Discovery of Evolutionary Divergence of Biological Nitrogen Fixation and Photosynthesis: Fine Tuning of Biogenesis of the NifH and the ChlL by a Peptidyl-Prolyl Cis/Trans Isomerase

Nara Gavini, Sinny Delacroix, Kelvin Harris Jr. and Lakshmi Pulakat
Department of Biological Sciences, Bowling Green State University, OH-40342, Bowling Green
Department of Biological Sciences, Mississippi State University, MS-39762, Mississippi State
Department of Internal Medicine, University of Missouri, MO-65212, Columbia

Abstract: Problem statement: Despite the structural and functional similarities between the nitrogenase that performs biological nitrogen fixation reaction and the Dark Protochlorophyllide Oxidoreductase (DPOR) that performs chlorophyll-biosynthesis, attempts to substitute nitrogenase-components with DPOR-components have hitherto failed. This investigation was undertaken to test if Chlamydomonas reinhardtii protochlorophyllide (Pchlide) reductase (ChlL) that shares some structural similarity with Nitrogenase Reductase (NifH) could complement the functions of NifH in biological nitrogen fixation of Azotobacter vinelandii. Approach: Genetic complementation studies were performed to test if the chlL gene and its mutants cloned under transcriptional control of nifH promoter (nifHp) in a broad-host range low copy plasmid pBG1380 could render a Nif+ phenotype to NifH-deficient A. vinelandii strains. Results: Expression of ChlL could render Nif+ phenotype to NifH-deficient A. vinelandii strains. The ChlL mutants Cys95Thr and Cys129Thr were unable to substitute for NifH. Thus, the conserved cysteine ligands of [4Fe-4S] cluster in ChlL are essential for successful substitution of NifH by ChlL. Since C-termini of NifH and ChlL demonstrated the least similarity and Pro258, a substrate for the PPlase activity of NifM, is located in the C-terminus of NifH, we posited that replacing the C-terminus of NifH with that of ChlL would render NifM-independence to NifH. The NifH-ChlL chimera could support the growth of NifH- and NifM-deficient A. vinelandii in nitrogen limiting conditions implying that it has acquired NifM-independence. Conclusion/Recommendations: Collectively, these observations suggest that NifM, an evolutionarily conserved nif-specific PPlase, could have contributed to the functional divergence of biological nitrogen fixation and photosynthesis during evolution by virtue of its ability to exert opposing effects on structurally similar substrates, ChlL and NifH.

Key words: Nitrogenase, NifH, ChlL, Dark Protochlorophyllide Oxidoreductase (DPOR), NifM, PPlase

INTRODUCTION

Functional divergence of biological nitrogen fixation and photosynthesis, the two fundamental biological processes that sustain life on earth, is still an enigma. Structural and functional similarities exist between nitrogenase that performs the biological nitrogen fixation reaction and Dark Protochlorophyllide Oxidoreductase (DPOR) that performs reduction of protochlorophyllide (Pchlide) to chlorophyllide during chlorophyll-biosynthesis (Brocker et al., 2008; Gavini et al., 2006; Georgiadis et al., 1992; Sarma et al., 2008; Tezcan et al., 2005; Watzlich et al., 2009; Yamamoto et al., 2009; 2008; Yamazaki et al., 2006a; 2006b; Nomata et al., 2006a; 2006b). Both nitrogenase and DPOR are oxygen sensitive two-component systems. However, attempts to substitute nitrogenase components NifH or NifDK by DPOR components have not been successful thus far.

Corresponding Author: Lakshmi Pulakat, Department of Internal Medicine, University of Missouri, MO-65212, Columbia Tel: 573-814-6000
Nitrogenase is dependent on a multitude of nif-specific accessory proteins for its maturation and assembly (Brocker et al., 2008; Gavini et al., 2006; Georgiadis et al., 1992; Yamazaki et al., 2006b; Betancourt et al., 2008; Chen et al., 1994; Christiansen et al., 2001; Curatti et al., 2007; Gavini and Burgess, 1992; Gavini et al., 1994; Finan, 2002; Howard et al., 1989; 1986; Howard and Rees, 1996; Jacobson et al., 1989a; 1989b; Lei et al., 1999; 1998; Peters and Sztajny, 2006; Petrova et al., 2002; Rubio and Ludden, 2005; Robinson et al., 1987). The DPOR component analogous to the nitrogenase component NifH is the BchL/ChlL protein encoded by the bchL/chlL genes of the photosynthetic bacteria.

Biogenesis of functional NifH is dependent on nif-accessory protein NifM in its natural system or in heterologous system (Finan, 2002; Howard et al., 1986; Jacobson et al., 1989a; Petrova et al., 2002). The NifM is a peptidyl-prolyl cis/trans isomerase (PPIase) belonging to the Parvulin family (Edlich and Fischer, 2006; Rahfeld et al., 1994). We have proposed that the NifM-mediated cis-to trans isomerization of one or more of the seven conserved prolines is needed for the generation of the functional NifH (Finan, 2002).

Other PPIases are unable to substitute for NifM unless their catalytic domain is fused to the N-terminal region of NifM. A comparison of ChlL with NifH shows four conserved prolines (Fig. 1) that are part of the nine conserved prolines among the ChlL peptides (Fig. 2). Additionally, superimposing a predicted model of ChlL onto the NifH template (PDB ID: 1NIP) (Georgiadis et al., 1992), using Swiss PDB (Deep View) protein modeling software (Fig. 3) shows that the ChL protein model thus generated has marked resemblance to the NifH protein.
Fig. 3: The NifH monomer structure (based on 1NIP crystallographic structure of the NifH peptide from A. vinelandii (Georgiadis et al., 1992) colored according to differences with the ChlL model. Deep View color for ChlL was white. The Deep View coloration of NifH was done according to root mean square deviation type of coloration (blue = maximum similarity; red = maximum dissimilarity) in comparison to ChlL. Bottom view shows a particularly dissimilar C-terminal region (red stretch) starting with Tyr230 and ending with Glu287 of NifH. The [4Fe-4S] cluster and the bound MgADP in NifH are indicated.

Since the ChlL protein showed such high structural similarity to the NifH protein, we hypothesized that ChlL would substitute for NifH in A. vinelandii and that NifM may play a role in modulating the functional properties of the NifH-like proteins such as ChlL/Bchl. The purpose of this study was to determine whether ChlL could substitute for NifH in biological nitrogen fixation in presence and absence of functional NifM.

MATERIALS AND METHODS

Construction of plasmid pBG2400: An 879bp DNA fragment encoding the ORF of chlL flanked by EcoRV and HindIII restriction enzyme sites was generated by PCR amplification using C. Reinhardtii chromosomal DNA as template and cloned into the pCR2.1 TOPO vector. The EcoRV- HindIII fragment encoding chlL ORF was subcloned into EcoRV- HindIII digested pBG1380 to generate pBG2434 that carries nifH gene under the transcriptional control of nifHp promoter (nifHp). The 129bp region that carries the last 42 amino acids at the C-terminus of NifH was removed via SalI digestion. Next, the DNA containing the last 55 codons of the chlL was PCR-amplified using a 5' primer that carries a SalI site (5'--GTCGACAATTCTACAGTAGGAGTGTC-3') and a 3' primer with a HindIII site (5'-AAGCTTTTAAATTTAAGATAGAAATC-3'). The resultant PCR product encoding the C-terminal region of ChlL protein (55 amino acids at the C-terminal end) was cloned into the Sall-HindIII digested pBG2434 (carrying the N-terminus of nifH) to generate a nifH-chlL chimeric gene in which the DNA encoding the C-terminal region of NifH (bp745-873) was replaced by the DNA encoding C-terminal region of ChlL (bp718-882).

RESULTS AND DISCUSSION

ChlL can substitutes for NifH in biological nitrogen fixation reaction only in the absence of NifM: Two NifH-deficient A. vinelandii strains, one NifM-positive (nifM+ A. vinelandii DJ54 (Gavini et al., 1994; Robinson et al., 1987) and one NifM-negative (nifM-:kan A. vinelandii BG98 (Gavini et al., 2006) respectively, were used to test the ability of the ChlL to substitute for the NifH in nitrogen fixation reaction by A. vinelandii. Both strains were transformed with pBG2400 that carries the C. reinhardtii chlL gene cloned under the transcriptional regulation of the nifHp and the ability of the transformants to grow under nitrogen limiting conditions was assessed as follows.
Since the plasmid carries a chloramphenicol resistance (Cm\(^R\)) marker, the transformants were originally selected on Burk’s Nitrogen plus (BN\(^+\)) medium supplemented with 72 µg mL\(^{-1}\) of chloramphenicol. Then, the ability of the chlL gene to render Nif\(^{+}\) phenotype to A. vinelandii strains DJ54 and BG98 was monitored by growing the transformants on Burk’s nitrogen free (BN\(^-\)) media containing molybdenum (Strandberg and Wilson, 1968) that imitates expression of alternate nitrogenases (Betancourt et al., 2008).

It was found that pBG2400 could not render a Nif\(^{+}\) phenotype to the A. vinelandii DJ54 (Fig. 4a), but it rendered Nif\(^{+}\) phenotype to the A. vinelandii BG98 (Fig. 4b).

Since the difference between the two strains is that DJ54 has an intact nifM, whereas BG98 has a disrupted nifM, we concluded that the ChlL could restore nitrogenase activity in the absence of functional NifH-but only if NifM was also absent. In summary, the ChlL can replace the NifH-function in biological nitrogen fixation by A. vinelandii. However, the NifM, the accessory PPIase essential for biogenesis of functional NifH, has a negative effect on the compensatory ability of ChlL. This interpretation is consistent with the observations that (a) the chlL gene could not complement the ΔnifH of nifM\(^{-}\) A. vinelandii DJ54 and (b) the BchL protein (similar in structure and function to the ChlL) isolated from nifM\(^{-}\) A. vinelandii was unable to substitute for the NifH protein in nitrogenase assay (Sarma et al., 2008).

**Cys95 and Cys129 of the ChlL are required for substitution of NifH by ChlL in biological nitrogen fixation reaction:** The cysteine ligands Cys97 and Cys132 of the NifH peptide are conserved in the ChlL peptide (Cys95 and Cys129 of the ChlL peptide respectively; Fig. 1). Replacing these conserved cysteines of NifH impairs its function (Howard et al., 1989). We posited that the Cys95 and Cys129 of the ChlL are required for participation in nitrogenase activity in the absence of functional NifH.

| Strain                                | Plasmid         | Codon/ amino acid for position 95 of ChlL | Codon/ amino acid for position 129 of ChlL | Growth on BN\(^*\) medium with Chloramphenicol | Growth on BN\(^*\) medium |
|---------------------------------------|-----------------|------------------------------------------|------------------------------------------|---------------------------------|--------------------------|
| A. vinelandii DJ54 (ΔnifH) NifM\(^*\) | pBG2400         | TGT/Cys                                  | TGT/Cys                                  | +++                             | ---                      |
| A. vinelandii BG98 nifM NifH-deficient| pBG2400         | TGT/Cys                                  | TGT/Cys                                  | +++                             | ---                      |
| A. vinelandii BG98 nifM NifH-deficient| pBG2406         | ACC/Thr                                  | TGT/Cys                                  | +++                             | ---                      |
| A. vinelandii DJ54 (ΔnifH) NifM\(^*\) | pBG2428         | ACC/Thr                                  | ACC/Thr                                  | +++                             | ---                      |
| A. vinelandii DJ54 (ΔnifH) NifM\(^*\) | pBG2428         | TGT/Cys                                  | ACC/Thr                                  | +++                             | ---                      |

(A) (B)
Fig. 5: (A) Comparison of the amino acid sequence of the NifH-ChlL chimera with the NifH and the ChlL. Conserved proline residues are marked by the open box. The C-terminal region of the NifH-ChlL chimera that corresponds to that of the ChlL peptide is highlighted in yellow. (B) Growth of the NifH-deficient \( \text{nifM} \) \( A. \text{vinelandii} \) strain BG98 carrying the parental plasmid pBG1380 (marked 2) and pBG1380-derivative expressing the nifH-chlL chimeric gene (marked 3) on BN\(^{-}\) medium is shown. Wild type \( A. \text{vinelandii} \) (marked 1) served as control. Thus, the NifH-ChlL chimera could support growth of \( \text{nifM} \) \( A. \text{vinelandii} \) strain BG98 in nitrogen limiting conditions indicating that replacement of the C-terminal region of the NifH with that of the ChlL resulted in partial relief from NifM-dependence. Experiments were repeated at least six times.

These observations suggested that the Cys95 and Cys129 of the ChlL could play roles analogous to that of Cys97 and Cys132 of NifH in stabilizing the [4Fe-4S] cluster of ChlL.

A \( \text{nifH-chlL} \) chimera could render \( \text{Nif}^+ \) phenotype to \( A. \text{vinelandii} \) BG98: As shown in Fig. 1 and 3, the C-termi­nus of the NifH and the ChlL are highly dissimilar. Therefore, the fact that NifH is not functional in the absence of NifM while ChlL is not functional in the presence of NifM might be traced to this region. We have shown previously that the Pro258 located in the C-terminus of the NifH is one of the substrates for the PPIase activity of NifM (Gavini et al., 2006).

Because the C-terminus of the ChlL is dissimilar to that of the NifH and the ChlL, the ChlL could substitute the NifH in the absence of the NifM in nitrogen fixation, it is conceivable that the C-terminus of the ChlL would render NifM-independence to the NifH. To test this idea, we analyzed the effect of replacing the C-terminus of NifH with that of ChlL. The DNA encoding the C-terminal region of NifH (bp745-873) was replaced by the DNA encoding C-terminal region of ChlL (bp718-882) to construct the \( \text{nifH-chlL} \) chimeric gene. Therefore, the resulting NifH-ChlL chimera did not contain Pro258 of the NifH. Amino acid sequence of the NifH-ChlL chimera is shown in Fig. 5a. \( A. \text{vinelandii} \) BG98 transformants expressing the \( \text{nifH-chlL} \) chimeric gene were capable of growing on BN\(^{-}\) medium (Fig. 5b). Thus, the \( \text{nifH-chlL} \) chimera could render partial NifM-independence to \( A. \text{vinelandii} \) BG98.

CONCLUSION

Our results show that the NifM, a NifH-specific PPIase that is essential for biogenesis of the NifH protein, has a role in disabling structurally similar ChlL from participating in the biological nitrogen fixation reaction. Significance of PPIase-substrate interactions are particularly highlighted in many pathological conditions. For example, overexpression of human Pin1 is implicated in the formation of Lewy bodies in Parkinson’s Disease, while the same protein has a beneficial effect in Alzheimer’s disease, since it regulates amyloid precursor protein processing and amyloid beta production (Pastorino et al., 2006; Ryo et al., 2006). Similarly, Macrophage Infectivity Potentiators (MIPs) are PPIases expressed by bacterial pathogens, however, they interact with host-cell proteins and alter their functions to establish infection (Kohler et al., 2003). These examples show that the molecular interactions between PPIases and proteins that share structural similarity to their natural substrates result in pathogenesis. The example that has emerged from this study is that of a PPIase which could have contributed to the functional divergence of two fundamental biological processes (nitrogen fixation and photosynthesis) during evolution. This is because this PPIase prototype (NifM) would render functionality to one substrate (NifH) and hinder the function of the other structurally similar substrate (ChlL) so that...
nitrogen fixation is favored under conditions that lead to NifM expression (such as nitrogen limitation). These findings represent a unique example of an accessory protein playing a vital part in the evolutionary divergence of biological processes.

The observation that ChlL mutants Cys 95Thr and Cys129Thr were unable to substitute for NifH further extends the structure-function similarity of the NifH and ChlL related to their mechanistic involvement in nitrogen fixation. Although the structure of ChlL is not yet solved, these observations strengthen the similarities in the role of Cys ligands of the (4Fe-4S) cluster of ChlL in electron transfer by the ChlL to that of the NifH. On the other hand, our studies also highlight the dissimilarity of the C-termini of the NifH and ChlL. The C-terminus of the NifH is involved in the NifM-dependence of the NifH due to the presence of Pro258 (Gavini et al., 2002). In contrast, the C-terminus of the ChlL could render NifM-independence to the NifH as shown by the functional NifH-ChlL chimera (Fig. 5). It is conceivable that a protein similar to the NifH-ChlL chimera could have served as a common ancestor for the NifH and the ChlL before the functional divergence of biological nitrogen fixation and photosynthesis during evolution.

ACKNOWLEDGMENT

We thank members of Gavini/Pulakat laboratories for valuable comments and technical help. Funding for this research was provided by NSF grant # MCB-1041718 to LP.

REFERENCES

Betancourt, D.A., T.M. Loveless, J.W. Brown and P.E. Bishop, 2008. Characterization of dichrotophs containing Mo-independent nitrogenases, isolated from diverse natural environments. Appl. Environ. Microbiol., 74: 3471-3480. PMID: 18378646

Brocker, M.J., D. Watzlich, F. Uliczka, M. Saggu and M. Christiansen, 2001. Mechanistic features of Mo-containing Nitrogenase. Annu. Rev. Plant. Physiology. Plant. Mol. Biol., 52: 269-295. PMID: 11337399

Curatti, L., J.A. Hernandez, R.Y. Igarashi, B. Soboh and D. Zhao et al., 2007. In vitro synthesis of the iron-molybdenum cofactor of nitrogenase from iron, sulfur, molybdenum and homocitrate using purified proteins. Proc. Natl. Acad. Sci. U S A, 104: 17626-17631. PMID: 17978192

Edlich, F. and G. Fischer, 2006. Pharmacological targeting of catalyzed protein folding: the example of peptide bond cis/trans isomerasers. Handb. Exp. Pharmacol., 172: 359-404. PMID: 16610367

Finan, T.M., 2002. Nitrogen Fixation: Global Perspectives. 1st Edn., CABI, Oxford, ISBN: 0851999518, pp: 553.

Gavini, N. and B.K. Burgess, 1992. FeMo cofactor synthesis by a nifH mutant with altered MgATP reactivity. J. Biol. Chem., 267: 21179-21186. PMID: 1400428

Gavini, N., L. Ma, G. Watt and B.K. Burgess, 1994. Purification and characterization of a FeMo cofactor-deficient MoFe protein. Biochemistry., 33: 11842-11849. PMID: 7918402

Gavini, N., N.S. Tungtur and L. Pulakat, 2006. Peptidyl-prolyl cis/trans isomerase-independent functional NifH mutant of Azotobacter vinelandii. J. Bacteriol., 188: 6020-6025. PMID: 16885471

Georgiadis, M.M., H. Komiva, P. Chakrabarti, D. Woo and J.J. Kornuc et al., 1992. Crystallographic structure of the nitrogenase iron protein from Azotobacter vinelandii. Science, 257: 1653-1659. PMID: 1529353

Howard, J.B. and D.C. Rees, 1996. Structural basis of biological nitrogen fixation. Chem. Rev., 96: 2965-982. PMID: 11848848

Howard, J.B., R. Davis, B. Moldenhauer, V. L. Cash and D. Dean et al., 1989. Fe; S cluster ligards are the only cysteines required for nitrogenase Fe-protein activities. J. Biol. Chem., 264: 11270-11274. PMID: 2500438

Howard, K.S., P.A. McLean, F.B. Hansen, P.V. Lemley and K.S. Koblan et al., 1986. Klebsiella pneumonia nifM gene product is required for stabilization and activation of nitrogenase iron protein in Escherichia coli. J. Biol. Chem., 261: 772-778. PMID: 3001082

Jacobson, M.R., K.E. Brigle, L.T. Bennett, R.A. Setterquist and M.S. Wilson et al., 1989a. Physical and genetic map of the major nif gene cluster from Azotobacter vinelandii. J. Bacteriol., 171: 1017-1027. PMID: 2644218

Jacobson, M.R., V.L. Cash, M.C. Weiss, N.F. Laird and W. E. Newton et al., 1989b. Biochemical and genetic analysis of the nifUSVWZM cluster from Azotobacter vinelandii. Mol. Gen. Genet., 219: 49-57. PMID: 2615765
Kohler, R., J. Fanghanel, B. König, E. Lüneberg and M. Froesch et al., 2003. Biochemical and functional analysis of the Mip protein: Influence of the N-terminal half and of peptidyl/prolyl isomerase activity on the virulence of Legionella pneumophila. Infect. Immun., 71: 4389-4397. PMID: 12874317

Lei, S., L. Pulakat and N. Gavini, 1999. Regulated expression of the nifM of Azotobacter vinelandii in response to molybdenum and vanadium supplements in Burk's nitrogen-free growth medium. Biochem. Biophys. Res. Commun., 264: 186-190. PMID: 10527862

Lei, S.H., L. Pulakat, K.C. Parker and N. Gavini, 1998. Genetic analysis on the NifW by utilizing the yeast two-hybrid system revealed that the NifW of Azotobacter vinelandii interacts with the NifZ to form higher-order complexes. Biochem. Biophys. Res. Commun., 244: 498-504. PMID: 9514861

Nomata, J., M. Kitashima, K. Inoue and Y. Fujita, 2006a. Nitrogenase Fe protein-like Fe-S cluster is conserved in L-protein (BchL) of dark-operative protochlorophyllide reductase from Rhodobacter capsulatus. FEBS Lett., 580: 6151-6154. PMID: 1706495

Nomata, J., T. Mizoguchi, H. Tamiaki and Y. Fujita, 2006b. A second nitrogenase-like enzyme for bacteriochlorophyll biosynthesis: reconstitution of chlorophyllide a reductase with purified X-protein (BchX) and YZ-protein (BchY-BchZ) from Rhodobacter capsulatus. J. Biol. Chem., 281: 15021-15028. PMID: 16571720

Pastorino, L., A. Sun, P.J. Lu, X.Z. Zhou and M. Balastik et al., 2006. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. Nature, 44: 528-534. PMID: 16554819

Peters, J.W. and R.K. Szilagyi, 2006. Exploring new frontiers of nitrogenase structure and mechanism. Curr. Opin. Chem. Biol., 2: 101-108. PMID: 16510305

Petrova, N., L. Gigova and P. Venkov, 2002. Dimerization of Rhizobium meliloti NifH protein in Saccharomyces cerevisiae cells requires simultaneous expression of NifM protein. Int. J. Biochem. Cell. Biol., 34: 33-42. PMID: 11733183

Rahfeld, J.U., K.P. Rucknagel, B. Schelbert, B. Ludwig and J. Hacker et al., 1994. Confirmation of the existence of a third family among peptidyl-prolyl cis/trans isomerases. Amino acid sequence and recombinant production of parvin. FEBS Lett., 352: 180-184. PMID: 7925971

Robinson, A.C., D.R. Dean and B.K. Burgess, 1987. Iron-molybdenum cofactor biosynthesis in Azotobacter vinelandii requires the iron protein of nitrogenase. J. Biol. Chem., 262: 14327-14332. PMID: 3477546

Rubio, L.M. and P.W. Ludden, 2005. Maturation of nitrogenase: A biochemical puzzle. J. Bacteriol., 187: 405-414. PMID: 15629911

Ryo, A., T. Togo, T. Nakai, A. Hirai and M. Nishi et al., 2006. Prolyl-isomerase Pin1 accumulates in lewy bodies of parkinson disease and facilitates formation of alpha-synuclein inclusions. J. Biol. Chem., 281: 4117-4125. PMID: 16365047

Strandberg, G.W. and P.W. Wilson, 1968. Formation of the nitrogen-fixing enzyme system in Azotobacter vinelandii. Can. J. Microbiol., 14: 25-31. PMID: 5644401

Suh, M.H., 2002. Engineering a simplified functional nitrogenase by gene fusion and DNA shuffling. Doctoral Dissertation, Bowling Green State University.

Tezcan, F.A., J.T. Kaiser, D. Mustafi, M.Y. Walton and J.B. Howard et al., 2005. Nitrogenase complexes: Multiple docking sites for a nucleotide switch protein. Science, 309: 1377-1380. PMID: 16123301

Watzlich, D., M.J. Brocker, F. Uliczka, M. Ribbe and S. Virus et al., 2009. Chimeric nitrogenase-like enzymes of (bacterio)chlorophyll biosynthesis. J. Biol. Chem., 284: 15530-15540. PMID: 19336405

Yamamoto, H., J. Nomata and Y. Fujita, 2008. Functional expression of nitrogenase-like protochlorophyllide reductase from Rhodobacter capsulatus in Escherichia coli. Photochem. Photobiol. Sci., 7: 1238-1242. PMID: 18846289

Yamamoto, H., S. Kurumiya, R. Ohashi, Y. Fujita, 2009. Oxygen sensitivity of a nitrogenase-like protochlorophyllide reductase from the cyanobacterium Leptolyngbya boryana. Plant. Cell. Physiol., 50: 1663-1673. PMID: 19643808
Yamazaki, S., J. Nomata and Y. Fujita, 2006a. Differential operation of dual protochlorophyllide reductases for chlorophyll biosynthesis in response to environmental oxygen levels in the cyanobacterium *Leptolyngbya boryana*. Plant. Physiol., 142: 911-922. PMID: 17028153

Yamazaki, S., J. Nomata and Y. Fujita, 2006b. Nitrogenase Fe protein-like Fe-S cluster is conserved in L-protein (BchL) of dark-operative protochlorophyllide reductase from *Rhodobacter capsulatus*. FEBS. Lett., 580: 6151-6154. PMID: 17064695