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Short Sequence-Paper

Nucleotide sequences of two hydrogenase-related genes (*hypA* and *hypB*) from *Bradyrhizobium japonicum*, one of which (*hypB*) encodes an extremely histidine-rich region and guanine nucleotide-binding domains

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Sequencing of a 1359-bp (*Nru*I-*Acc*I) DNA fragment located approximately 5.2 kb downstream from the end of the hydrogenase structural genes of *Bradyrhizobium japonicum* revealed two open reading frames designated *hypA* and *hypB*, encoding polypeptides with predicted molecular masses of 12.3 and 32.8 kDa, respectively. Both *hypA* and *hypB* showed strong homology with other genes in hydrogenase-containing bacteria. Two 'C-X-X-C' motifs were contained in the deduced amino acid sequence of *hypA*, a motif that is present in all known products homologous to HypA. The deduced product of *hypB* contains an area remarkably rich in histidine residues at the N-terminus (24 histidines within a 39 amino acid stretch). The deduced HypB also contains GTP-binding domains. We postulate that the product of *hypB* is involved in nickel binding and accumulation, and may utilize energy (GTP) to mobilize nickel for its subsequent incorporation into hydrogenase.

Bradyrhizobium japonicum, the N_2-fixing symbiont of soybeans, possesses an Ni-containing hydrogenase [1,2]. The enzyme couples the H_2 evolved by nitrogenase into the respiratory electron transport chain, generating energy for N_2 fixation. The purified hydrogenase of *B. japonicum* consists of two subunits (33 and 65 kDa) [3,4] and contains nickel. The cosmid pSH22 contains essential Hup (hydrogen uptake) determinants (including the hydrogenase structural genes) and it complements many Hup$^-$ mutant strains of *B. japonicum* [5]. In addition to the hydrogenase structural genes, other genes and loci on pSH22 located both downstream and upstream of the structural genes have been found to be involved in hydrogenase regulation [6], processing [7] and nickel incorporation [8,9] into the enzyme.

To further understand the genetic requirements for H_2 oxidation in *B. japonicum*, a 2.5-kb *Eco*RI fragment located approximately 5.0 kb from the end of the hydrogenase structural genes was isolated from pSH22 and bidirectionally cloned into the *Eco*RI site of pBluescript KS II(+) (Stratagene, La Jolla, CA). The resulting plasmids (pKS2.5E2 and pKS2.5E4) were used for making nested deletions by using the Exo/Mung bean sequencing kit from Stratagene. Sequencing was carried out in both orientations.

Analysis of the nucleotide sequence of a 1359-bp segment (*Nru*I-*Acc*I fragment) within the insert DNA revealed the presence of two open reading frames, termed *hypA* and *hypB* based on their sequence homologies to other named genes from hydrogenase-containing bacteria. Complete nucleotide and deduced amino acid sequences are shown in Fig. 1. *hypA* and *hypB* encode polypeptides with predicted molecular masses of 12.3 and 32.8 kDa, respectively, and all are oriented in the same direction as the hydrogenase structural genes, *hupSL*. Both *hypA* and *hypB* start at an initiation codon ATG preceded by a putative ribosome-binding site and they terminate at a stop codon TGA (Fig. 1). They are closely linked, and have overlapping stop and start codons.

Gene *hypA* encodes a polypeptide of 12.3 kDa (113 aa). The product of the *hypA* gene (HypA) showed significant homology with *Rhizobium leguminosarum* HypA [10], *Azotobacter vinelandii* ORF4 [11], *Azotobacter chroococcum* HupA [12], *Rhodobacter capsulatus* HypA [13,14], and *Escherichia coli* HypA [15]. Interestingly, HypA of *B. japonicum* also showed homology to

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the product of ORFB from an operon adjacent to the furmarate reductase operon of *Proteus vulgaris* [16]. Alignment of HypA with the homologous products is shown in Fig. 2A. All seven proteins contain a 'C-X_2-C-X_{12-13}-C-X_2-C' motif near the C-terminus (Fig. 2A). This stretch of amino acids is usually characteristic of metal-binding domains within non-heme iron proteins [17] and can play roles in the binding of Fe-S clusters [18]. The specific (metal-metabolism) functions of these products are unknown.

Gene *hypB* encodes a polypeptide of 32.8 kDa (302 aa). HypB showed 56, 54, 53, 54 and 49% identity with the *R. leguminosarum* HypB [10], *A. vinelandii* ORF5 [11], *A. chroococcum* HupB [12], *R. capsulatus* HypB [13,14], and *E. coli* HypB [15], respectively. Alignment of HypB of *B. japonicum* with the homologous products is shown in Fig. 2B. All six proteins contained an 'MCTXCGC' motif at the N-terminus, suggesting that this motif is functionally important. Most notably, the HypB protein contained an extremely histidine-rich region near the N-terminus (24 histidines in a 39-amino acid stretch). The histidine-rich areas span positions 16 to 54 and includes two runs of hist residues, one each of six and five sequential residues. The area of interest is HAHDHHHDHGHDHDHGHDGHHHHH-HGHDQDHHHHHDHAH (Figs. 1 and 2B). This histidine-rich region contributes a highly hydrophilic domain based on the Kyte-Doolitle scale [19]. Histidine-

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  NruI
TCGCGAGGCCGGCCATGCATGAGATGGCGCTGTGCGAAGGCATCATTGGCATCGTCGAGGAGGAAGCGCGCA    72
        HupA M H E M A L C E G I I G I V E E E A R

AGCGCGCTTTCGCCAAGGTGAACGTCGTCTGCCTCGAGATCGGTGCGCTCAGCCATGTCGCGCCGGAGGCCC   144
K R A F A K V N V V C L E I G A L S H V A P E A

TGCAATTCTGCTTCGAGGCCGTCGCCGCGCGGACCATCGCGCAAGGCGCCAAACTCGAGATCGTCGAGACGC   216
L Q F C F E A V A A R T I A Q G A K L E I V E T

CGGGCACGGCGTGGTGCATGGCCTGTTCCAAAAGCGTCGAGATCAAGCAGCGATACGAACCGTGTCCGTCCT   288
P G T A W C M A C S K S V E I K Q R Y E P C P S

GCGGCGGCTATCAATTGCAGGTGACGGGCGGCGAAGAGATGCGCGTGAGGGAATTGGAGGTCGACTGATGTG   360
C G G Y Q L Q V T G G E E M R V R E L E V D *M C
                                          HupB
TACGGTCTGCGGCTGCAGCGACGGCAAGGCGTCCATCGAACATGCCCATGATCATCATCATGATCACGGGCA   432
  T V C G C S D G K A S I E H A H D H H H D H G H

TGACCACGATCATGGTCACGACGGGCACCATCACCACCACCACGGTCATGACCAAGATCACCATCATCACCA   504
  D H D H G H D G H H H H H H G H D Q D H H H H H

CGATCACGCCCACGGCGATGCCGGACTGCTCGACTGCGGCGCTAATCCGGCCGGCCAGAAGATCACCGGCAT   576
  D H A H G D A G L L D C G A N P A G Q K I T G M

GAGCAGCGACCGCATCATCCAGGTCGAGCGCGACATCCTCGGCAAGAACGATAGGCTCGCCGCCGACAATCG   648
  S S D R I I Q V E R D I L G K N D R L A A D N R

CGCCCGCTTCCGCGCCGACGAGGTGCTTGCCTTCAACCTTGTCTCGAGCCCCGGCGCCGGCAAGACCTCGCT   720
  A R F R A D E V L A F N L V S S P G A G K T S L

CTTGGTCCGTGCCGTCTCCGAGCTGAAGGACAGTTTTGCGATCGGCGTGATCGAAGGCGACCAGCAGACCTC   792
  L V R A V S E L K D S F A I G V I E G D Q Q T S

CAACGATGCCGAGCGCATTCGCGCGACCGGCGTGCCGGCGATCCAGGTCAACACCGGCAAGGGCTGCCATCT   864
  N D A E R I R A T G V P A I Q V N T G K G C H L

CGATGCCGCCATGGTCGGCGAGGCCTATGACCGGCTGCCTTGGCTGAACGGCGGGCTGCTTTTCATCGAGAA   936
  D A A M V G E A Y D R L P W L N G G L L F I E N

TGTCGGCAACCTCGTTTGCCCGGCAGCGTTCGATCTTGGCGAAGCCTGCAAGATCGTGGTGTTCTCGACCAC  1008
  V G N L V C P A A F D L G E A C K I V V F S T T

CGAGGGCGAGGACAAGCCGTTGAAATATCCTGACATGTTCGCCGCCTCGTCCCTGATGCTGATCAACAAGAT  1080
  E G E D K P L K Y P D M F A A S S L M L I N K I

CGATCTCGCCTCGGTGCTCGATTTCGATCTCGCCAGGACCATCGAATATGCGCGGCGGGTCAACCCGAAGAT  1152
  D L A S V L D F D L A R T I E Y A R R V N P K I

CGAGGTGCTGACGCTGTCGGCGCGCACCGGCGAAGGCTTTGCGGCGTTCTATGCCTGGATCCGCAAGCGGAT  1224
  E V L T L S A R T G E G F A A F Y A W I R K R M

GGCGGCGACGACGCCGGCCGCGATGACGGCGGCGGAATGACAATGGGCGGCGAGGCTACAGCACTGGACGGA  1296
  A A T T P A A M T A A E *
                                                    AccI
CGGCGGCTGCGCCTGCATGTGCGCGGCGCGGTGCAGGGTGTCGGCTTTCGTCCCTATGTCTAC            1359
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Fig. 1. Nucleotide and deduced amino acid sequences of the *B. japonicum hypA* and *hypB* genes. Potential ribosome-binding sites are underlined. The putative translation start codons are in bold. The asterisks indicate stop codons. The histidine-rich region is also underlined.

rich motifs were also found at the N-termini of the HypB products of *R. leguminosarum* (21 hist out of 55 residues) and *R. capsulatus* (26 hist out of 96 residues) (Fig. 2B); but the histidine residues of *B. japonicum* HypB are more clustered than the corresponding genes' products of *R. leguminosarum* and *R. capsulatus*. Although the *A. chroococcum* HupB and *A. vinelandii* ORF5 products had fewer histidines than *B. japonicum* HypB, *R. leguminosarum* HypB, and *R. capsulatus* HypB, 12 histidines were closely clustered in their N-termini. Such histidine-rich motifs likely function as nickel ligands. All six proteins, including the *B. japonicum* HypB, had clear guanine nucleotide-binding domains (Fig. 2B, residues indicated by asterisks) [20].

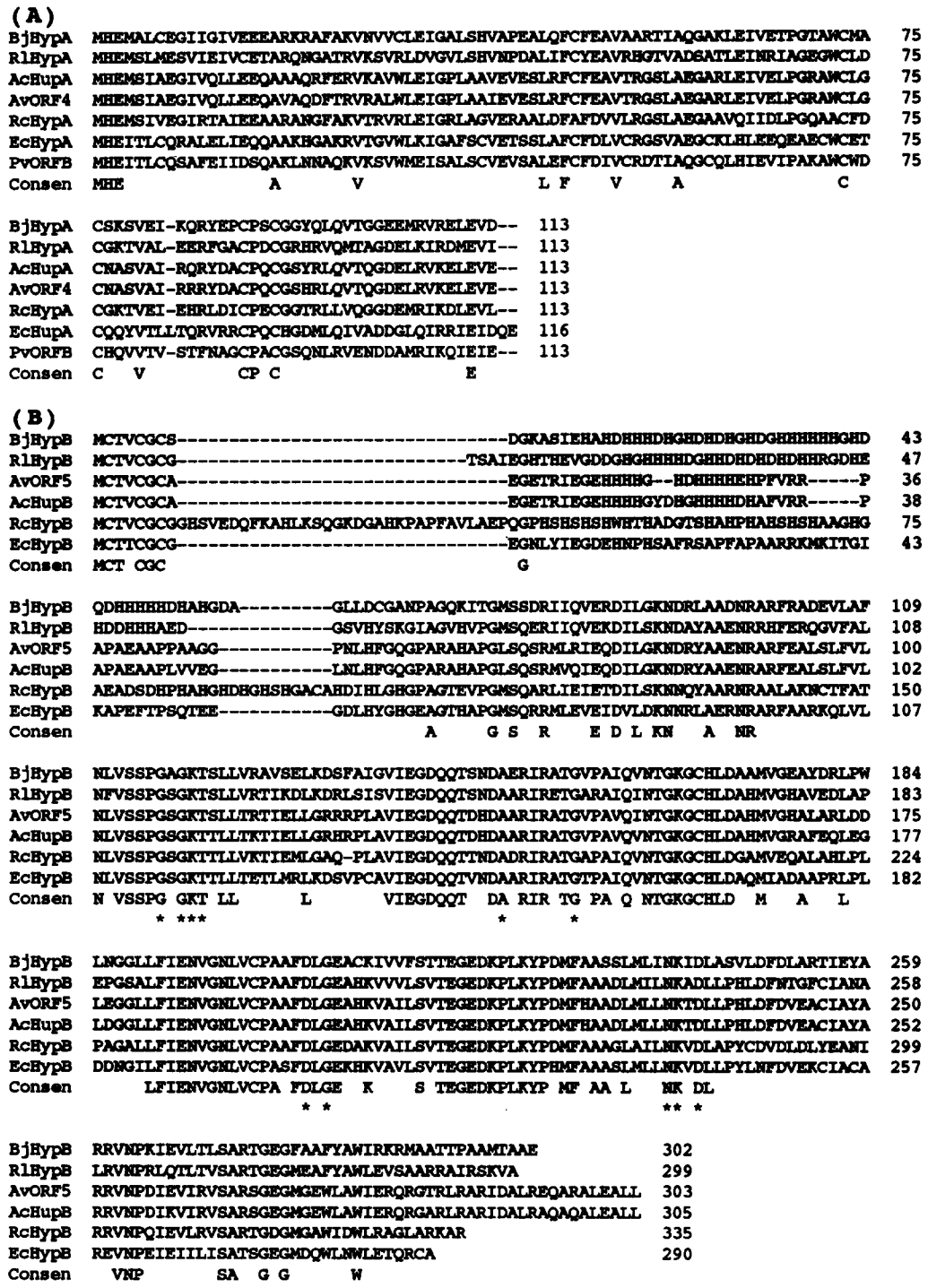

Fig. 2. Alignment of the deduced amino acid sequences of the HypA (A) and HypB (B) of *B. japonicum* with the homologous products from *R. leguminosarum* (Rl), *A. vinelandii* (Av), *A chroococcum* (Ac), *R. capsulatus* (Rc), *E. coli* (Ec) and *Proteus vulgaris* (Pv). Consensus sequence (consen) is shown at the bottom. G-binding domains are indicated by asterisks.

The ureE protein of the *Klebsiella aerogenes* urease gene operon contains a histidine-rich region near its C-terminus (10 of 15 residues are histidines) [21]. The protein was demonstrated to bind nickel and subsequently provide nickel for urease activity [22]. The product of the *hypB* gene, a guanine nucleotide-binding protein, was reported to be required for nickel incorporation into *E. coli* hydrogenase [23]. It was suggested that GTP hydrolysis took place during nickel transfer between proteins [23]. Due to these previous studies, compared to the now-characterized *B. japonicum hypB*, we propose that HypB ligands to and accumulates nickel, and in a GTP-requiring reaction, mobilizes the metal for its subsequent incorporation into hydrogenase.

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