化学生物学的アプローチによる

新たな生理学計測法

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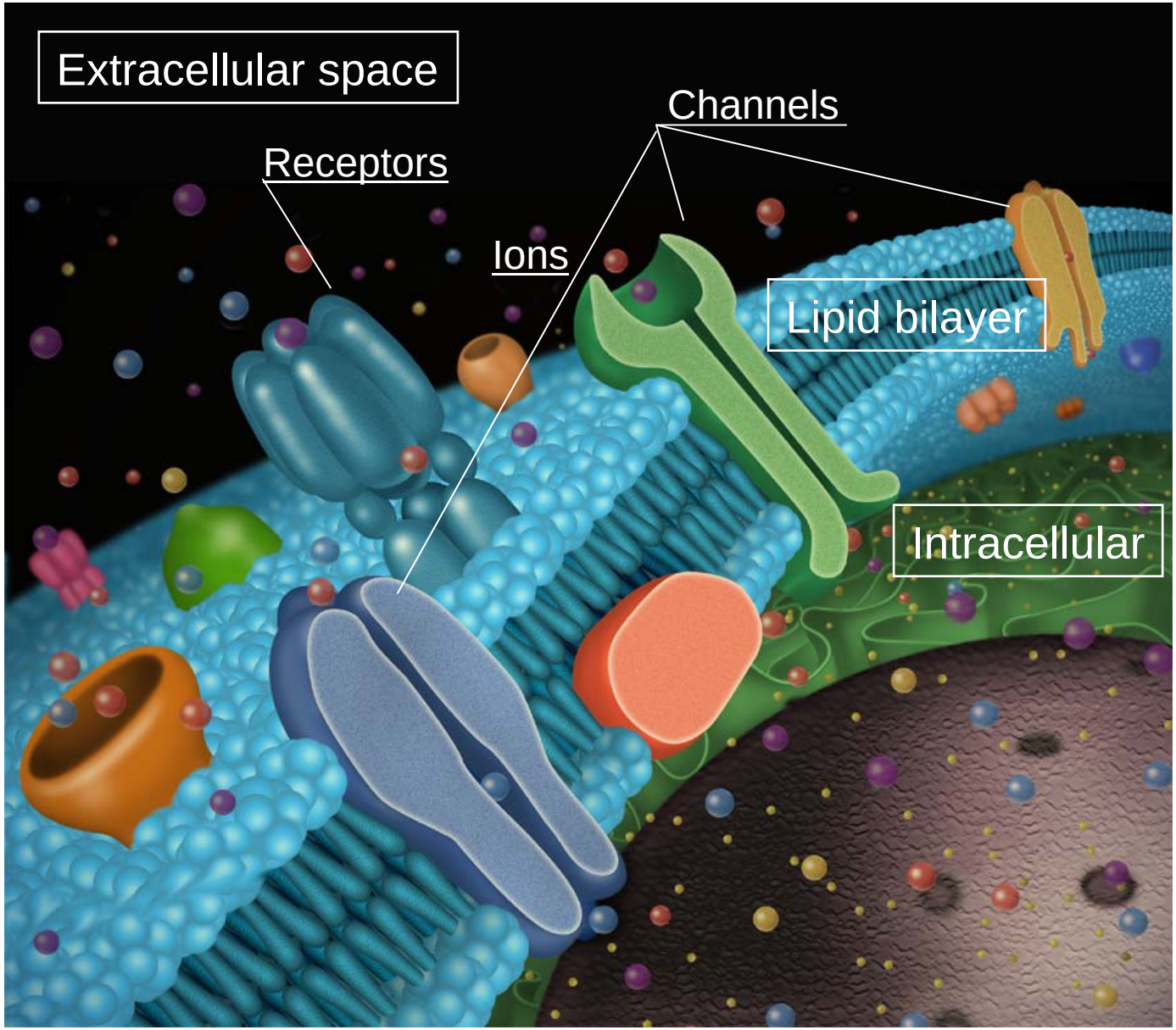
A cartoon showing the cell structure near the plasmamembrane



What are ion channels?

・Hydrophilic pores comprised of transmembrane proteins.

・Transport ions across membrane with extremely high efficiency at 10^6~10^8 ions/sec（activation energy for transport reaction is low and ~3kcal/mol for K^+ channels）.

・Induce large changes in membrane voltages as well as rapid changes in intracellular ionic concentrations.

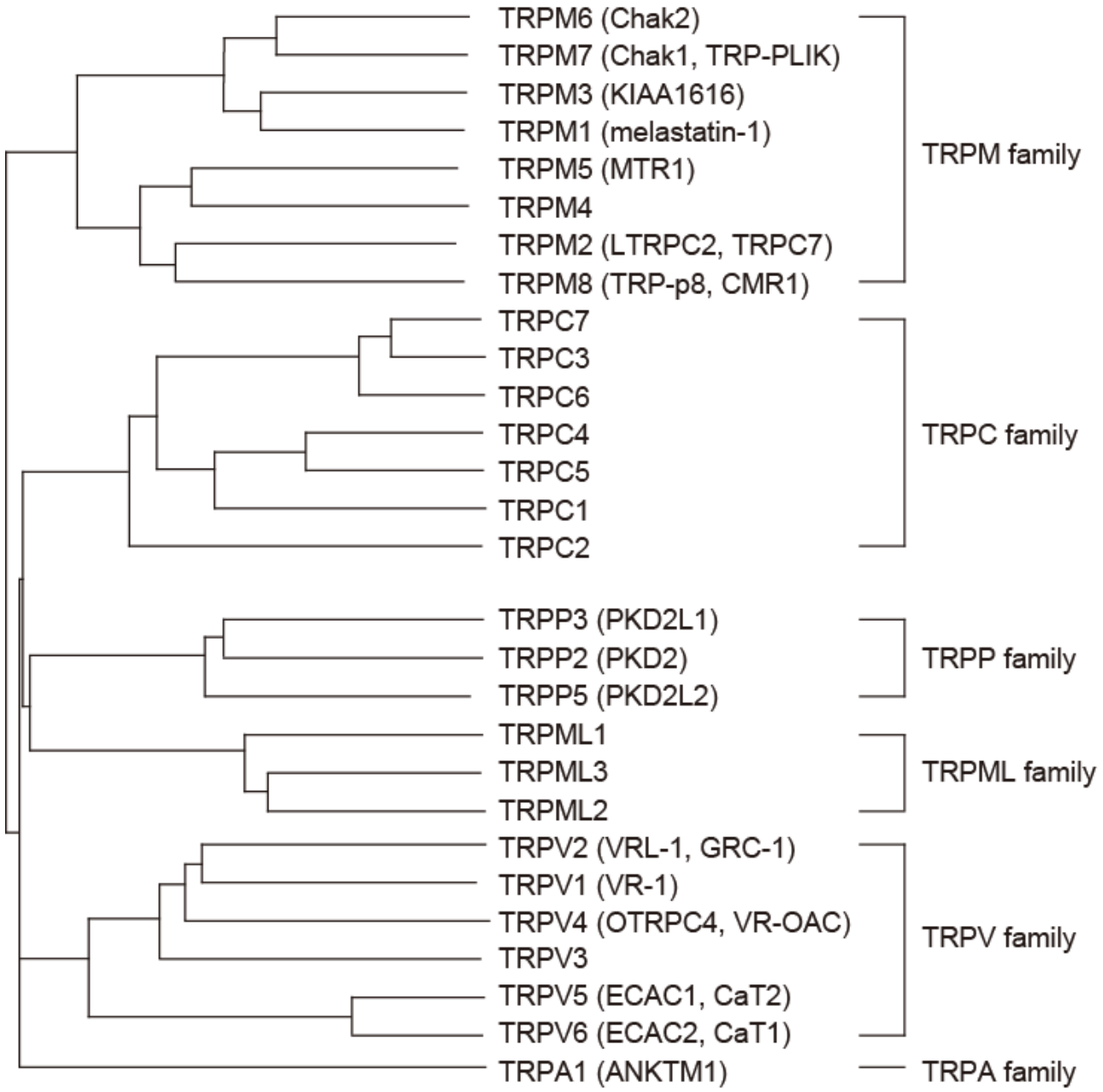
・Each ion channel shows selectivity to permeating ions and activation triggers.

＝ Diverse ion channels are formed by proteins encoded by different genes to play essential roles in various biological responses.

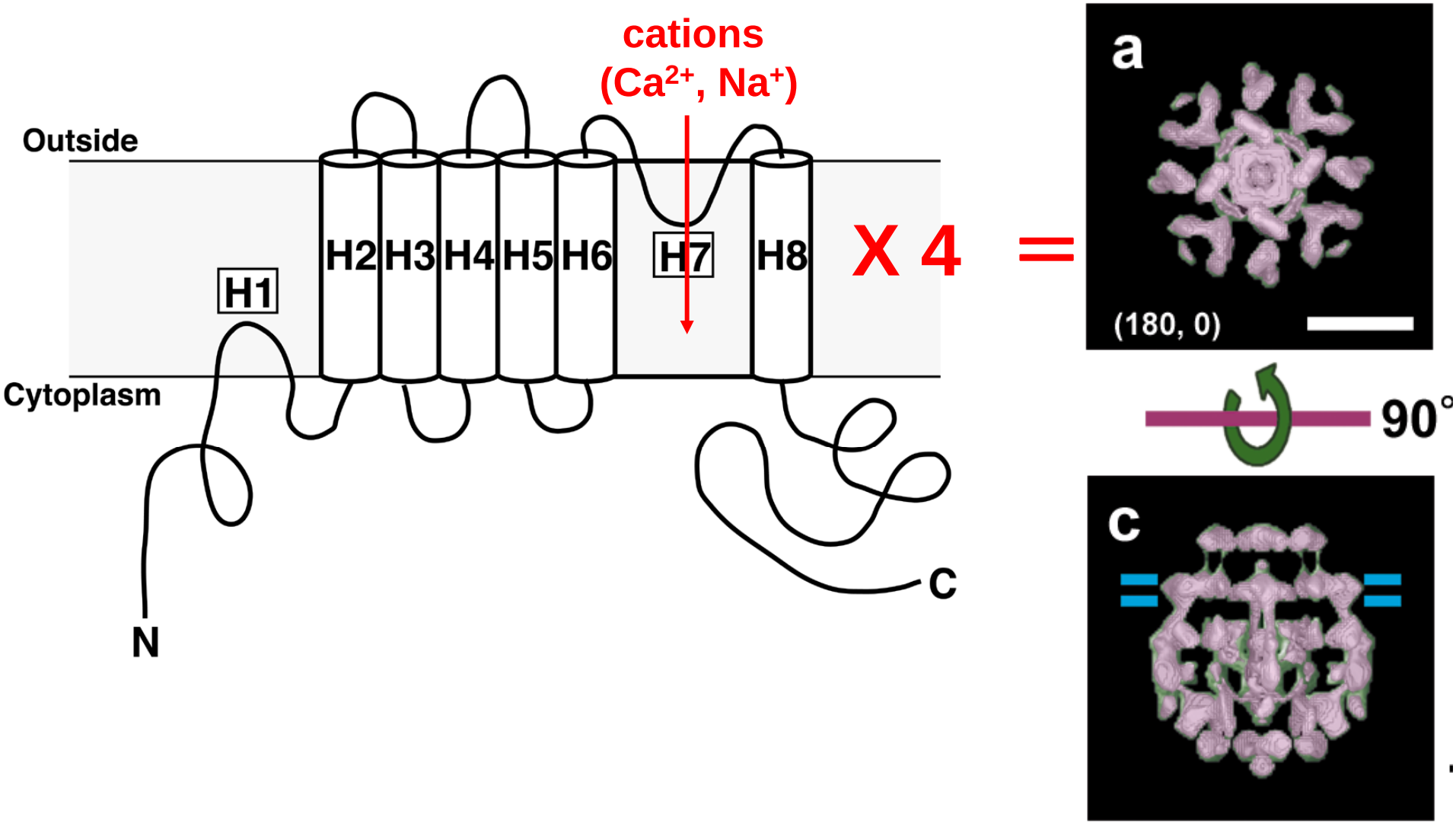
TRP

transient receptor potential

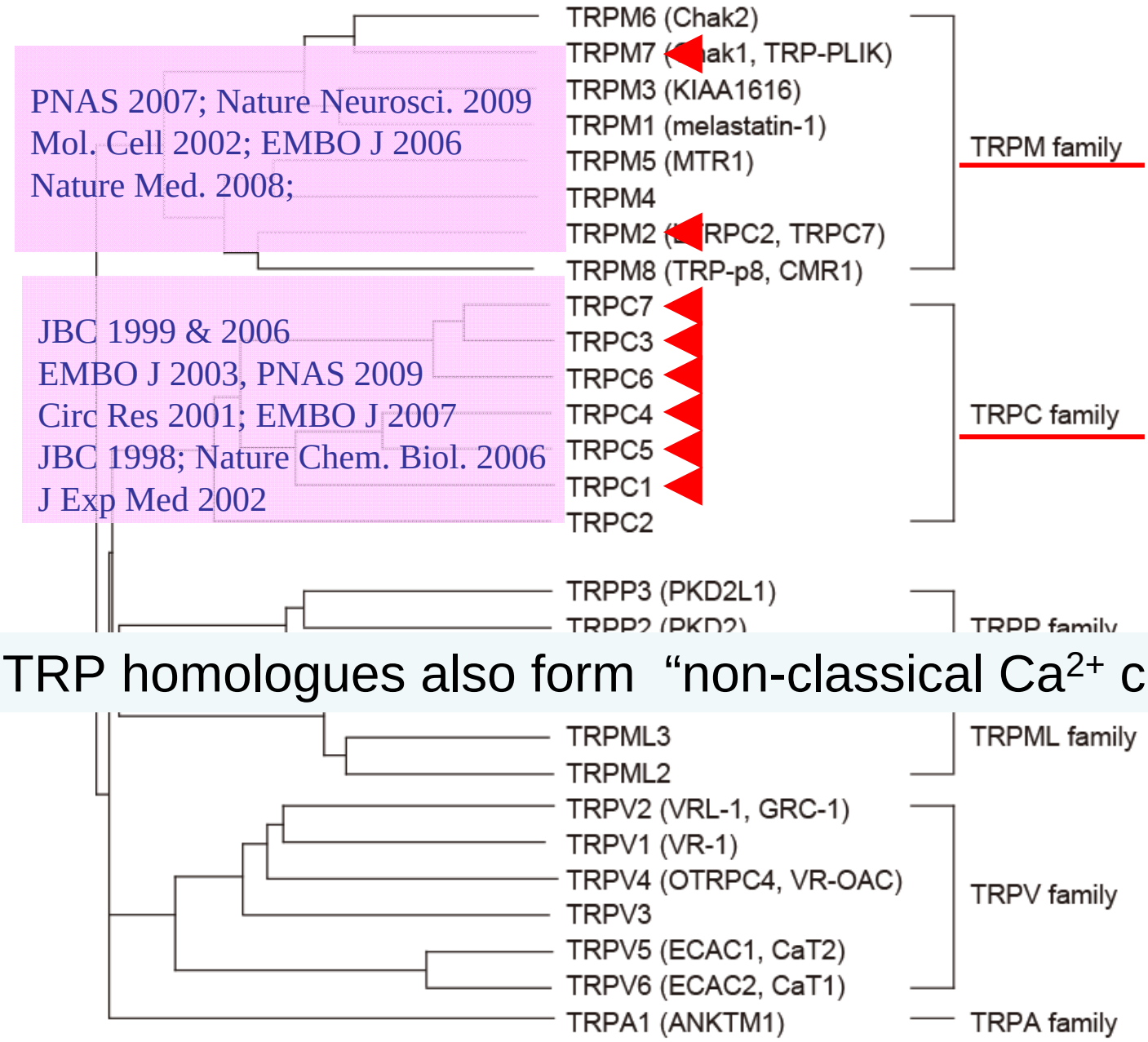
Phylogenetic tree of TRP proteins



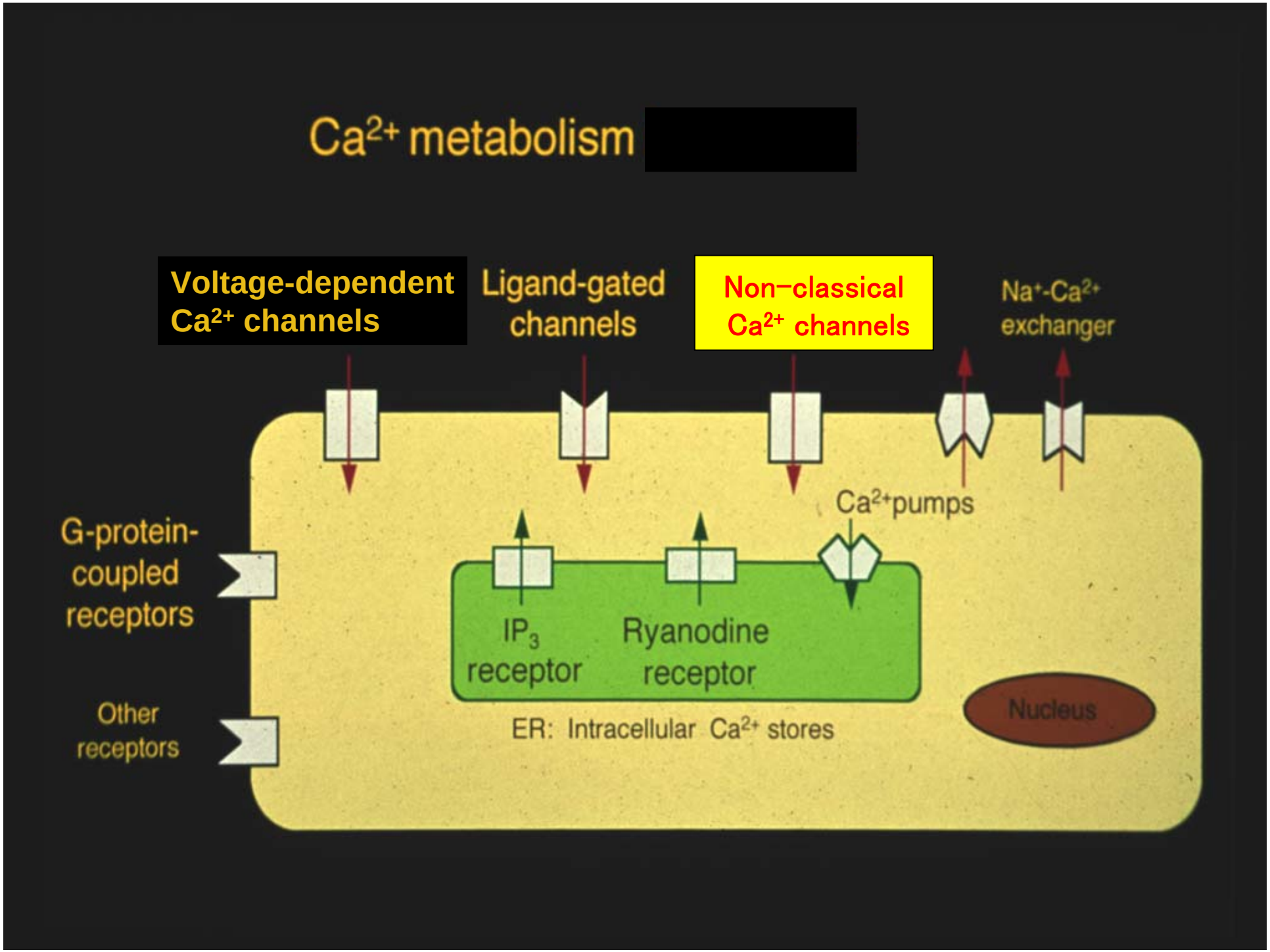
Putative transmembrane topology of TRP channels



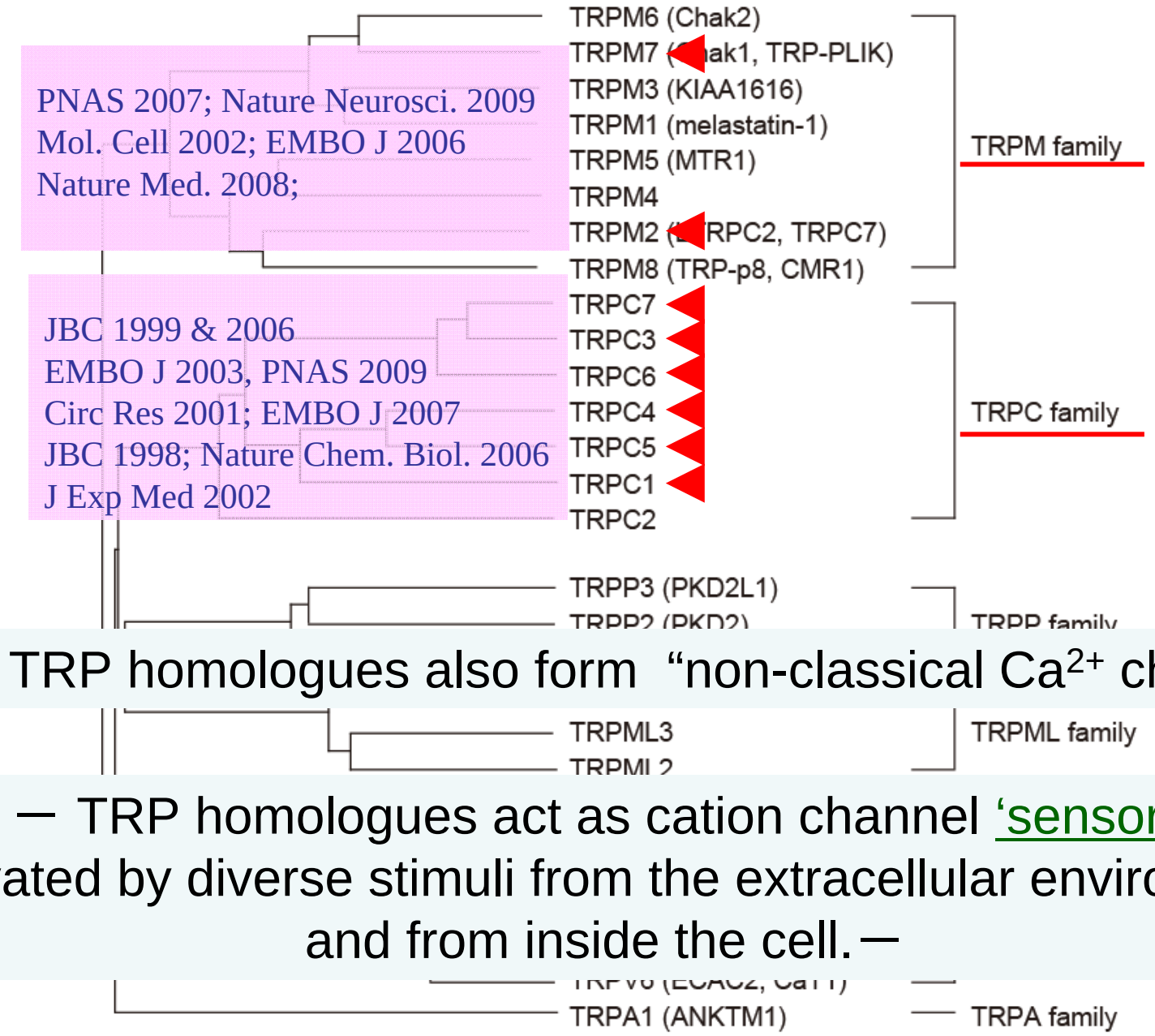
Phylogenetic tree of TRP proteins



Many TRP homologues also form "non-classical Ca^{2+} channels".

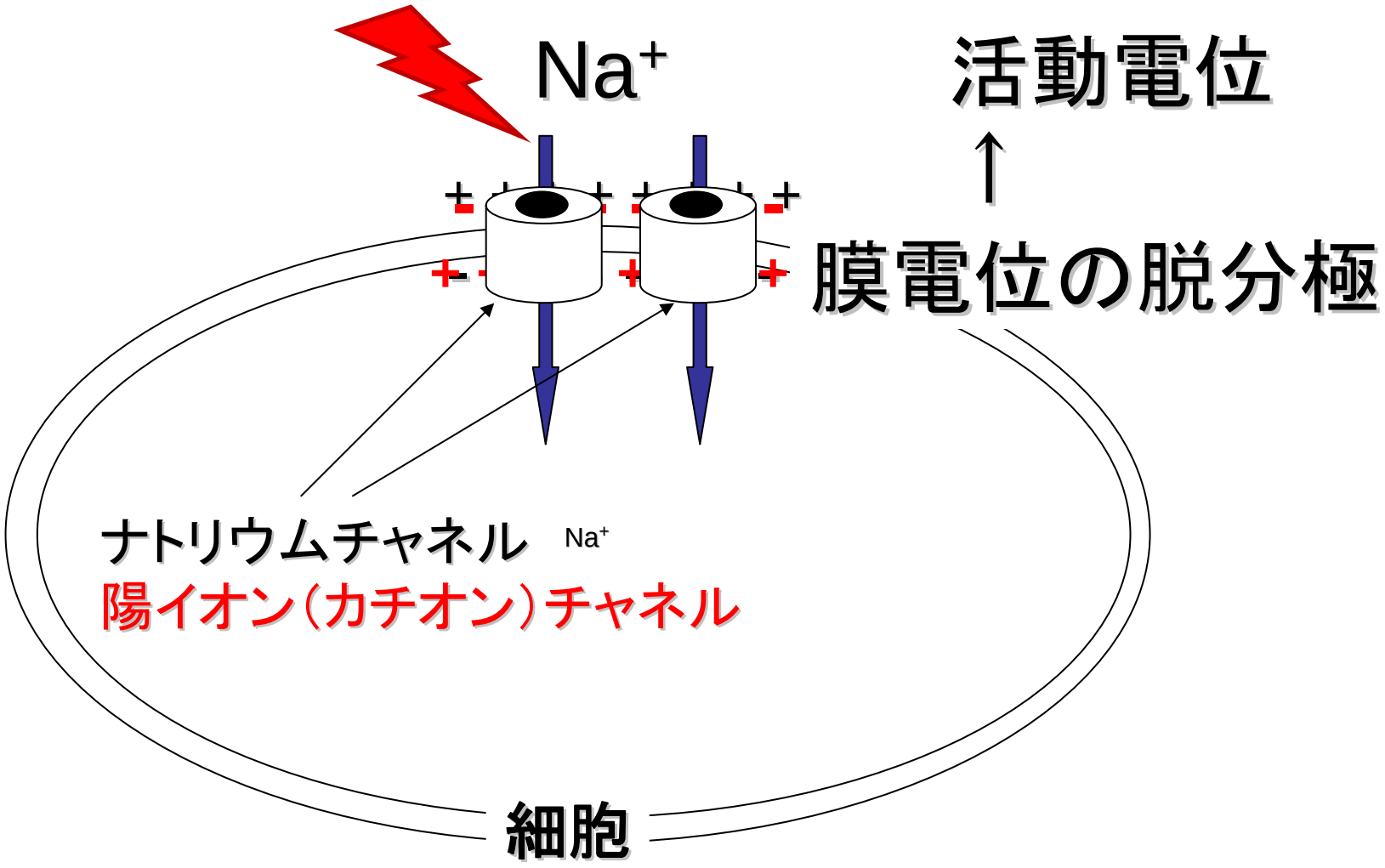

Ca2+ metabolism
Voltage-dependent Ca2+ channels
Ligand-gated channels
Non–classical Ca2+ channels
Na+-Ca2+ exchanger
Ca2+pumps
G-protein-coupled receptors
Other receptors
IP3 receptor
Ryanodine receptor
ER: Intracellular Ca2+ stores
Nucleus

Phylogenetic tree of TRP proteins

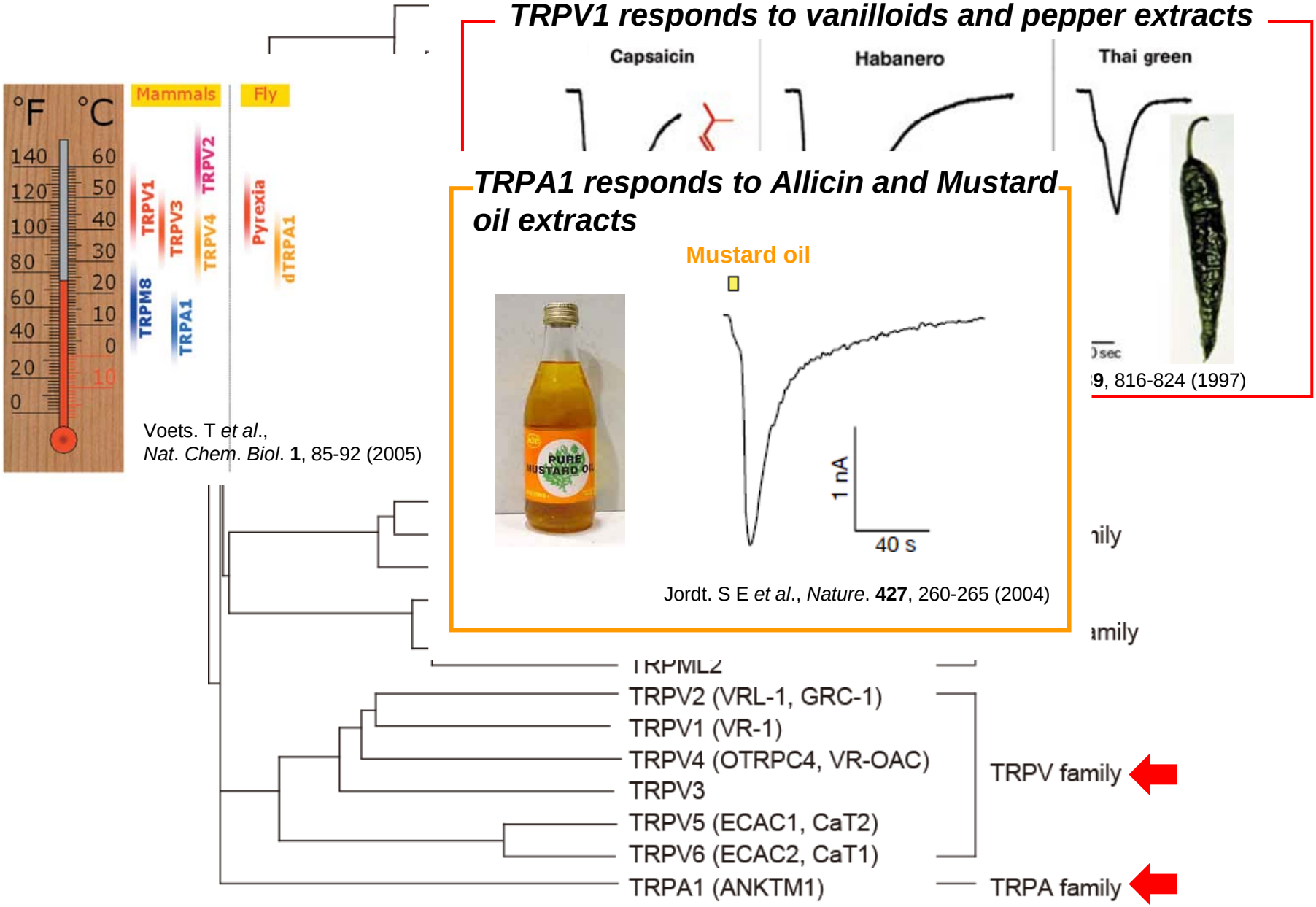


Many TRP homologues also form "non-classical Ca^{2+} channels".

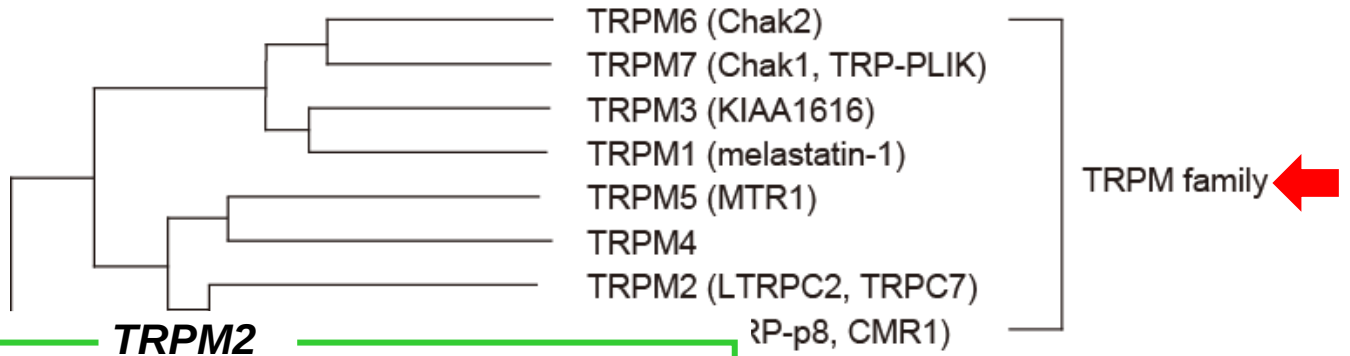
– TRP homologues act as cation channel 'sensors' activated by diverse stimuli from the extracellular environment and from inside the cell. –


Na+
活動電位
膜電位の脱分極
ナトリウムチャネル Na+
陽イオン（カチオン）チャネル
細胞

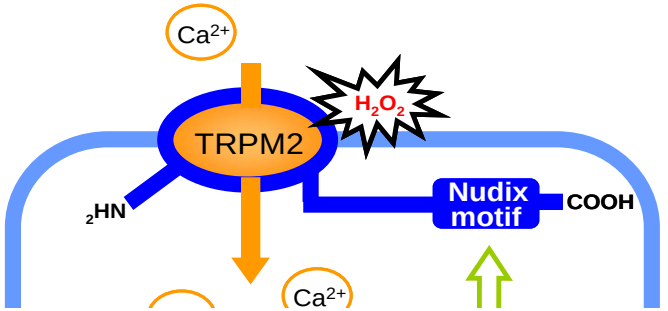
Phylogenetic tree of TRP proteins

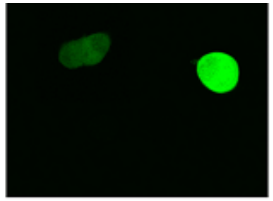


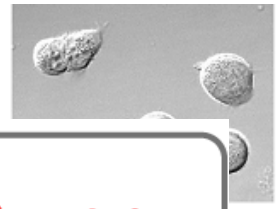
Phylogenetic tree of TRP proteins



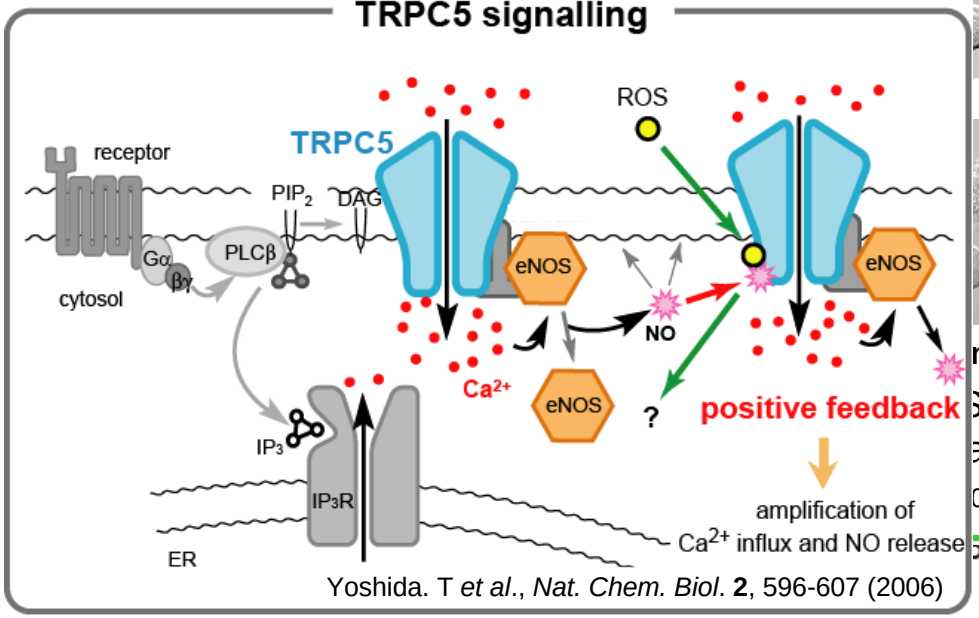
TRPM2







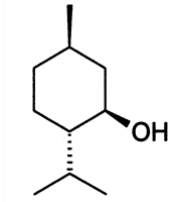
TRPC5 signalling



Yoshida. T *et al*., *Nat. Chem. Biol*. **2**, 596-607 (2006)

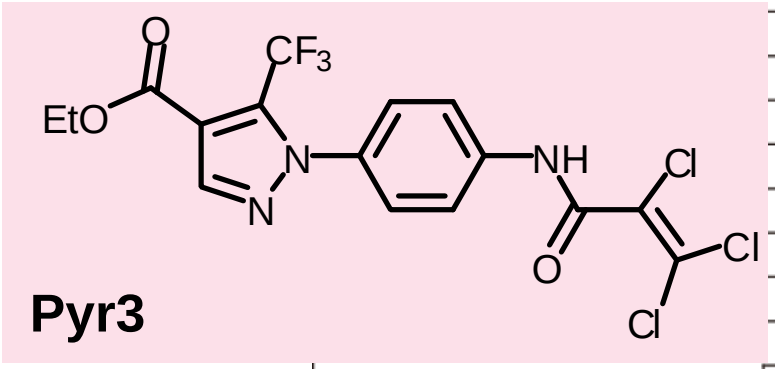
TRPM8 responds to Menthol

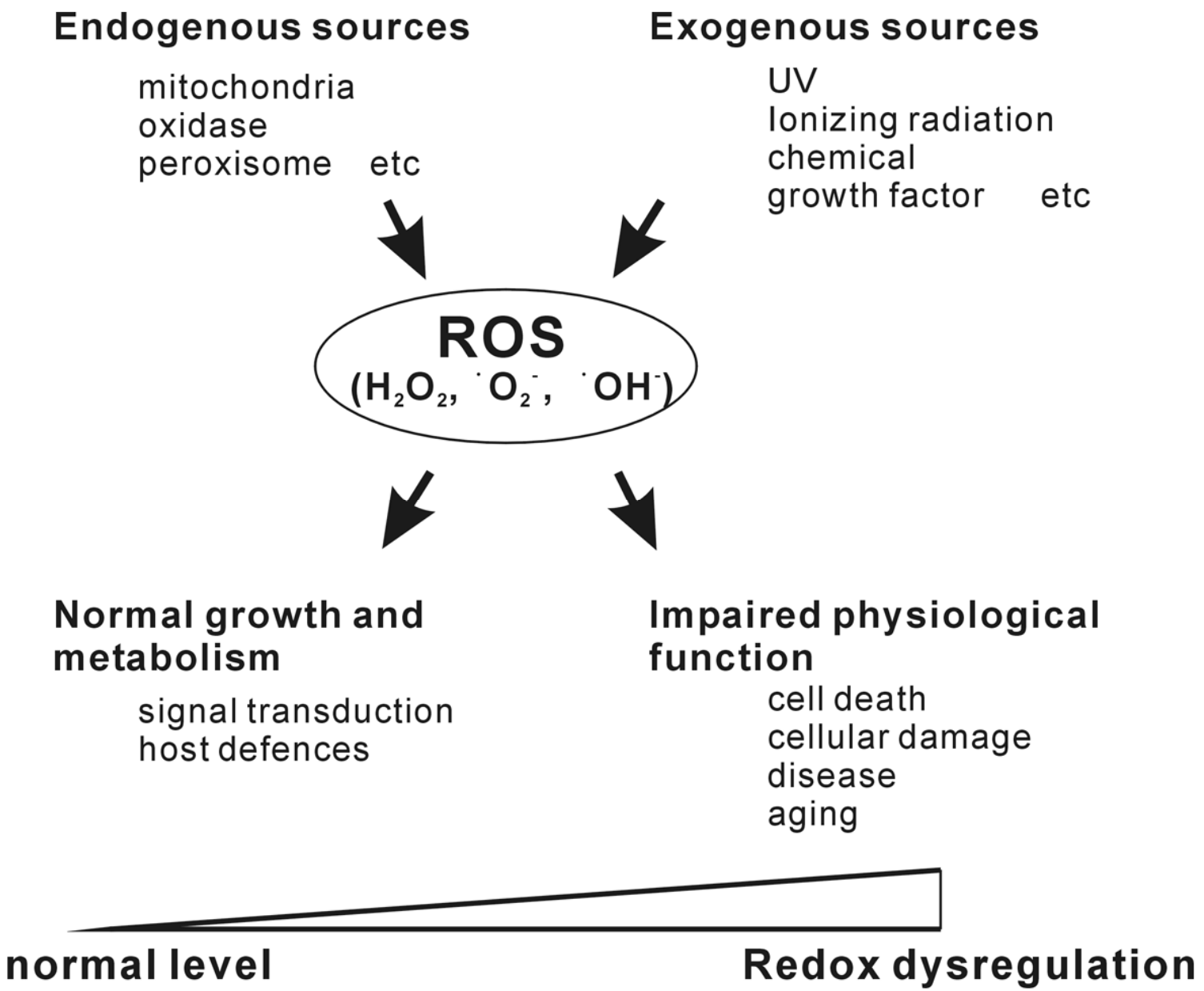
L-Menthol



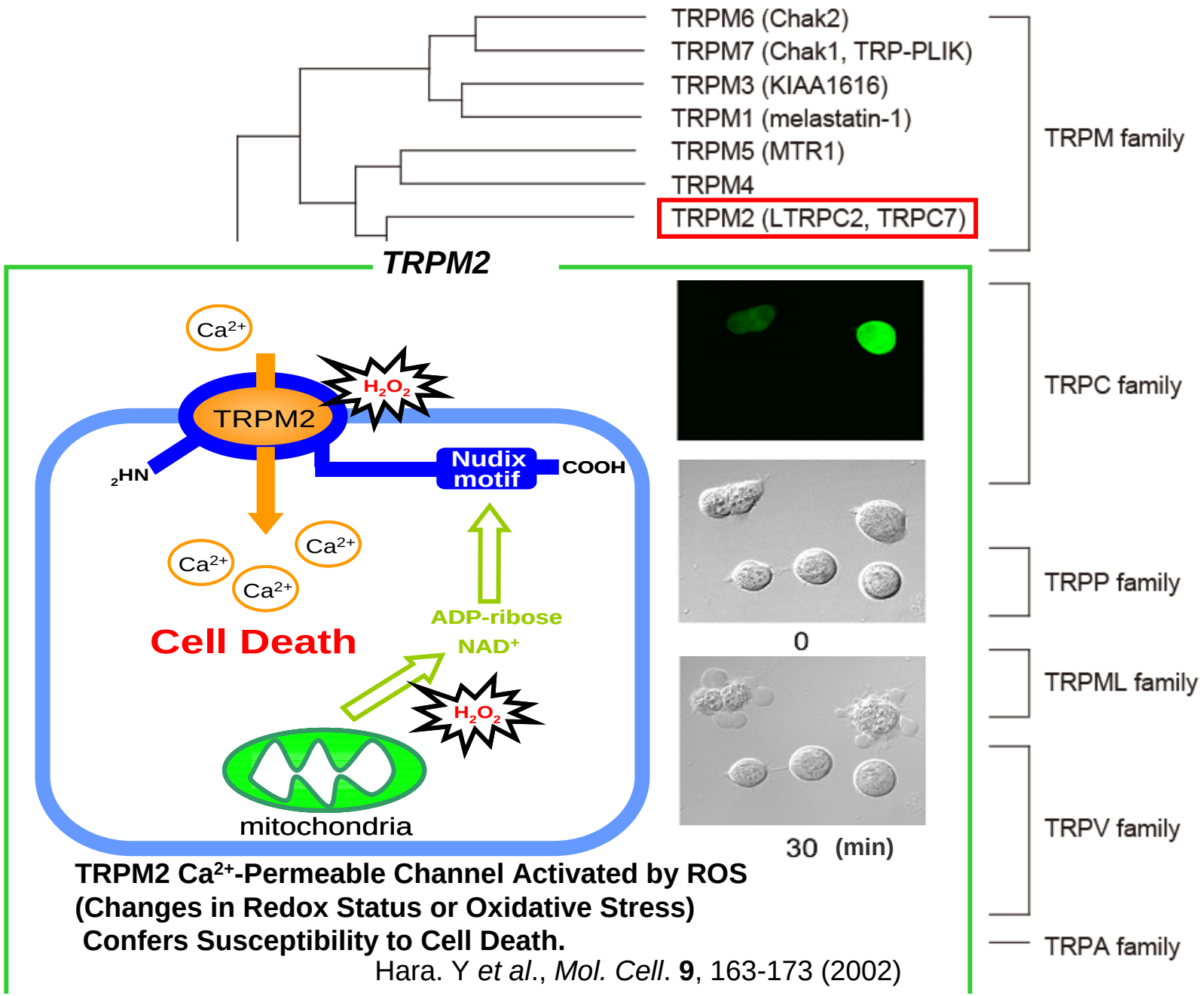
Peier. A M *et al*., *Cell*. **108**, 705-715 (2002)

Phylogenetic tree of TRP channels




Endogenous sources
mitochondria
oxidase
peroxisome etc
Exogenous sources
UV
Ionizing radiation
chemical
growth factor etc
ROS
(H2O2, ·O2-, ·OH-)
Normal growth and metabolism
signal transduction
host defences
Impaired physiological function
cell death
cellular damage
disease
aging
normal level
Redox dysregulation

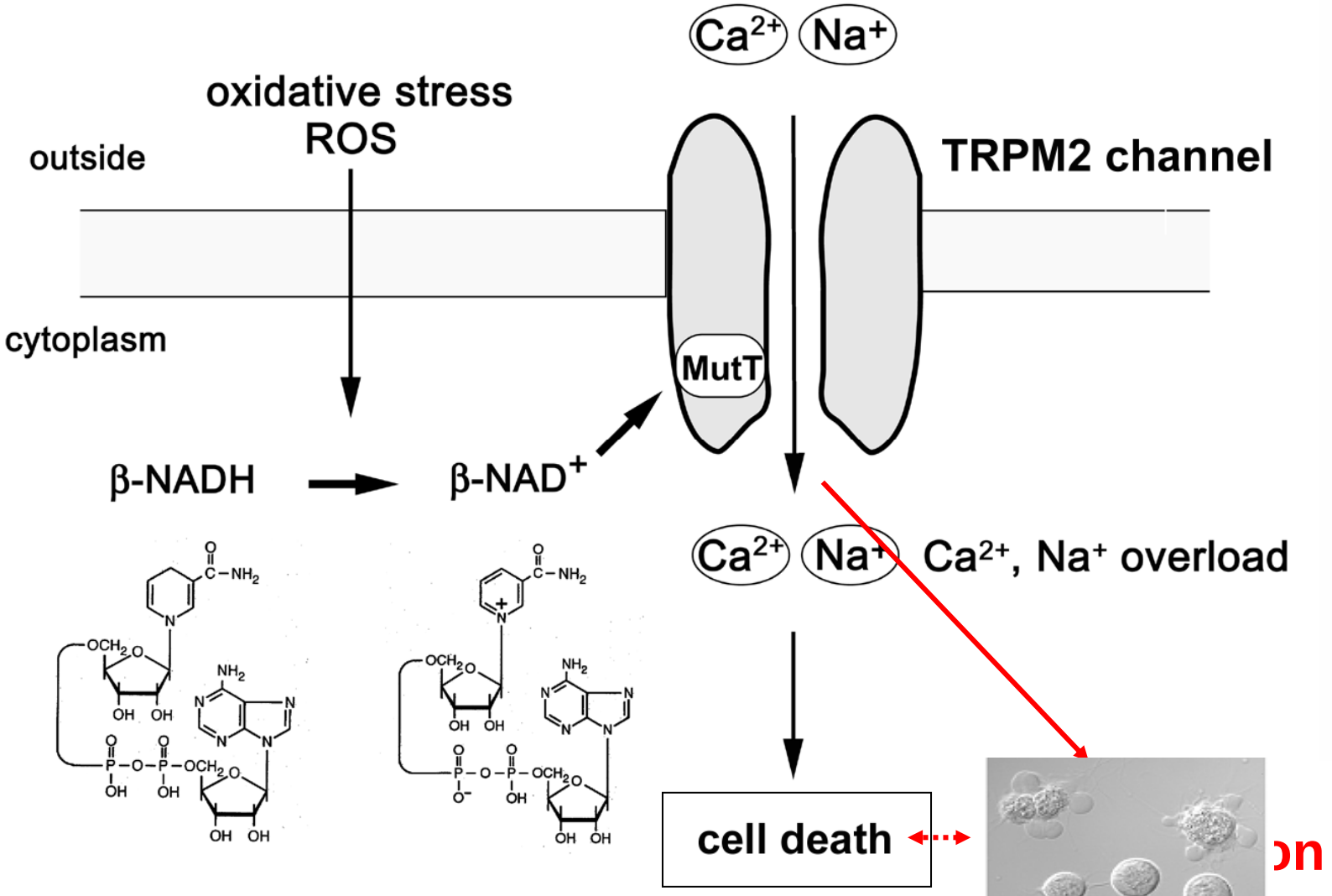
Phylogenetic tree of TRP proteins



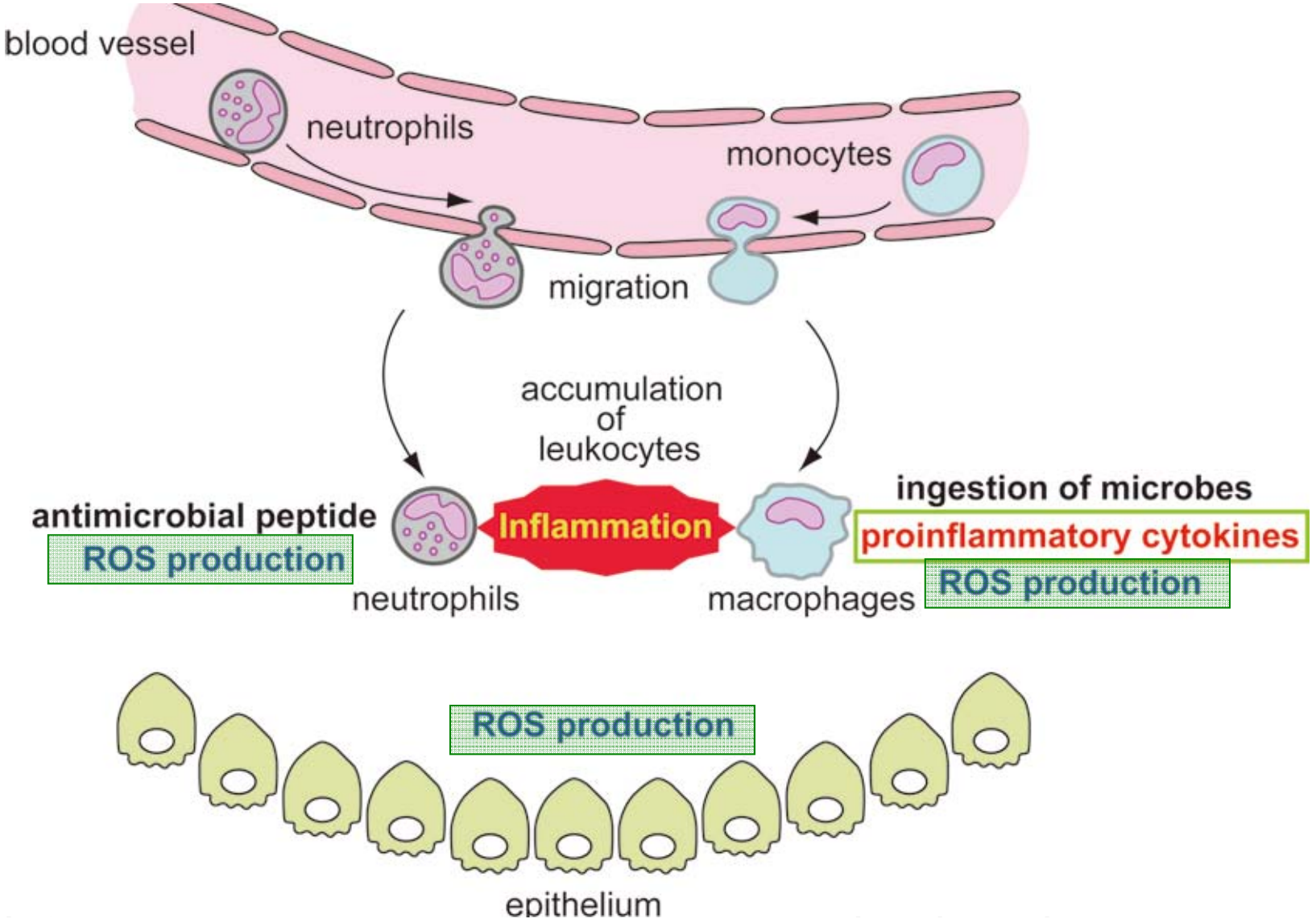
TRPM2 Ca^{2+}-Permeable Channel Activated by ROS (Changes in Redox Status or Oxidative Stress) Confers Susceptibility to Cell Death.

Hara. Y *et al*., *Mol. Cell*. **9**, 163-173 (2002)

Activation mechanism of TRPM2



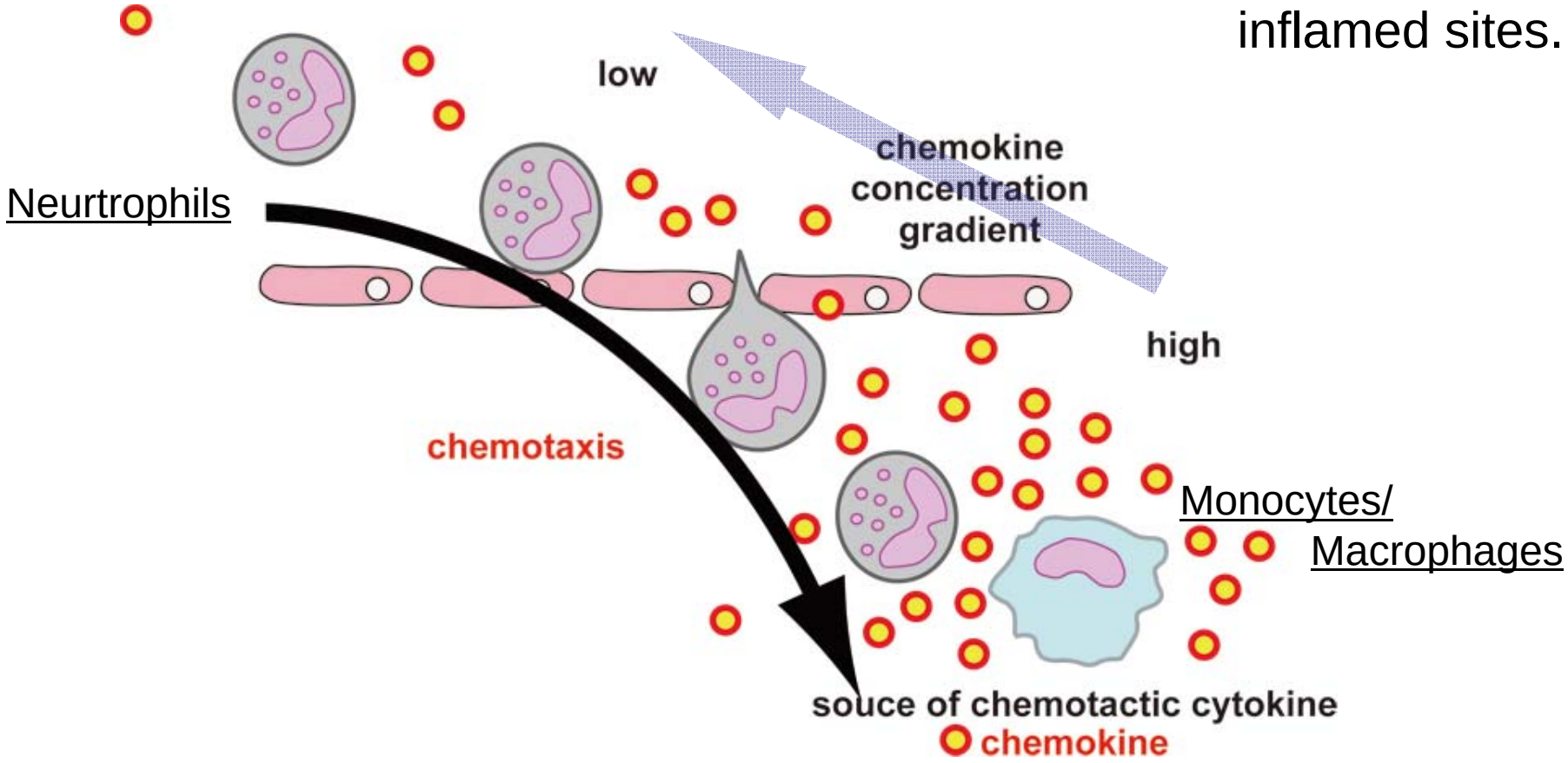
Hara. Y *et al*., *Mol. Cell*. **9**, 163-173 (2002)



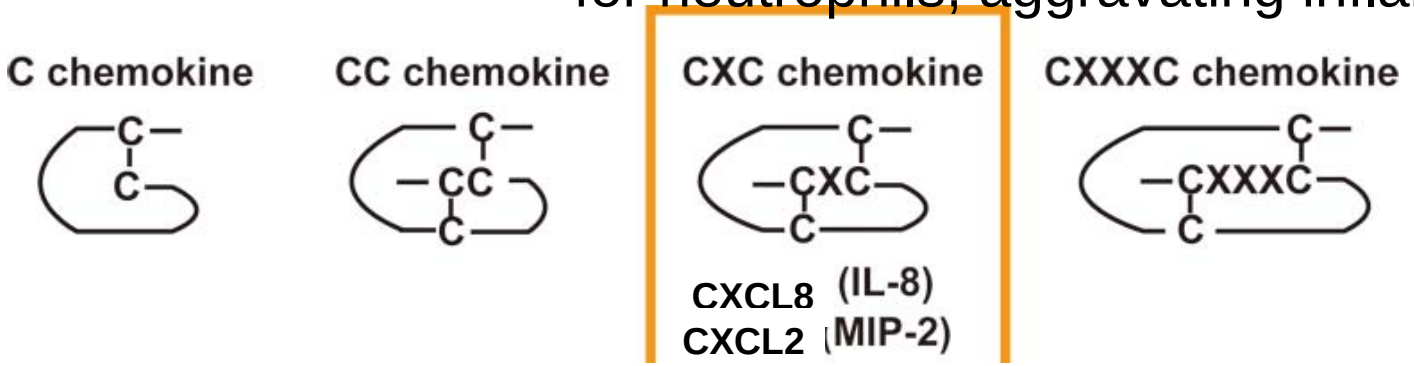
The biological significance of inflammation:

to bring fluids, proteins and inflammatory cells from blood into the damaged tissues to eliminate the injuring substances and trigger the healing and repairing processes.

Chemokines play a key role in recruiting inflammatory cells migrating to inflamed sites.



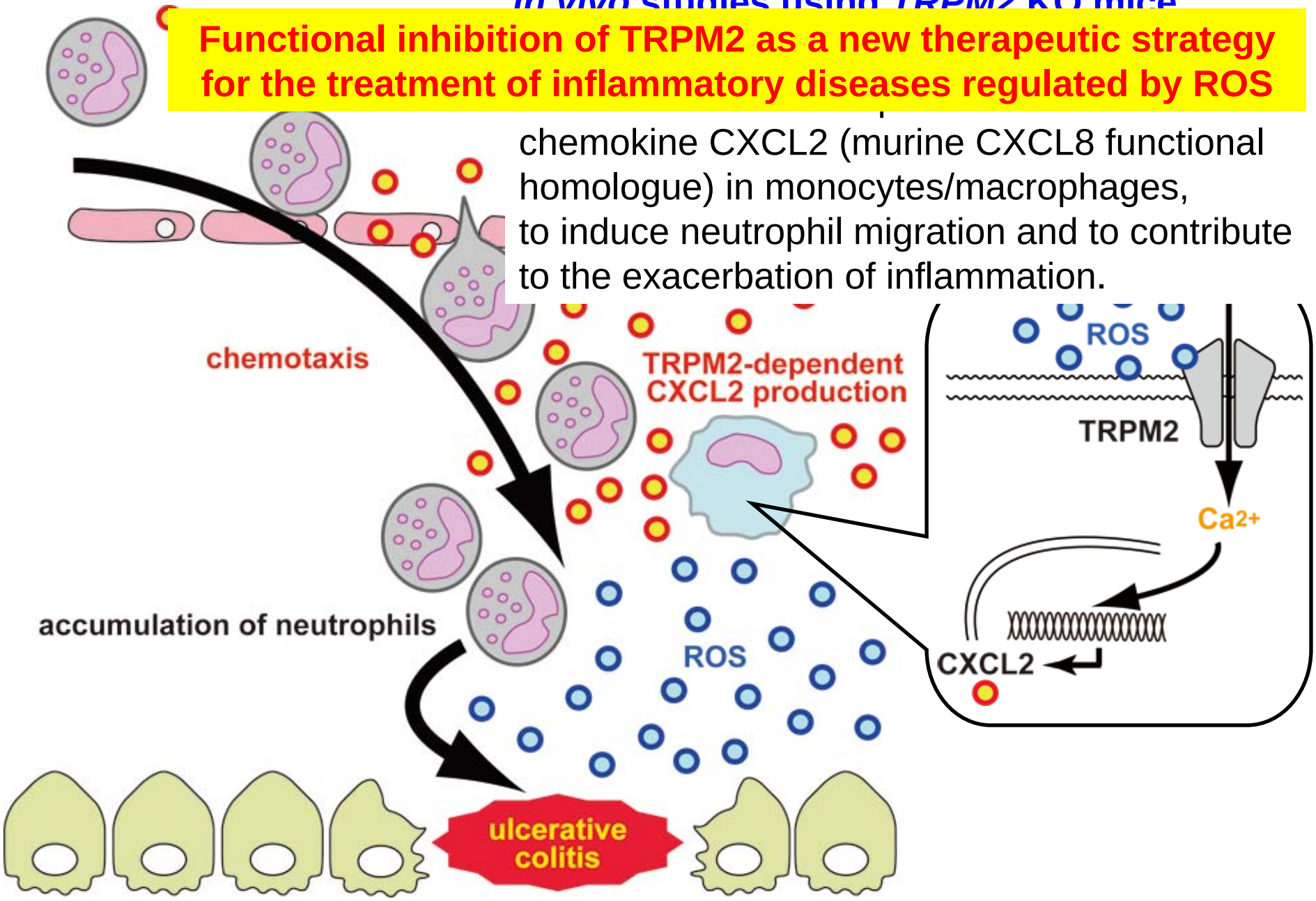
CXC chemokines, CXCL8 and CXCL2, exhibit potent chemotactic activity for neutrophils, aggravating inflammation.



in vivo studies using *TRPM2* KO mice

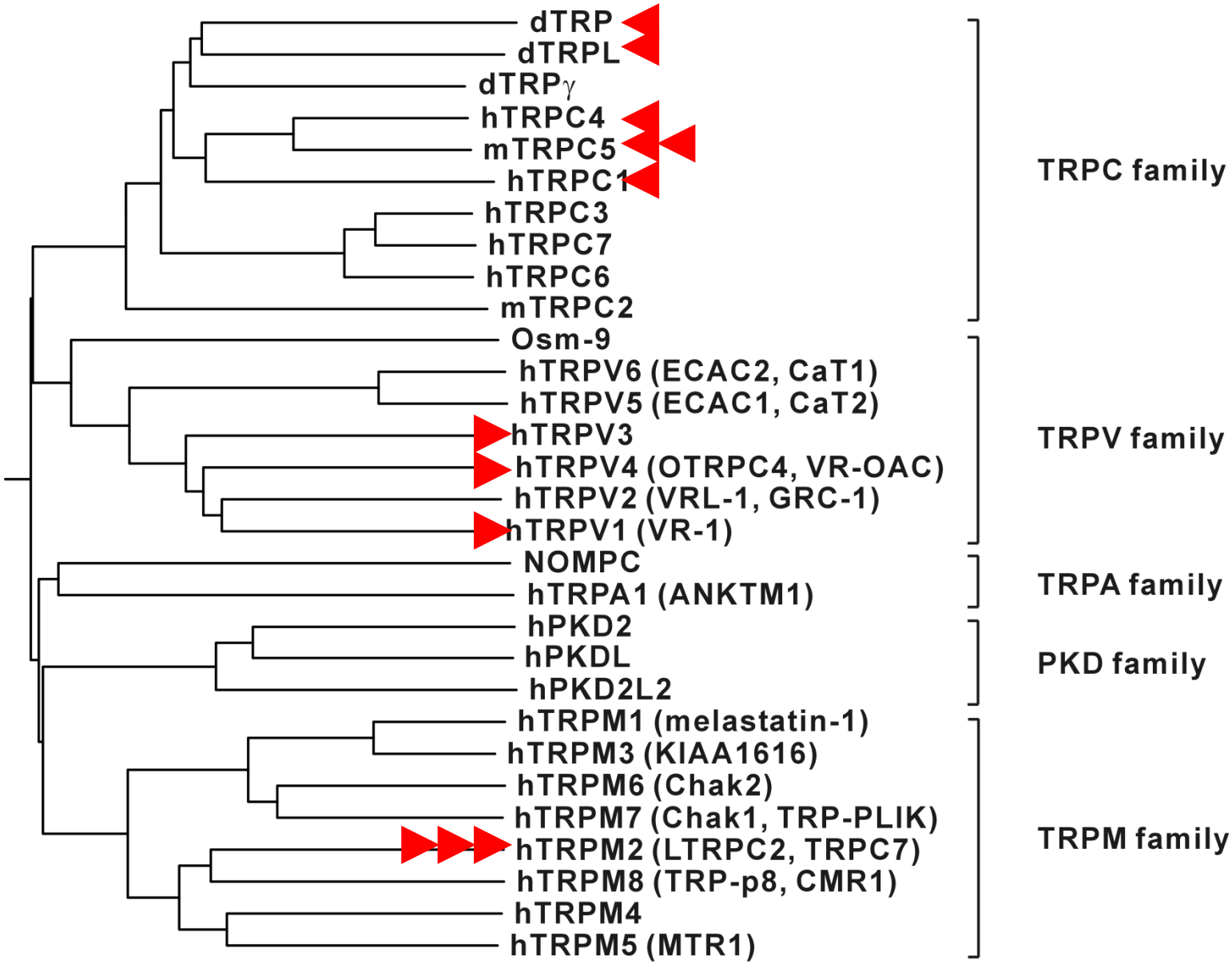
Functional inhibition of TRPM2 as a new therapeutic strategy for the treatment of inflammatory diseases regulated by ROS

chemokine CXCL2 (murine CXCL8 functional homologue) in monocytes/macrophages, to induce neutrophil migration and to contribute to the exacerbation of inflammation.



Yamamoto *et al*., Nature Med. (2008)

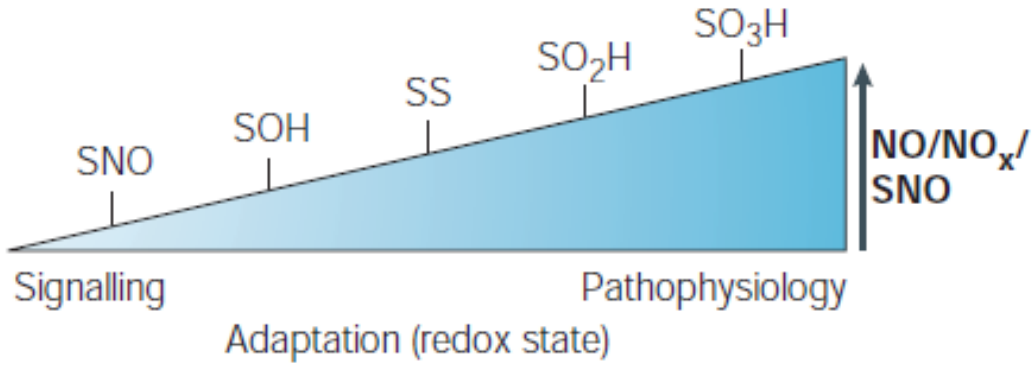
Redox-sensitive TRP channels



(ref; Hess et al., *Nat Rev Mol Cell Biol* **6**, 150-166, 2005).

-based modification of protein cysteine thiols

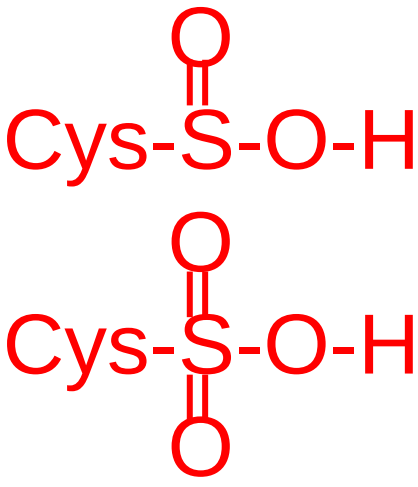
Cys-SH) can undergo a range of nitric oxide
t electrophilic and oxidative modifications as
pendent oxidation by reactive oxygen species
odifications can be viewed as a continuum
relates levels (amount, origin, spatio-
ution) of reactive NO/higher NO oxides
ne form and consequences of modification.
ctive, the progression from *S*-nitrosylation
l (SOH)/disulphide (SS), to sulphinic (SO_2^-) and
signalling functions through adaptation to NITRO
n be internal, or mixed between proteins or betv
ch as tyrosine nitration can also occur.) It shoul
of NO toxicity (nitrosative stress)[8,116,117,144] tha
ns are likely.
oxidative stress that results from increased or dy
red by several cellular mechanisms, which are tr
proteins that directly, or through transcriptiona
ed and/or extreme redox-related stress is associ
cal sequelae. Therefore, reversible modifications
kages (intramolecular, or mixed disulphides inc
he homeostatic maintenance of the cellular redo
some sulphinic acids and of sulphonic acids, ar
ious possible nitrosative/oxidative modification



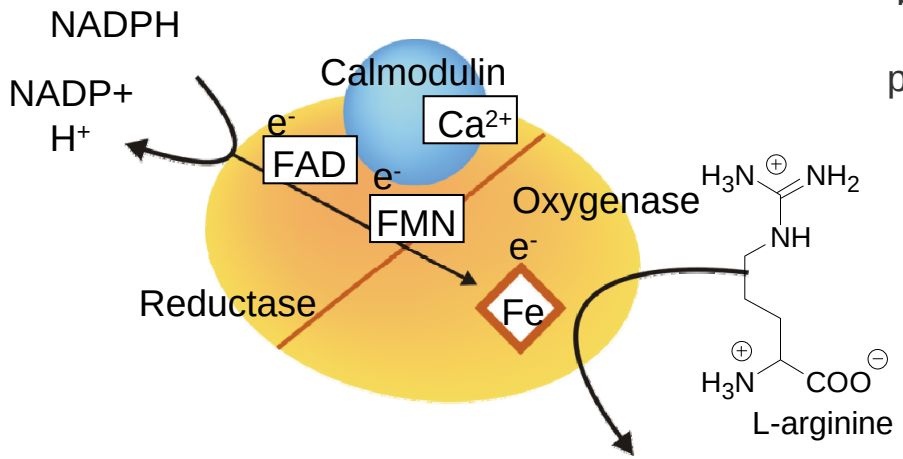
Cys-S-H → Cys-S-N=O

Cys-S-O-H

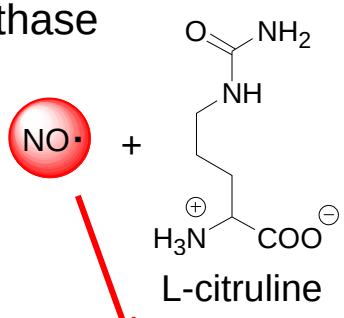
Cys-S-S-Cys



Physiological importance of NO

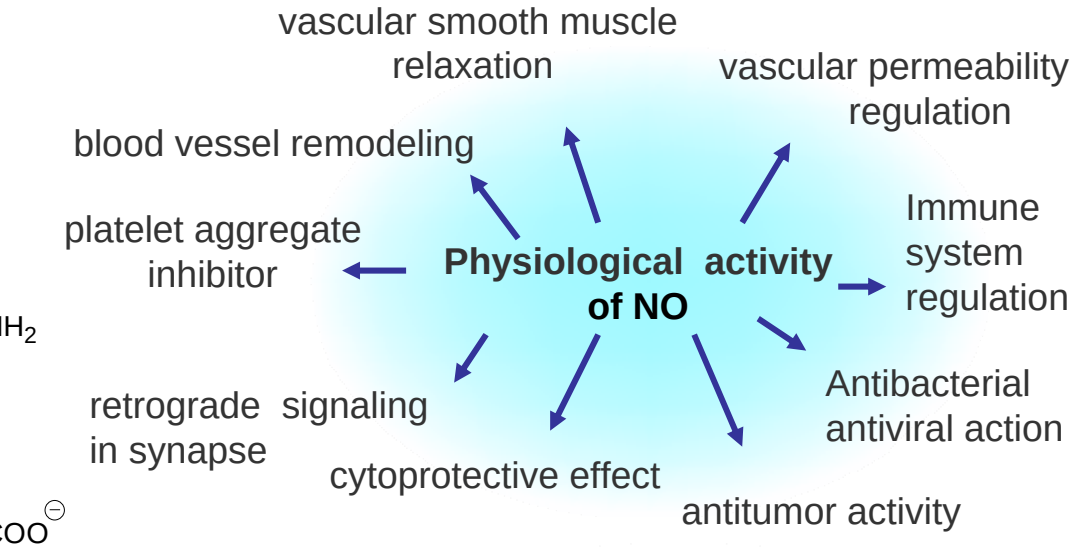


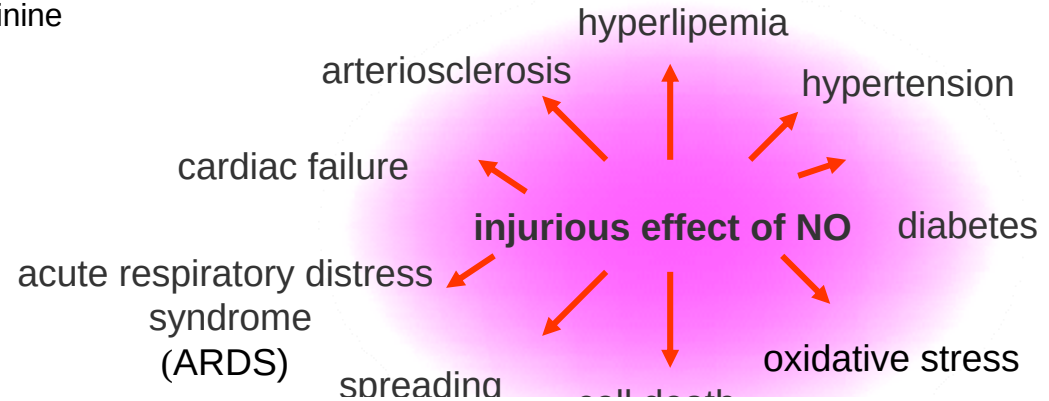
Nitric oxide synthase
NOS



Guanylate cyclase
cGMP
G-kinase

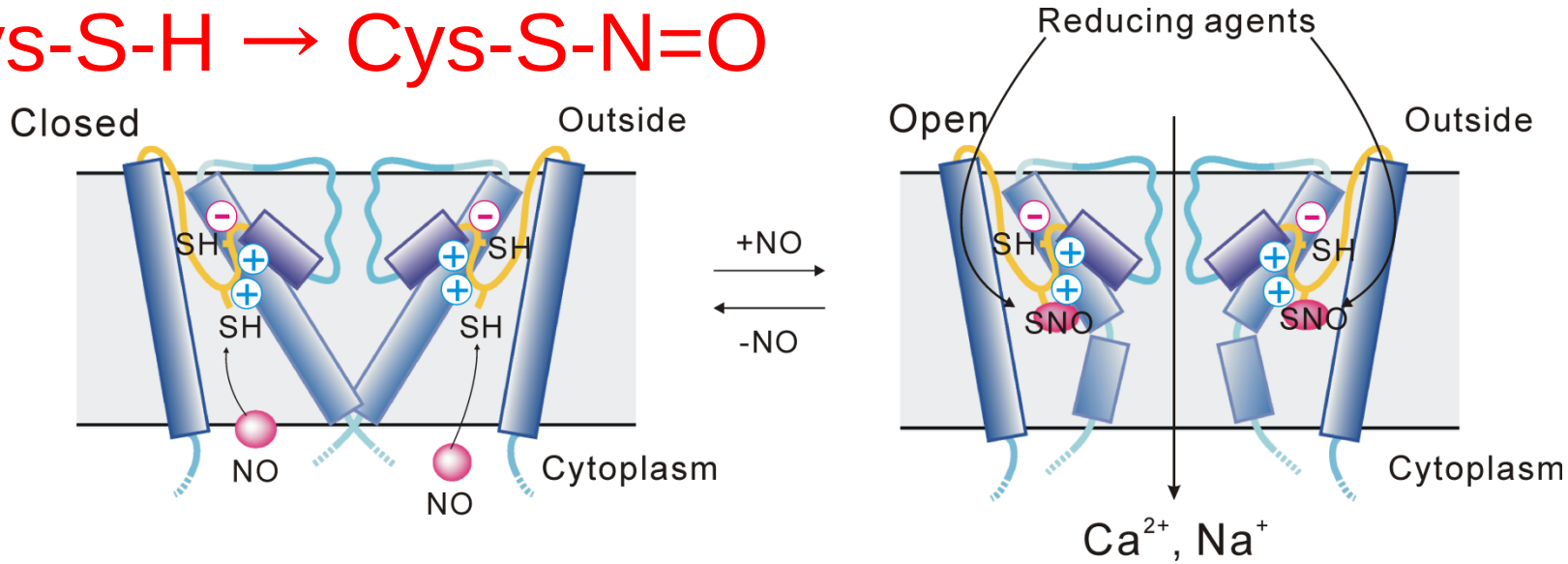
Cysteine S-nitrosylation of proteins





Cys-S-H → Cys-S-N=O

Cys-S-H → Cys-S-N=O



Alignment of various TRPs with the C553/C558-containing S5-S6 TRPC5 sequence

```
                                  553   558
mTRPC5  LYFY-YETRAIDE--PNNCKGIRCEKQNN------AFSTLFETLQSLF
mTRPC4  LYFY-YEETKG-----LSCKGIRCEKQNN------AFSTLFETLQSLF
mTRPC1  LYDKGYTSKEQ-----KDCVGIFCEQQSND-----TFHSFIGTCFALF
mTRPC2  IYVPYQ---ESE----------------------KLGNFNETFQFLF
mTRPC3  LYSYYLGAKVNP---------------------AFTTVEESFKTLF
mTRPC6  LYSYYIGAKQNE---------------------AFTTVEESFKTLF
mTRPC7  LYSYYRGAKYNP---------------------AFTTVEESFKTLF
mTRPM2  ILIHNESRVDWIFRGVVYHSYLTIFGQIP-----TYIDGVNFSMDQCS
mTRPM7  ILYPHEEPSWSL---------------------AKDIVFHPYWMIF
mTRPV1  LIEDGKNNSLPVESPPHKCRGSACRPGN-------SYNSLYSTCLELF
mTRPV4  LLNPCTNMKVCDEDQ-SNCTVPTYPACR-------DSETFSAFLLDLF
dTRP    LLWY-YAELEKNKCYHLHPDVADFDDQEKACTIWRRFSNLFETSQSLF
dTRPL   LLWY-FAALEKSKCYVLPGGEADWGSHGDSCMKWRRFGNLFESSQSLF
```

Receptor-activated channels

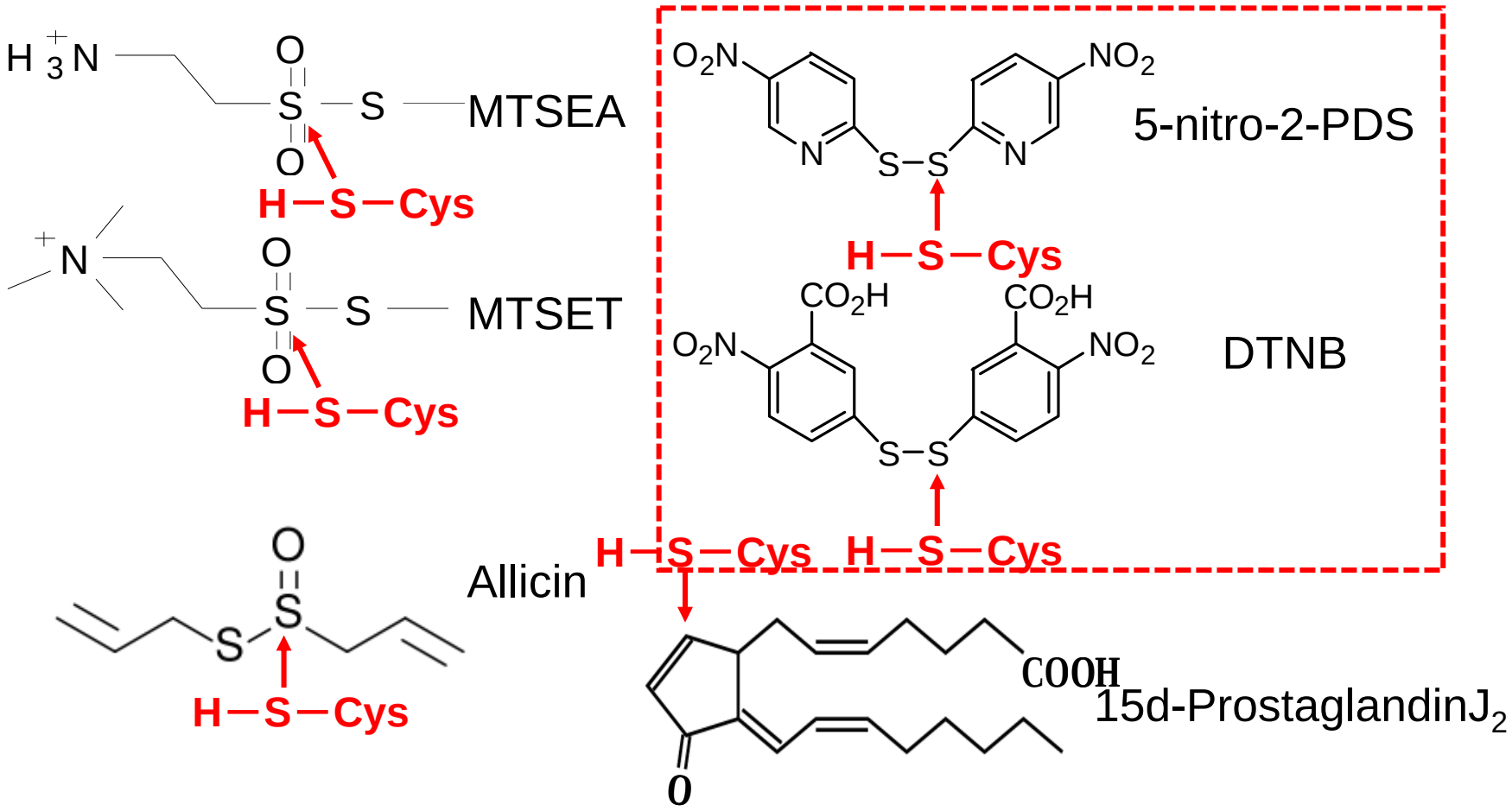
Thermosenor channels

+ TRPV3

Yoshida *et al*., Nature Chem. Biol. (2006)

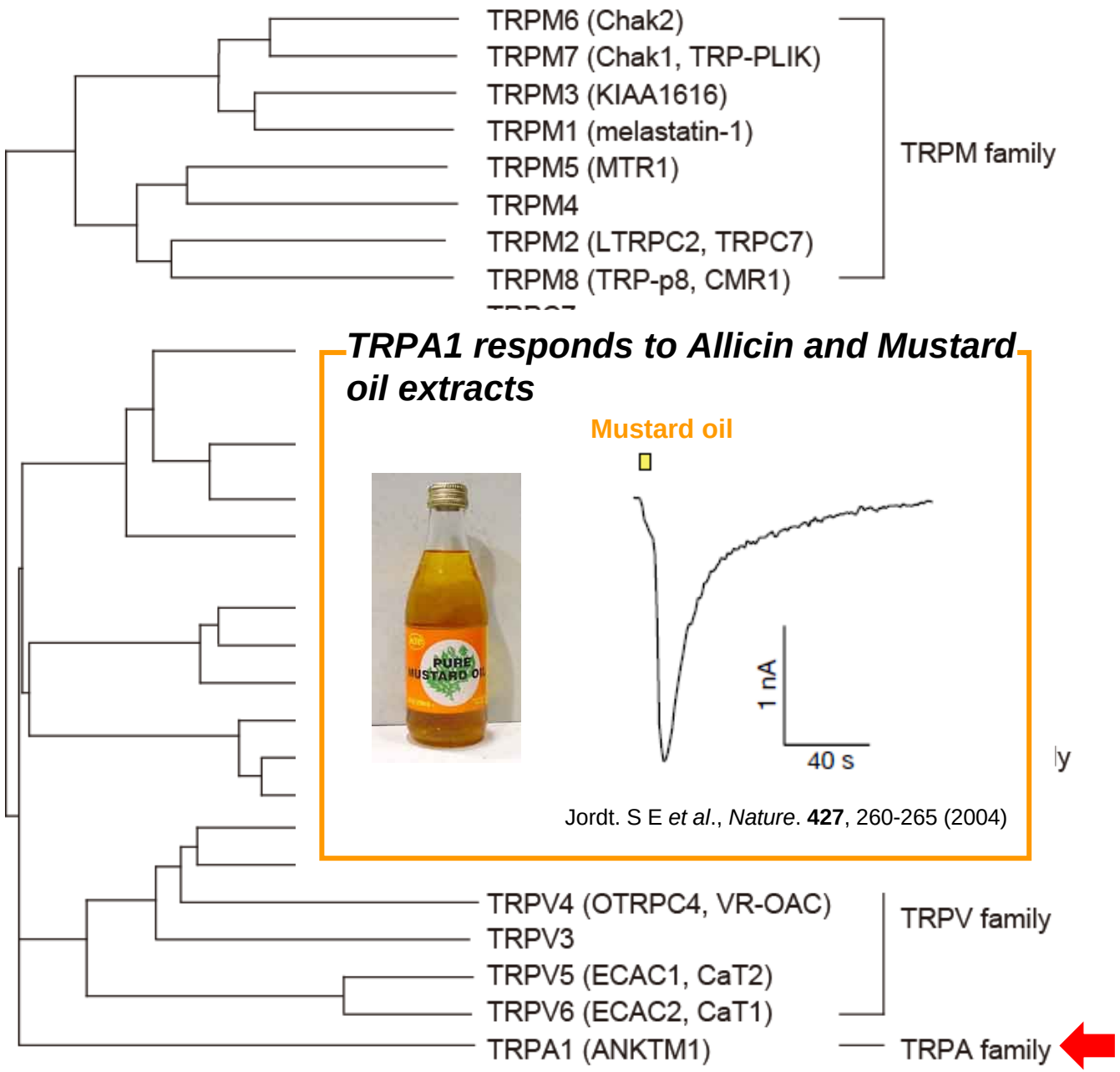
The TRP channels that respond to NO!

Cysteine-targetting reactive compounds and disulfides



Theses compounds activate TRPs by modification of the same Cys as NO, and other TRP channels as well!

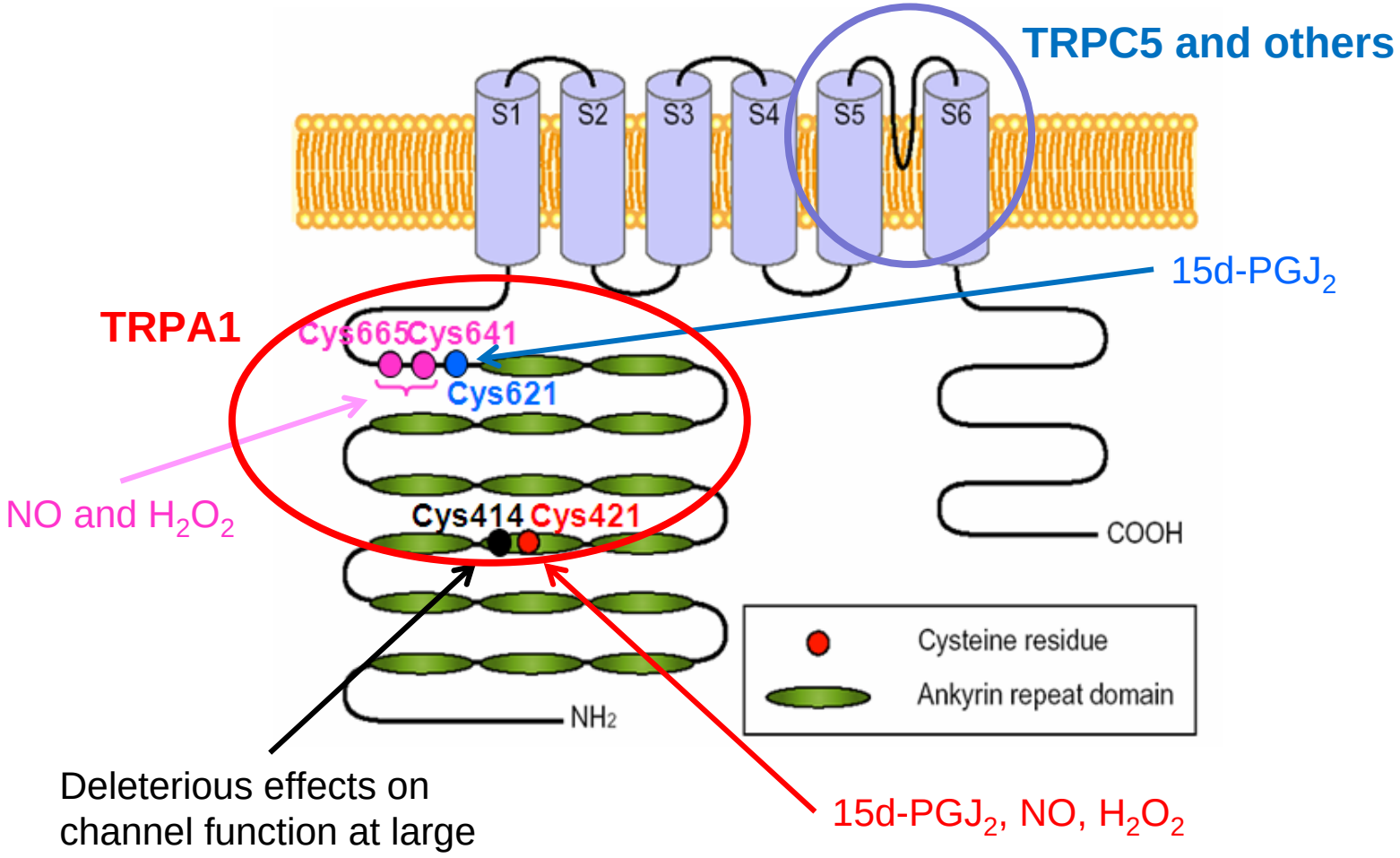
Phylogenetic tree of TRP proteins



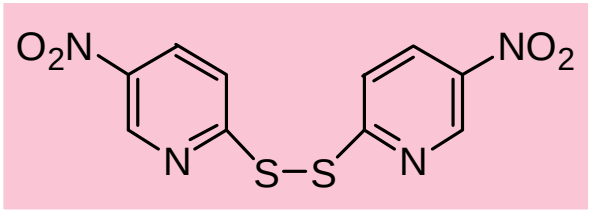
TRPA1 is activated through oxidative modification of Cys residues

TRPA1 is targeted by an array of inflammatory mediators to elicit inflammatory pain in the nervous system.

The sites of action of 15d-PGJ_2 are partly different from those of NO and H_2O_2.



Redox-sensitive TRP channels



Reactive disulfides are powerful tools to assess oxidation sensitivity of proteins and downstream biological responses