Signaling via cCMP and cUMP

A short overview and appetizer for the research community, particularly prospective students

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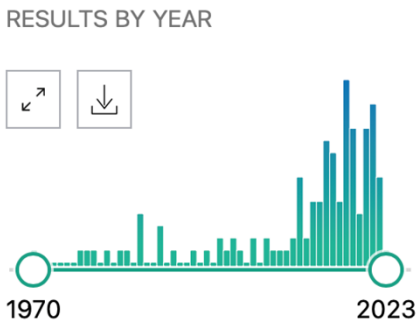
Problems in the cCMP and cUMP field

- Start of the field with artifacts and methodological problems in the 1970s
- → Low reputation
- Very few groups have been working in the field since then
- Biological functions as yet poorly understood
- **Mass spectrometry key for identification and quantification**
- Researchers are hesitant to enter the field (high risk,
  no known specific cCMP- or cUMP effectors)
- → Progress very slow until recently……



PubMed search for “cUMP“ (16.1.2023)

cNMP analysis by mass spectrometry

Exact mass of protonated cCMP 306.0492 Da

Tandem-mass spectrometry

Low mass selectivity 0.7 Da (~ 2000 ppm), **but high sensitivity**!

Masses in a range of 305.35 and 306.75 Da are detected as protonated cCMP

→ **quantification**

Time of flight mass spectrometry

High mass selectivity 5 - 15 ppm, **but lower sensitivity!**

Masses in a range of 306.0445 and 306.0454 Da are detected as protonated cCMP

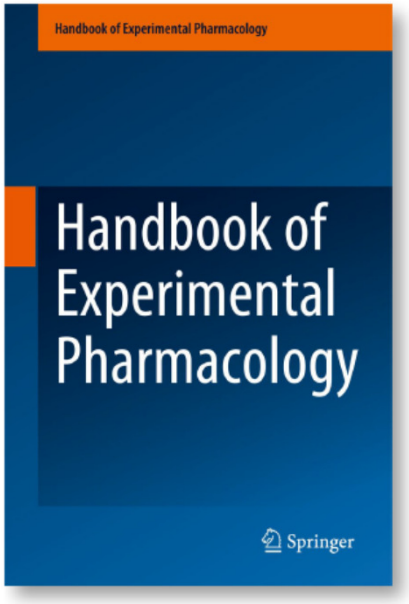
→ **identification verification**

Handbook of Experimental Pharmacology

Volume 238

Volume title: **Non-Canonical Cyclic Nucleotides**

Volume Editor: **Roland Seifert**



Part I: cCMP and cUMP as second messengers

Part II: cIMP, 8-nitro-cGMP, 2',3'-cNMP and cyclic dinucleotides as signaling molecules

Part III: Methods/tools for cNMP research

18 chapters written by leading experts; published 2017

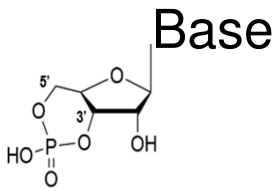
Canonical and non-canonical cNMP

Canonical cNMP:

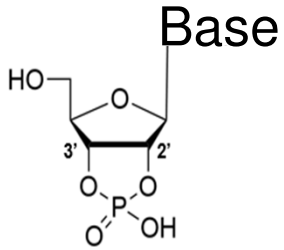
- 3‘,5‘-cAMP (long-established second messenger)
- 3‘,5‘-cGMP (long-established second messenger)

Non-canonical cNMP:

- **3‘,5‘-cCMP (newly established second messenger)**
- **3‘,5‘-cUMP (newly established second messenger)**
- 2‘,3‘-cNMP (just emerging second messenger)

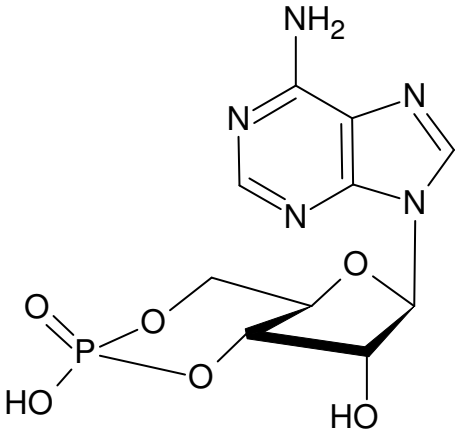


3’,5’-cNMP

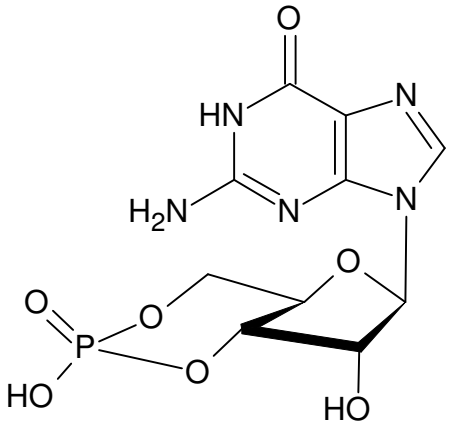


2’,3’-cNMP

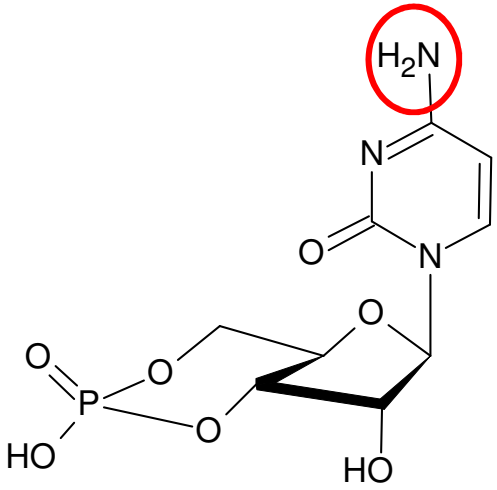
Cyclic purine and pyrimidine nucleotides



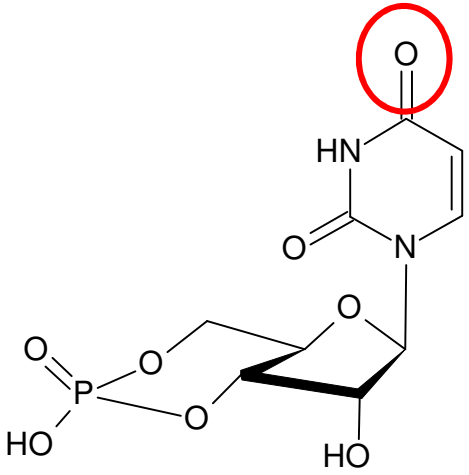
cAMP



cGMP



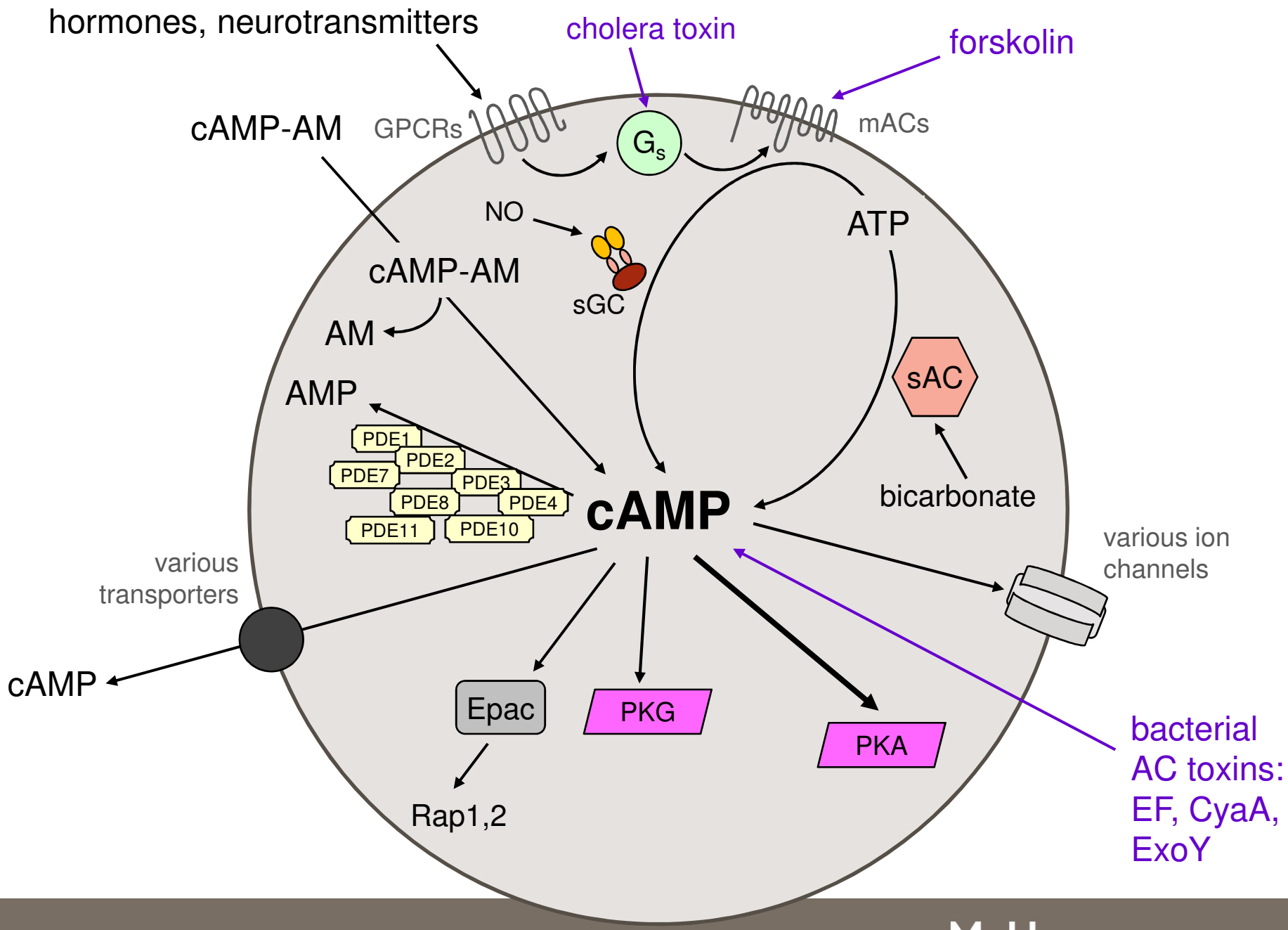
cCMP

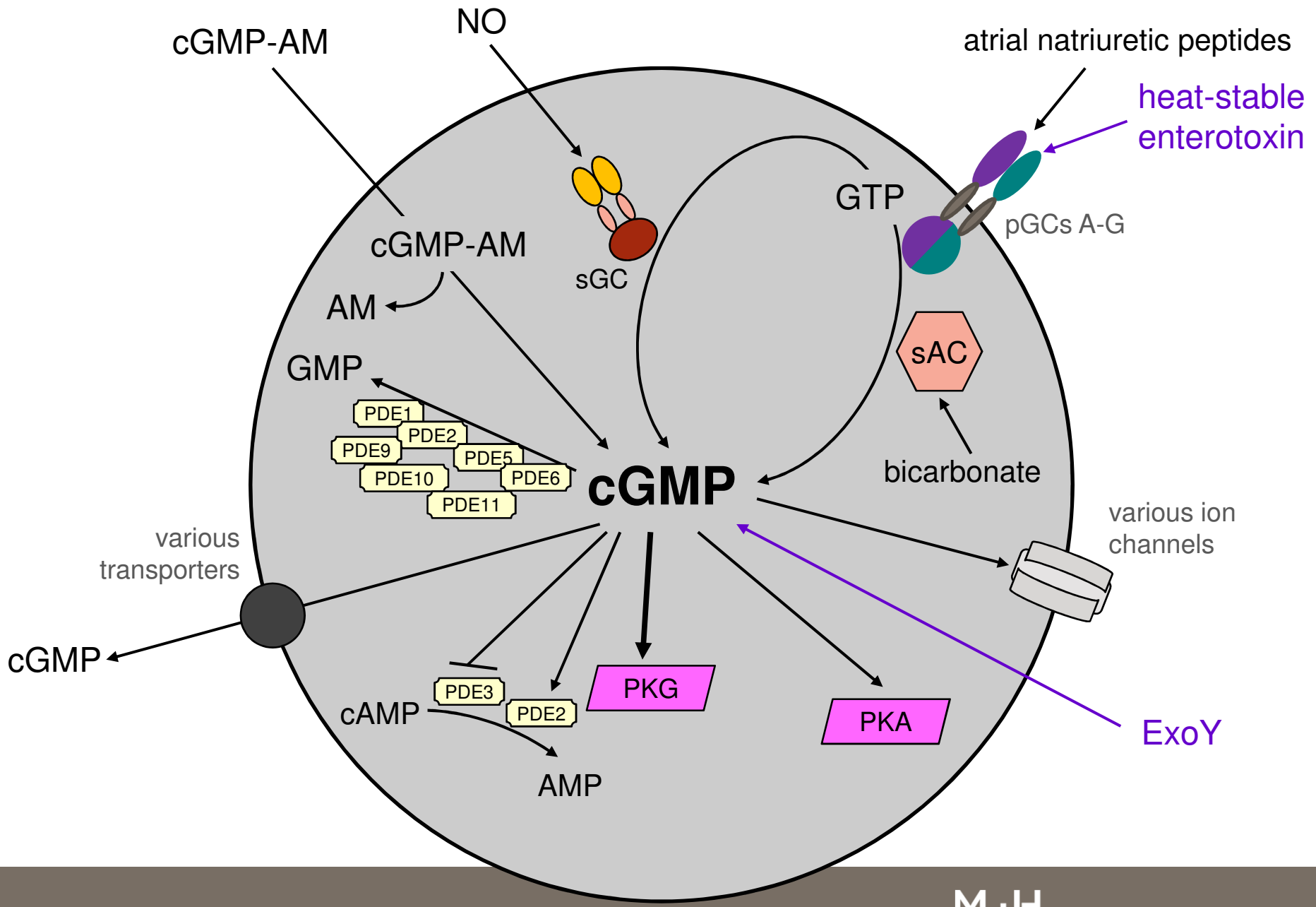


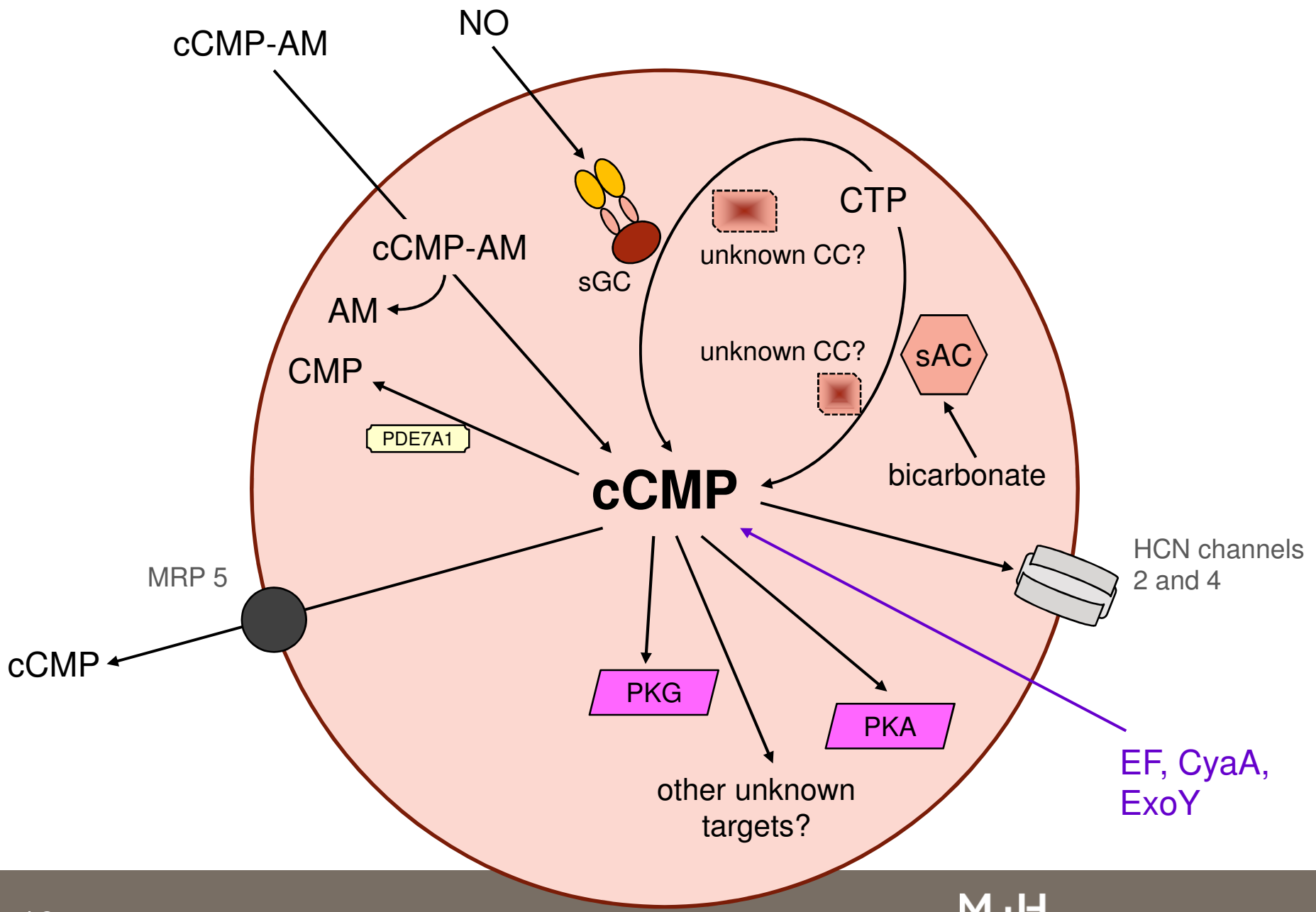
cUMP

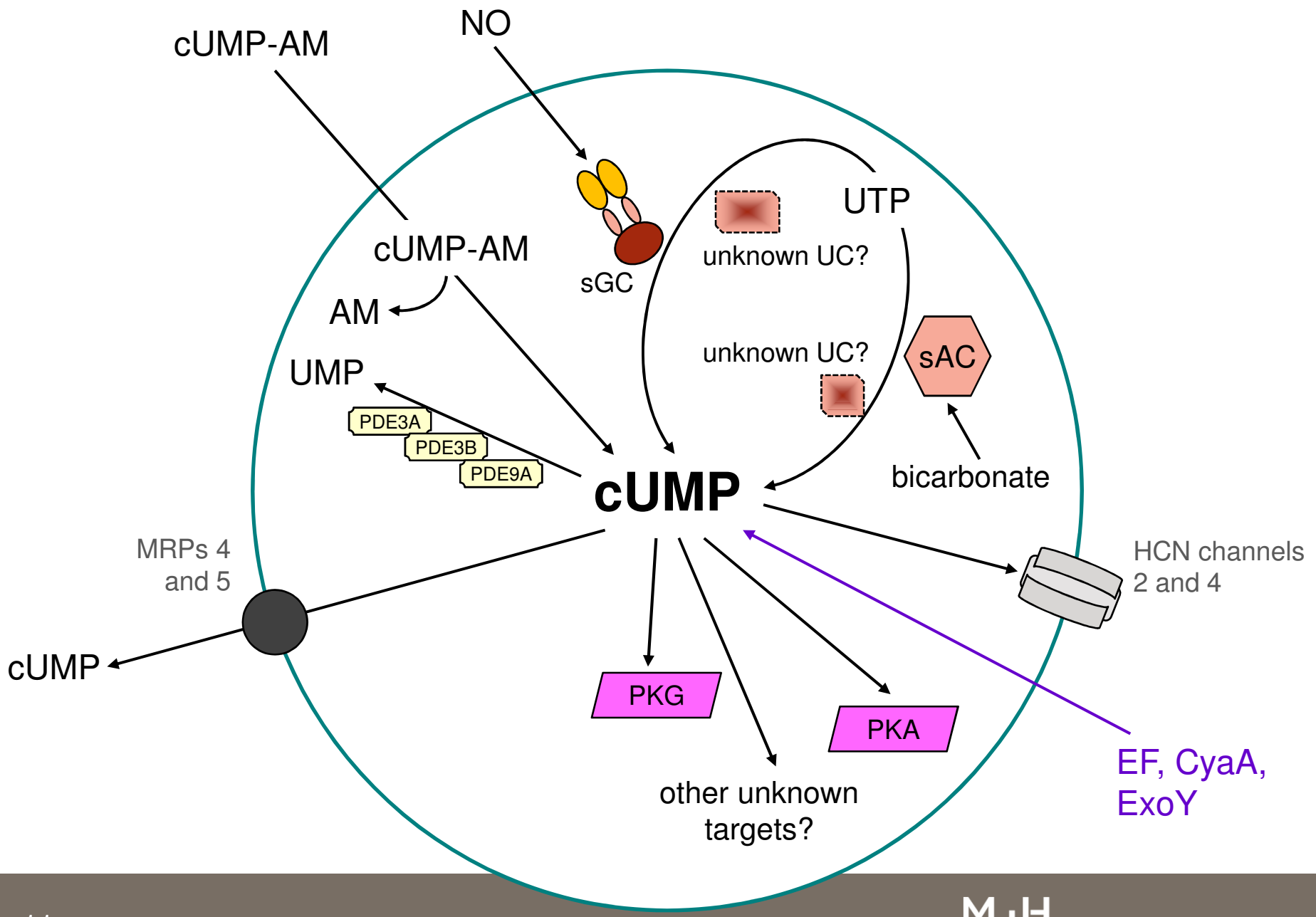
cNMPs as second messengers

Criterion	cAMP	cGMP	cCMP	cUMP
Generators	mACs 1-9, sAC (sGC, pGC-A)	sGC, pGCs A-G, (sAC)	sGC, sAC, **other cyclases?**	sGC, sAC, **others cyclases?**
Effectors	PKA, Epac, HCN channels, (PKG)	PKG, (PKA), PDEs, ion channels	PKA, PKG, HCN channels 2 and 4, **others targets?**	PKA, PKG, HCN channels 2 and 4, **other targets?**
Biological functions	Virtually every cell is regulated by cAMP	Many cell functions, e.g. vascular tone, platelet aggregation, metabolism, vision	Heart pacing, immune cell function, apoptosis, reproductive function? **Other functions**	Heart pacing, apoptosis, neuronal homeostasis? **Other functions?**
Inactivation	cAMP-specific PDEs, but also cAMP/cGMP- and cGMP-specific PDEs; MRPs 4 and 5	cGMP-specific PDEs, but also cAMP/cGMP-specific and cAMP-specific PDEs, MRPs 4 and 5, OAT2	PDE7A1, MRP5	PDEs 3A, 3B and 9A, MRPs 4 and 5
Membrane-permeable analogs	DB-cAMP, cAMP-AM and others	DB-cGMP, cGMP-AM	DB-cCMP, cCMP-AM	cUMP-AM
Mimicry by toxins	Cholera toxin, EF, CyaA, (ExoY)	Heat-stable enterotoxin, ExoY	ExoY, (CyaA, EF)	**ExoY**, (CyaA)

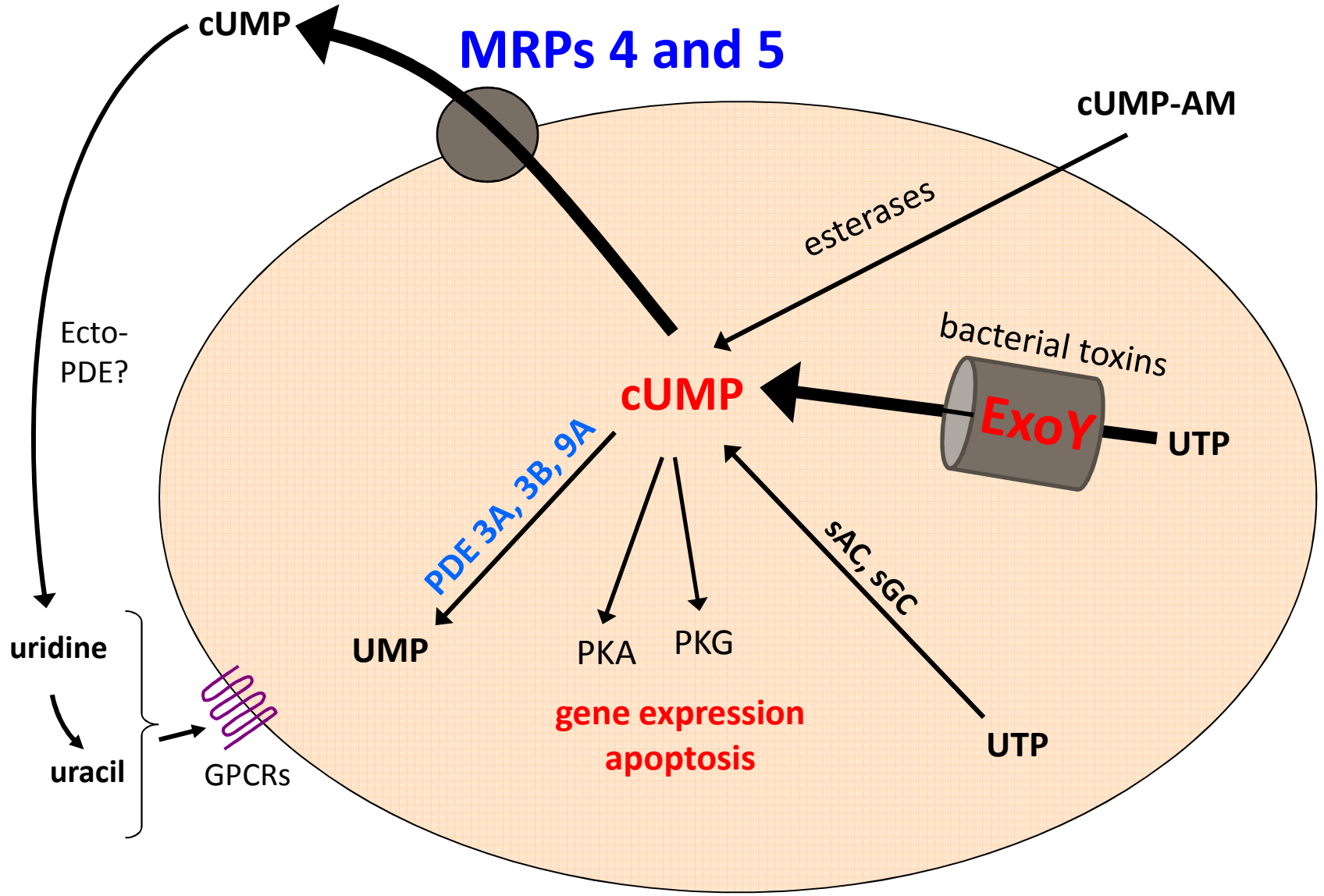

hormones, neurotransmitters
cholera toxin
forskolin
cAMP-AM
GPCRs
mACs
G_s
NO
ATP
cAMP-AM
sGC
AM
AMP
sAC
PDE1
PDE2
PDE7
PDE3
PDE8
PDE4
PDE11
PDE10
cAMP
bicarbonate
various ion channels
various transporters
cAMP
Epac
PKG
PKA
Rap1,2
bacterial AC toxins: EF, CyaA, ExoY


cGMP-AM
NO
atrial natriuretic peptides
heat-stable
enterotoxin
GTP
pGCs A-G
cGMP-AM
sGC
AM
GMP
sAC
PDE1
PDE2
PDE9
PDE5
PDE10
PDE6
PDE11
cGMP
bicarbonate
various ion
channels
various
transporters
cGMP
PDE3
PDE2
PKG
PKA
cAMP
AMP
ExoY


cCMP-AM
NO
cCMP-AM
sGC
CTP
unknown CC?
unknown CC?
sAC
AM
CMP
PDE7A1
cCMP
bicarbonate
HCN channels
2 and 4
MRP 5
cCMP
PKG
PKA
other unknown
targets?
EF, CyaA,
ExoY

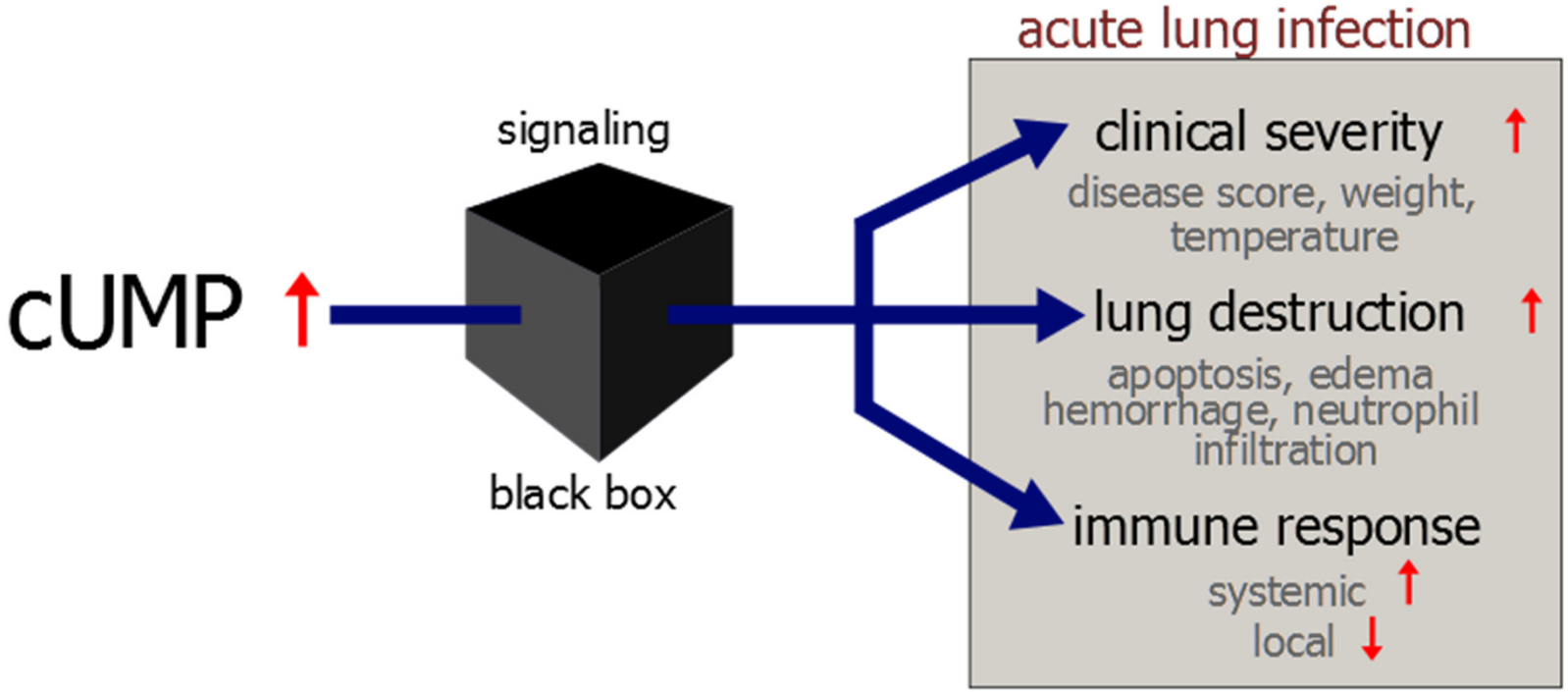

cUMP-AM
NO
cUMP-AM
sGC
UTP
unknown UC?
AM
unknown UC?
sAC
UMP
PDE3A
PDE3B
PDE9A
cUMP
bicarbonate
MRPs 4
and 5
HCN channels
2 and 4
cUMP
PKG
PKA
EF, CyaA,
ExoY
other unknown
targets?

Export of cUMP by MRPs more relevant than degradation by PDEs?

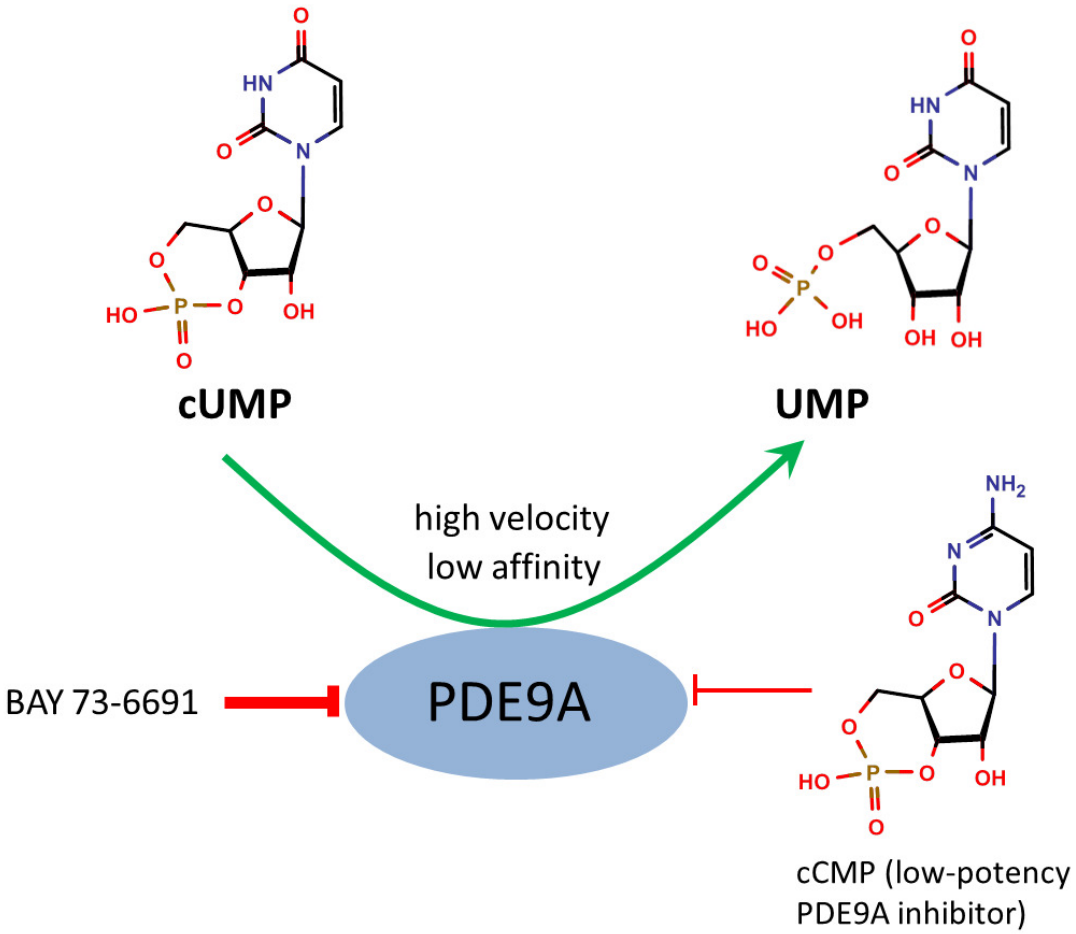


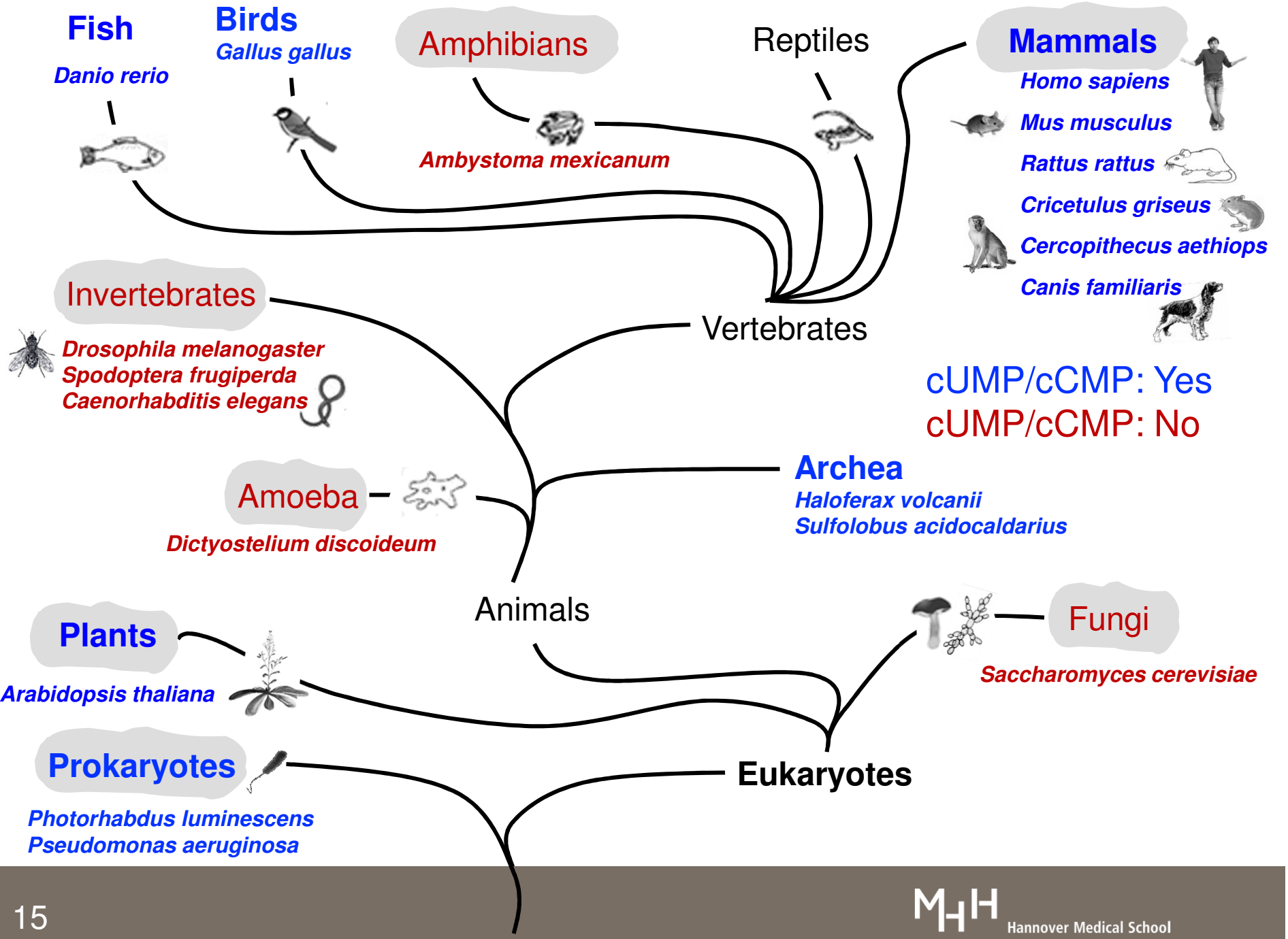
Summary of ExoY

- Nucleotidyl cyclase activity of ExoY leads to a more severe lung infection.
- cUMP is the most prominent cNMP during early infection.
- Establishment of effective (innate) immune response in the lung may be hindered by ExoY (TNF / IL-1β ↓) during early infection

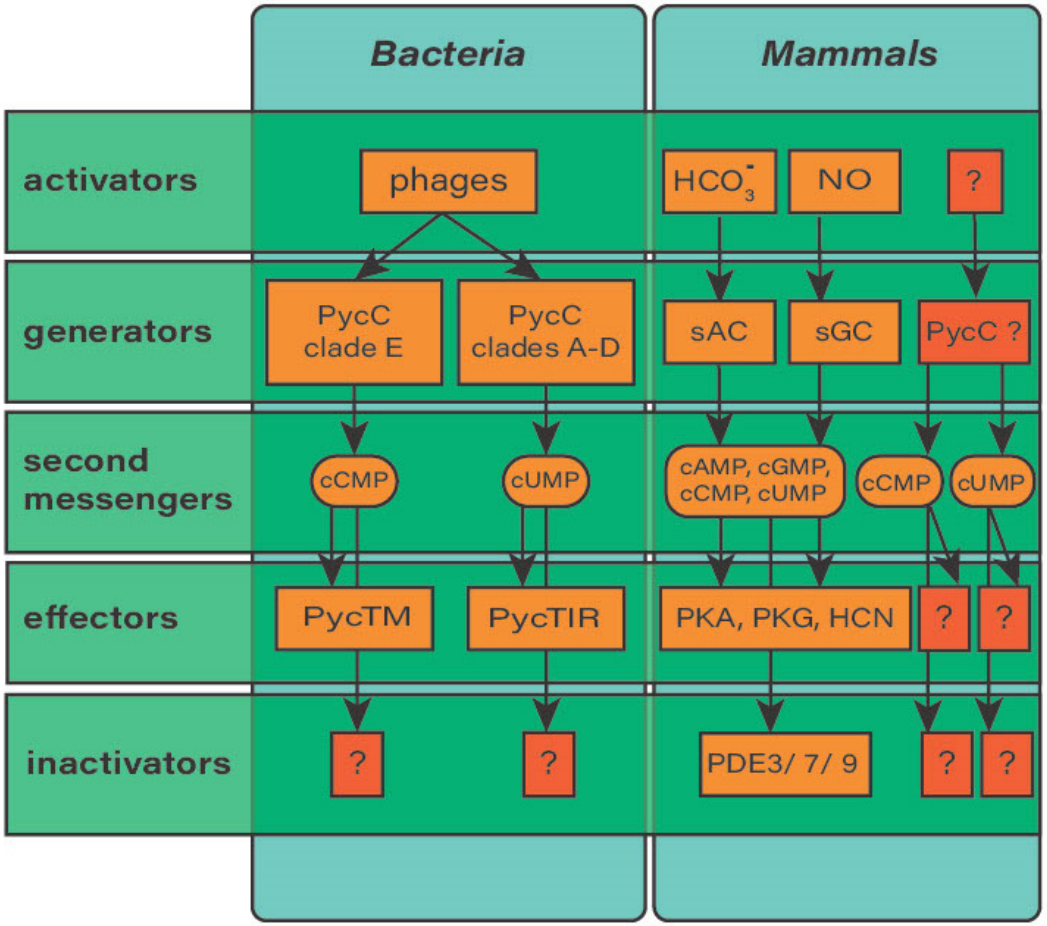


PDE9A as cCMP-inhibited cUMP-hydrolyzing PDE




Fish
Danio rerio
Birds
Gallus gallus
Amphibians
Ambystoma mexicanum
Reptiles
Mammals
Homo sapiens
Mus musculus
Rattus rattus
Cricetulus griseus
Cercopithecus aethiops
Canis familiaris
Vertebrates
Invertebrates
Drosophila melanogaster
Spodoptera frugiperda
Caenorhabditis elegans
cUMP/cCMP: Yes
cUMP/cCMP: No
Amoeba
Dictyostelium discoideum
Archea
Haloferax volcanii
Sulfolobus acidocaldarius
Animals
Plants
Arabidopsis thaliana
Fungi
Saccharomyces cerevisiae
Prokaryotes
Photorhabdus luminescens
Pseudomonas aeruginosa
Eukaryotes

Selective PycC, cCMP and cUMP effectors



- Tal N….. Kranzusch PJ, Sorek R. Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. *Cell* **184:**5728 (2021)
- Seifert R, Schirmer B. cCMP and cUMP come into the spotlight, finally. *Trends Biochem Sci* **47:**461 (2022)

Take-home messages

- New large families of bacterial UC and CC (PycC)
- cCMP and cUMP in many kingdoms of life
- cCMP and cUMP as second messengers
- Different regulation of cNMPs (2‘,3‘ and 3‘,5‘) under different living conditions
- Viral cCMP/cUMP-degrading PDE („nucleases“) as defence weapons
- Promiscuous mammalian NC and bacterial NC toxins