Nitrate reduction in bacteria

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Date: 15 Dec 2015

Outline

- Nitrogen and nitrate reduction
- Nitrate reductase in bacteria
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    2. Membrane-bound respiratory (Nar)
    3. Cytoplasmic assimilatory (Nas)
- Conclusions and perspectives

Nitrogen

Nitrogen is a basic element for life because it is a vital component in essential biomolecules:

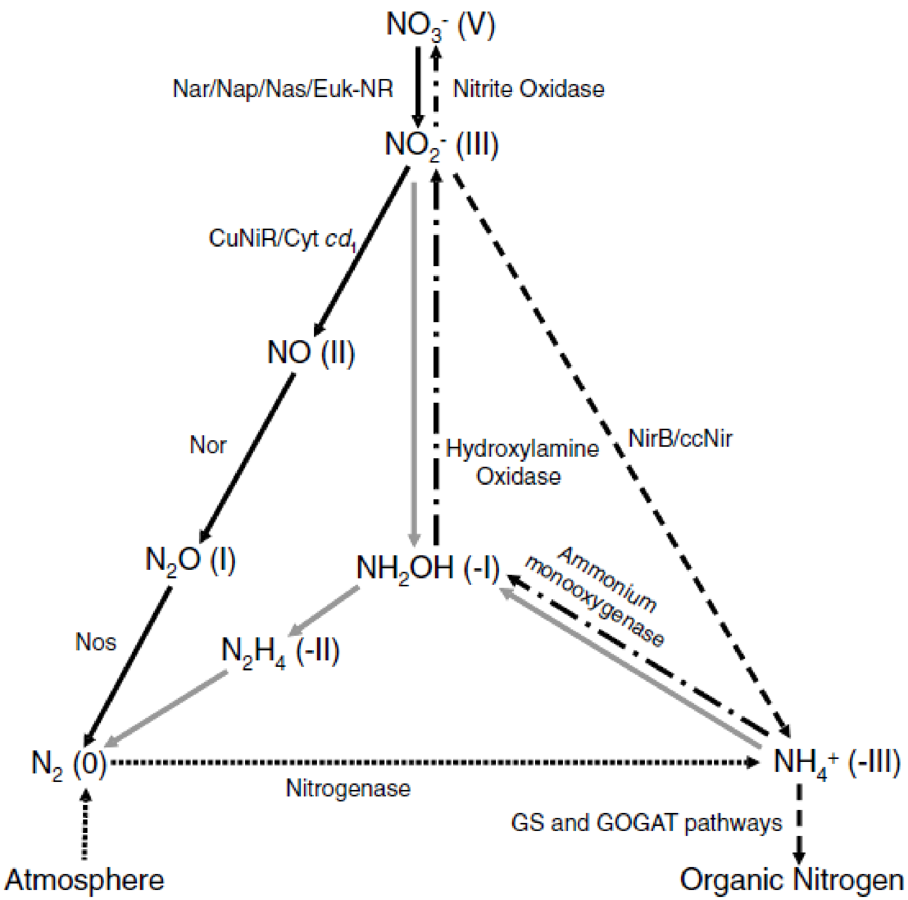
- nucleic acids (DNA, RNA)
- amino acids
- cell wall components
- organic cofactors

Nitrogen forms compounds with a wide range of oxidation states, from -3 to +5.

Oxidation state	**-3**	**-2**	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**+5**
Example	NH_4^+	N_2H_4	N_2H_2	N_2	N_2O	NO	NO_2^-	NO_2	NO_3^-

Reduction ←

Nitrogen cycle



The inorganic nitrogen cycle

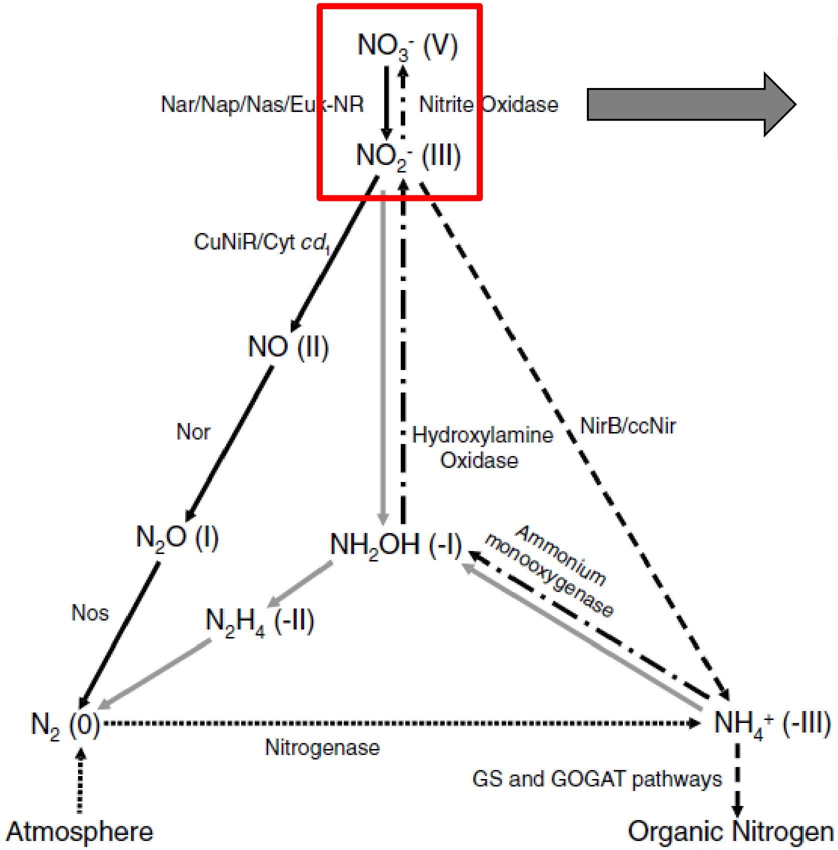
Gonzalez et al. J Inorg Biochem, 2006

Nitrogen cycle

Nitrogen metabolism in different species			
	Bacteria	**Plants**	**Animals**
Nitrogen fixation	Yes	No	No
Nitrate/nitrite reduction	Yes	Yes	No
Nitrification	Yes	No	No
Denitrification	Yes	No	No
Ammonia assimilation	Yes	Yes	Yes
Amino acid synthesis	Yes	Yes	Yes

- ✓ This cycle involves a number of redox reactions.
- ✓ Bacteria play a predominant role in nitrogen cycle.
- ✓ Bacteria have the reductive or oxidative enzymes carrying out these biological processes.

Nitrate reduction reaction



The inorganic nitrogen cycle

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \ (E^0 = +420\,\mathrm{mV})$$

Nitrate reduction: the initial reductive step of all the reductive branches in the nitrogen cycle.

- the conversion of nitrate to nitrite
- consumption of two electrons

Gonzalez et al. J Inorg Biochem, 2006

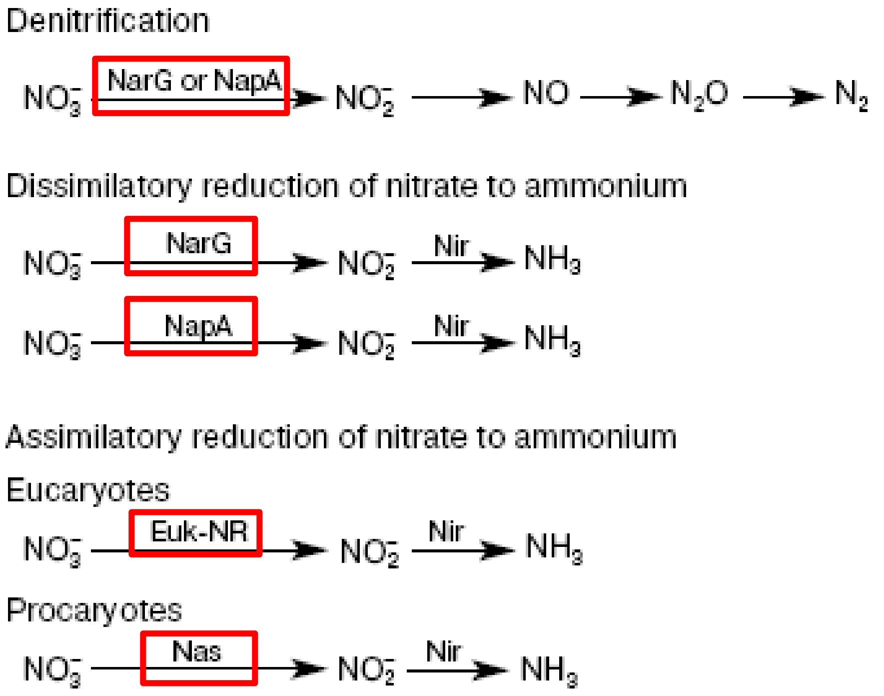
Nitrate reductase (NR)

Due to the different cellular location, molecular properties and function, nitrate reductases have been classified into four types:

1. eukaryotic assimilatory NR (Euk-NR)
2. cytoplasmic assimilatory NR (Nas)
3. membrane-bound respiratory NR (Nar)
4. periplasmic dissimilatory NR (Nap)

Three different bacterial enzymes:

Nas Nar Nap

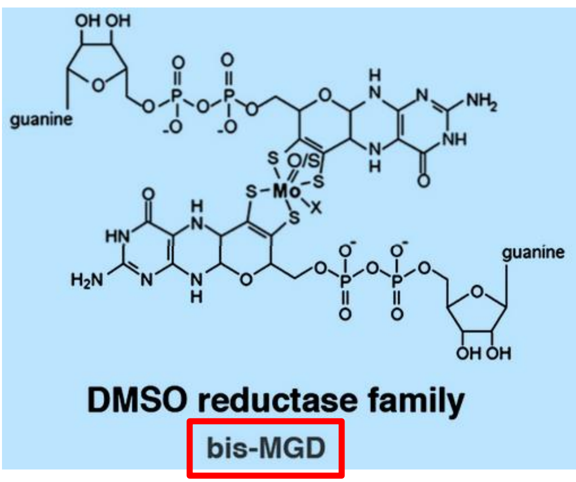


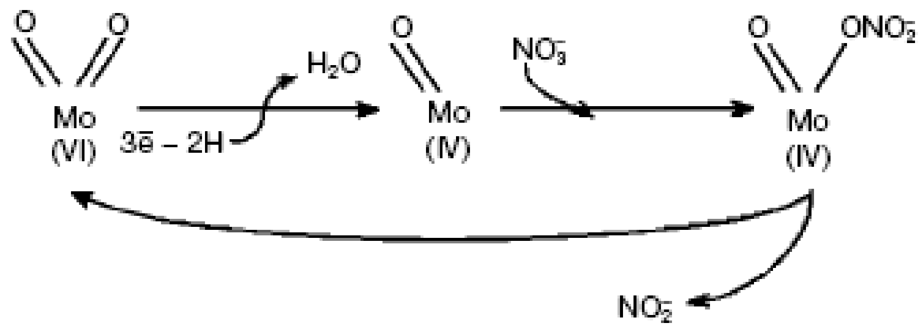
Reactions of nitrogen reduction in pro- and eukaryotes

Morozkina et al. Biochemistry (Mosc), 2007

Catalytic reaction in the active center

All studied bacterial NR share a common property--the presence of bis-MGD cofactor (one form of molybdenum [Mo] cofactor) at the enzyme active center.





eg. In the Nap-mediated nitrate reduction, nitrate molecule binds to Mo in the reduced state (+6) and undergo reduction to nitrite.

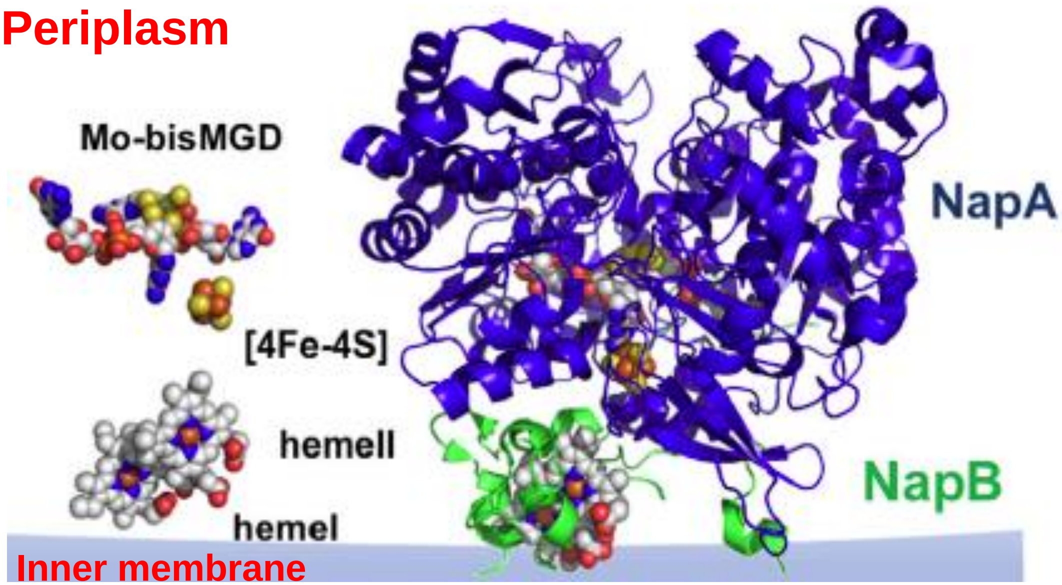
Morozkina et al. Biochemistry (Mosc), 2007
Iobbi-Nivol et al. BBA Bioenergetics, 2013

Periplasmic dissimilatory nitrate reductase (Nap)

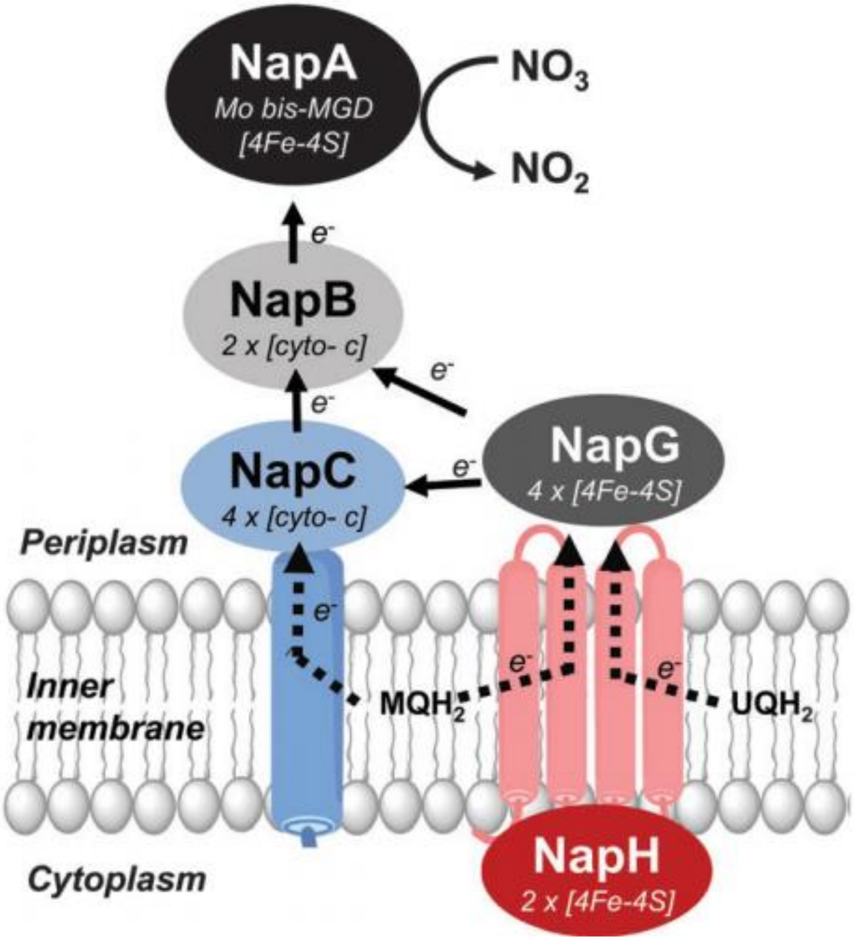
Almost all Nap proteins are heterodimers (NapAB) (except the monomeric NapA from *Desulfovibrio desulfuricans*), locating in the periplasm compartment of the cell.

NapA:
- catalytic subunit
- bis-MGD
- [4Fe-4S]

NapB:
- e^- transfer subunit
- 2 c-type hemes



Coelho et al. Protein Sci, 2015



Electron donor: quinol pool (MQH2 or UQH2)

No energy generation

Nitrate dissimilation:
the dissipation of excess reducing energy for redox balance

Electron transfer pathways: Electrons can be transferred from menaquinone (MQH2) or ubiquinone (UQH2) with in the inner membrane by a quinone oxidase (NapC or NapH).

Sparacino-Watkins et al. Chem Soc Rev, 2014

nap operon

Genes organization : The *nap* genes are clustered in an operon in many bacteria.

Escherichia coli K12	*napFDAGHBC*
Rhodobacter sphaeroides	*napKEFDABC*
Paracoccus pantotrophus	*napEDABC*
Wautersia eutropha	*napEDABC*
Bradyrhizobium japonicum	*napEDABC*
Pseudomonas G-179	*napEFDABC*
Campylobacter jejunii	*napAGHBLD*
Wollinella succinogenes	*napAGHBFLD*
Haemophylus influenzae	*napFDAGHBC*
Shewanella oneidensis MR1	*napDAGHB*
Desulfitobacterium hafniense	*napDGAH*

***nap* operon**

Regulation of *nap* expression:

O_2	NO/yes
NH4+	NO
NO3-/NO2-	NO/yes

eg. E. coli nap operon is induced during anaerobic conditions, via the Fnr regulator, and by nitrate or in a lesser extent by nitrite, via the regulator NarLP.

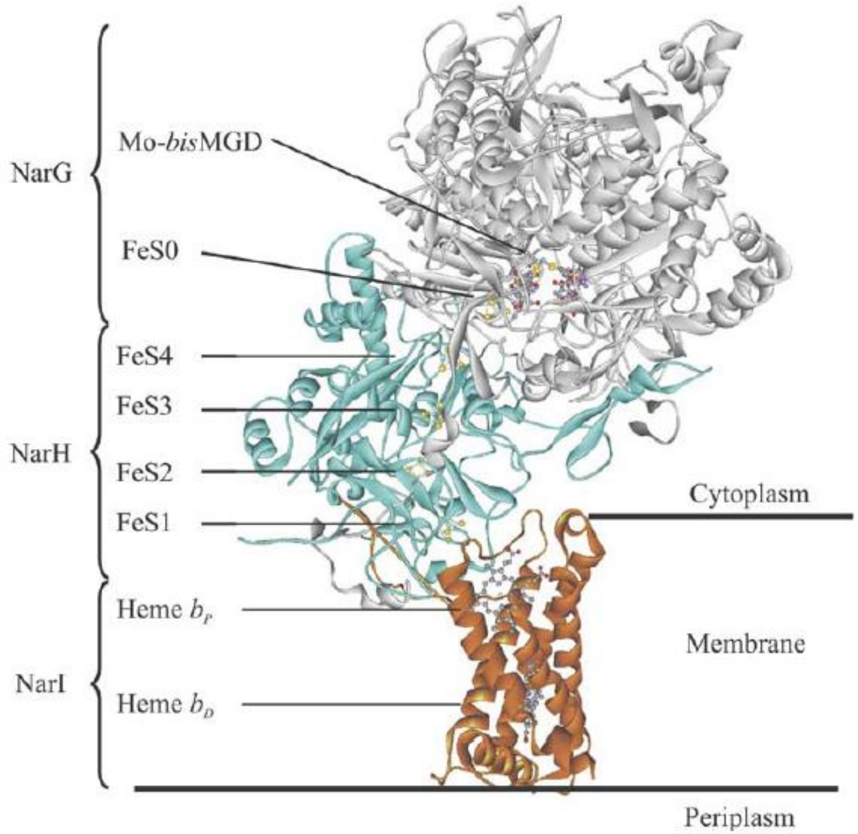
Morozkina et al. Biochemistry (Mosc), 2007

Membrane-bound respiratory nitrate reductase (Nar)

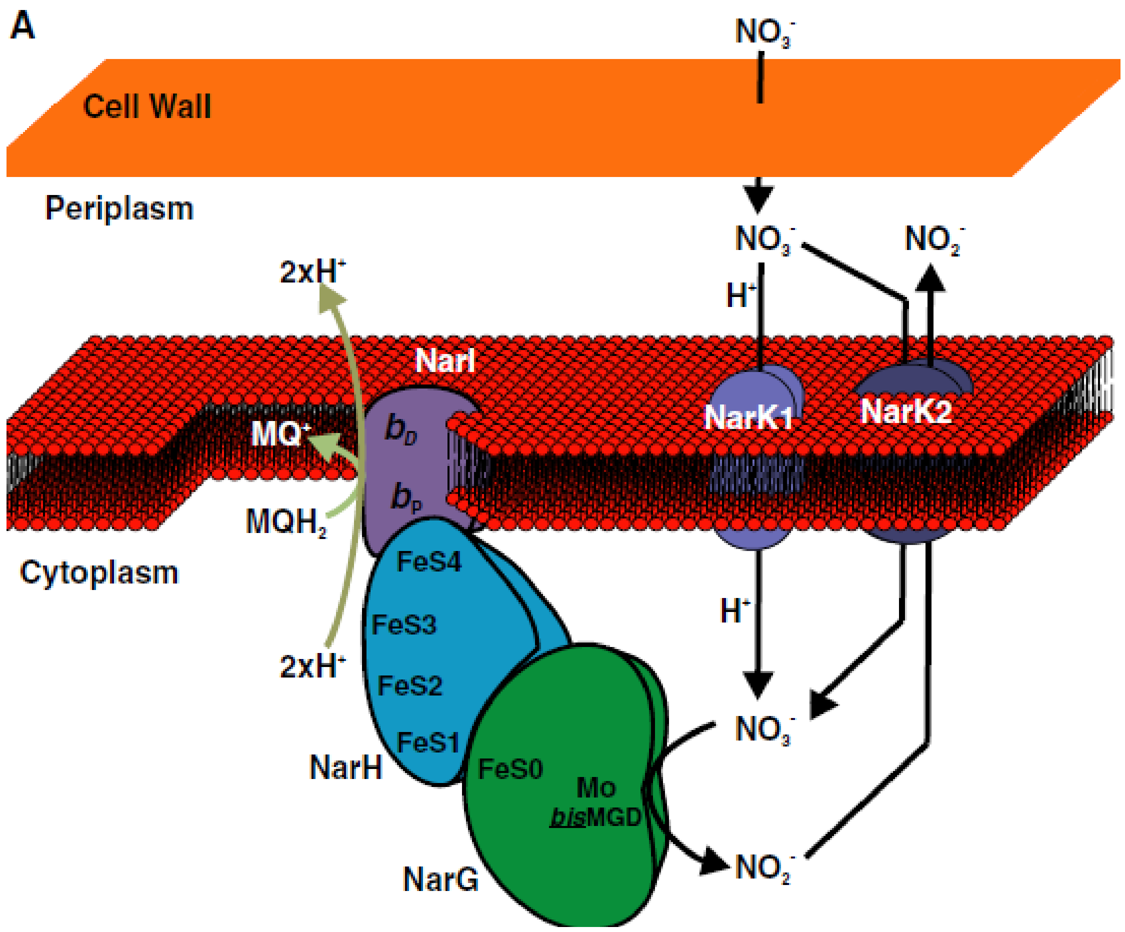
Nar enzymes are heterotrimeric proteins composed of three subunits NarG, NarH and NarI. The soluble NarGH dimer (assembled by NarJ) in the cytoplasm is anchored to the membrane through NarI.

Catalytic subunit

e^- transfer subunit



Morozkina et al. Biochemistry (Mosc), 2007



The nitrate respiration pathway

Electron donor: quinol pool (MQH2)

the terminal electron acceptor: nitrate

Function: nitrate respiration
Generation of proton motive force (for ATP synthesis)

Morozkina et al. Biochemistry (Mosc), 2007

nar operon

Genes organization : The *nar* genes are clustered in an operon with a nitrate transporter gene *narK*.

Escherichia coli K12	*narLX-//-K-//-GHJI*
	narU-//-ZYWV
Paracoccus denitrificans	*narK-//-GHJI*
Paracoccus pantotrophus	*narK-//-GHJI*

Regulation of *nar* expression:

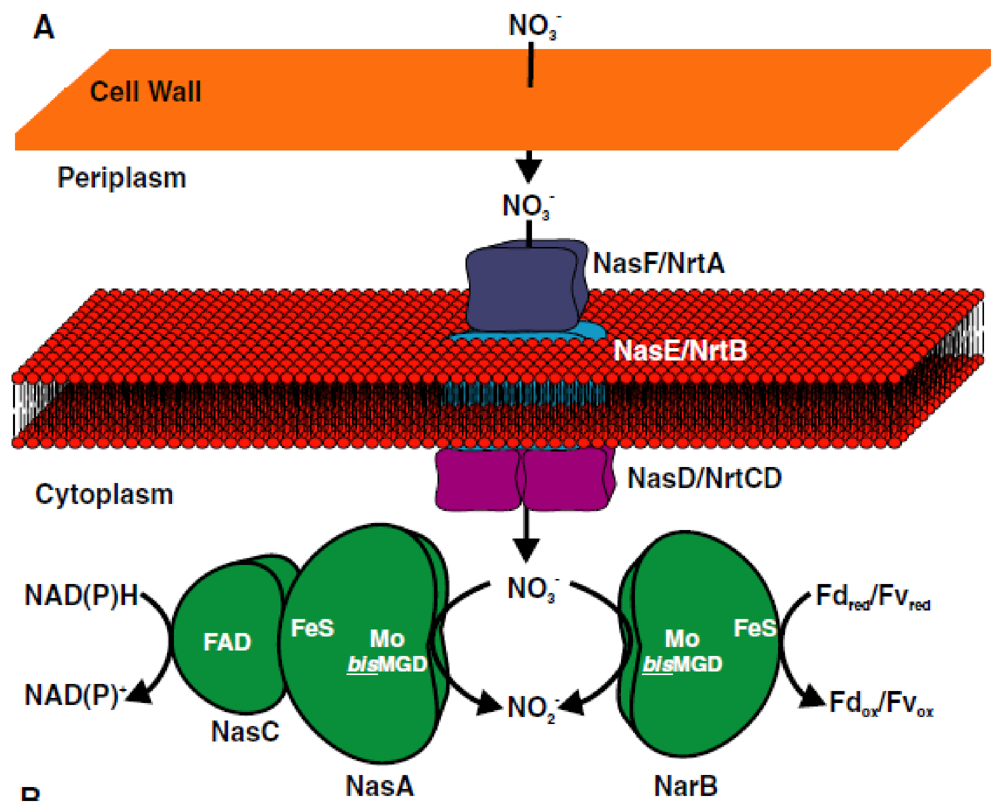
O_2	Yes
NH4+	NO
NO3-/NO2-	Yes

eg. Under anaerobic conditions, nitrate or nitrite affects the gene expression of *E. coli nar,* via a two-component signal system NarLX-NarPQ.

Morozkina et al. Biochemistry (Mosc), 2007

Cytoplasmic assimilatory nitrate reductase (Nas)

Two classes of assimilatory nitrate reductases are found in bacteria: the ferredoxin- or flavodoxin-dependent Nas and the NADH-dependent Nas.



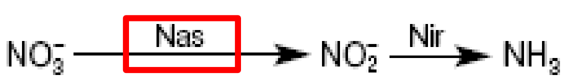
NADH-dependent Nas:

1) catalytic subunit NasA
   bis-MGD
   FeS center
2) e- transfer subunit NasC
   FAD cofactor

Fd/Fv-dependent Nas (monomeric):

bis-MGD
FeS center

Function: nitrate assimilation



Morozkina et al. Biochemistry (Mosc), 2007

nas operon

Genes organization : The *nas* genes are clustered in an operon with nitrogen-related genes such as transporter gene, nitrite reductase gene in many bacteria.

Klebsiella pneumoniae	*nasRFEDCBA*
Bacillus subtilis	*nasABCDEF*
Synechococcus sp. PCC7942	*ntcB-nirBA-nrtABCD-narB*
Synechococcus sp. PCC6803	*nrtABCD-narB-//-nirA*

Regulation of *nas* expression:

O_2	NO
NH4+	Yes
NO3-/NO2-	Yes

eg. Ammonium-promoted repression and positive regulation of nitrate assimilation by nitrate or nitrite have also been reported for photosynthetic bacteria.

Morozkina et al. Biochemistry (Mosc), 2007

Conclusions

1. Bacterial nitrate reduction relies on three different types of nitrate reductases, which are clearly different at the level of cellular location, structure, biochemical properties, gene organization and regulation.

TABLE 1. Prokaryotic nitrate reduction

Characteristic	Assimilatory, NO_3^- assimilation	Dissimilatory	
		NO_3^- respiration	NO_3^- reduction
Nitrate reductase	Assimilatory Nas	Respiratory Nar	Dissimilatory Nap
Location	Cytoplasm	Membrane	Periplasm
Reaction catalyzed	$NO_3^- \Rightarrow NO_2^-$	$NO_3^- \Rightarrow NO_2^-$	$NO_3^- \Rightarrow NO_2^-$
Structural genes	*nasCA*[a]/*narB*[b]	*narGHI*	*napAB*
Prosthetic groups	FAD[c], FeS[d], MGD	cyt*b*[e], FeS, MGD	cyt*c*, FeS, MGD
Nitrate transport	Yes	Yes	No
Function	Biosynthesis of N compounds	PMF (nitrate respiration and denitrification)	2H ⇓[f] and denitrification
Regulation[g]			
O_2	No	Yes	No/yes
NH_4^+	Yes	No	No
NO_3^-/NO_2^-	Yes	Yes	No/yes

Moreno-Vivian et al. J Bacteriol, 1999

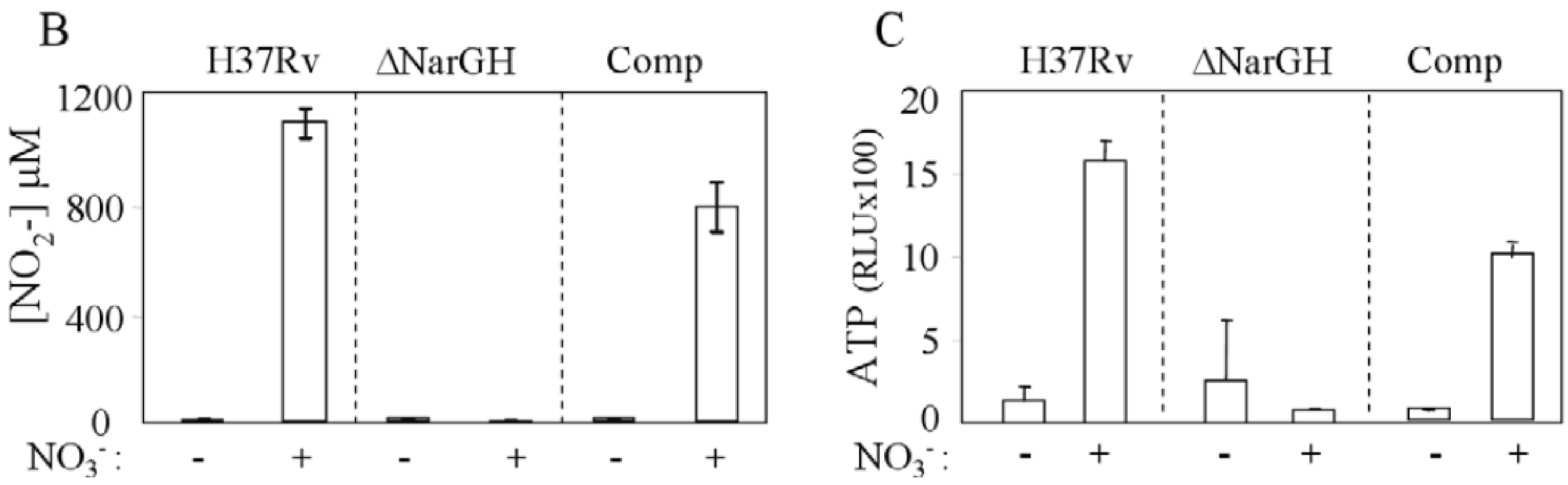
Conclusions

2. Nitrate reduction has several functions:
   - nitrate assimilation (Nas): the utilization of nitrate as a nitrogen source for growth
   - nitrate respiration (Nar): the generation of metabolic energy by using nitrate as a terminal electron acceptor
   - nitrate dissimilation (Nap): the maintenance of redox balance

3. The enzymes may play distinct roles under different metabolic conditions to facilitate a rapid and better adaptation to the unfavorable environments.

Function of nitrate reductase in certain bacteria

eg. NarGHI of *Mycobacterium tuberculosis* (Mtb)



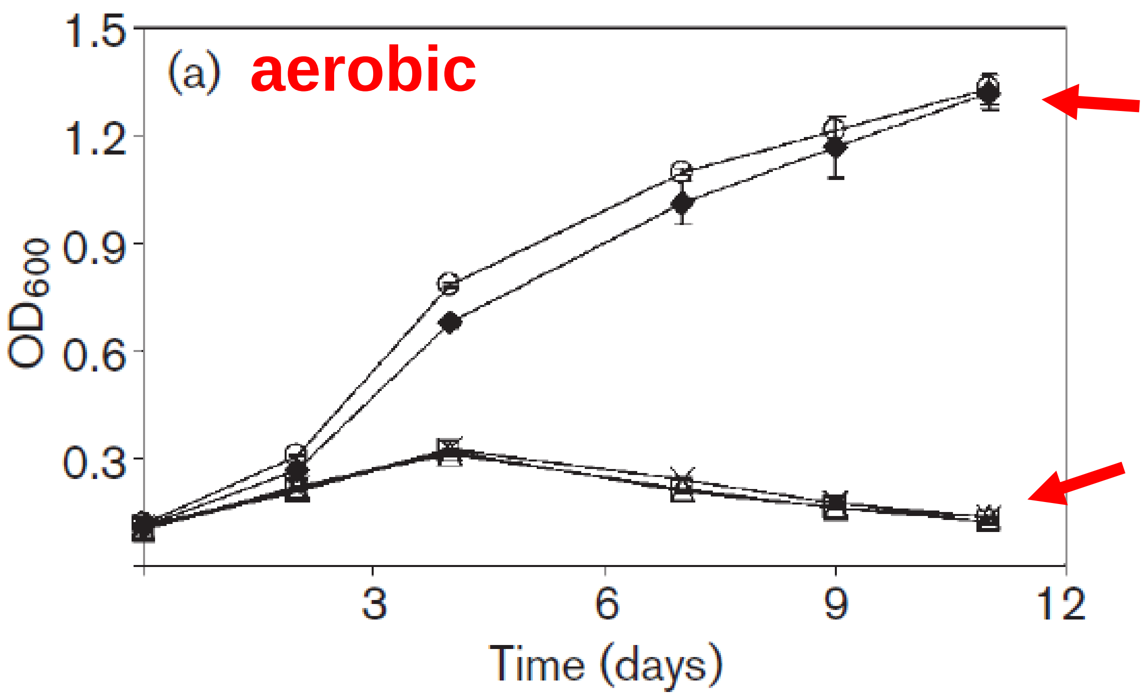
Nitrite and ATP production of Δ*narGH* under **hypoxic** acidic conditions

Function of Mtb NarGHI: nitrate respiration

Tan et al. Plos One, 2010

Function of nitrate reductase in certain bacteria

eg. NarGHI of *Mycobacterium tuberculosis* (Mtb)



Mtb H37Rv and complemented strain

ΔnarG

Function of Mtb NarGHI: nitrate assimilation

Growth of Δ*narG* on nitrate as the sole nitrogen source

Malm et al. Microbiology, 2009

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Thank you