Neuronal nitric oxide synthase contains a discs-large homologous region (DHR) sequence motif

ZO-1, the major protein component of tight junctions, PSD-95/SAP90, a constituent of synaptic junctions, and the *Drosophila* discs-large tumour-suppressor gene product DlgA, present in septate junctions, are all membrane-associated guanylate kinase homologues [1]. Besides the guanylate kinase-like domain, these proteins all contain an SH3 domain and, at their N-terminus, three copies of an 80-amino-acid motif called GLGF repeat [2] or DHR (for Discs-large Homologous Region [1]). This motif was suggested to play a role in targeting the protein to the sub-membranous cytoskeleton or in regulating the intrinsic activity of the enzyme [2]. Recently, murine (PTP-BL) (W. Hendriks, J. Schepens, D. Bächner, J. Rijss, P. Zeeuwen, U. Zechner, H.

Hameister and B. Wieringa, unpublished work), bovine (BA14) [3] and human (PTP-BAS) [4] cDNA clones have been obtained that encode a protein-tyrosine phosphatase containing five such DHR motifs in between a band-4.1-like domain and the catalytic phosphotyrosine phosphatase domain. The band-4.1-like domain, also present in the neurofibromatosis type 2 tumour suppressor and the *Drosophila* tumour suppressor expanded [5], is regarded as being a plasma-membrane-binding sequence, and the band-4.1-like sequence of PTP-BL had indeed been shown to target the protein to the cytoplasmic side of the cell surface membrane (W. Hendriks, J. Schepens, D. Bächner, J. Rijss, P. Zeeuwen, U. Zechner, H. Hameister and B. Wieringa, unpublished work). Expression of only the five DHR motifs from PTP-BL revealed a more general association to cytoplasmic faces of (intra)cellular membranes.

To detect other proteins that contain DHR motifs, sequence

Figure 1 Comparison of DHR-containing proteins

(a) Schematic representation of DHR-motif-containing proteins. DHR motifs (dotted circles), band 4.1-like domains (hatched bars), tyrosine phosphatase domains (black boxes), SH-3 domains (open bars), guanylate kinase domains (cross-hatched bars), and calmodulin (C), FAD (F), FMN (FM) and NADPH (N) binding sites are indicated. Abbreviation: aa, amino acid. (b) Multiple sequence alignment of DHR from various proteins. DHR-containing proteins were identified in the Swiss-Prot database (release 28) by using the pattern GLGFx(I,L,V)xGGx{16,30}DGx(L,I)xGDx(I,L,V), inferred from known proteins with DHR motifs (DlgA, ZO-1, ZO-2, PSD95, p55 and PTP-BL), allowing four mismatches. The indicated segments of *Drosophila* DlgA (accession no. P31007), mouse ZO-1 (D14340), dog ZO-2 (L27152), rat PSD-95 (P31016), human p55 (Q00013), mouse PTP-BL (Z32740), human PTPH1 (P26045), human MEG (P29074) and human neuronal NOS (P29475) were used. The alignment obtained of the nNOS segment with DHR motifs were judged as highly significant ($P < 0.00005$) by using a sophisticated shuffling method derived from program RDF2 [6]. On average, 26% sequence identity with other DHR motifs was observed for the nNOS DHR motif, and sequence similarity ranged from 38% to 51%. The highest similarity for the nNOS DHR motif was with the third motif in PTP-BL (38% identity). Database searches, alignments and consensus calculations were all done with the GCG-Wisconsin package. Amino acid residues that contribute to the consensus are shown in capitals, and are in the one-letter code. Dots indicate the gaps introduced due to the alignment procedure.

databases were searched, and three additional proteins were found (Figure 1). The protein-tyrosine phosphatases MEG [7] and PTPH1 [8] were also found to contain a previously unnoticed single DHR motif located in between the band-4.1-like domain and the catalytic phosphatase domain. The third protein was neuronal nitric oxide synthase (NOS). Three NOS isoforms have been identified to date: neuronal NOS, endothelial NOS and macrophage NOS [9,10]. NOS transforms arginine into citrulline and the powerful biological messenger NO. NO is a simple gas with free-radical chemical properties and corresponding short biological half-life and diffusion distance. Production of NO by NOS close to the membrane could increase the efficiency in exerting paracrine effects on adjacent cells. Indeed, endothelial NOS is membrane-associated, due to N-terminal myristoylation [11]. The other two NOS isoforms do not contain a putative N-myristoylation consensus sequence. Data are accumulating, however, that neuronal NOS is also membrane-bound [12], although the nature of the membrane association remained unclear. The single DHR motif present in the N-terminal extension of neuronal NOS is absent from the other isoforms, and might be responsible for this direct or indirect association

with the membrane, thereby enabling the enzyme to produce NO close to its potential targets.

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