Significance of Nif genes regarding root nodulation in legumes

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Abstract

Nif genes encode the nitrogenase enzyme complex that converts inert atmospheric nitrogen into usable ammonia inside legume root nodules.
Role of Nif Genes in Nitrogen Fixation

- Nitrogenase Production: Nif genes (such as nifH, nifD, and nifK) provide the genetic code for the structural components and cofactors of the nitrogenase enzyme.
- Ammonia Conversion: The expressed enzyme reduces atmospheric nitrogen gas
- Differentiation from Nod Genes: While nod genes govern the initial structural signaling and physical creation of the root nodule, nif genes operate later to power the actual chemical fixation of nitrogen once the symbiotic bacteroids form.

- Agricultural and Ecological Impact
- Nutrient Enrichment: This process naturally enriches soil fertility, reducing or eliminating the need for synthetic nitrogen fertilizers.
- Metabolic Coordination: The plant supplies essential carbon and energy sources (like malate) to the bacteria to sustain the high ATP demands of the nif-encoded nitrogenase reaction.

Keywords- Nif genes, nitrogen fixation,nitrogenase, nod genes, symbiosis

Introduction

The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms. The primary enzyme encoded by the nif genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N2) to other nitrogen forms such as ammonia which the organism can use for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. The nif genes are found in both free-living nitrogen-fixing bacteria and in symbiotic bacteria associated with various plants. The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment of host plants). The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned in the early 1980s by Gary Ruvkun and Sharon R. Long in Frederick M. Ausubel's laboratory.[1,2,3]
In most bacteria, regulation of nif genes transcription is done by the nitrogen sensitive NifA protein. When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the

nif genes. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated: NifL. NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation. NifL is regulated by the products of glnD and glnK. The nif genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids or symbiosis islands with other genes related to nitrogen fixation (such as the nod genes).

Discussion

The expression and regulation of nif genes, while sharing common features in all or most of the nitrogen-fixing organisms in nature, have distinct characters and qualities that differ from one diazotroph to another. Examples of nif gene structure and regulation in different diazotrophs include:

Klebsiella pneumoniae—a free-living, facultatively anaerobic nitrogen-fixing bacterium. It contains a total of 20 nif genes located on the chromosome in a 24-Kb region. nifH, nifD, and nifK encode the nitrogenase subunits, while nifE, nifN, nifU, nifS, nifV, nifW, nifX, nifB, and nifQ encode proteins involved the assembly and incorporation of iron and molybdenum atoms into the nitrogenase subunits. nifF and nifJ encode proteins related to electron transfer taking place in the reduction process and nifA and nifL are regulatory proteins in charge of regulating the expression of the other nif genes.[4,5,6]

Rhodospirillum rubrum—a free-living anaerobic photosynthetic bacterium which, in addition to the transcriptional controls described above, regulates expression of the nif genes also in a metabolic way through a reversible ADP-ribosylation of a specific arginine residue in the nitrogenase complex. The ribosylation takes place when reduced nitrogen is present and it causes a barrier in the electron transfer flow and thereby inactivates nitrogenase activity. The enzymes catalyzing the ribosylation are called DraG and DraT.[3][4]

Rhodobacter capsulatus—a free-living anaerobic phototroph containing a transcriptional nif gene regulatory system. R. capsulatus regulates nif gene expression through nifA in the same manner described before, but it uses a different nifA activator which initiates the NtrC. NtrC activates a different expression of nifA and the other nif genes.[3][4]

Rhizobium spp.—Gram-negative, symbiotic nitrogen fixing bacteria that usually form a symbiotic relationship with legume species. In some rhizobia, the nif genes are located on plasmids called 'sym plasmids' (sym = symbiosis) which contain genes related to nitrogen fixation and metabolism, while the chromosomes contain most of the housekeeping genes of the bacteria. Regulation of the nif genes is at the transcriptional level and is dependent on colonization of the plant host.[7,8]

Results

Biological nitrogen fixation occurs in more than 100 genera distributed among several of the major phylogenetic divisions of Bacteria and Archaea [1].

Bacterial nif genes are known to encode the components of the nitrogenase enzyme complex. The structural subunit of dinitrogenase reductase and the 2 subunits of dinitrogenase are encoded by the nifH, nifD, and nifK genes, respectively. In many diazotrophs like Azotobacter vinelandii [2], Herbaspirillum seropedicae [3], Pseudomonas stutzeri [4], and Bradyrhizobium japonicum [5], these proteins have similar sequences and common structures and functions. Furthermore, genetic and biochemical analyses revealed that many additional nif genes, including nifE, nifN, nifX, nifQ, nif W, nifV, nifA, nifB, nifZ, and nifS, play roles in the regulation of nif genes and maturation processes of inactive products, such as electron transport and FeMo-cofactor biosynthesis and assembly [6], [7]. However, the linkage and arrangement of nif gene cluster vary considerably in many diazotrophs. The apparent conservation of gene arrangement suggests that they serve some important function, perhaps in regulation of nitrogen fixation[8]. In addition, the fixABCX genes first identified in Rhizobium meliloti [9] and subsequently in other diazotrophs were reported to encode a membrane complex participating in electron transfer to nitrogenase [6,7]

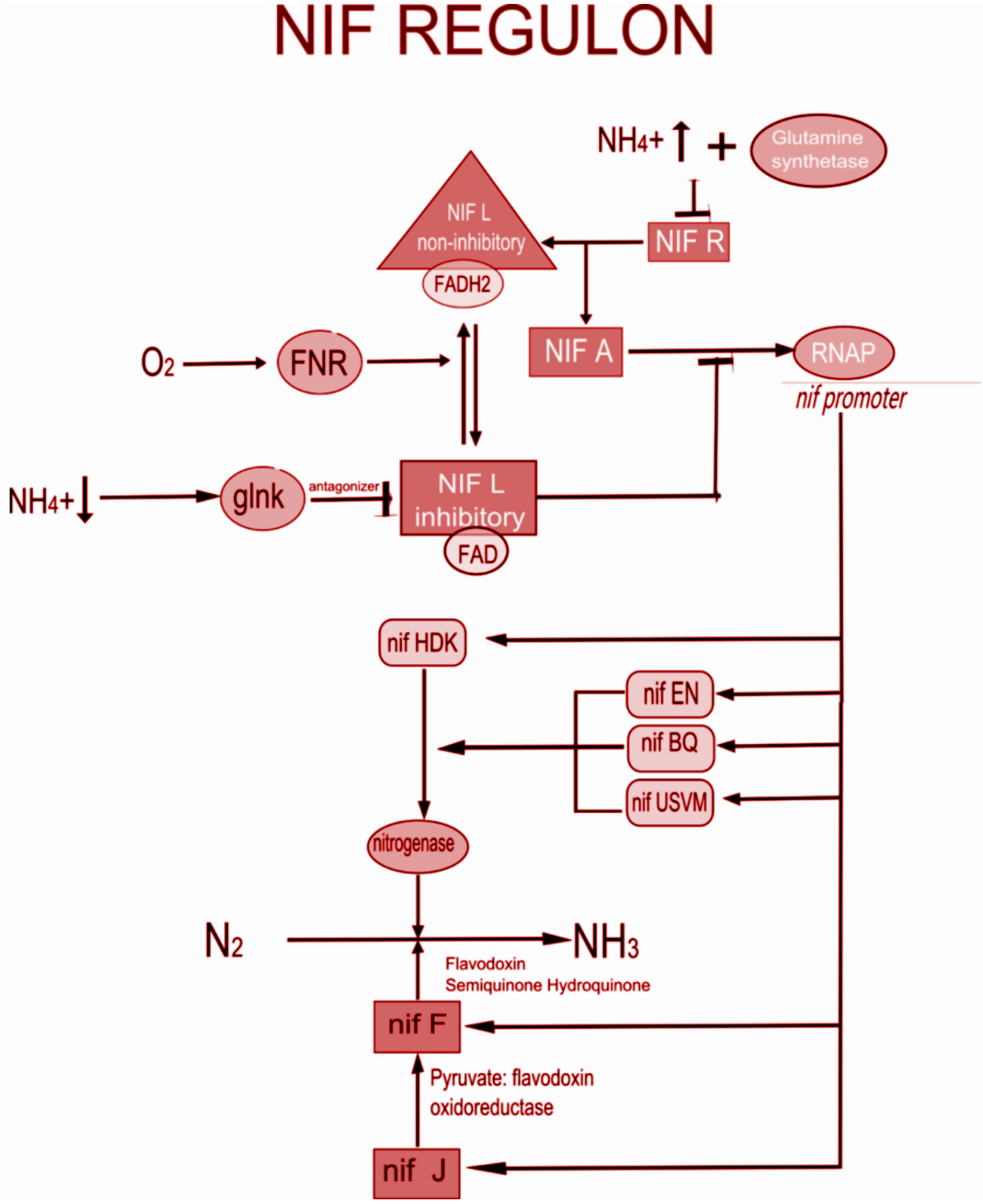
Acid mine drainage (AMD) is the outflow of acidic water from metal or coal mines, which causes worldwide environmental problems. Despite the extreme acidity, heat, and high concentrations of toxic metals, a wide variety of microorganisms populate AMD environments. These organisms can sustain by the oxidation of sulfide minerals, CO2, O2, and N2 derived from air, and phosphate liberated by water-rock interaction [11]. Since the input of externally-derived fixed nitrogen is negligible, biological nitrogen fixation is an important function of AMD microbial communities. However, a few microorganisms in AMD environments are represented by isolates that have been cultivated and described [12] and only several species in three genera (Acidithiobacillus, Leptospirillum and Methylacidiphilum) have been proved to have nitrogen-fixing ability until now [13], [14]. Little is known about the diversity and structure of nitrogen-fixing genes of AMD microbial community.

Metagenomic sequencing and metagenomic libraries recently provide powerful tools to isolate novel genes or gene clusters from unexploited gene pools in uncultured microorganisms [15]. In this study, we found evidences of novel nif genes present in acid mine drainage from Dexing Copper Mine, China, using metagenomic sequencing. We also present the phylogenetic analysis of nif genes in AMD community. To understand the organization of nif gene clusters of uncultured microorganisms in AMD community, a metagenome microarray was constructed to screen nif-containing fosmids. A comparative analysis of the nitrogen-fixing gene cluster in different species revealed the presence of novel nitrogen-fixing gene cluster in AMD community. These results confirmed that there are more novel diazotrophs surviving in AMD community.[4]
More advances in our understanding of the roles of the various nif-specific gene products in biological nitrogen fixation have occurred in the 2+ years since the

Knoxville meeting than in any period following the original designation of gene product relationships by Roberts et al. (1978). Several nif gene products have been isolated, the biochemical properties of some have been determined and the roles of a number of the products have been established or proposed. Table 1 lists the known nif genes from K. pneumoniae and provides a brief description of the roles that each have been shown or proposed to play in the nitrogenase catalysis or the assembly of those gene products involved in catalysis. Information regarding the role of nif gene products in assembly, maturation or stability of the nitrogenase complex has come primarily from the Azotobacter vinelandii and Kiebsiella pneumoniae systems, but homologs for genes encoding the proteins assigned to various functions have been found in several organisms. As the structural information regarding the nitrogenase proteins becomes more definitive, our attention will turn to how those structural entities are assembled, how they function and how they are maintained in the cell.

The rise in the global population and the growing demand for agricultural products have become a matter of grave concern for the present generation. To address this current challenge, it is vital to provide adequate amounts of nitrogen in a form that is easily accessible to the crop. The roles of the nitrogen-fixing (nif) genes in microorganisms are very important because commercially prepared nitrogen is not sufficient enough to meet the nitrogen demand. The present review provides an insight into the world of nif genes, their associated roles, and how they are regulated. The evolution of nif genes, structural and functional role of each nif gene, the gradual development in research of various genes, and its related clusters identified in different crops and microbes are discussed. The review also sheds light on the current research and developments on nif genes across the globe and areas where more research must be encouraged.[5,6]

The ‘Nif’ gene for nitrogen fixation in cereal crops like wheat, jowar, etc., is introduced by cloning the bacteria Rhizobium melloti. Rhizobium melloti a gram-negative bacterium that forms a symbiotic relationship with legumes from the genera Medicago, Melilotus, and Trigonella. The bacteria belong from the Rhizobiaceae family. This symbiosis (a mutual association benefitting both the organisms) results in the formation of a new plant organ termed as root nodule and is deemed symbiotic as it leaves excess nitrogen behind for the plant.



Conclusion

Gary Ruvkun and Sharon R. Long cloned the first Rhizobium genes for nitrogen fixation (Nif) and nodulation (nod) in the early 1980s, in Frederick M. Ausubel's laboratory.

Cloning is the process of producing individuals with identical DNA, either naturally or artificially. Clones can be produced by asexual reproduction, found in many organisms

The expression of 'Nif' genes is induced as a response to low concentrations of fixed nitrogen and oxygen (the low oxygen concentrations are actively maintained in the root environment of host plants).

In the case of free bacteria the 'Nif' genes can be found on the bacterial chromosomes, but in symbiotic bacteria, they are often found on plasmids.[8]

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