# Regulation of nitrogenase by combined nitrogen sources

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\* When added to  $N_2$  fixing organisms, ammonium ( $NH_4^+$ ) usually inhibits nitrogenase activity markedly whereas nitrate ( $NO_3^-$ ) and nitrite ( $NO_2^-$ ) is often less effective in inhibiting  $N_2$  fixation.

#### Nitrogenase enzyme synthesis is regulated at transcriptional level by presence of other sources of nitrogen.

- Nif genes are the genes encode for nitrogenase enzyme.
- Regulation of nif genes transcription is done by the nitrogen sensitive NifA protein.
- When there isn't enough fixed nitrogen available NtrC protein triggers NifA expression.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein NifL is activated.
- NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.

## **Post-translational regulation**

- During energy limiting or nitrogen sufficient condition, the nitrogense compex is rapidly, reversibly inactivated by ADP-ribosylation of Fe protein.
- It occurs at a specific arginine residue, i.e. Arg<sub>101</sub>.
- The presence of ADP ribose group prevents association of Fe protein with Mo-Fe protein.
- Thus it results in regulating the nitrogen fixation.

#### Effect of ammonia or ammonium salts

- Ammonia or ammonium salts can inhibit nitrogenase activity:
- by interfering with the supply of reductant to nitrogenase.
- through covalent modification of the Fe-protein of nitrogenase.
- a more general effect of NH<sub>4</sub><sup>+</sup> on N<sub>2</sub> fixation is exerted through inhibition of nitrogenase synthesis. The true inhibitor is either NH<sub>4</sub><sup>+</sup> itself or an assimilatory product derived from NH<sub>4</sub><sup>+</sup>.

## **Effect of nitrate and nitrite**

#### Nitrate (NO<sub>3</sub>-)

- The reduction of nitrate by the enzyme nitrate reductase produces nitrite.
- Subsequently nitrite is converted to NH<sub>4</sub><sup>+</sup> by the enzyme nitrite reductase.
- If nitrates are present in plentiful amount, diazotrophs prefer the second pathway for the synthesis of amino acids.
- In through this way  $NO_3^-$  inhibits nitrogenase synthesis.

#### Nitrite (NO<sub>2</sub><sup>-</sup>)

In Anabaena variabilis, for example, nitrite (NO<sub>2</sub><sup>-</sup>) inactivated nitrogenase directly, whereas in Azotobacter chroococcum, inhibition of N<sub>2</sub> fixation by NO<sub>2</sub><sup>-</sup> was indirect, caused by one or more assimilatory product(s) of NH<sub>4</sub><sup>+</sup> which, in turn, arose as a result of the action of nitrite reductase on NO<sub>2</sub><sup>-</sup>.

## Questions

- Explain regulation of nitrogenase by combined nitrogen sources.
- Explain how availability of ammonium salts, nitrate and nitrites regulates nitrogenase enzyme activity?