Title
Special Issue on Nitrogenases and Homologous Systems.

Journal
Chembiochem : a European journal of chemical biology, 21(12)

ISSN
1439-4227

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Publication Date
2020-06-01

DOI
10.1002/cbic.202000279

Peer reviewed

Published in final edited form as:

Chembiochem. 2020 June 15; 21(12): 1668–1670. doi:10.1002/cbic.202000279.

Special Issue on Nitrogenase and Homologous Systems

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Graphical Abstract

The nitrogenase superfamily comprises homologous enzyme systems that carry out fundamentally important processes, including the reduction of N_2 and CO, and the biosynthesis of bacteriochlorophyll and coenzyme F430. This special issue provides a cross-disciplinary overview of the ongoing research in this highly diverse and unique research area of metalloprotein biochemistry.

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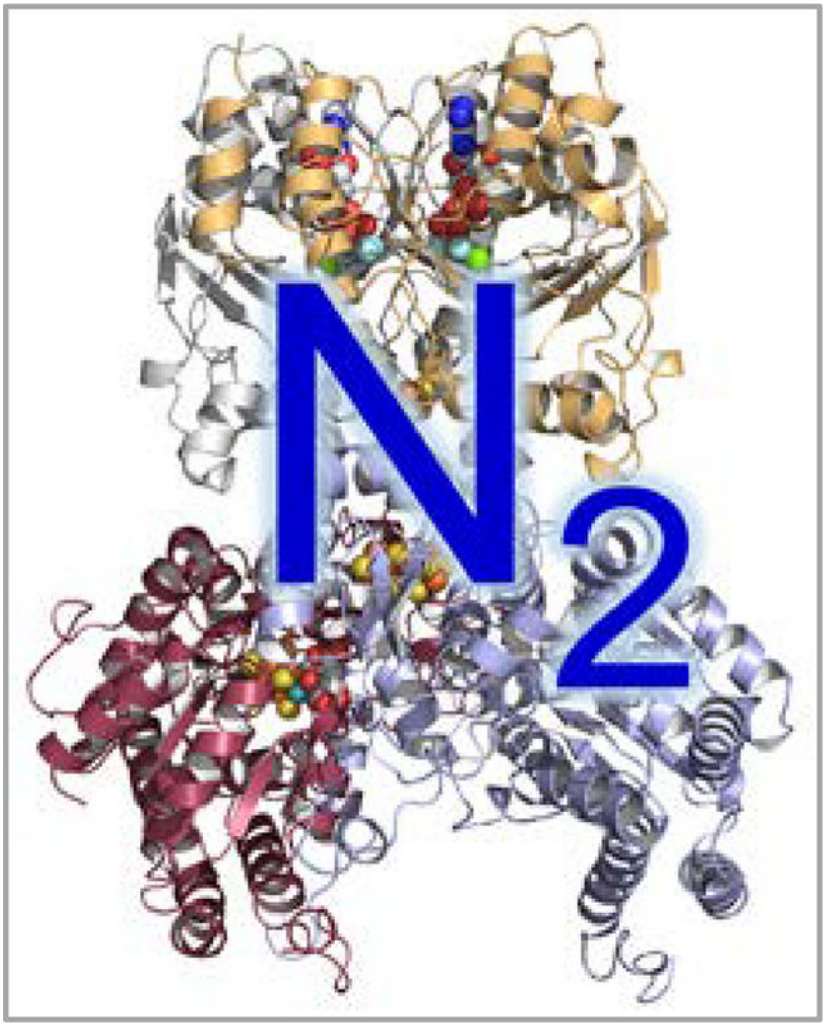
This editorial is part of a special Issue on nitrogenase and homologous systems.



Keywords

Nitrogen Fixation; Nitrogenase; Bacteriochlorophyll; Coenzyme F430; Double-cubane cluster

One can’t help but wonder if Hellriegel, Wilfarth and Beijerinck expected that their discovery of biological nitrogen fixation in the late 18th century[1–2] would capture the attention of generations of scientists from a wide range of disciplines in the next 140 years to come? The progress in this area, however, was very slow early on due to the lack of appropriate methods and techniques to tackle the questions related to the complex process, which forced many scientist to leave this field and seek topics more amenable to the approaches available at this time. It took only more than half a century until 1942, when Burris provided conclusive evidence that ammonia (NH_3) represented the key intermediate formed from the reduction of the atmospheric dinitrogen (N_2) in this process,[3] and that NH_3 was eventually incorporated into the proteins in the bacterial cells to provide the source of the essential element nitrogen for the entire global food chain.[4] It took another half a

century until the intrinsic biochemical and structural beauty of nitrogenase, the complex metalloenzyme underlying the biological nitrogen fixation, truly unfolded.[5–12]

Driven by the realization that the Mo-dependent nitrogenase is a two-component system, and using proper anaerobic techniques to purify its iron (Fe) protein and molybdenum iron (MoFe) protein components, Thorneley and Lowe conducted a series of kinetic experiments of Mo-nitrogenase and postulated the classic mechanistic framework of N_2 reduction by this nitrogenase.[5–8] Designated the Lowe-Thorneley model, this mechanistic proposal depicts the kinetics of the chemical transformations that occur on the Fe protein and MoFe protein components during nitrogenase catalysis, and the delivery of proton and electron equivalents to the catalytic MoFe protein component, where electrons and protons accumulate for the reduction of N_2 (Figure 1). While this model has been updated over the years by others (most notably, by the team of Seefeldt, Hoffman and Dean[13]), it still represents the essence of how we think nitrogenase achieves its biological function today.

The other astonishing event in nitrogenase research occurred in 1992, when Douglas Rees reported the crystallographic analysis of the catalytic MoFe protein component of the Mo-nitrogenase and provided the first structural depiction of its two unique metallocofactors: the P-cluster ($[Fe_8S_7]$) and the active site M-cluster [(*R*-homocitrate)$MoFe_7S_9$] (Figure 2).[10,11] Arguably the most complex metalloclusters found in nature, the P- and M-clusters are biologically important and chemically unprecedented. In particular, the structure of the M-cluster is so unique that it took the nitrogenase community nearly two decades after the first structural depiction of the M-cluster to realize the presence of a carbide (C^{4-}) atom in the center of this cluster, which led to an update of the stoichiometry of the M-cluster as [(*R*-homocitrate)$MoFe_7S_9C$].[14–16] Both the M- and P-clusters have evaded chemical synthesis so far, although topologs of these clusters have been successfully synthesized by Dick Holm and others,[17] which provided crucial insights into the structure-function relationship of these unique metalloclusters.

Still, the nitrogenase storybook proved to be far from completion with the discovery of the alternative vanadium (V)- and iron (Fe)-only nitrogenases.[18–20] The V- and Fe-only nitrogenases share high degrees of sequence and structural homologies with the Mo-nitrogenase.[21] However, other than the ability to reduce N_2 to NH_3, the alternative nitrogenases demonstrate the ability to reduce CO and/or CO_2 to various hydrocarbons at considerably higher efficiencies than their classic, Mo counterpart.[22–25] While the physiological function of these reactions remain unclear, the observation of the capability of nitrogenase to convert CO to hydrocarbons, such as C_3H_8 and C_4H_{10}, is of significant importance, as it represents the first and the only biological process that mirrors the Fischer–Tropsch reaction[26] that is used for the industrial production of synthetic fuel from CO.

The recent decades witnessed a whole new development of the nitrogenase field as the community started to realize the wide distribution of nitrogenase homologs among bacteria and archaea, include surprising microbial hosts unable to perform biological nitrogen fixation.[27] Recent biochemical and structural studies revealed the involvement of these nitrogenase homologs in the biosynthesis of bacteriochlorophyll and coenzyme F430,[28–31] thereby establishing a nitrogenase superfamily comprising several homologous enzymatic

systems in distinct phylogenetic clades (Figure 3).[32] An intriguing and complex class of metalloenzymes, the nitrogenase superfamily provides a whole new avenue for a highly diversified and cross-disciplinary research community.

The intention of this special issue is to provide a quick glimpse of this newly emerged field, showcasing the breadth of methods applied to the investigations of the individual members of the nitrogenase superfamily while highlighting the intrinsic correlation between the various members of this homologous group of enzymes:

Emphasizing on the genetic and evolutionary aspects of this research area, Steven Mansoorabadi provides an overview of the members of the nitrogenase superfamily,[33] Patricia Dos Santos gives a detailed account of alternative nitrogenases among microbes,[34] and Sanfeng Chen discusses the advances in transferring the prokaryotic nitrogen fixation genes into non-diazotrophic prokaryotic and eukaryotic hosts.[35]

The topic of nitrogenase mechanism is discussed by Ian Dance, who uses computational approaches to probe this intriguing process;[36] whereas Shelley Minteer and Jenny Yang provide the experimental insights into the reactivties of nitrogenase by applying electrochemistry to the reactions catalyzed by the nitrogenase enzyme[37] and its extracted cofactors.[38]

The topic of nitrogenase biosynthesis is discussed by Brian Hales, who transfers the knowledge of nitrogenase assembly to nitrogenase catalysis to further our mechanistic understanding of the nitrogenase enzyme.[39] Yilin Hu demonstrates how a nitrogenase hybrid containing an unnatural M-cluster variant can be used to modulate nitrogenase reactivity and provide insights into the mechanism of this enzyme.[40] Gunhild Layer and Jürgen Moser illustrate what chimeric systems comprising components of different members of this enzyme superfamily can help us gain a better understanding of their mechanistic properties,[41] and Yuichi Fujita reports an unexpected reactivity of an enzyme involved in the biosynthesis of bacteriochlorophyll.[42]

Last but not least, Holger Dobbek further expands our horizon in this research area by reviewing an astounding enzyme family that does not show sequence homology to the nitrogenase enzyme, but contains a double-cubane [Fe_8S_9] cluster with similar catalytic features to those of the nitrogenase clusters.[43] It seems to be just a matter of time for more unexpected members of the nitrogenase enzyme superfamily to surface, the investigation of which will keep us busy for at least another 140 years.

Acknowledgments

We would like to thank all authors who contributed manuscripts to this special issue. We would also like to thank the funding agencies that support the work in our groups, including the NIH-NIGMS grant GM67626 (to M.W.R. and Y.H.), which funds research related to the assembly of nitrogenase; the Department of Energy grants DOE (BES) DE-SC0016510 (to Y.H. and M.W.R.) and DE-SC0014470 (to M.W.R. and Y.H.), which fund work related to the mechanistic investigation of ammonia and hydrocarbon formation, respectively, by nitrogenase and related variants; and the NSF grants CHE-1904131 (to M.W.R. and Y.H.) and CHE-1651398 (to Y.H.), which fund work related to CO and CO_2 activation by nitrogenase and its Fe protein component, respectively.

Biography

Markus W. Ribbe received a B.S. degree in Biology, a M.S. degree in Microbiology, and a Ph.D. degree in Microbiology from the University of Bayreuth, Germany. He was a postdoctoral fellow at the University of California, Irvine, and is now Chancellor's Professor at the same institute. During the past 25 years, he has been focusing on the mechanistic investigation of nitrogenase catalysis and biosynthesis using a combination of genetic, biochemical, spectroscopic and structural approaches.

Yilin Hu received a B.S. degree in Genetics from Fudan University, China, and a Ph.D. degree in Biochemistry from Loma Linda University, USA. She was a postdoctoral fellow at the University of California, Irvine, and is currently Associate Professor at the same institute. During the past 17 years, she has been focusing on the structural-functional studies of nitrogenase and homologous systems using genetic, biochemical, spectroscopic and structural approaches.

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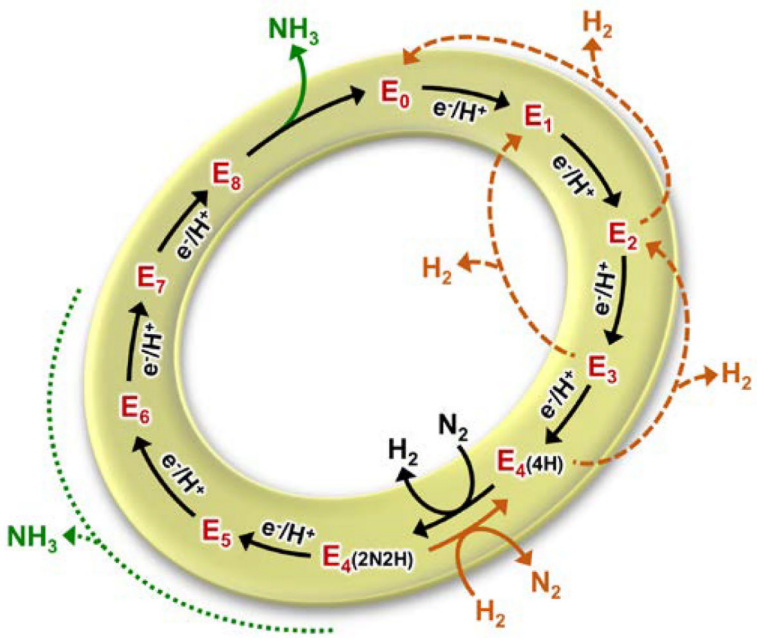

Figure 1.

The Lowe-Thorneley model of the Mo-nitrogenase. E_n depicts one αβ-dimer of the tetrameric MoFe protein component that has accumulated n electrons, with the resting-state MoFe protein designated as E_0. The steps at which NH_3 and H_2 are released are indicated.

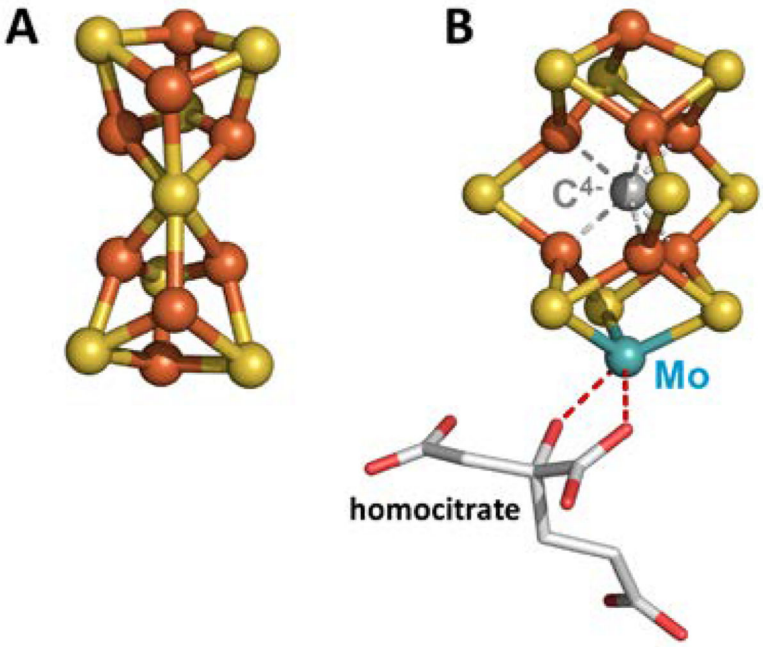

Figure 2.
Structures of the P-cluster (A) and the M-cluster (B) of the Mo-nitrogenase. Color code of atoms: Fe, orange; S, yellow; O, red; C, gray; Mo; cyan.

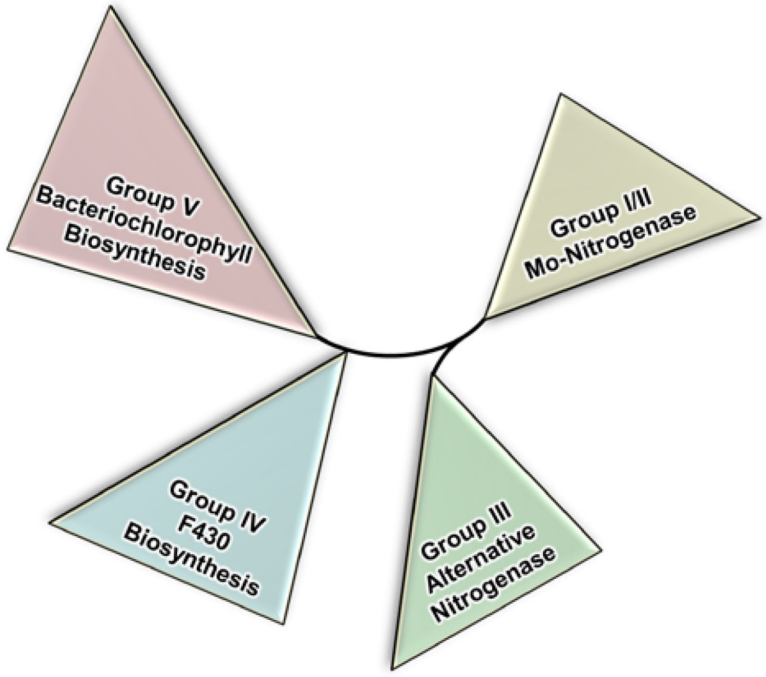

Figure 3.

Overview of the phylogenetic groups of the nitrogenase superfamily. Shown is a simplified schematic presentation of a phylogenetic tree comprising the Fe protein and MoFe protein homologs found in the available microbial genomes.[32]