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histidine, stabilizing the motif into a single folded domain[12,14]. As well as the conservation of the Zn^{2+} ligands, there are additional conserved residues between MDM2 and the rest of the RING-finger family, including a number of hydrophobic residues implicated in stabilizing the RING-finger fold (Fig. 1a; Ref. 14). Interestingly, two of the proteins shown in the alignment are implicated in the control of cell fate, namely IAP[15] and DROC3H[16]. ICPO is involved in gene regulation[14] whereas the functions of CELRO5 (a *Caenorhabditis elegans* protein)[17] and RING1 (Ref. 12) are not known. MDM2 and CELR05 show significant sequence homologies outside the RING finger, and both can be aligned (17% identity over 491 residues; Fig. 1b). To further assess the significance of the alignment, the zinc finger region of MDM2 (434–491) was used to search the OWL database (version 22.1) with the program PROSRCH[18] using PAM matrices of 100 and 250. Statistically significant matches (excluding itself) were observed only between MDM2 and IAP, DROC3H, CELR05 and ICPO. Furthermore, no matches were observed in the top 100 alignments between MDM2 and any C2H2 or C4 zinc-finger-containing protein, supporting the proposal that MDM2 contains a RING finger rather than two tandemly arranged zinc fingers as previously postulated[8–10].

The extensive RING-finger family contains a number of known oncoproteins including, for example, MEL18, BMI-1, RFP, PML and T18 (reviewed in Ref. 13). A large number of proteins in this family are also implicated in the control of cell growth, differentiation and development[13]. The presence of the RING-finger domain in MDM2 may infer a common functionality with other members of the RING-finger family. This function may involve protein–protein or protein–nucleic acid interaction, which would be distinct from the proposed inactivation of p53, but necessary for the other important biological activities of MDM2.

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The modular structure of NifU proteins

The *nif* cluster contains genes responsible for nitrogen fixation in several prokaryotes[1–3]. With the exception of the essential genes coding for and regulating the expression of the nitrogenase subunits, the functions of other *nif* genes appear to be associated with cofactor biosynthesis, amino acid interconversion and regulation of ammonia storage. However, not all the biochemical activities of the *nif* gene products have been identified yet. Some of these proteins might represent specialized versions of general metabolic enzymes; thus their presence cannot be precluded from non-nitrogen fixing organisms. A recent example is the identification of the NifS family as pyridoxal-phosphate-dependent aminotransferases[4].

Here we report the identification by sequence analysis of two distinct domains in the NifU protein family (Fig. 1a). Although the biochemical function of NifU proteins remains unknown, it has been shown that NifU exists as a homodimer, containing one redox 2Fe–2S cluster per subunit[5].

The carboxy-terminal domain, known to be the only common region of NifU between nitrogen-fixing bacteria and several rhodobacterial species[6,7], is shown here to be present in: the open reading frame (ORF) YKL253 of chromosome XI of yeast[8]; a hypothetical 22 kDa protein from *Haemophilus influenzae*[9]; and a short ORF (ORF2) in *Azotobacter vinelandii*[10] (Fig. 1b). Thus, *A. vinelandii* has two NifU-like proteins, one of which contains only the carboxy-terminal domain (Fig. 1a, b).

An internal domain of NifU proteins from nitrogen-fixing bacteria, not present in the so-called NifU proteins from two *Rhodobacter* species or the NifU-like protein from yeast, shows significant sequence similarity (Fig. 1c) to two internal repeats of nitrite reductases from *Klebsiella pneumoniae* (NasB)[11], *Escherichia coli* (NirB)[12], *Emericella nidulans* (NiiA)[13] and *Neurospora crassa* (Nit-6)[14], as well as to a single carboxy-terminal domain of nitrate reductase of *K. pneumoniae* (NasA)[11], the carboxyl terminus of NifE from *Bradyrhizobium japonicum*[15] and a short ORF (gp64)[16] from *E. coli*. Interestingly, the domain is present in NifE of *B. japonicum* at its extreme carboxy-terminal end and is absent from the other NifE homologues. In *E. nidulans*, the domain is encoded by the fifth exon of the *niiA* (nitrite reductase) gene[13]. This domain contains two pairs of conserved cysteines that might participate in the formation of the 2Fe–2S cluster in NifU and the other proteins identified (Fig. 1a). This cluster might serve as an electron carrier in the nitrate and nitrite reductases.

Modular proteins, frequently found in higher eukaryotes, are rare in prokaryotes, probably because of a lack of appropriate genetic shuffling mechanisms (see Refs 17, 18 and references therein). The mobility of the NifU-like domains in both prokaryotes and lower eukaryotes therefore comes as a surprise. It will be a challenge to identify the genetic mechanism(s) that enabled the various species to acquire the two domains. In addition, knowledge of the modular structure of NifU-like proteins might contribute towards elucidating the functions of their domains.

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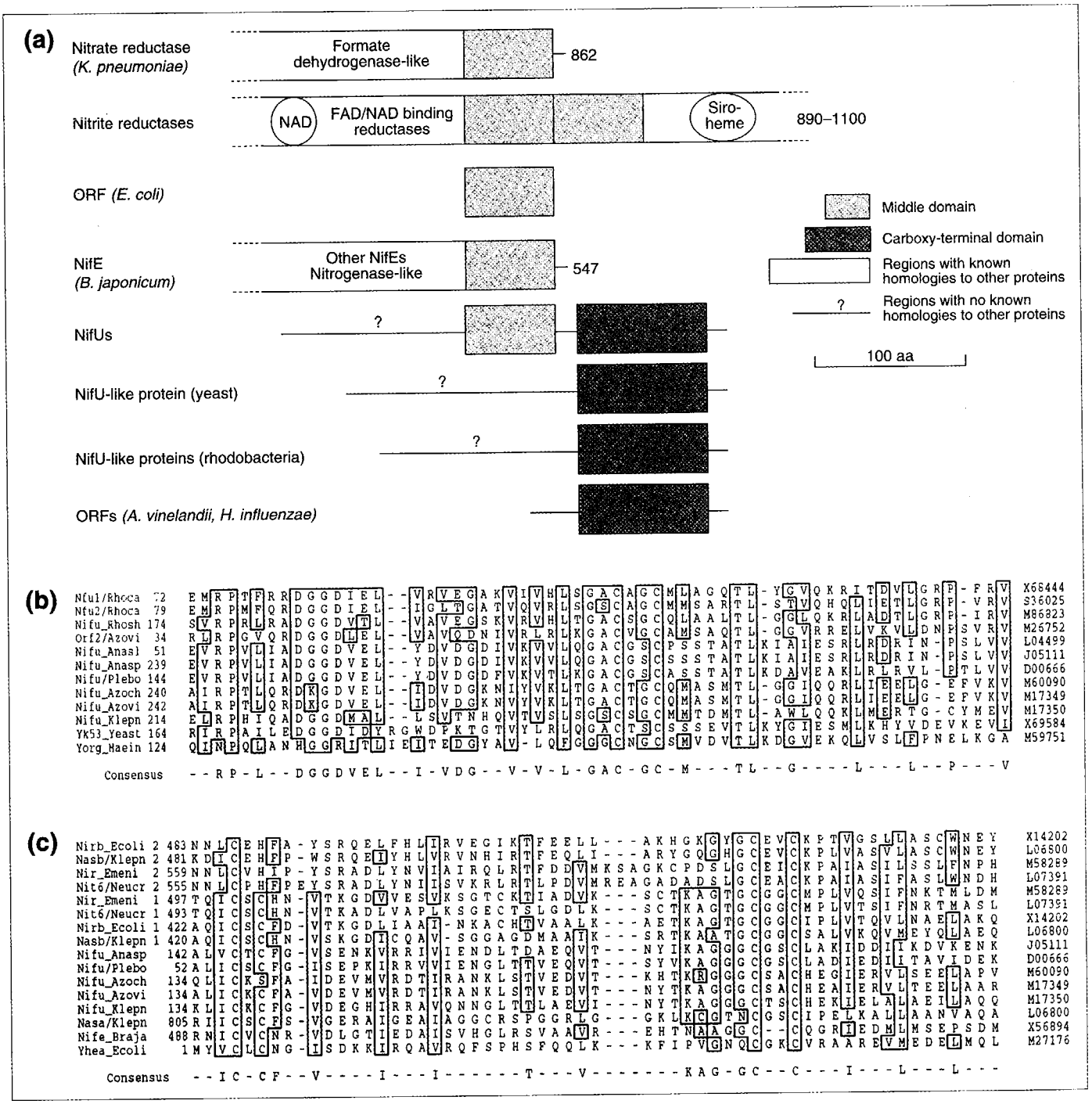

Figure 1

(a) Modular architecture of proteins that contain NifU-like domains. For large proteins (fragments only shown), the length of sequence shown is indicated. **(b)** Alignment of the approximately 65-residue carboxy-terminal domain. Proteins are indicated by their Swissprot names if available [these contain an underscore (_) in the entry code]. Nfu1/Rhoca and Nfu2/Rhoca, *R. capsulatus* Nfu1 and Nfu2; Nifu_Rhosh, *R. sphaeroides* Nifu; Orf2/Azovi, *A. vinelandii* ORF 2; Nifu_Anas1, Nifu_Anasp, Nifu/Plebo, Nifu_Azoch, Nifu_Azovi and Nifu_Klepn, NifU proteins from *Anabaena* (two species), *Plectoneme boryanum*, *A. chroococcum*, *A. vinelandii* and *K. pneumoniae*, respectively; Yk53_Yeast, yeast NifU-like protein; Yorg_Haein, *H. influenzae* hypothetical 22 kDa protein encoded by ORF G. The position of the domain within the corresponding proteins is indicated, as is the EMBL database accession number. Residues conserved in more than half of the sequences are boxed and displayed as consensus (bottom line). The newly identified domains show significant sequence similarity to known NifU proteins (amino acid identity: Yk53_Yeast, 28–36%; Yorg_Haein, 24–33%; Orf2/Azovi 35–50%). Database searches with the candidate proteins result in BLASTP P-values[19] that are below $10e \times 10^{-6}$ for at least one NifU. In order to verify these similarities, various sequence analysis methods were used including profile and pattern searches[20]. **(c)** Alignment of the approximately 60-residue middle domain. Nirb_Ecoli, *E. coli* nitrite reductase B; Nasb/Klepn, *K. pneumoniae* nitrite reductase B; Nir_Emeni, *E. nidulans* nitrite reductase; Nit6/Neucr, *N. crassa* nitrite reductase; Nasa/Klepn, *K. pneumoniae* nitrite reductase A; Nife_Braja, *B. japonicum* Nife protein; Yhea_Ecoli, *E. coli* hypothetical protein encoded by the *bfr* 3'-region. The newly identified domains show significant sequence similarity to known NifU proteins as follows: Nasa/Klepn, 19–30%; Nirb_Ecoli domain 1 (as an example of nitrite reductases from prokaryotes and fungi), 21–38%; Yhea_Ecoli, 19–26%; Nife_Braja, 22–38%. The similarities were verified as in **(b)**.

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