



# Neuroglobin and Cytochrome:

“Fresh blood” for the  
vertebrate globin gene family

Thomas Hankeln & Thorsten Burmester

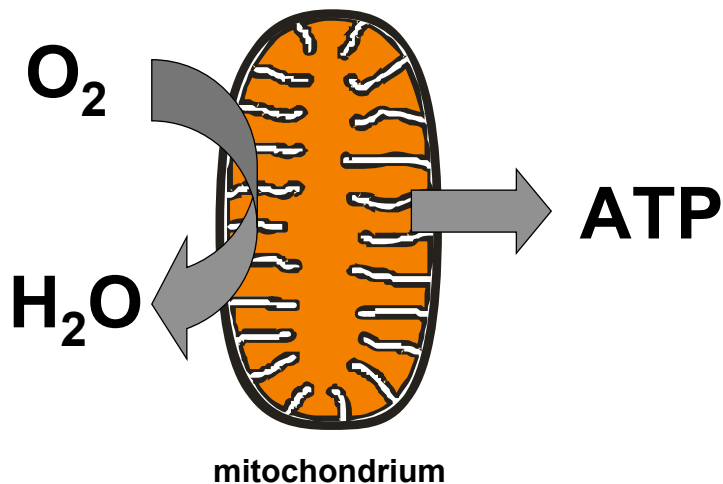


Institut für Molekulargenetik,  
Johannes Gutenberg Universität Mainz

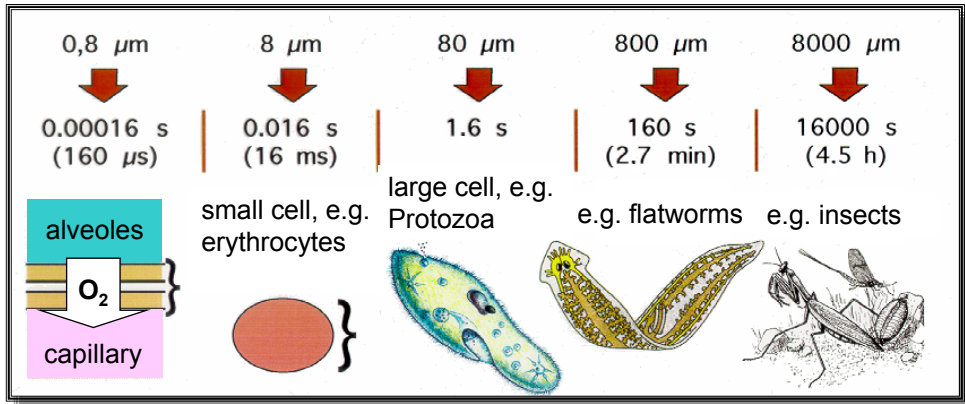
Institut für Zoologie  
Uni Hamburg



## The task...

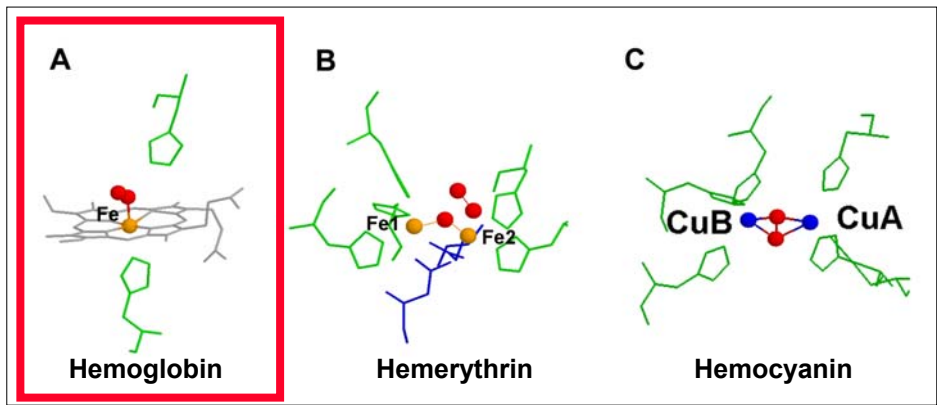


# The problem...







10 x distance > 100 x time

## One solution (among others)... Respiratory proteins



↳ in bacteria, protists, fungi, plants, invertebrates, vertebrates

# The Respiratory Proteins of Vertebrates (until 2000)

Tissue	Globin	Function
 blood	<b>Hemoglobin</b> 	<ul style="list-style-type: none"> <li>▪ O<sub>2</sub>-transport</li> <li>▪ CO<sub>2</sub>-transport</li> <li>▪ NO-detoxification</li> <li>▪ NO-transport (???)</li> </ul>
 muscle	<b>Myoglobin</b> 	<ul style="list-style-type: none"> <li>▪ O<sub>2</sub>-storage + supply</li> <li>▪ Intracellular O<sub>2</sub>-diffusion (?)</li> <li>▪ NO detoxification</li> </ul>

## The power of

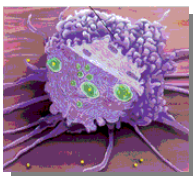
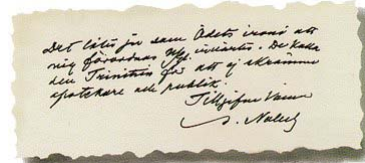


Signal molecule in neurons

NO inhalation against high blood pressure



NO treatment of Angina pectoris

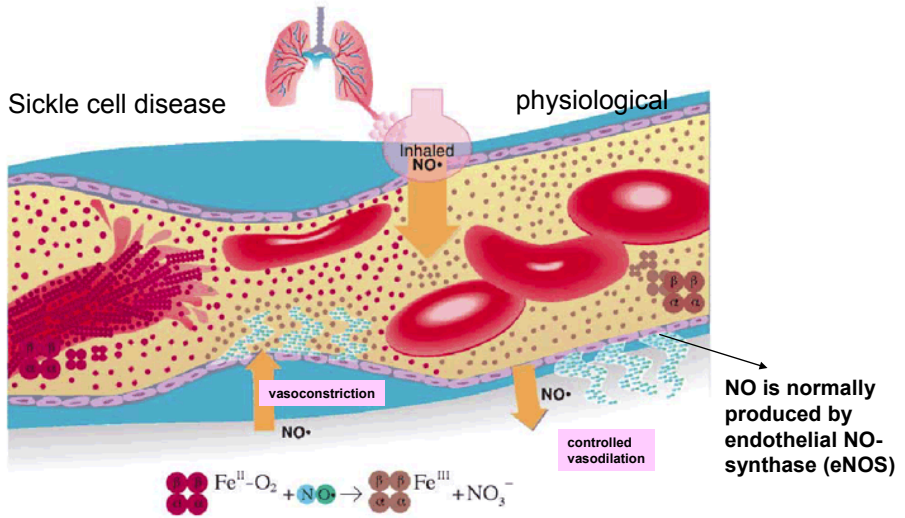


Antibacterial defense in makrophages

"It sounds like the irony of fate that I have been prescribed nitroglycerine internally. They have named it Trinitrin in order Your affectionate friend,

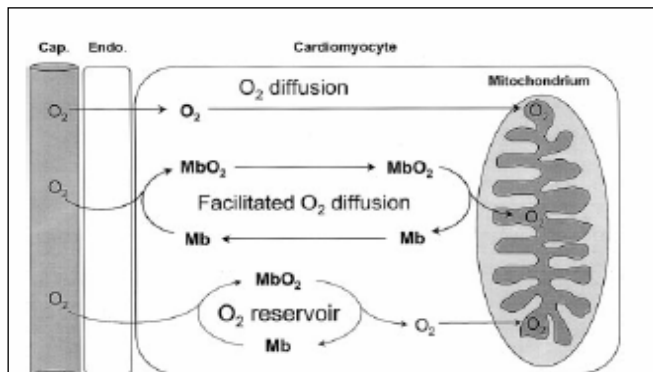
A. Nobel"

# Hb functions in NO homeostasis



## Myoglobin facilitates oxygen diffusion<sup>1</sup>

MARC W. MERX, ULRICH FLÖGEL, THOMAS STUMPE, AXEL GÖDECKE,  
 ULRICH K. M. DECKING, AND JÜRGEN SCHRADER<sup>2</sup>  
 Institut für Herz-und Kreislaufphysiologie, Heinrich-Heine-Universität Düsseldorf, Germany

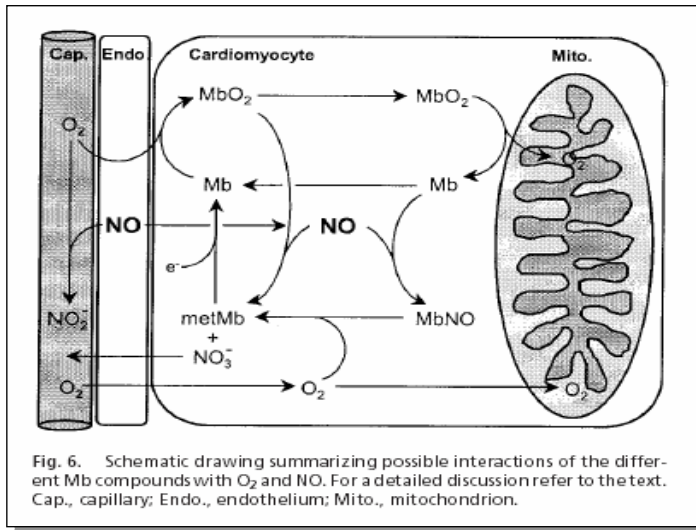


**Figure 3.** Myoglobin-facilitated oxygen diffusion. Myoglobin is loaded with oxygen at the sarcolemma where PO<sub>2</sub> is high and sheds oxygen at the mitochondria where PO<sub>2</sub> is low.

# Myoglobin: A scavenger of bioactive NO

Ulrich Flögel, Marc W. Merx, Axel Gödecke, Ulrich K. M. Decking, and Jürgen Schrader\*

Institute for Cardiovascular Physiology, Heinrich-Heine-University, 40225 Düsseldorf, Germany



Some of the human disease genes that are absent in *Drosophila* reflect clear differences in physiology between the two organisms. For instance, none of the hemoglobins, which are mutated in thalassemias, have orthologs in *Drosophila*. In flies, oxygen is delivered directly to tissues via the tracheal system rather than by circulating erythrocytes. Similarly, several genes required for normal rearrangement of the immunoglobulin genes do not have *Drosophila* orthologs.

See:  
Burmester  
& Hankeln  
1999



# BLAST: *Drosophila*-Hb vs. Mouse ESTs

Database: GenBank Mouse EST entries 1,758,095 sequences; 629,105,611 total letters

**NCBI BLAST Search Results** BLAST Entrez ?

Sequences producing significant alignments:

dbj AU036042.1 AU036042	AU036042 Sugano mouse brain mncb Mu...	(bits)	value
dbj BE648697.1 BE648697	UI-M-BG1-aid-e-09-0-UI.r1 NIH_BMAP_M...	37	0.045
gb AW548186.1 AW548186	L0032E08-3 Mouse E12.5 Female Mesone...	32	0.89
gb AW546198.1 AW546198	L0005A02-3 Mouse E12.5 Female Mesone...	32	0.89
gb AW548428.1 AW548428	L0036F07-3 Mouse E12.5 Female Mesone...	32	1.1
emb AL362383.1 AL362383	AL362383 ICRPp 522 and 523 Mus musc...	32	1.3

etc..

Alignments>

[dbj|AU036042.1|AU036042](#) AU036042 Sugano mouse brain mncb Mus musculus cDNA clone MNCb-7114.  
 Length = 740 Score = 40.8 bits (126), Expect = 0.003  
 Identities = 33/154 (21%), Positives = 63/154 (40%), Gaps = 5/154 (3%)  
 Frame = +3

Query: 1 MNSDEVLIKKTWEIPVATPTDGAAILTQFFNRPFSNLEKFFPRDVPL---EELSGNAR 57  
 M E +LI+++W + +P + G + + F PS L F + E+ +  
 Sbjct: 156 MERPESELIRQSRWVRSRSPLEHGTVLFARLFALEPSLLPLFYQNGRQFSSPEDCLSSPE 335

Query: 58 FRAHAGRIIRVFDESIQVLGQDGDLEKLEIWTKIAVSHIPRTVSKESYNQLKGVILDVL 117  
 F H +++ V D ++ + DL L+E T + H V S++ + +L +L  
 Sbjct: 336 FLDHIRKVMLVIDAAVTV---EDLSSLEEYLSLGRKRAVGVRLSSFTVSGESLLYML 506

Query: 118 TAACSLDESQA--ATWAKLVHDHYAIIKKAIDDD 149  
 D A W++L V ++ D +  
 Sbjct: 507 EKCLGPDFIPAIRTANSRLYGAVVQAMSRGWGDE 608 etc...

=> with BLOSUM45 matrix to identify distant proteins

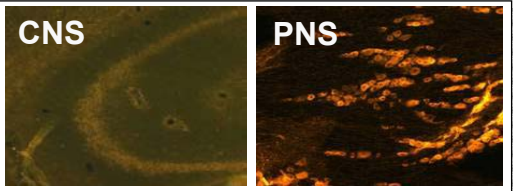
## 2000: Neuroglobin

MmuNgb --MERPESELIRQSRWVRSRSPLEHGTVLFARLFALEPSLLPLFYQNGRQFSSPEDCLSPFLDIRMLVIDAVT 77  
 HsaNgb --NERPDEIRQSRWVRSRSPLEHGTVLFARLFALEPSLLPLFYQNGRQFSSPEDCLSPFLDIRMLVIDAVT 77  
 HsaHb --RGGGGLGGLNIVVAVADIAAGTAVGIDLSTHSEKDKK--HSEEDMKKEDLKK--STL--AGTIK 78  
 MmuHb --RGGGGLGGLNIVVAVADIAAGTAVGIDLSTHSEKDKK--HSEEDMKKEDLKK--STL--AGTIK 78  
 HsaHBA --VYVADITNKAAGGQJAHAGTAVGIDLSTHSEKDKK--HSEEDMKKEDLKK--STL--AGTIK 72  
 HsaHbA --VYVADITNKAAGGQJAHAGTAVGIDLSTHSEKDKK--HSEEDMKKEDLKK--STL--AGTIK 72  
 HsaHBB MVHTEDEGATLAKGV--VVDVGLGGLLVVYV--GRF--G--TPDAVMNPKK--AG--GLG--TSDGA 77  
 MmuHbb MVHTEDEGATLAKGV--VVDVGLGGLLVVYV--GRY--G--TPDAVMNPKK--AG--GLG--TSDGA 77

MmuNgb NYEELSSCYEASGRKRAVG--KLSSTVSGSLYVHCKCLSDA--DTRTWSRLYGAVVQAMSRGWGDE--- 151  
 HsaNgb NYEELSSCYEASGRKRAVG--KLSSTVSGSLYVHCKCLSDA--DTRTWSRLYGAVVQAMSRGWGDE--- 151  
 HsaHb KKGQ--HEATKTP--AGS--TKPKPVYLEFISCTICIQSRKRG--GADAG--H--KELFRKDN--K--KELGFQG 154  
 MmuHb KKGQ--HEATKTP--AGS--TKPKPVYLEFISCTICIQSRKRG--GADAG--H--KELFRKDN--K--KELGFQG 154  
 HsaHBA --L--MHPAESA--D--H--G--D--V--K--K--H--C--L--V--T--A--A--P--A--T--P--V--H--L--D--P--A--G--T--V--S--T--S--H-- 142  
 MmuHbA --L--MHPAESA--D--H--G--D--V--K--K--H--C--L--V--T--A--A--P--A--T--P--V--H--L--D--P--A--G--T--V--S--T--S--H-- 142  
 HsaHBB --L--MHPAESA--D--H--G--D--V--K--K--H--C--L--V--T--A--A--P--A--T--P--V--H--L--D--P--A--G--T--V--S--T--S--H-- 142  
 MmuHbb --L--MHPAESA--D--H--G--D--V--K--K--H--C--L--V--T--A--A--P--A--T--P--V--H--L--D--P--A--G--T--V--S--T--S--H-- 142

B12.2 ↑  
 E11.0 ↑  
 G7.0 ↑

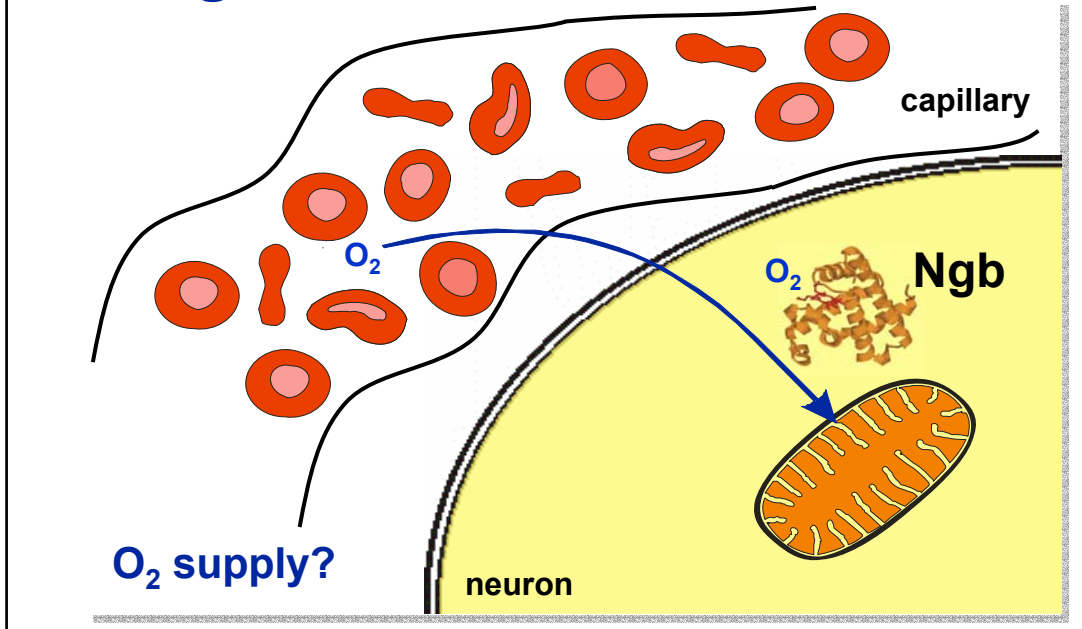
▪ < 25% identity with myoglobin or hemoglobin



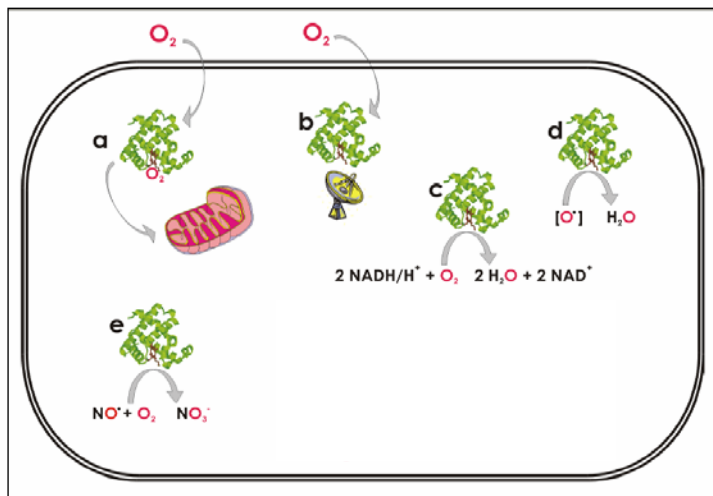
▪ mainly expressed in CNS, PNS + endocrine systems

Burmester et al. (2000) *Nature* 407, 520-523  
 Reuss et al. (2002) *Neuroscience* 115, 645-656  
 Wystub et al. (2003) *Neurosci. Lett.* 346, 114-116

# Neuroglobin function?



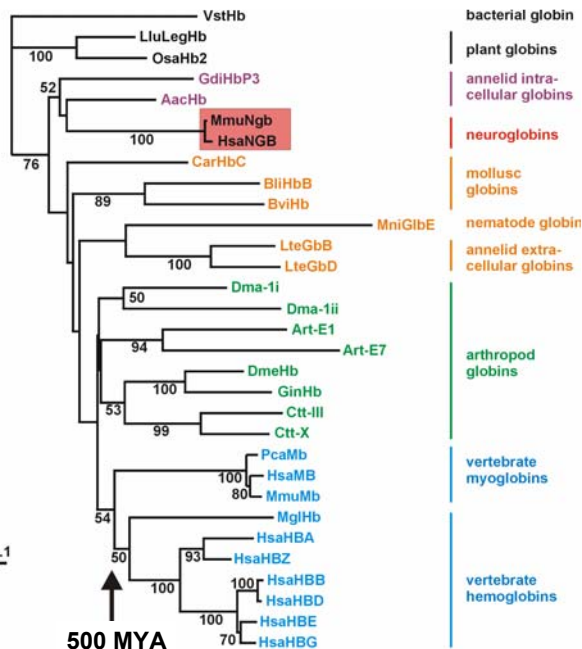
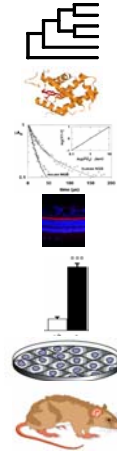
# Neuroglobin functions?



- a. O<sub>2</sub>- supply
- b. O<sub>2</sub>-sensor
- c. terminal oxidase
- d. ROS detoxification
- e. NO-dioxygenase

# How to assess Neuroglobin function?

- phylogeny
- structure
- kinetics
- localisation
- expression regulation
- cell culture
- KO + transgenics
- etc...



## Neuroglobin evolution

- Ngb groups with "Nerve-Hb" of *Aphrodite* (Annelida)

- Ngb is much older than 500 million years

- evolution rates:

Ngb:  $\sim 0.4 \times 10^{-9}/\text{aa}/\text{year}$

Hb:  $\sim 1.2 \times 10^{-9}/\text{aa}/\text{year}$

Mb:  $\sim 1.1 \times 10^{-9}/\text{aa}/\text{year}$



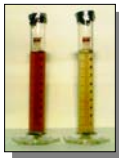
# Invertebrate nerve hemoglobins



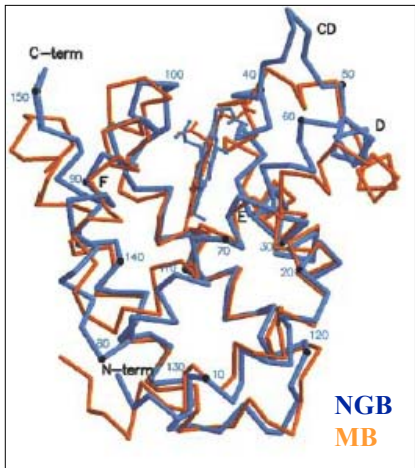
	Where?	P <sub>50</sub> (O <sub>2</sub> )
- <i>Aphrodite aculeata</i> (Annelida)	glia cells (+)	1.1 Torr
- <i>Cerebratulus lacteus</i> (Nemertini)	glia cells (+)	2.9 Torr
- <i>Spisula solidissima</i> (Bivalvia)	glia cells (+)	2.3 Torr
- <i>Tellina alternata</i> (Bivalvia)	glia cells (+)	1.3 Torr
- <i>Aplysia depilans</i> (Gastropoda)	neurons	4 Torr
- <i>Lymnaea stagnalis</i> (Gastropoda)	glia cells (+)	
- <i>Planorbarius spec.</i> (Gastropoda)	glia cells (+)	
- <i>Helix pomatia</i> (Gastropoda)	neurons	
- <i>Cepaea nemoralis</i> (Gastropoda)	neurons	

(+) = high concentrations

Review: Wittenberg 1992



## NGB is myoglobin-like



	Ngb	Mb
structure	monomer	monomer
MW	17 kDa	16 kDa
O <sub>2</sub> affinity (P <sub>50</sub> )	~ 1 Torr	~ 1 Torr

Pesce et al. (2003) *Structure* 11, 1087-1095  
 Dewilde et al. (2001) *JBC* 276, 38949-38955

# Ngb expression is neuronal

rat primary hippocampal cell culture (17/18 dpc)

**Ngb**

**NeuN**

**Ngb**

**GFAP**

**Ngb**

**GFAP**

**NRSE**

Laufs et al. (2004) *Neurosci.Lett.* 362, 83-86

# Neuroglobin in the retina

**Ngb Western blot**

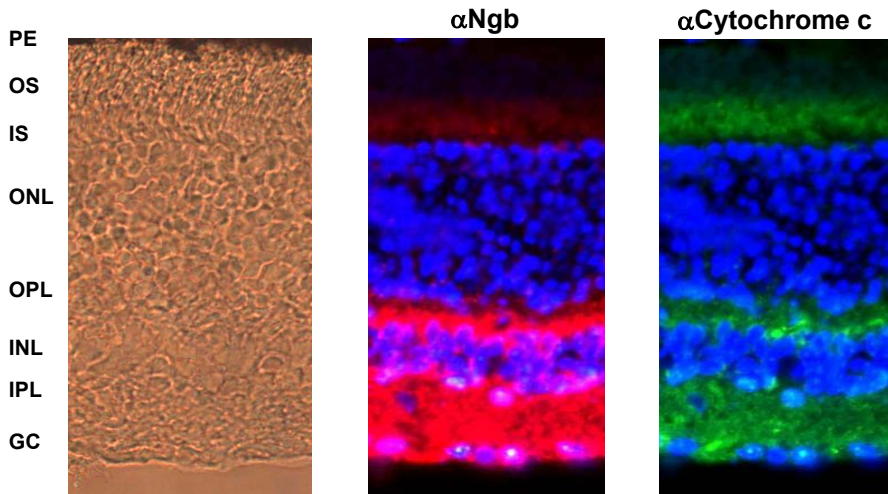
total retina extract:  
> 100 µM !

**mRNA**

**Protein**

Schmidt et al. (2003) *JBC* 278, 1932-1935

# Neuroglobin and mitochondria



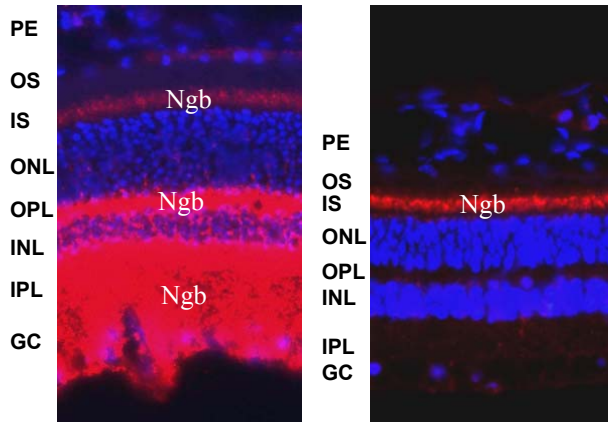
# Ngb in the avascular retina



vascular



avascular

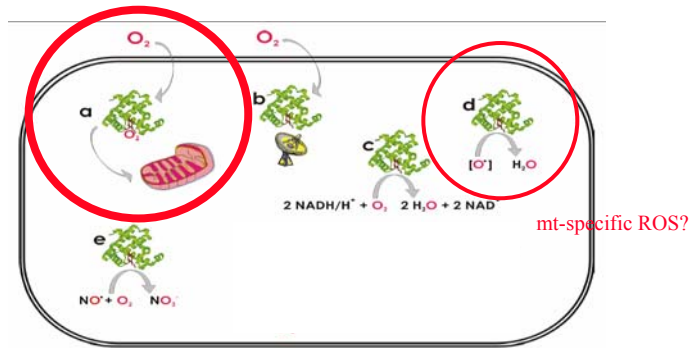


Bentmann et al. (2005)  
JBC 278, 1932-1935



# Ngb function in the retina

- expressed strongly enough to possibly sustain intracellular  $O_2$  supply!
- localized near, but not in mitochondria



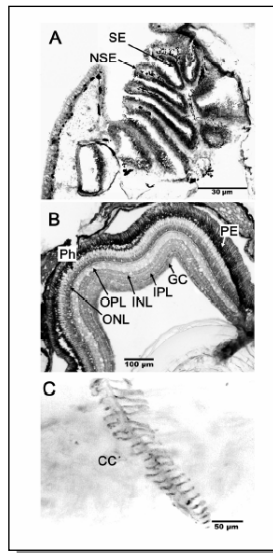
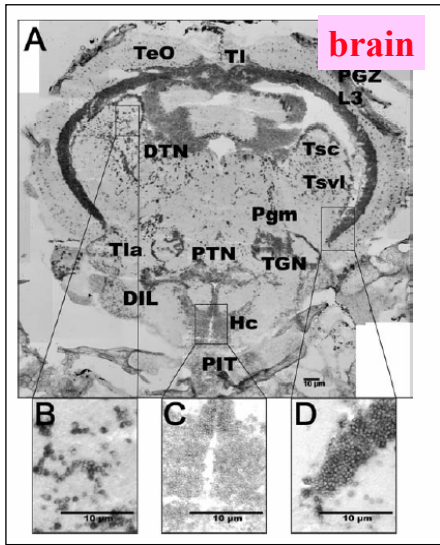
## ...and Ngb in the brain?



„Ngb must be a pseudogene, because as we all know, our brain is white“

*an anonymous Reviewer*

# Ngb expression is conserved in fish



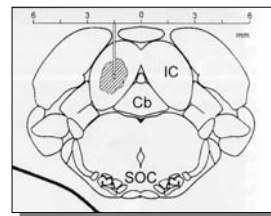
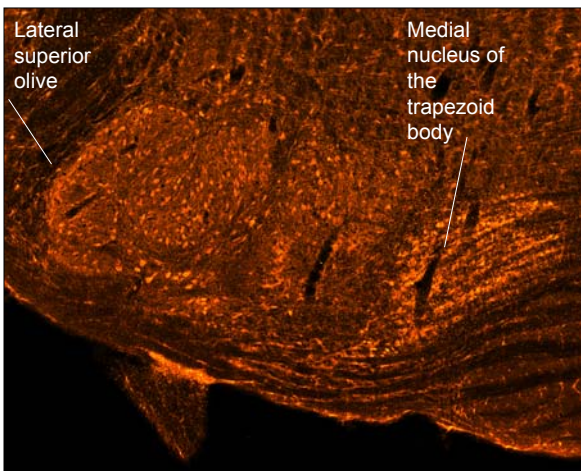
olfactory  
bulb

retina

gills

Christine Fuchs, Valeska Heib, Anja Roesner

# Ngb expression correlates with high metabolic activity



Stefan Reuss, Marco Hill



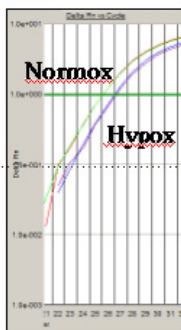


# What happens to Ngb under pathophysiological stress?

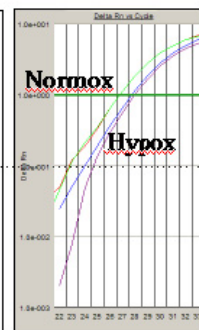
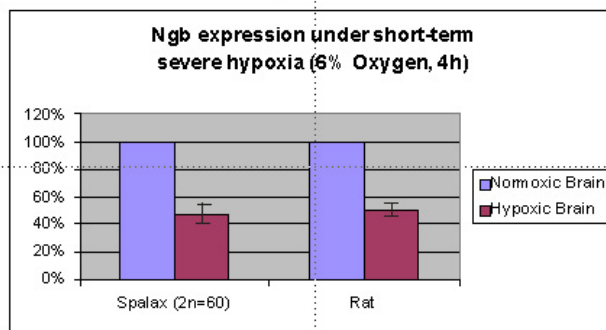
- hypoxia *in vivo*?
- ischemia/reperfusion *in vivo*?
- severe ROS stress *in vitro*?



Ngb is slightly **down**-regulated upon hypoxia in brain



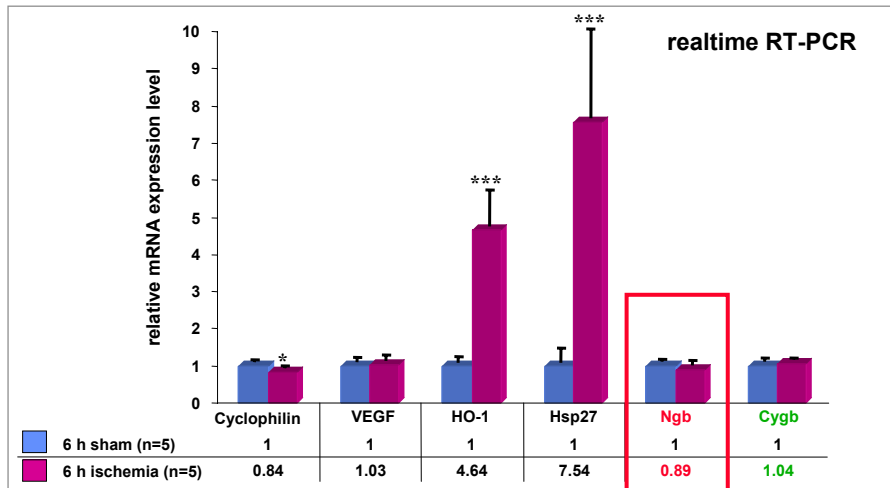
Spalax



Rat



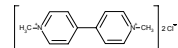
# Global brain ischemia



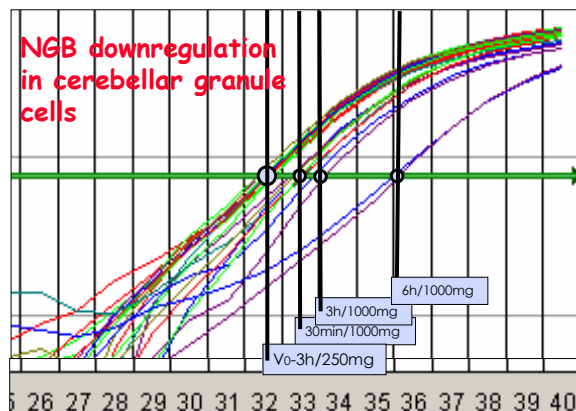
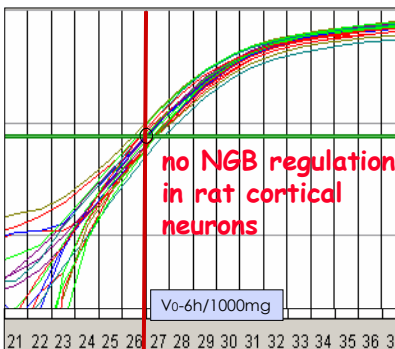
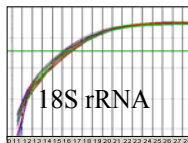
15 min global ischemia  
6 h reperfusion

Fabian Büttner,  
cooperation O. Kempfski

# NGB and severe ROS stress



Paraquat



# Conclusions so far...

Neither severe artificial, nor pathophysiologically relevant ROS stress after ischemia-reperfusion have any *stimulating* effect on Ng2 regulation in brain.

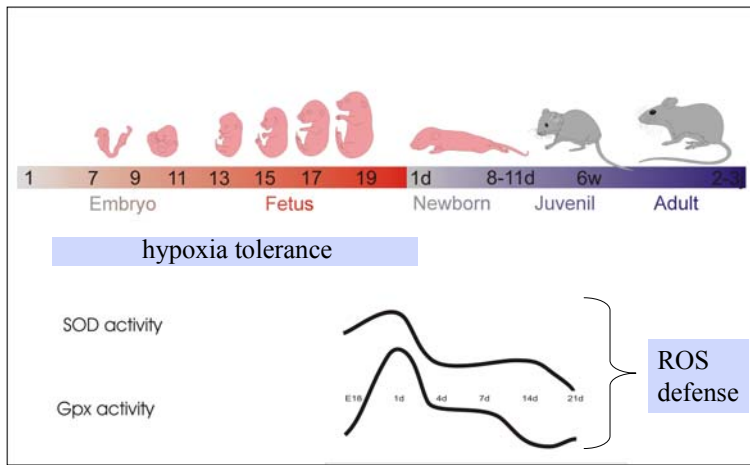
Neither does have systemic hypoxia.

This may not be too much surprising after all, since our brain has not evolved to fight these challenges very efficiently!



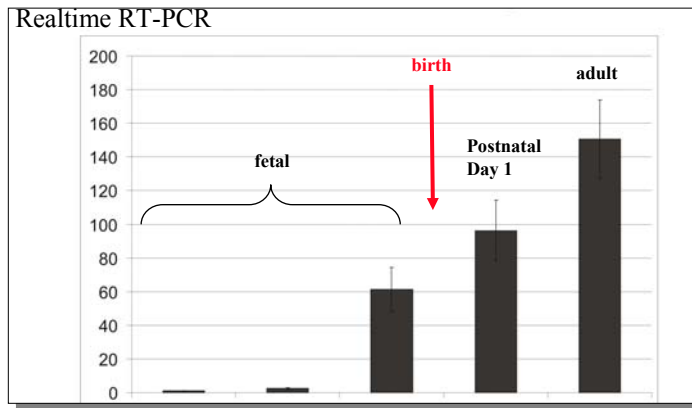
What about more ,natural' challenges by hypoxia or ROS?

# What happens during mouse brain development?

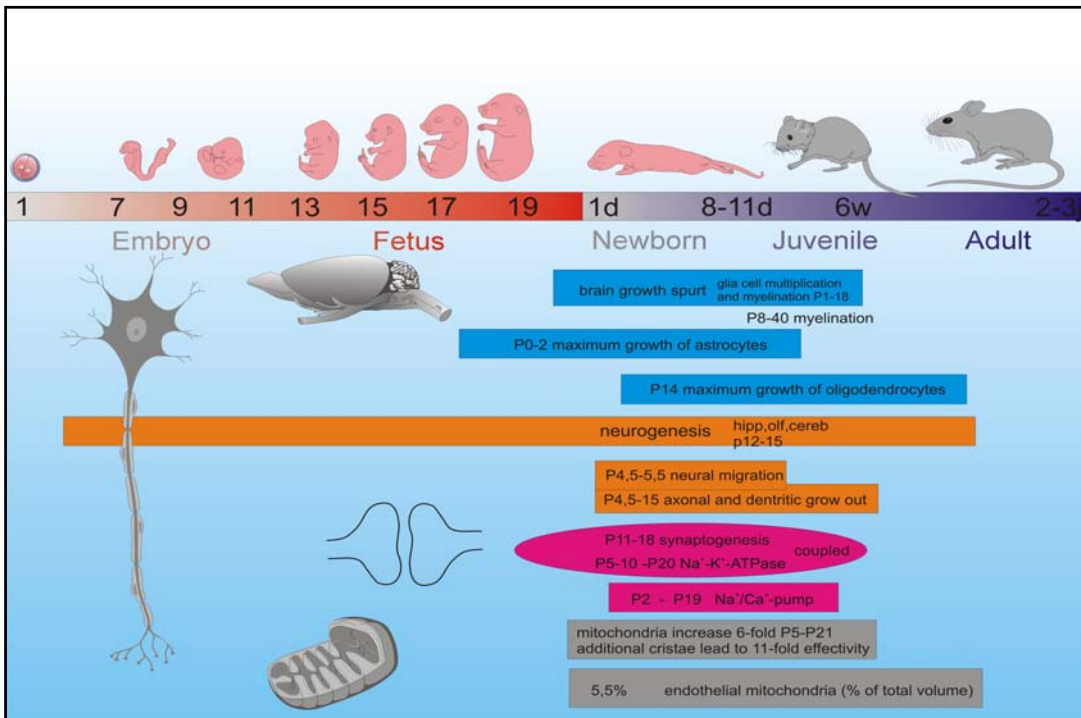




# Ngb expression in mouse development



Timmi Laufs



# Ngb in the hypoxia-resistant mole rat *Spalax*

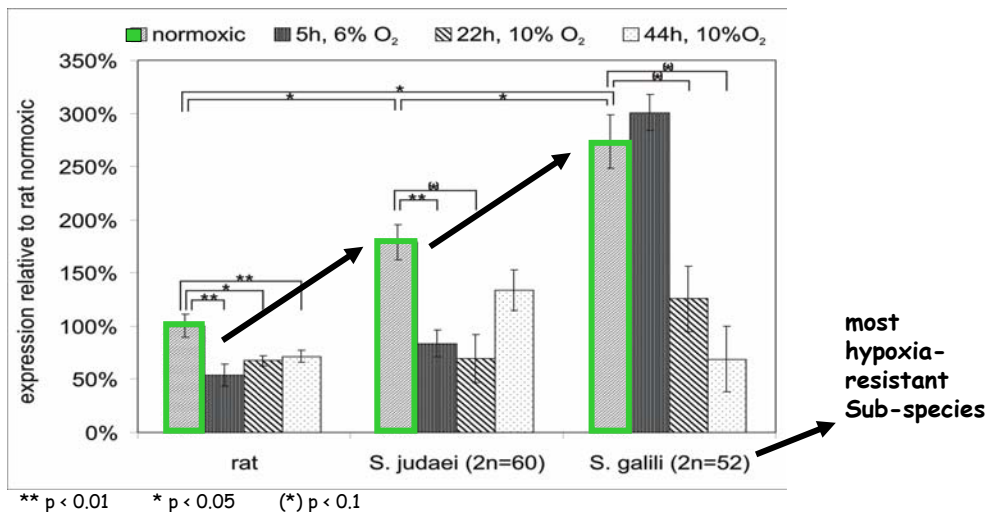


- 7% O<sub>2</sub> and 6% CO<sub>2</sub> in underground burrows!
- survives 3% O<sub>2</sub> for >14 hrs without damage!

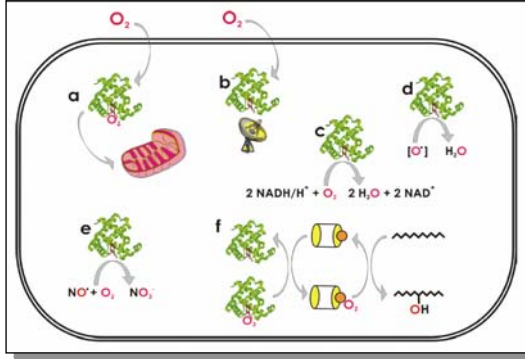
Frank Gerlach    Coop. Aaron Avivi, Eviatar Nevo (Haifa)



## Spalax constitutively expresses more Ngb mRNA in the brain!



# Ngb function(s)...



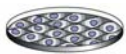
...have been strongly selected for by evolution

...are +/- constitutively working (= house-keeping)

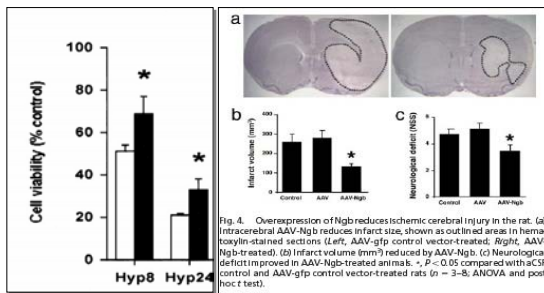
...occur predominantly in metabolically most active neurons

...are coupled to the presence of mitochondria and oxidative metabolism

regional short-term O<sub>2</sub> buffer **or**  
safeguard of mitochondria against ROS or NO

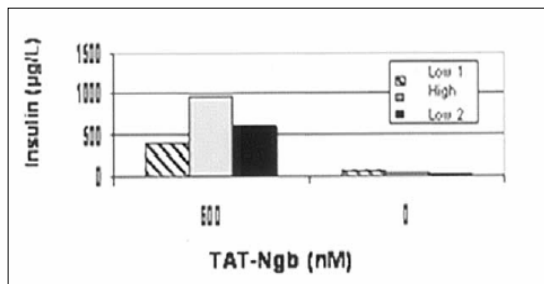


## Ngb is cyto-protective



■ **Ngb** protects neuronal cells from hypoxic stress

Sun *et al.* (2001) *PNAS* 98, 15306-15311  
Sun *et al.* (2003) *PNAS* 100, 3497-3500



■ **Additional Ngb** enhances viability of isolated islet cells

Mendoza *et al.* (2005) *Transpl Proc* 37, 237-240



# Go back, Jack, do it again!

(Steely Dan, 1975)

NCBI Basic BLAST BLAST Entrez ?

Clear Input Advanced BLAST

Message of the day ...  
Sequence submissions to GenBank: gb\_sub@ncbi.nlm.nih.gov

Click here for a [description](#) of the BLAST queuing system

Choose program to use and database to search:  
Program: tblastn Database: mouse\_ests

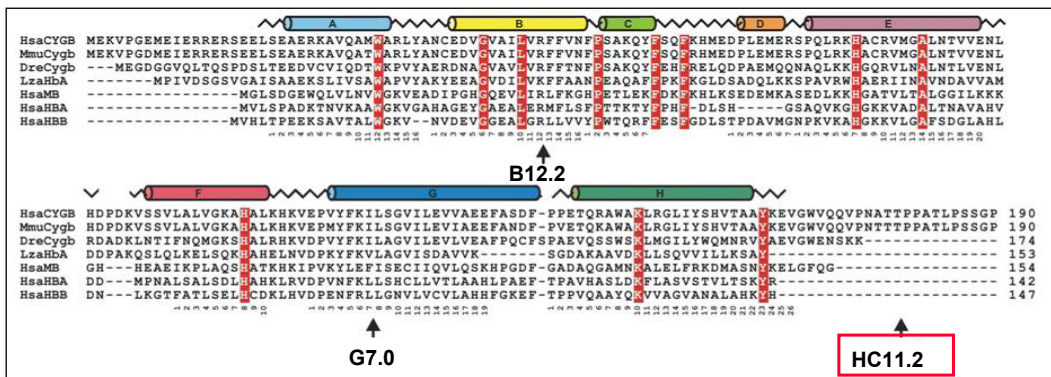
**NEW** To search the Human Genome sequences, go to the [human genome blast page](#)

Perform ungapped alignment  
 Perform CDD Search (proteins only)

The query sequence is  filtered for low complexity regions by default.  
Enter here your input data as: Sequence in FASTA format Search

```
MNSDEVQLIKKTWEIPVATPTDSGAAILTOFFNRFPNLEKFFPRDVPLEELSGNARFRAH
AGRIIRYFDESILQVLGQDGDLEKLDEIWTIKIAVSHIPRTYSKESYNQLKGVILDVLTAACS
LDESQAATWAKLVDRHVYGIIFKAIDDDGNAK
```

## 2001: Cytoglobin

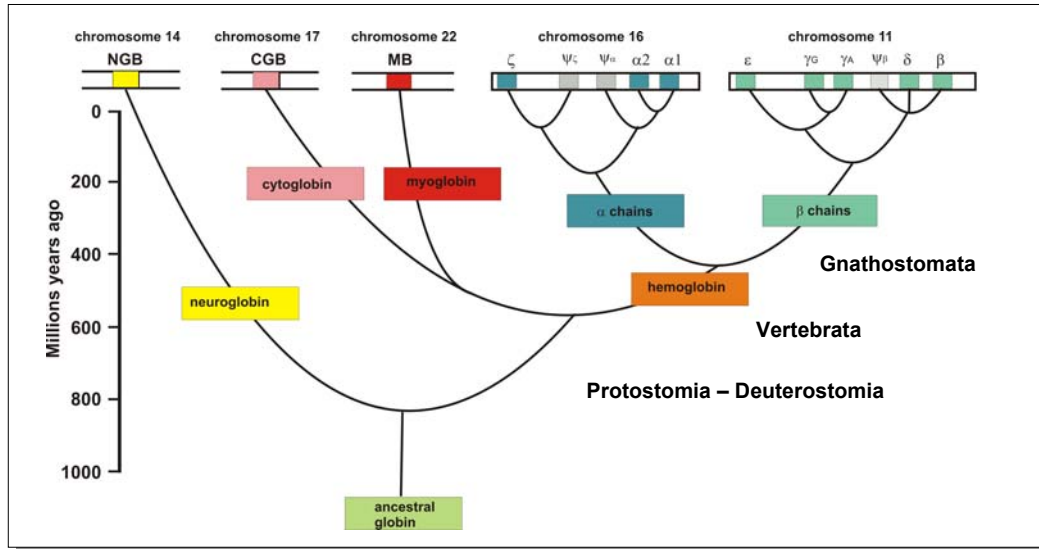


< 32% identity with myoglobin  
< 30% identity with hemoglobin

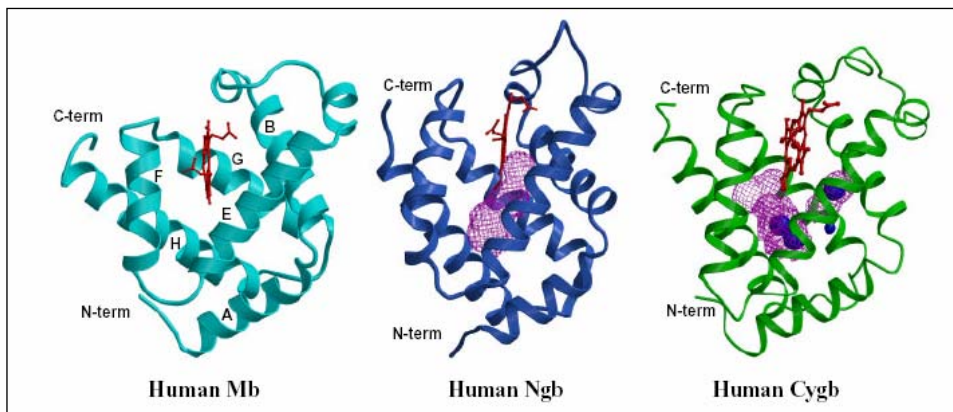
additional exon at C-terminus!

Kawada et al. (2001) *JBC* 276, 25318-25323  
Burmester et al. (2002) *MBE* 19, 416-421  
Trent & Hargrove (2002) *JBC* 19538-19545

# Evolution of Globins



# Cygb structure & ligand binding

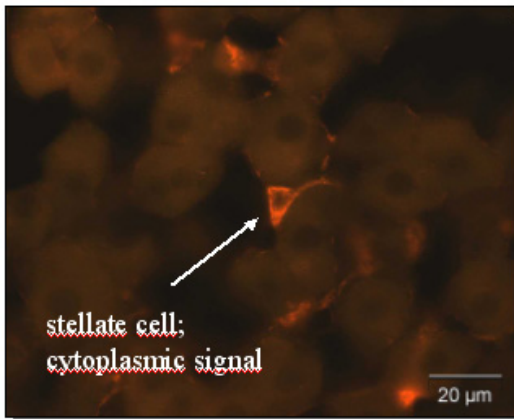


Monomeric, 16 kDa  
 $P_{50}(O_2)$  ca. 1 Torr

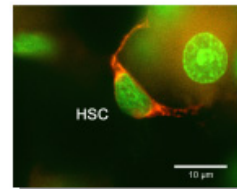
Monomeric, 17 kDa  
 $P_{50}(O_2)$  ca. 1 Torr

**dimeric, 2x 21 kDa**  
 **$P_{50}(O_2)$  ca. 1 Torr**

# Cygb expression pattern is cell type-specific

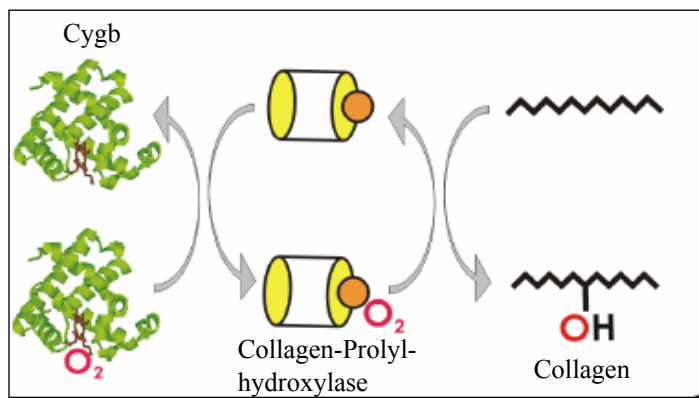


Cygb is expressed in hepatic stellate cells, but not in hepatocytes!



Schmidt *et al.* (2004) *JBC* 279, 8063-8069

## A possible function for Cygb in fibroblast-like cells?

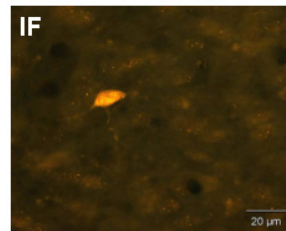
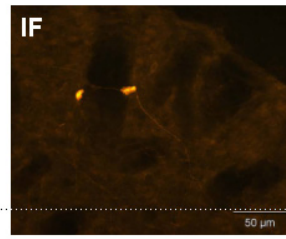
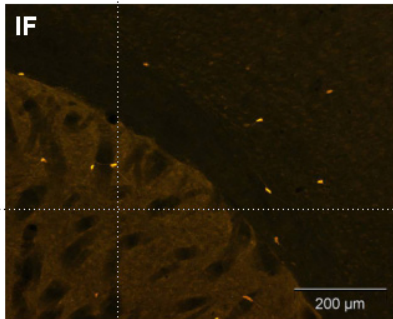


Does Cygb supply the prolyl-hydroxylases with  $\text{O}_2$ ?

Hankeln *et al.* (2005) *J. Inorg. Biochem.* 99, 110-119



# A second function for Cygb in the CNS?

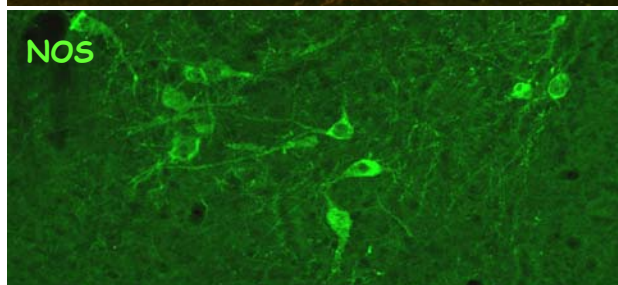
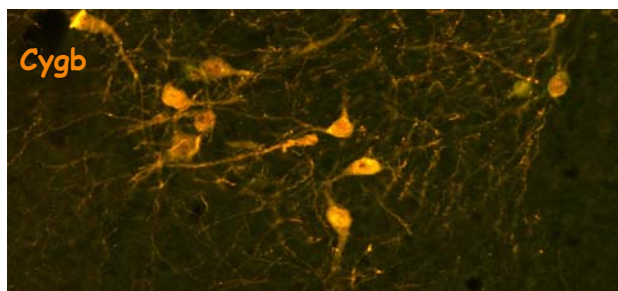


Cygb is expressed in some (but not all) neurons of the CNS

=> Cytoplasmic and nuclear staining!

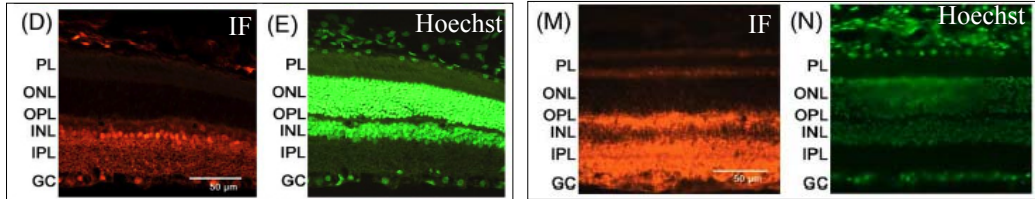
Schmidt *et al.* (2004) *JBC* 279, 8063-8069

# Cygb co-localizes with nNOS



S. Reuss,  
S. Wystub

# Cygb in the retina



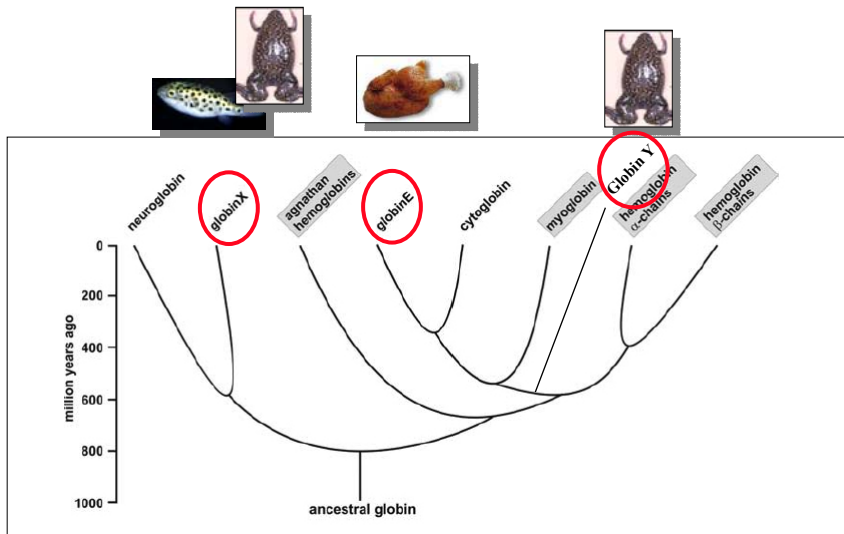
Cygb  $\neq$  Ngb

↓  
no indication for respiratory function!

Schmidt et al. (2005) *Neurosci. Lett.* 279, 8063-8069



# Novel Vertebrate Globins



Anja Roesner, Christine Fuchs



# Thank you...



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**Frank Gerlach**  
**Bettina Ebner**  
**Christine Fuchs**  
**Tina Hinkelmann**  
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**Supported by DFG, EU & Land Rheinland-Pfalz**