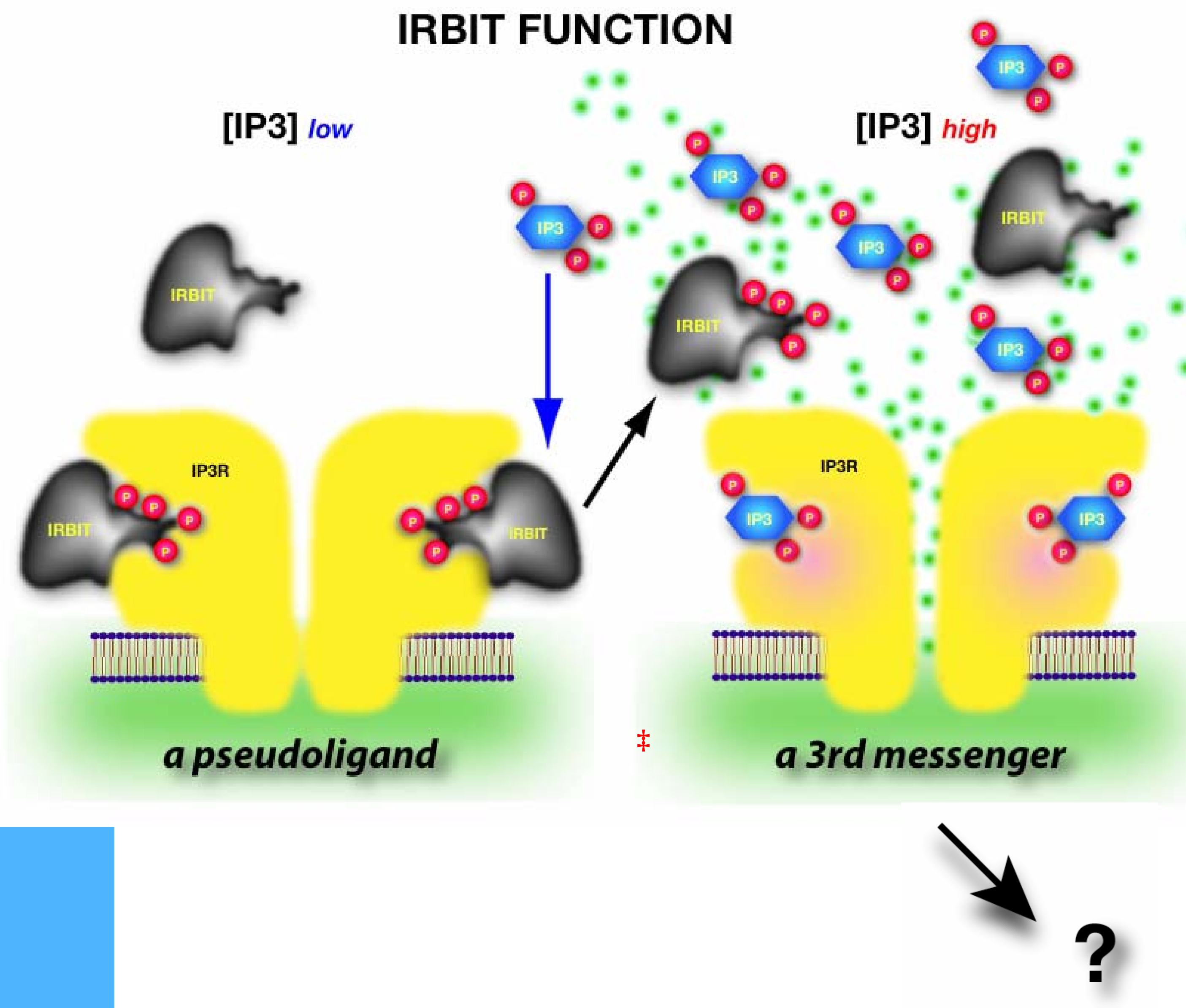
Molecular mimicry ‡





Mimicry

Two functions of IRBIT

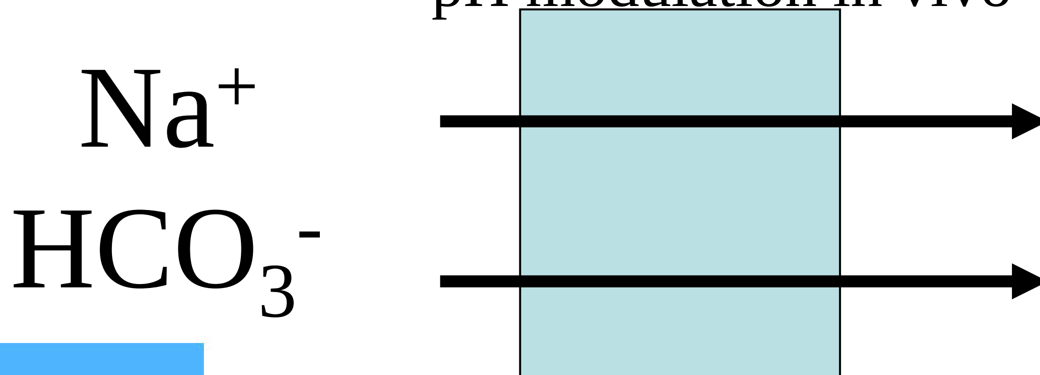


Target molecule of IRBIT:

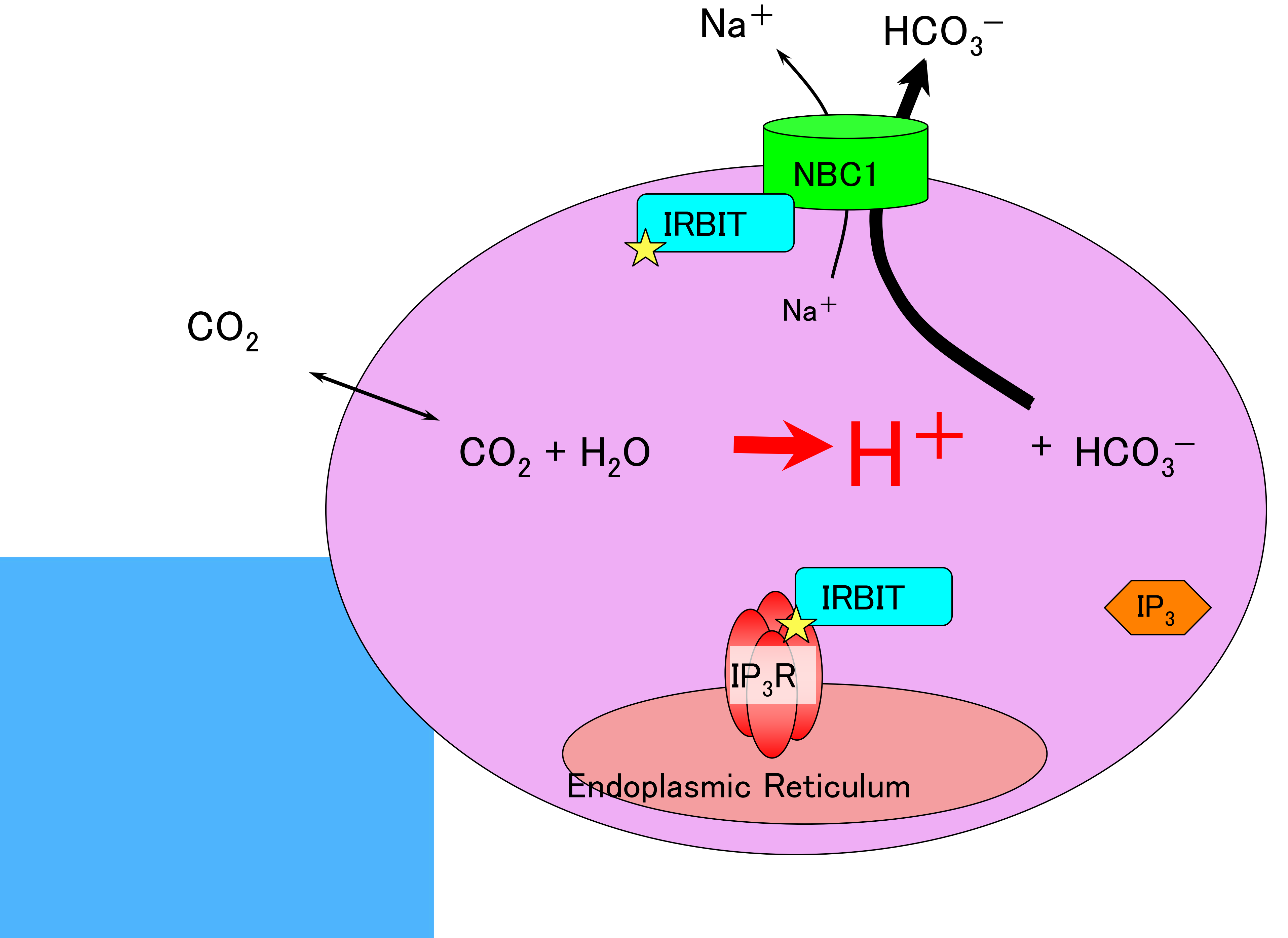
Na^+, Bicarbonate$^-$ cotransporter (NBC1)

１０times transmembrane protein　($CO_2+H_2O \rightleftharpoons H^+ + HCO_3^-$)

pH modulation in vivo

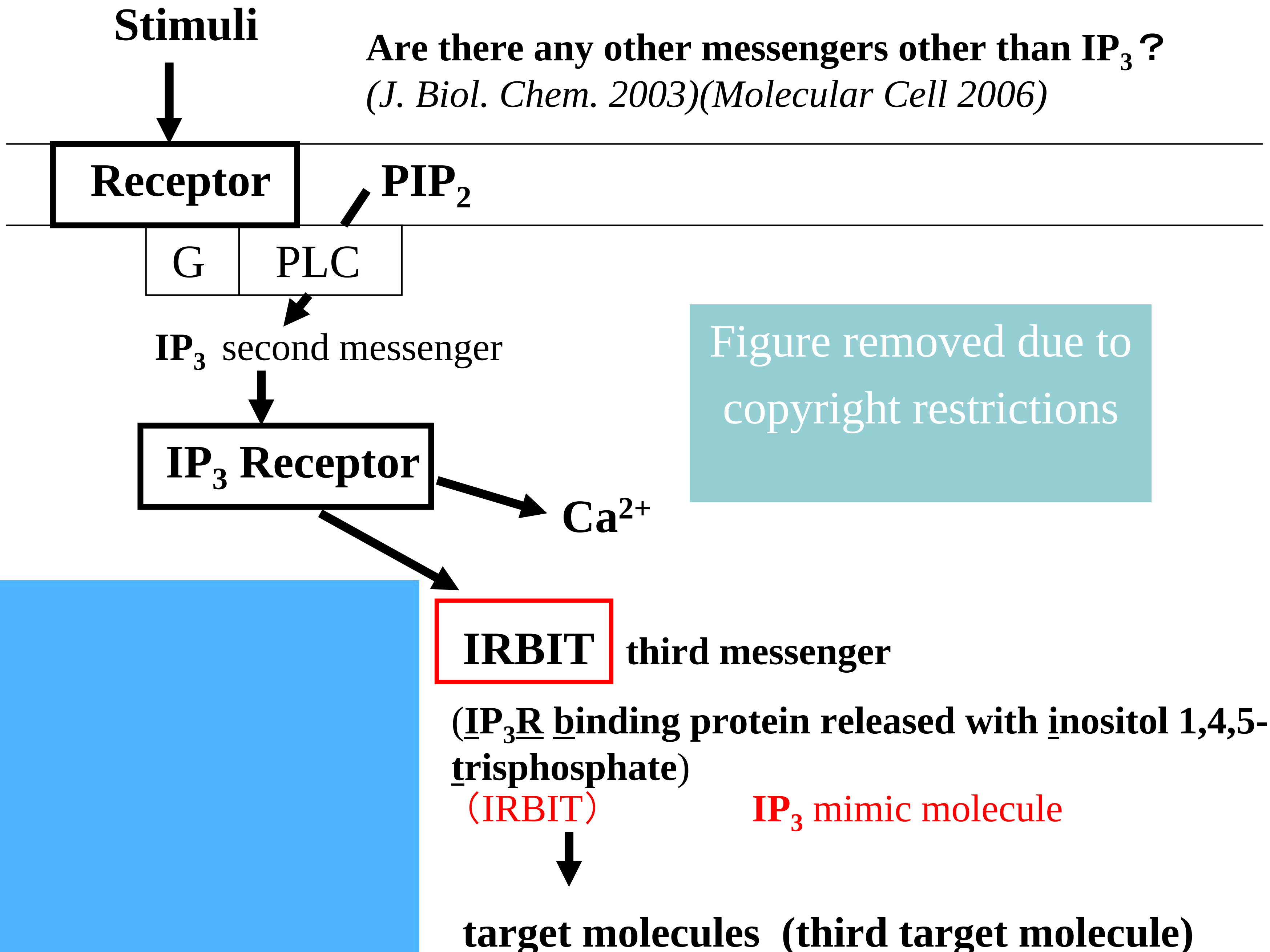


IRBIT activates NBC1*(Proc. Natl. Acad. Sci. 2006)*
phospholylation of IRBIT is needed for activation
Responsible gene for proximal RTA is NBC1
（associated with glaucoma, cataracta, low body height, retardation）
pNBC1 is highly contained in nerve system, especially in pancreas


Na+
HCO3−
NBC1
IRBIT
Na+
CO2
CO2 + H2O
H+
+ HCO3−
IRBIT
IP3
IP3R
Endoplasmic Reticulum

Are there any other messengers other than IP_3?

(J. Biol. Chem. 2003)(Molecular Cell 2006)



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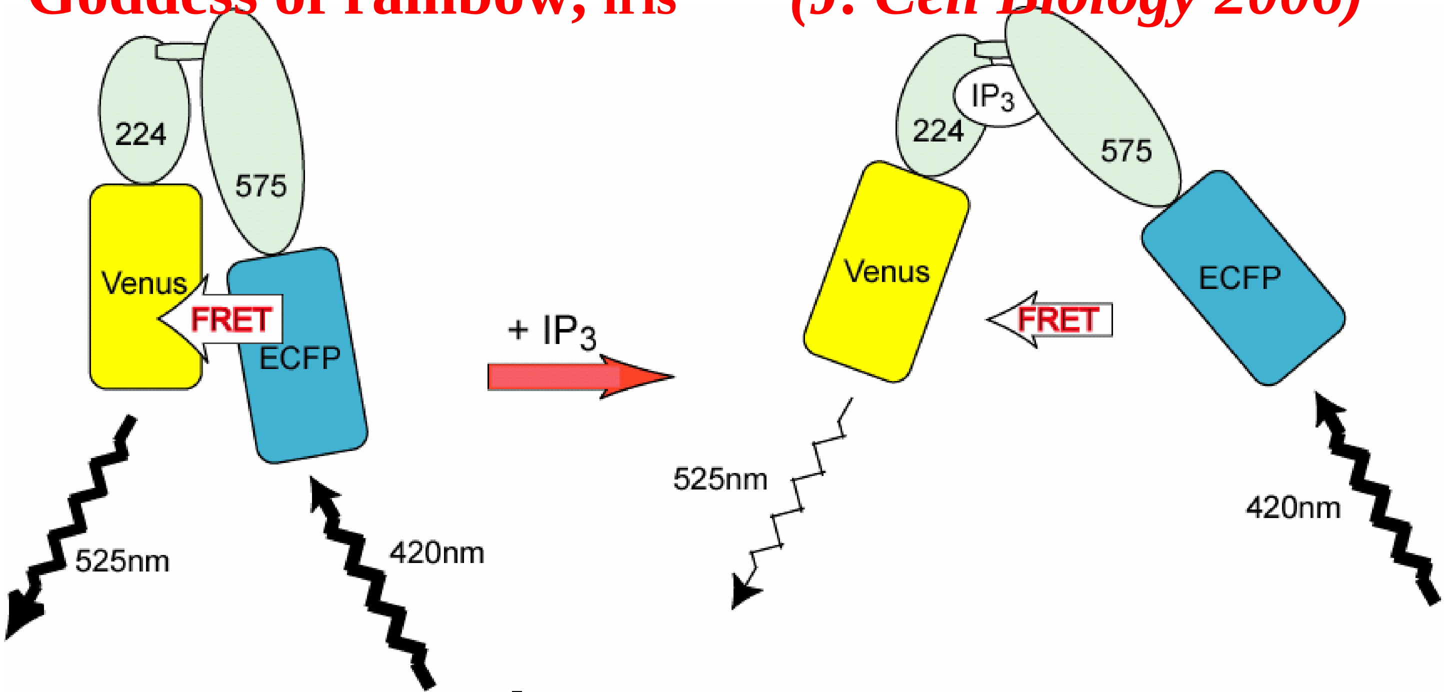
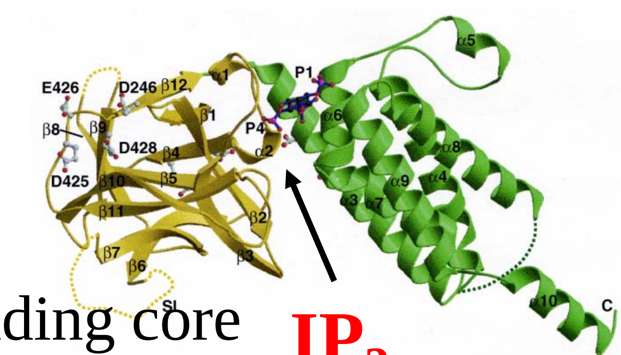
IP3R-based IP$_3$ sensor (IRIS) is a new IP$_3$ indicator

Goddess of rainbow, iris *(J. Cell Biology 2006)*



Fluorescence Resonance Energy Transfer (FRET)

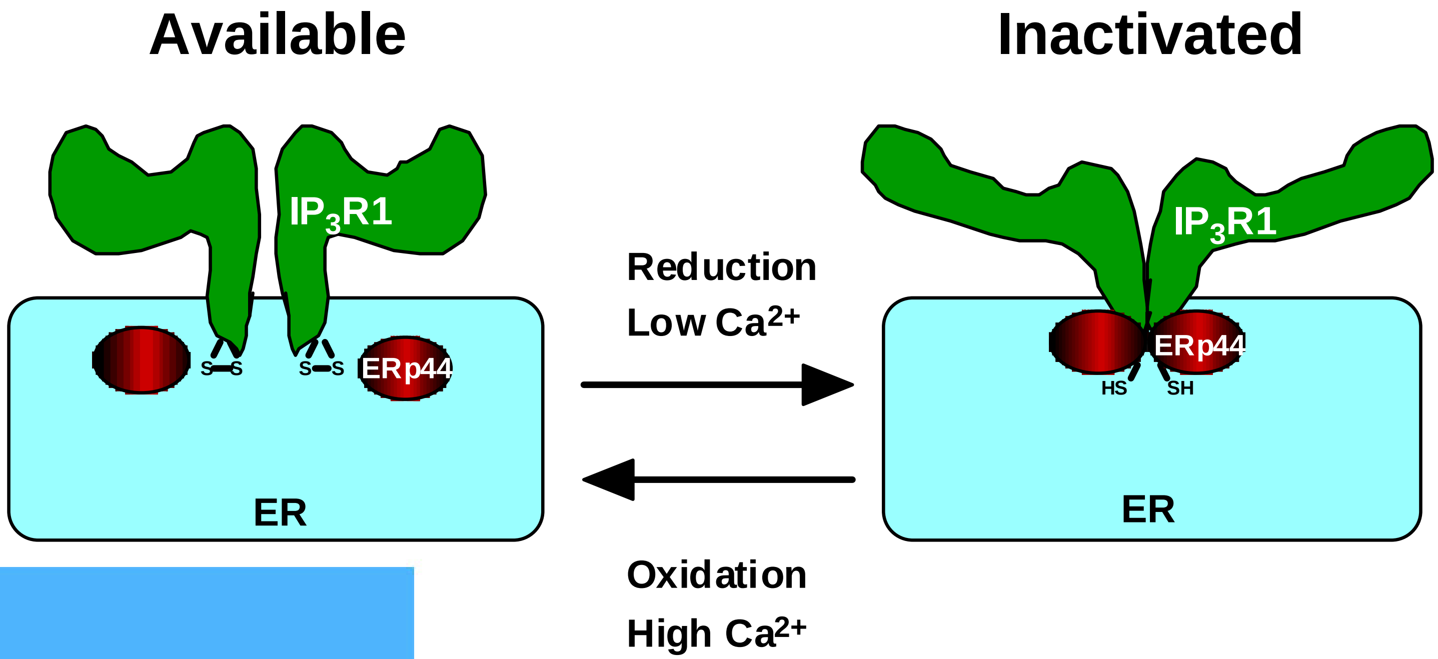


IP$_3$ binding core
(Nature 20002)

IP$_3$

redox
regulation and calcium

ERP$_{44}$ in ER(Thioredoxin family) senses redox status and modulates calcium signaling *(Cell 2005)*



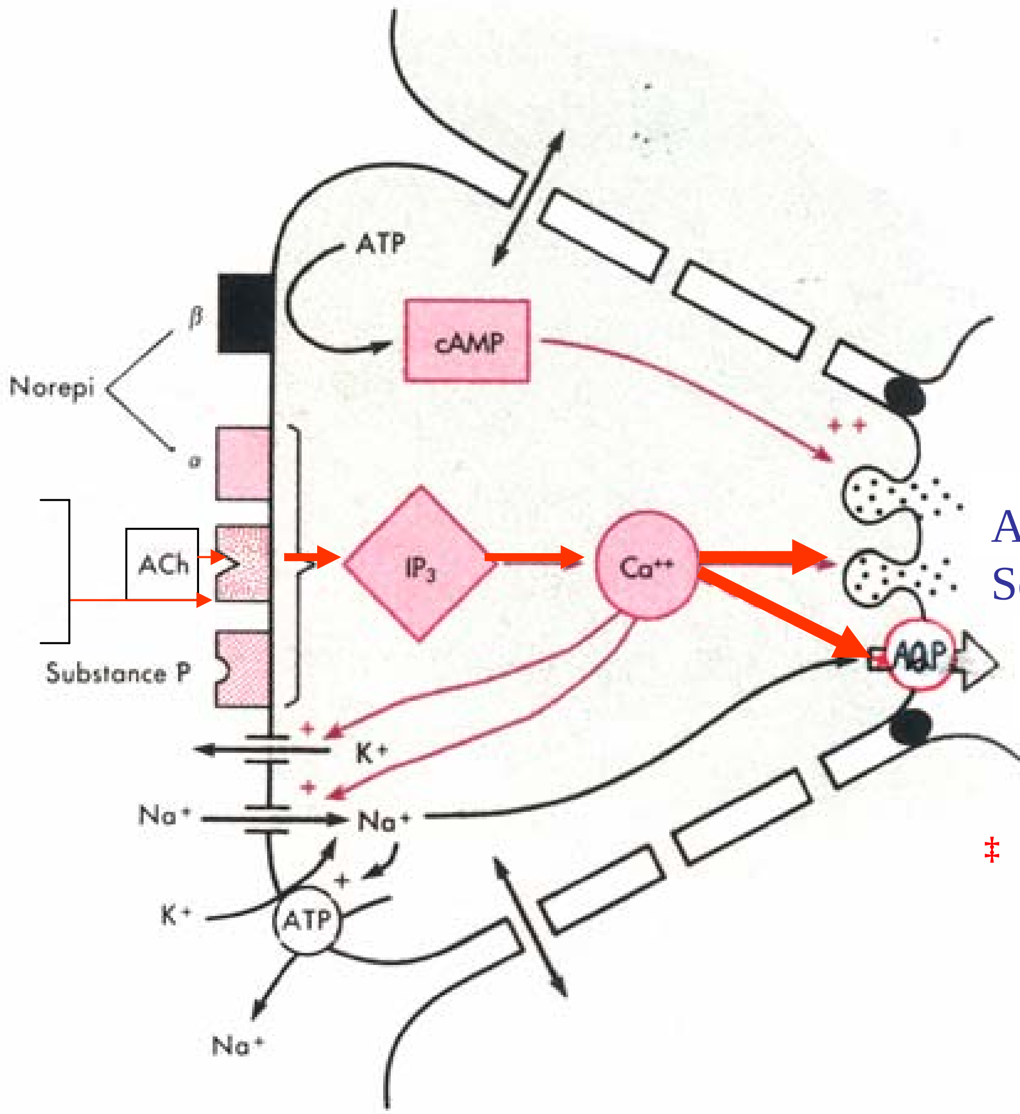
Ectocrine and **IP_3** receptor

Cellular Mechanisms of Fluid Secretion and Exocytosis in Salivary Glands

Agonists

Pilocarpine

Carbachol (CCh)

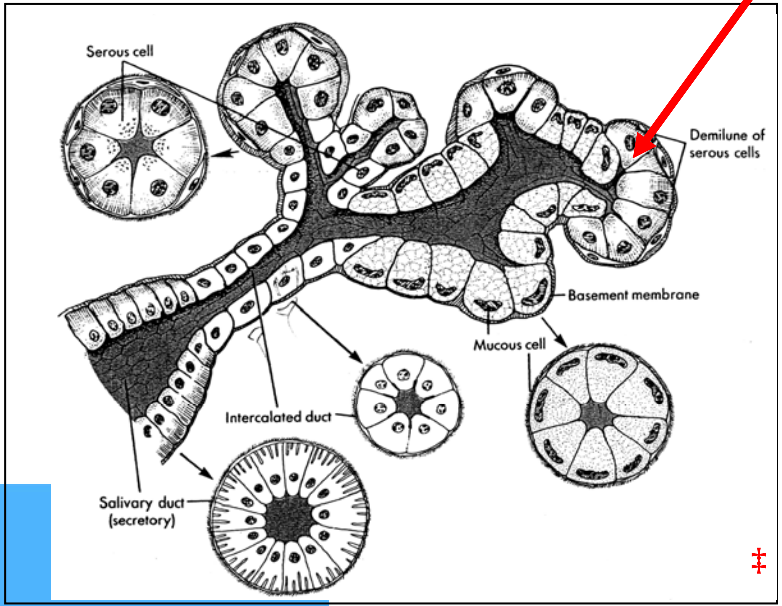


Amylase Secretion

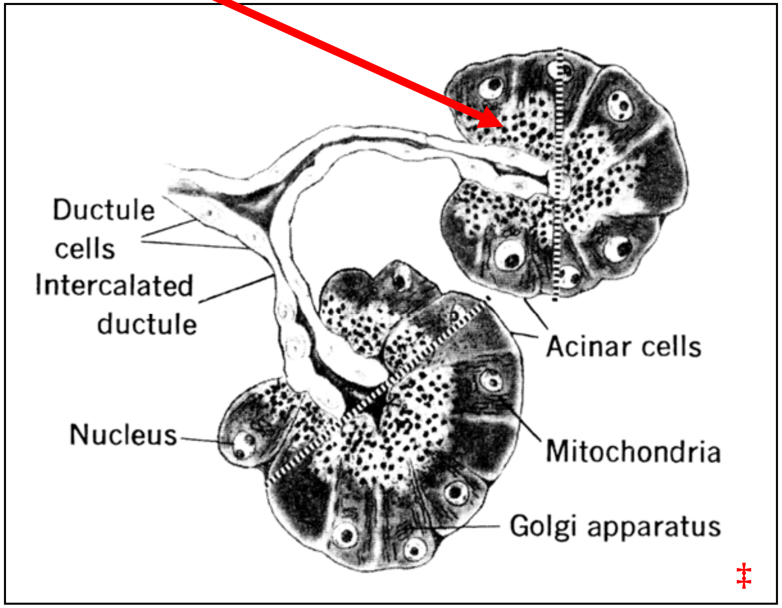
Fluid (Water) Secretion

Type 2 and type 3 IP_3 receptors play key roles in Ca^{2+} signaling and secretory function of exocrine glands.

IP3R2 & IP3R3

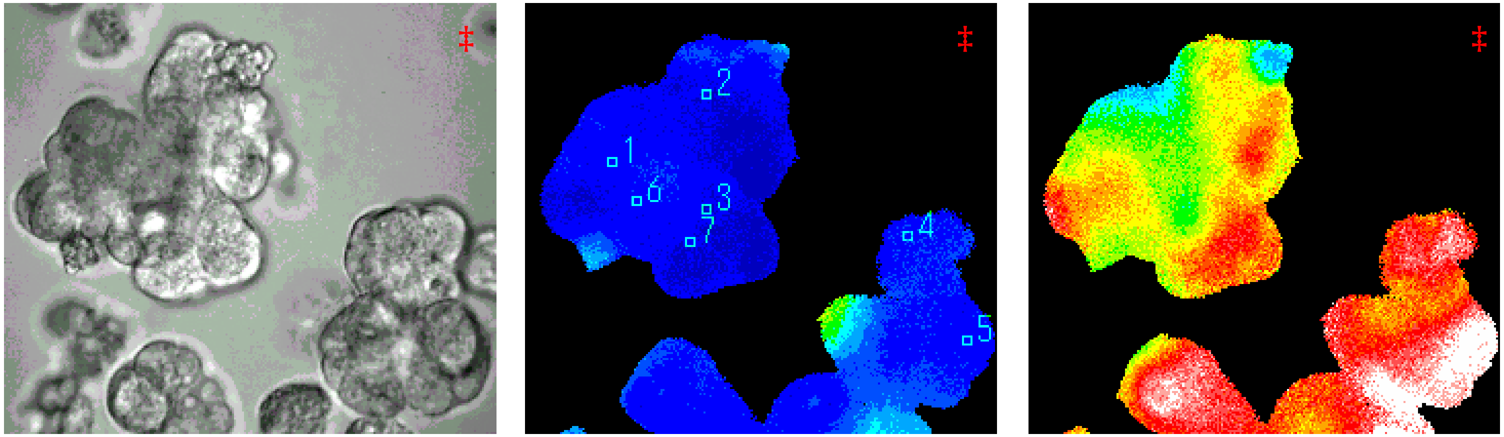


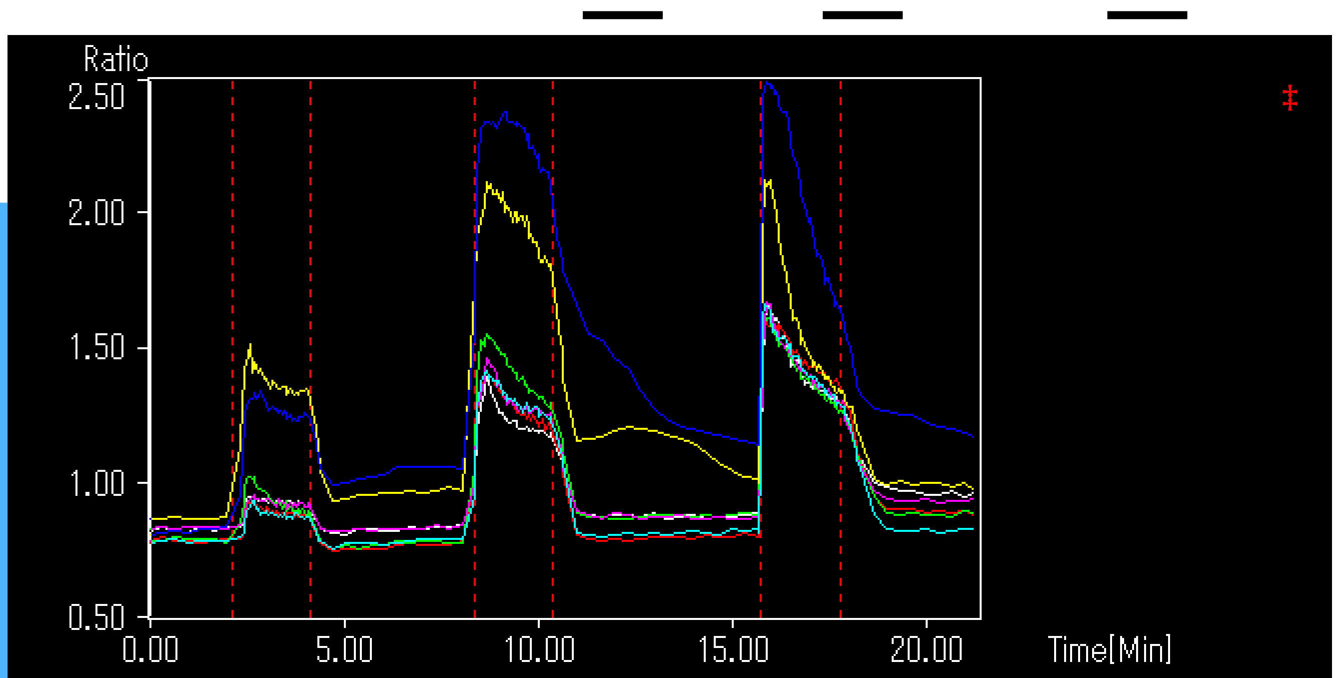
Salivary gland



Pancreas

WT

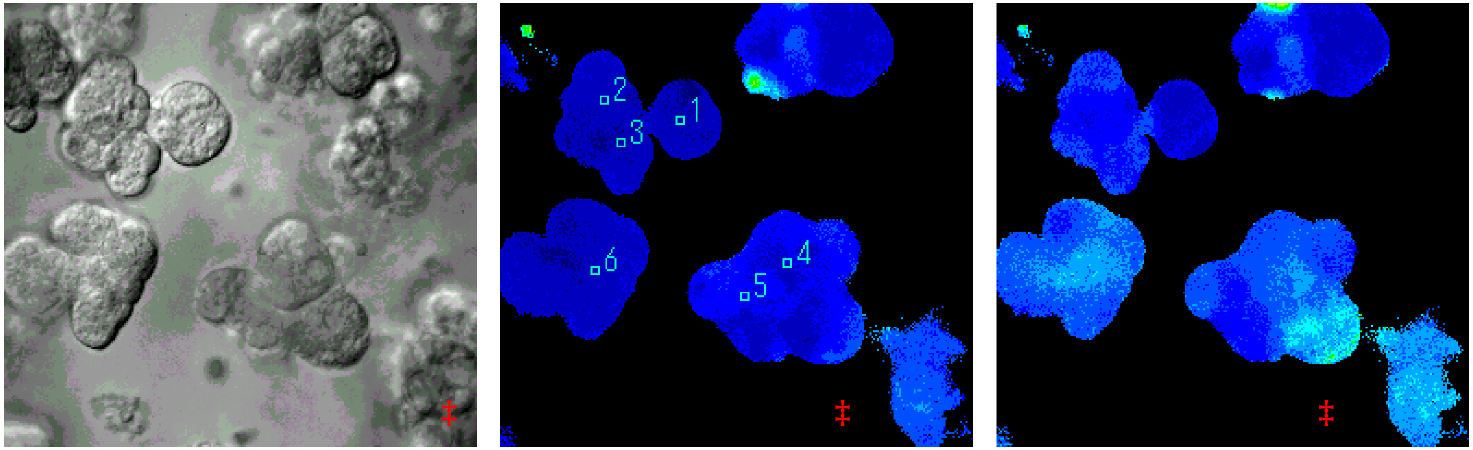

resting
30μM CCh
1
2
3
4
5
6
7


CCh
0.3μM
3μM
30μM
Ratio
2.50
2.00
1.50
1.00
0.50
0.00
5.00
10.00
15.00
20.00
Time[Min]

O/O

resting

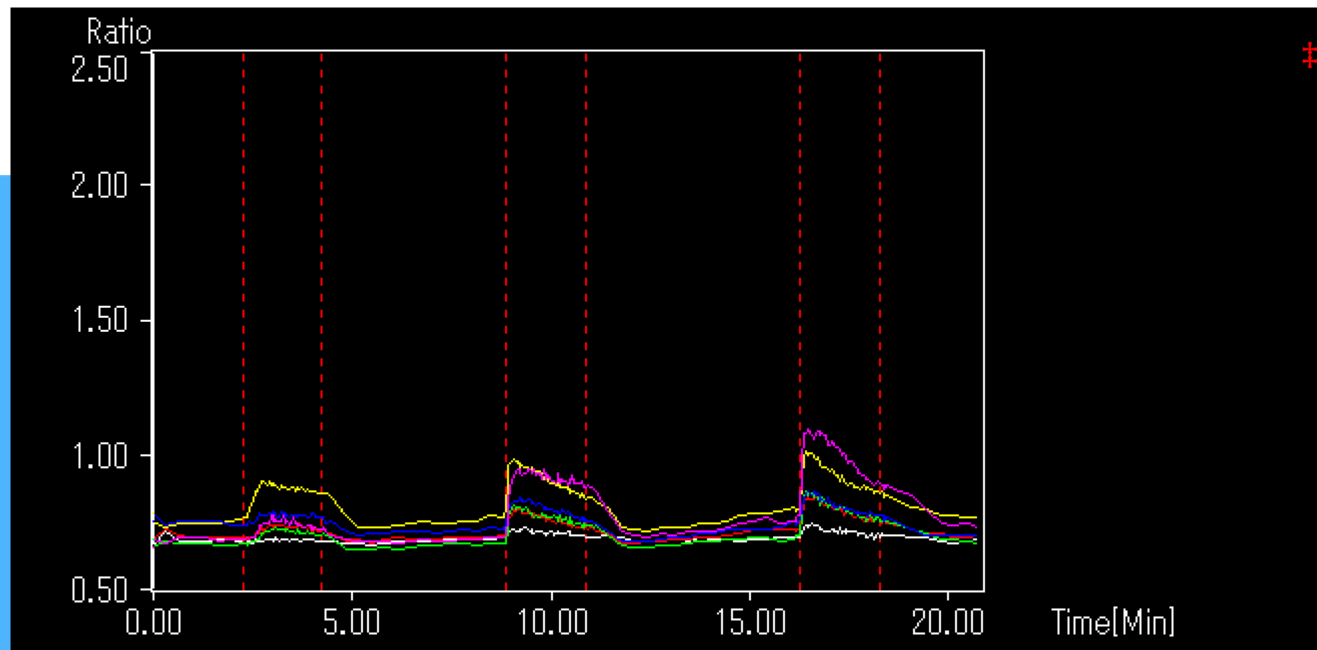
30μM CCh



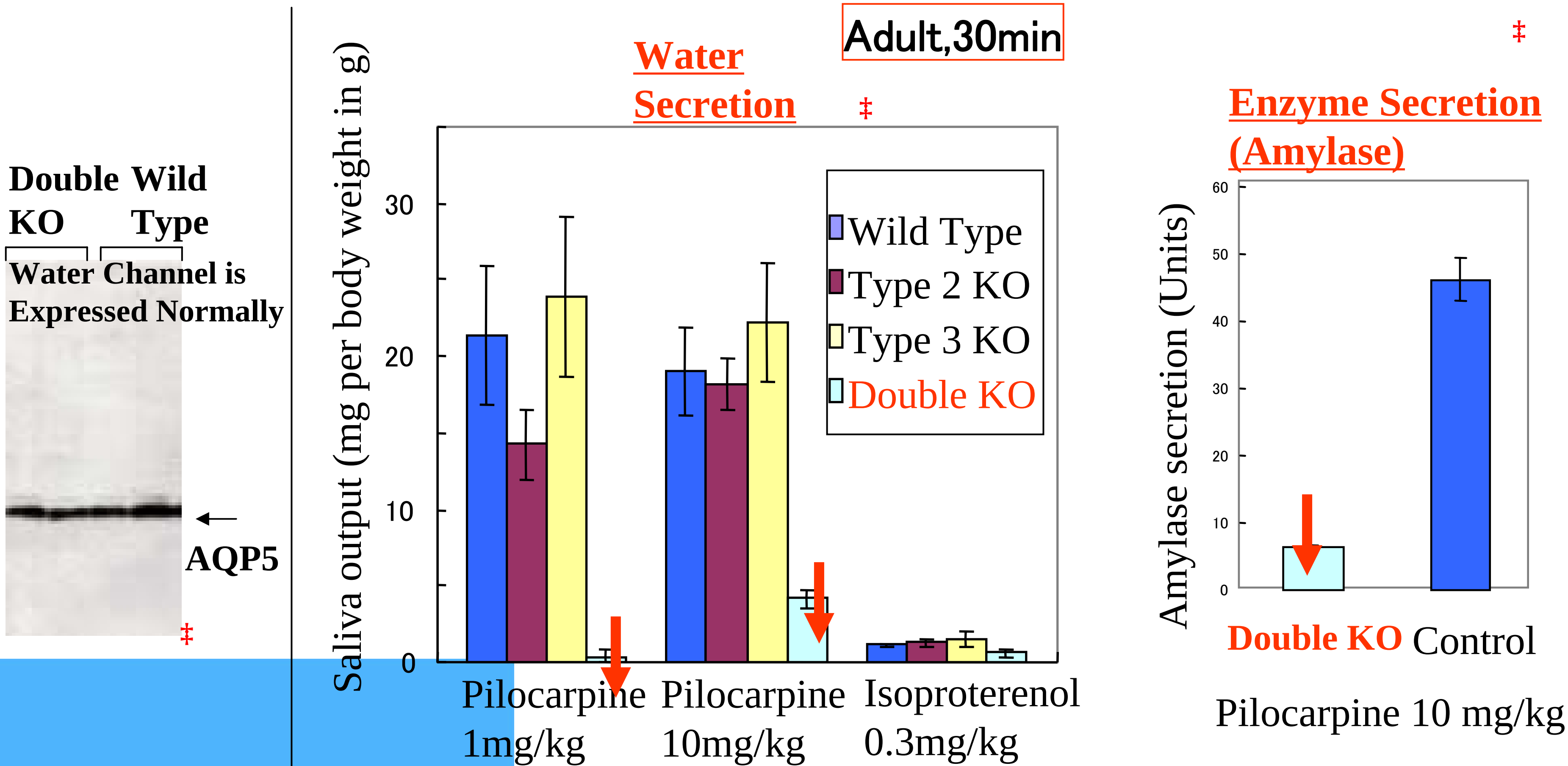
CCh
0.3μM

3μM

30μM



Defective Saliva Secretion in IP_3R2/IP_3R3 Double Knockout Mice

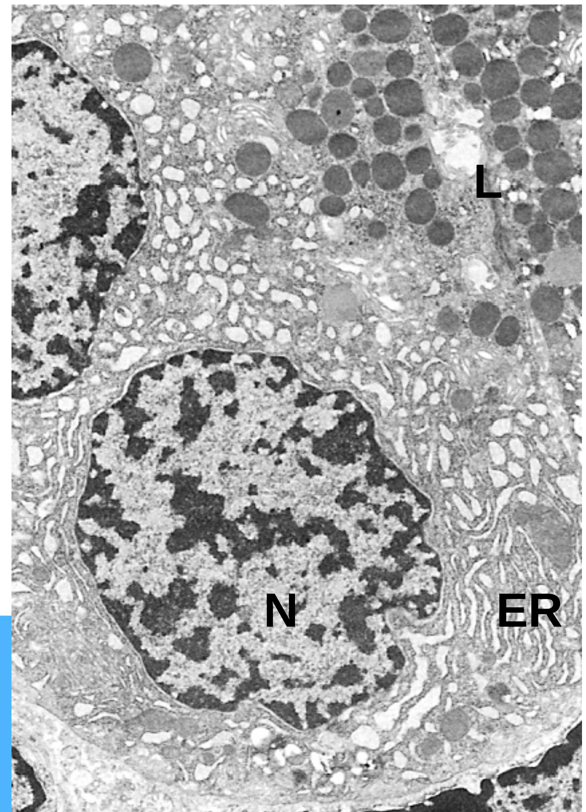


P21 mice gave similar results.

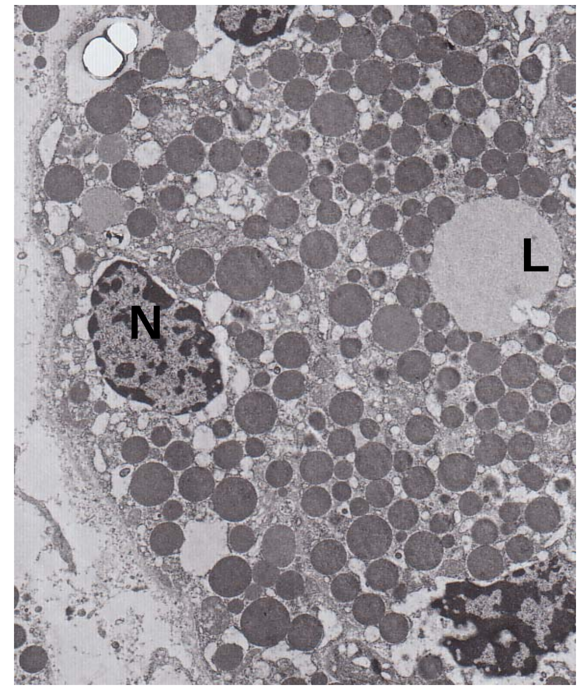
Electron Micrographs of Lacrimal Gland of Wild-type and $IP_3R2^{-/-}$-$IP_3R3^{-/-}$ Mouse

L: lumen
ER: rough endoplasmic reticulumn
N: nucleus

Wild ‡



$IP_3R2^{-/-}$-$IP_3R3^{-/-}$ ‡



(X2,000)

Abnormal accumulation of secretory granules was observed in acinar cells of $IP3R2^{-/-}$-$IP3R3^{-/-}$ mouse.

A. Discovery of IP_3 receptor・determination of primary structure・calcium channel

１． Discovery of IP_3 receptor
(Brain Res. 1977)(Dev. Neurosci. 1979) （Nature 1989) (EMBO J. 1990）
２． Determination of whole primary structure of IP_3receptor （Nature 1989）
３． IP_3 receptor is Ca^{2+} channel （J.Biol..Chem.1991,1995）
4. intercellular Ca^{2+} oscillator (Science 1992)

B. Discovery of role in development /differentiation/plasticity of nerve system

1. elucidation of dorsalization mechanism which makes nerve （Science 1987, Nature 2002）
2. a role in neurite elongation （Science 1998) (J.Neurosci. 2006)
3. knockout mouse shows cerebellar ataxia, and have epileptic seizure （Nature 1996）
4. proof that IP_3 receptor is involved in synapse plasticity
   １） involvement in long-term depression of cerebellum （J.Neurosci. 1998）
   ２） involvement in long-term potentiation of hippocampus （Learning & Memory 2000）
   ３） regulate specificity of synapse （Nature 2000）

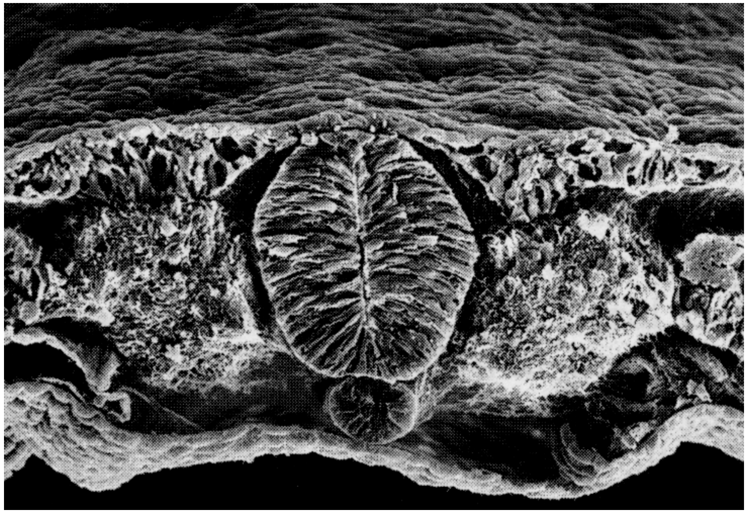
C. Discovery of new signaling mechanism

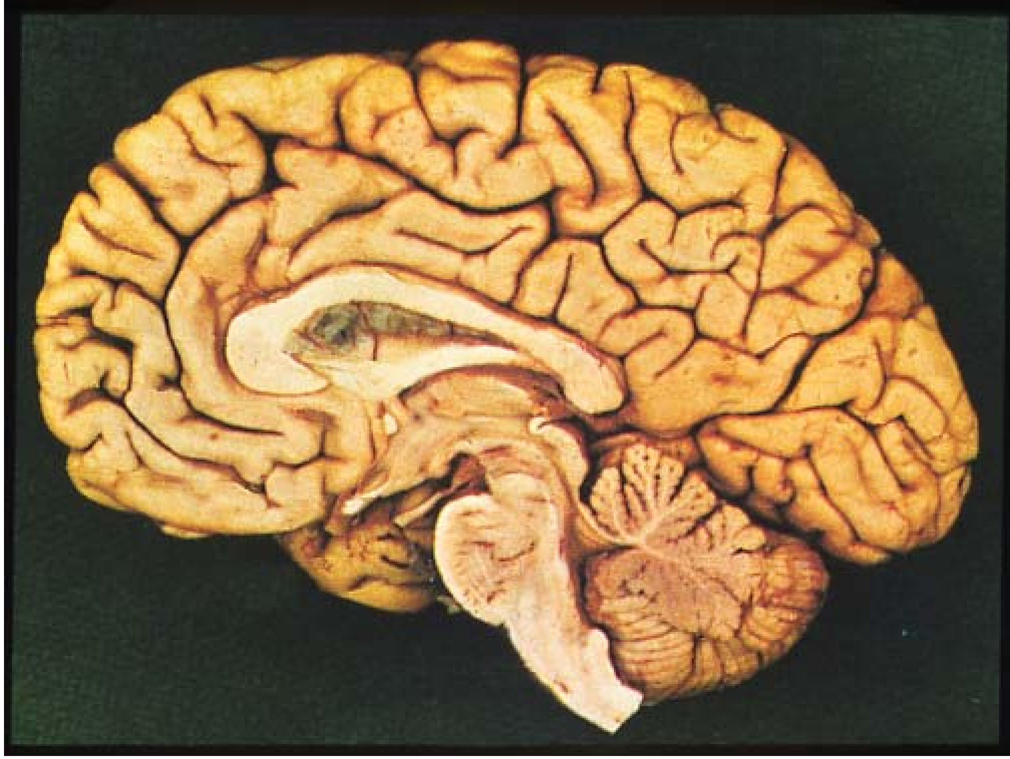
1. New IRBIT is released when IP_3 binds to receptor. (J.Biol.Chem 2003) (Molecular Cell 2006)
   IRBIT modifies acid/base balance (PNAS 2006)
2. Various unique cell biological traits
   (J.Biol Chem.a,b,c,d 2004)(J. Cell Sci. 2004)
3. Redox and calcium signal is linked. (Cell 2005)
4. IP3R2,3 are essential for ectocrine (Science 2005)

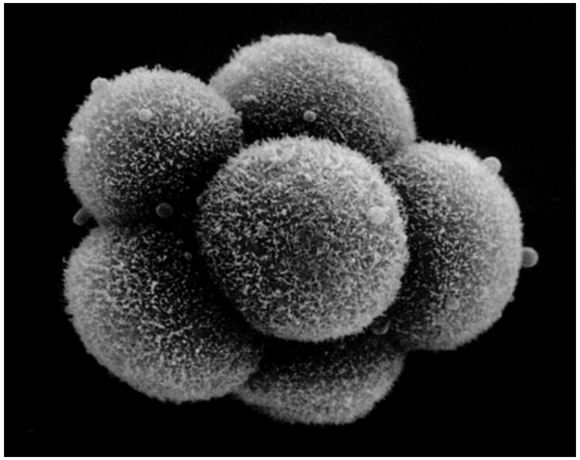
D. ３ dimensional structure

1. Determination of IP_3 ３D Ｘ-lay crystal structure(2.2Å) of IP_3 recognition site （Nature 2002）
2. 3D construction of IP_3 receptor, conversion of allosteric structure, elucidation of gating mechanism (J.Biol.Chem. 2003） (J. Mol. Biol. 2003) (Molecular Cell 2005)
3. Development of IP_3 indicator (J. Cell Biology 2006)

Understanding the molecular basis of neural development







Molecular biology,
Biochemistry,
Biophysics,
Structure biology

- ***Normal — Abnormal***

- ***Health — Disease***

Figure removed due to copyright restrictions

cerebellar ataxia mutant mouse

Possible to link molecular disorder—morphologic disorder—functional disorder

1. Positioning of nerve cells and formation of wrinkles in the brain
2. Discovery & analysis of Zic gene
3. Discovery & analysis of IP_3 receptor

Research

1. New viewpoint and a fresh dimension
2. Breaking convention & fixed idea
3. Departure from same old pattern
4. Uniqueness, freeness, richness of idea
3. Free choice of materials
4. Introduction of new technology
5. Discovery of new technology

Human resource

1. Personnel from diverse fields
2. Unique personnel
3. Personnel that transmit information and attract people

——————————

cultivate one’s independent spirit

Toru Ikegami
Takafumi Inoue
Miwako Iwai
Tomohiro Hisagaki
Naoko Ogawa
Yoko Ueno
Akihiro Mizutani
Zhang S.
Takaaki Michikawa
Keiko Uchida
Akinori Kuruma

Kozo Hamada
Junichi Goto
Ayuko Kurokura
Hiroko Sakauchi
Toru Matsuura
Takeyasu Higo
Akio Suzuki
Helene Kiefer
Roman Hudec
Shoichiro Ozaki
Osamu Shinohara

Akinobu Suzuki
Dai Tsuzurugi
Kyoko Nakamura
Hideki Nakamura
Takako Nishida
Naohide Yamaguchi
Mika Yamazaki
Yoshiyuki Yamada
Emiko Yamamoto
Hideaki Ando
Masahiro Enomoto
Akito Nagayoshi
Katsuhiro Kawai
Akitoshi Miyamoto
Sayako Tamamushi
Yukoko Kuroda
Kanayo Sato
Mai Sato
Akiko Terauchi
Nagisa Matsumoto
Kazumi Fukatsu
菅原　健之

Sachiko Ishida
Ichiro Fujimoto
Mitsunori Nomura
Misa Arizono
Chika Shimizu
Kotomi Sawaguchi
Kyoko Shirakabe
Toshimi Sasaji
Yoshinori Shimizu
山口博世
Toshio Ohshima
Miyuki Ogawa
Seiichi Tomonda
Kei Futaki
Hiroyuki Kabayama
Yasuko Abe
Mizuki Yamada
Tetsuya Minagawa
Katsunori Hironaka
Naoko Tokushige
Makoto Takeuchi
Hiromi Suzuki
Etsuko Ebisui

Tomoko Adachi
Nanaho Fukuda
Minoru Hatayama
Yukari Ochi
Miko Ikebe
Miwa Takamura
Yuko Aihara
Atsuo Aoyama
Jun Ariga
Ryoko Ando
Kazuhiro Ikenaka
Mizuho Ishiwata
Kunitatsu Izumi
Rie Ito
Hidetoshi Inase
Takafumi Inoue
Keiji Ibata
Takao Imai
Hiroshi Imogawa
Hirohide Iwasaki
Yasuno Iwasaki
Iwatsuki Masato

Go/Aya Uchiyama
Sachiko Oota
Keiko Ohata
Yasuko Ooya
Hideyuki Okano
Mieko Okano(Oguro)
Keiko Okabe(Maeda)
Masataka Okabe
Taro Okamoto
Yukiyo SUda
Masahiko Kaitsu
Tetsushi Kagawa

Hiroko Kasahara
Toshiko Kato (Isono)
Tatsuro Kaminaga
Masaaki Kawasaki
Eiko Kanno
Kiyomi Kikuchi
Tetsuya Kitaguchi
Kenichiro Kubo
Shoen Kume
Taruho Kuroda
Yukako Kuroda
Goro Kuwajima

Tsuyoshi Koide
Kazuhisa Koda
Wataru Kokubo
Toshio Kojima
Yasuhiro Kosuge
Yoshio Koyabu
Tetsuro Kondo
Wakana Saikawa
Weihua Cai
Tetsuichiro Saito
Miyuki Saito
Chika Saegusa
Keiko Sakai
Shinichi Sakakibara
Tetsushi Sadakaga
Takero Saneyoshi
Kazunobu Sawamoto
Naomi Sawamoto
Chiyo Shioda
Reiko Hoshi(Shiba)
Ko Shu
Yoko Yamaguchi
(Shiroishi)
Ryong-Moon Shin
Kei Suga

Maki Suga(Yano)
Tomoyasu Sugiyama
Noboru Suzuki
Kosuke Kakuta
Lee G. Sayers
Takuya Takahashi
Naoko Takahashi
Chitoshi Takayama
Yoko Takiyama
Kotaro Takei
Hiroshi Takeshita
Hiroyuki Takeda
Kuniko Takemiya
Yasuko Takeyama
Naoko Tate
Yoko Tateishi
Hidenori Tabata
Takaaki Tamura
Emi Tsujimura
Ikuko Kakuta
Hisae Tsuboi (Asai)
Akio Tsuboi
Asako Tsuchii
Tsutomu Tokuyama

Shuhei Tomita
Junji Nakao
Naomi Nagaoka
Toshiyuki Nakagawa
Kazunori Nakajima
Kenji Nagai
Eiichiro Nagata
Katsunori Nakata
Masatsugu Nakade
Setsuko Nakanishi
Yoko Nakano
Kensuke Nakahira
Yoshie Nakamata
Makoto Nakamura
Takahiro Nakayama
Tomohiro Nakayama
Toru Natsume
Michio Niinobe
Mitsuhiro Hashimoto
Koji Hattori
Fumiaki Hamasato
Tomohiro Hamada(Ohnishi)
Yuki Hamanaka

Kanehiro Hayashi
Masami Hayashi
Yasuko Hisano
Chie Hirashima
Atsuko Hiratsuka
Junji Hirota
Mitsunori Fukuda
Yuko Fukuda (Aizawa)
Shigemoto Fukui
Ichiro Fujino
Hirofumi Funamizu
Teiichi Furuichi

Daisuke Furutama
Akemi Hoshino
Jun Hoshino
Kazunaga Chiba
Nobuaki Maeda
Nobuko Maeda
Fumio Matsushita
Reiko Matsumura
Mineo Matsumoto
Julie M.Matheson

Hiroshi Yurugi
Masayuki Miura
Atsushi Mikami
Hideaki Mizuno
Fuyuki Mitsuyama
Hiroshi Miyauchi
Takaki Miyata
Takeshi Miyamura
Ayumi Miyamoto
宮本　一世
Atsushi Miyawaki
Aya Muto
Satoshi Moriguchi
Noriyuki Morita
Mitsuhiro Morita
Toshifumi Morimura
Toshiaki Kadokawa
Masato Yasui
Daisuke Yasutomi
ベイン(旧 柳田）知花
Kazushi Yamauchi

Yoshihide Yamaguchi
Akiko Yamazaki
山田　憲彦
Maki Yamada
Hideyuki Yamamoto
Miki Yamamoto (Hino)
Noriko Yamazaki
Michisuke Yuzaki
Naoki Yokota
Rei Yokoyama
Kayoko Sakamoto (Yoshikawa)
Shingo Yoshikawa
Kaori Yoshikawa(Kawamoto)
Fumio Yoshikawa
Manabu Yoshida
Hiroyuki Yonejima
李　敏
李　勝天
Yoshimi Ryo