neurons derived from early-postnatal mouse cerebellum and cultured on a layer of poly-L-lysine or laminin. Neurite outgrowth was inhibited considerably in both systems when high-mannose-type oligosaccharides were added to the culture medium[8]. The inhibitory activity of a preparation of high-mannose-type neoglycolipids was greater than that of the free oligosaccharides, in accord with the multivalent micellar state that neoglycolipids adopt in solution. No inhibitory effects were observed in the presence of unrelated oligosaccharides: galactose–*N*-acetylglucosamine–mannose (tested also as a neoglycolipid); the galactose-terminating tetrasaccharide lacto-*N*-tetraose; and glycopeptides derived from asialofetuin, which contain complex-type galactose-terminating oligosaccharides, were all negative. If, as implied[8], carbohydrate-mediated binding of NCAM to L1 is a key requirement for processes leading to neuronal outgrowths, then this *cis*-interaction (co-aggregation) system could be one of the first tangible examples of networks or operational units[14] within cell membranes based on lectin–carbohydrate interactions. These interactions could turn out to mediate cell-signalling pathways[15] with points of control at the level of availability of binding proteins and correct glycosylation of target proteins.

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FMN- or DNA-binding?

A highly significant similarity between *Escherichia coli* tryptophan repressor-binding protein (WrbA), yeast protein YCP4 (Ycr4w), p25 from *Schizosaccharomyces pombe* and a putative protein from rice has recently been reported, and a modified helix–turn–helix (HTH) DNA-binding domain has been tentatively identified at their carboxyl termini[1]. However, our systematic analysis of yeast chromosome III gene products suggested a different activity for these proteins[2]. We found that the above four proteins show significant similarity to flavodoxins, with three conserved motifs, including the flavin mononucleotide (FMN)-binding site (Fig. 1). In order to further explore the conservation of the FMN-binding site, we constructed a position-dependent weight matrix from the respective portion of the alignment of the WrbA family with four related flavodoxins (Fig. 1). An iterative database search (R. L. Tatusov, S. F. Altschul and E. V. Koonin, unpublished) using this matrix showed that, in addition to known flavodoxins, the FMN-binding motif is conserved in two putative proteins from *E. coli*: MioC and YihB (Fig. 1). Our subsequent database screening using BLAST[3] showed that MioC clusters with the flavodoxin domains of cytochrome P450 reductases

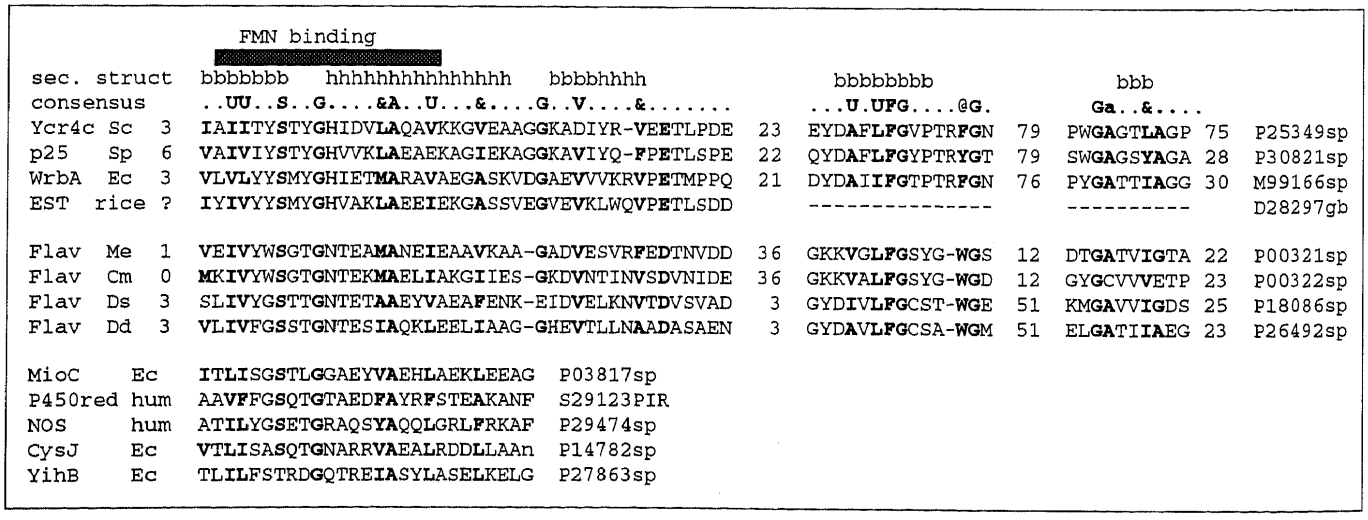

Figure 1

Conserved sequence motifs in the WrbA-family proteins and other flavodoxin domains. For the proteins of the WrbA family and selected flavodoxins from Gram-negative and Gram-positive eubacteria and Archaea, the alignment of three conserved motifs is shown. For the two amino-terminal motifs, the probability of obtaining the alignment by chance was below 10^{-7} as computed using the MACAW program[6]. The consensus includes amino acid residues that are conserved in all or all but one of the aligned sequences [U, aliphatic residues; @, aromatic residues; &, hydrophobic residues (either aliphatic or aromatic); dot, any residue]. The location of the FMN-binding site and the secondary structure are from the experimentally determined three-dimensional structure of clostridial flavodoxin (3FMN in PDB: b, β-strand; h, α-helix). Numbers indicate the distances between the motifs and the distances from the protein termini. P450red is cytochrome P450 reductase; NOS is nitric oxide synthetase; and CysJ is sulfate reductase. A database accession number is given for each sequence (sp, Swissprot; gb, Genbank). EST, Expressed Sequence Tag; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Ec, *E. coli*; Cm, *Clostridium mp*; Me, *Megasphaera elsdenii*; Ds, *Desulfovibrio salexigenes*; Dd, *Desulfovibrio desulfuricans*.

and nitric oxide synthetases, with a probability of about 10^{-7} of matching the sequence of human P450 reductase by chance (Fig. 1; data not shown). The *mioC* gene is located near the *oriC* site and has been implicated in the regulation of chromosomal replication initiation, although no direct functional information on the protein is available[4]. These findings emphasize the variety of flavodoxin domains and their possible functions.

We found it difficult to confirm the presence of the HTH domain in the proteins of the WrbA family. A position-dependent matrix constructed from the respective portion of the alignment of WrbA, YCP4 and p25 failed to select any of the HTH proteins from the database; conversely, a matrix derived from 28 HTH domains[5] did not detect any of these proteins. In addition, the two positions around the conserved glycine (see figure in Ref. 1) are occupied by polar residues, unlike the HTH proteins, all of which contain hydrophobic residues. Thus, the WrbA-family proteins do not contain typical HTH domains.

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Grandori and Carey reply

In a paper recently submitted to *Protein Science*, we report findings in considerable agreement with those of Drs Koonin and Bork. By profile analysis[1], we identified the same group of six new flavodoxin-like sequences. We extended the analysis to the entire sequence length and to structural prediction, finding strong support for the hypothesis that these proteins share the common core of the three-dimensional structure of flavodoxins. Although our model does not make a unique prediction about the conformation of the carboxy-terminal region of the WrbA family, and FMN- and DNA-binding functions need not be considered mutually exclusive, we agree that the collective new evidence does not support a DNA-binding function for the WrbA family.

However, we note a difference between our results and those of Drs Koonin and Bork with implications for structural and functional prediction: one region of the proposed new flavodoxin-like sequences (DAIIFG in WrbA) corresponds to strand β4 of the known structures of Flav Cm[2] and Flav Me[3] in their alignment, but to β3 in ours. This difference is most probably due to ambiguities deriving from the presence of a similar sequence pattern in strands β3 and β4 of flavodoxins. Indeed, the analyses by ourselves and others[4–6] suggest that residues of Flav Ds and Flav Dd in that region of their alignment can be assigned to strand β3. This difference affects prediction of the residues that would contact FMN. Analysis of flavodoxin structures indicates that the FMN-binding site is not segregated to residues of the amino-terminal region, and that the loops following strands β3 and β4 play a critical role, providing the residues (M56 and W90 in Flav Cm; Ref. 2) that bracket the isoalloxazine ring from the protein and the solvent sides, respectively[7]. The alignment by Drs Koonin and Bork does not imply an M56 homolog in the WrbA family, and suggests that F79 of WrbA corresponds to W90. Our alignment suggests that R78 of WrbA corresponds to M56, and Y142 to W90.

Nevertheless, our interpretation stresses that the reported sequence similarities to flavodoxins lead to structural, more than functional, insights. This point applies particularly to the WrbA family, as MioC and YihB show better conservation of the critical residues contacting FMN. As Drs Koonin and Bork also indicate, only one of three invariant hydroxyls that form hydrogen bonds to the phosphate group of FMN in flavodoxins (S7, T9 and T12 in Flav Cm; Ref. 2) is conserved in all six new sequences (S9 in WrbA). Substitution of these functionalities among known FMN-binding proteins indicates that alternative networks of hydrogen bonds are likely, but at the same time this incomplete conservation weakens the power of functional prediction by sequence analysis alone. Our study also suggests that residues W90 in Flav Cm and Y142 in WrbA, which would serve the same function, probably map to topologically nonequivalent loops, deriving, in the WrbA family, from a proposed additional β-strand. Still, we think that the major argument supporting prediction of an FMN-binding function in the WrbA family is the overall sequence similarity to flavodoxins, and that demonstration of an FMN-binding site may reveal interesting differences from the flavodoxins.

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