substrates, including leuteinizing hormone, substance P and extracellular matrix proteins. It can be seen from Fig. 1 that the MATH domain of meprins is located in a region of unknown function between the epidermal growth factor (EGF)-like domain and the MAM domain (a motif found in meprins, neuropilin and receptor tyrosine phosphatases).

As meprins are extracellular and TRAFs are intracellular, it is curious that the MATH domain has been conserved. In TRAF proteins, the TRAFC (MATH) domain appears to be a protein–protein interaction motif required for the binding of TRAFs to TNFR2, CD40 and TRADD, as well as for TRAF–TRAF interactions. This suggests that the conserved MATH domain of meprins might also act as a protein–protein interaction motif. Thus, the MATH domain might allow dimerization (in addition to the disulphide linkages), or else be involved in the binding of meprins to other, as yet unidentified, partners, some of which might turn out to resemble the proteins that bind TRAFs.

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Pleckstrin's repeat performance: a novel domain in G-protein signaling?

Recognition of two repeats in pleckstrin, a major protein kinase C (PKC) substrate in platelets, and the subsequent identification of numerous pleckstrin-homology (PH) domains in a diverse range of signaling and cytoskeletal proteins has initiated numerous functional studies on PH domains and their host proteins. PH domains are now known to bind lipids and/or G-protein β/γ subunits and/or PKC isoforms (reviewed in Ref. 1). Functions for the pair of PH domains in pleckstrin have been suggested[2], but little is known about the intervening region of the protein. We report here the identification, by sequence analysis, of a globular domain in the central portion of pleckstrin that also can be found in a variety of other proteins involved in signaling pathways (Fig. 1a). As the novel domain is present in well-studied proteins such as *dishevelled* gene product (dsh) and egl-10, as well as pleckstrin, we shall refer to it as the DEP domain.

Drosophila dsh, like pleckstrin, is hyperphosphorylated[3] and appears to have a general role in signal transduction,

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2D structure:          eeee      eeee      hHHHHHHHHH          hhHHHHHHHHHHHHHh eeEEe              eEEEe    EEEEE
P47_Human      136 TEKGIKEL-NLEKDKKIFNHCFTGNCVIDWLVSNQS-------VRNRQEGLMIASSLLNEGYLQPAGDMSKSAVDG---TAENPFLDNPDAFYYFPD  sw:P08567
Plel/Pig         ? SSSGIRPS-PNMEQGSTYKKTFIGSSLVDWLISNSF-------AASRLEAVTLASMLMEENFLRPVGARSTGAIRSGDLVEQVSWMT-SRALYTFXX  em:F14527
Yj9g_Yeast    1198 GEDRITLV-NRKWHWKKHEKCFVGSEMVNWLIRNFS------DIDTREDAIKYGQKVMKEGLFVHV------------LNKHNFLD-GHYFYQFSP  sw:P47170
T08a11/Caeel  1492 PYVGLKNTNEKQMSVAHPANMFVVYDFALWLRYNVE------EISKFEIAVNLIRRLADSKFIQVITSKGKYGSESS--LNSKKMFK-FKAEYGFQL  em:Z50875
Dvl-2/Mouse    433 PESGLEVR-DRMWLKITIPNAFLGSDVVDWLYHHVE------GFPERREARKYASGLLKAGLIRHT------------VNKITFSE--QCYYVFGD  em:U24160
Rack8/Human      ? PESGLEVR-DRMWLKITIPNAFIGSDVVDWLYHNVE------GFTDRREARKYASNLLKAGFIRHT------------VNKITFSE--QCYYIFGD  em:U48252
xdsh/xenla     428 PESGLEVR-DRMWLKITIPNAFLGSDVVDWLYHHVE------GFQDRREARKFASNLLKAGFIRHT------------VNKITFSE--QCYYIFGD  em:U31552
Dvl-1/Human    400 PDSGLEIR-DRMWLKITIANAVIGADVVDWLYTHVE------GFKERREARKYASSLLKHGFLRHT------------VNKITFSE--QCYYVFGD  em:U46461
Dvl-1/Mouse    421 PDSGLEIR-DRMWLKITIANAVIGADVVDWLYTHVE------GFKERREARKYASSMLKHGFLRHT------------VNKITFSE--QCYYVFGD  em:U28138
Dsh/Drome      401 PDSGLEIR-DRMWLKITIPNAFIGADAVNWVLENVE------DVQDRREARRIVSAMLRSNYIKHT------------VNKLTFSE--QCYYVVNE  em:U02491
T05c12/Caeel   427 PDSGLAVK-NRKWLKIPIPMSFLGTDLVEWLVKHVQ------GIHNRKKARIYAARLLAAGLIRHV------------VSKLTFTE--KCYYVFGD  em:Z66500_5
C34f11/Caeel   597 PGSGLDIK-NRTWLKIPIPMSFLGSDLVEWLLDHVE------GLRERKTARNFAADLLKLKYIAHV------------VNKVTFTE--QCYYVLGD  em:U46753_10
C27a2.6/Caeel  488 EGSGLDIK-DRYWFKIPIPMSFLGTDLVEWLVKHVQ------GLETKKKAREFAEEMLKLGYIRPG------------VGKQSFTK--ECYYVMGD  wo:CE04106
Ymf4_Caeel      23 FRSNLSLK-TNRRGLATAKETFSGRAAVGFLMIEIP-KMIPNKVPERDKMQKFVEFMVDMNVISEAYPKK--------DQKRRPFSN--ARIYLFMK  sw:P34464
Ya8c_Schpo     232 PVYSVSSPSPKDSLSKVTKYQMFGIDIAEWLMCNTM-------LLDWSEMETVASDLLIHSYIAYENNSETPL--------KFSYAK-GVSYFLTGK  sw:Q09777
F1ba_Emeni     427 VGVKMARE--RKVGDKICANTFTGKAAVDWLMDCST-------TIEPRETVLIAELFVKYGLITVLQEDRSMPQV---ENSLVAFQPSKNAIYAITE  sw:P38093
Sst2_Yeast     279 LFENKTFG---TSKKIVIKYTFTTKAIWQWIMDCTD-------IMHVKEAVSLAALFLKTGLIVPVLLQPSRT-----DKKKFQISR--SSFFTLSK  sw:P11972
Egl10/Caeel     37 AEAGVPIK-TVKSFLSKVPSVFTGQDLIGWIMKNLE-------MTDLSDALHLAHLIASHGYLFQIDDHVLTVKND----GTFYRFQ---TPYFWPS  em:U32326
Rgs7/Human       ? ...........SFLSKIPSVFSGSDIVQWLIKNLT-------IEDPVEALHLGTLMAAHGYFFPISDHVLTLKDD----GTFYRFQ---TPYFWPS  em:R17653*
Ynx5_Caeel     431 DNHQVIRD--ITTEHTRVQNCMIGAEMIDWLLTLFVSTSTTCSSLSRIQMSAIWQVLLNNGLISHIDG----------EHQFLDKTN---SYYRWVQ  sw:P34578
H29208/Human     ? HSSGMEFQ-XHRYWLRTHPNCIVGXELVNWLIRNGH------IATRYSXLRRFXYYLXXLNXXXYL--------------KIVRVT---DF.....  em:H29208
Ya2i_Schpo     215 QEIPLQDY--RVPILGTYPNTCSGNIIVSWLQENLP-------VPTLVAAEAFGQDLIAQGFLRHMGVGG-SFVNS--TNFHYQWKD---KAFQFAG  sw:Q09697
Yfe7_Yeast     218 PKTDYKLP----LISYSLSNTNNGGEITKFLLDHMS-------LKDIDQAETFGQDLLNLGFLKYCNGVGNTFVNS--KKFQYQWKN---TAYMFAN  sw:P43556
F14996/Human     ? .....................GCDLVNWLIEVGL-------ASDRGEAVIYGDRLVQGGVIQHI------------TNEYEFRD---XYLFYRF  em:F14996
consensus:          tttLth     h hhhht shhGtthhtWLht h        h th th  hht hhttthh                 th h t   shYahtt
```

Figure 1

Alignment of DEP domains from diverse signaling proteins. First column: names/species (P47, pleckstrin; Plel, pleckstrin-like; Dvl, mammalian homologues of *Drosophila* dsh); second column: position of the displayed regions in their respective sequences; right column: database accession numbers; em, EMBL; sw, SWISS-PROT; wo, WORMPEP. The secondary (2D) structure elements were predicted using the PhD server[11]: H/h denotes an α-helix and E/e, a β-strand with an expected accuracy higher than 82% (upper case)/72%(lower case). Amino acids conserved in at least 60% of the sequences are in red; hydrophobic residues conserved in all but two sequences are in green. The consensus line summarizes residue properties conserved in at least 80% of all sequences (t, turn-like or polar; h, hydrophobic; s, small). The conserved glycine at the amino cap of the first predicted helix is mutated in an allele of egl-10 with defective function[6]. Complementary methods (for details see Ref. 5) including iterative profile and motif searches using SEARCHWISE[12] and MoST[13] identified the DEP domains shown. Despite the wide species range, analysis using MACAW[14] indicated the significance of the alignments, e.g. two distinct sequence blocks yielded P-values of 2×10^{-11} and 6×10^{-5} in six of the most divergent family members (FlbA, Dvl-1, Yj9g, Ya2i, pleckstrin and Ynx5). The sequence indicated with an asterisk has been combined with U32439.

PII: S0968-0004(96)30027-3

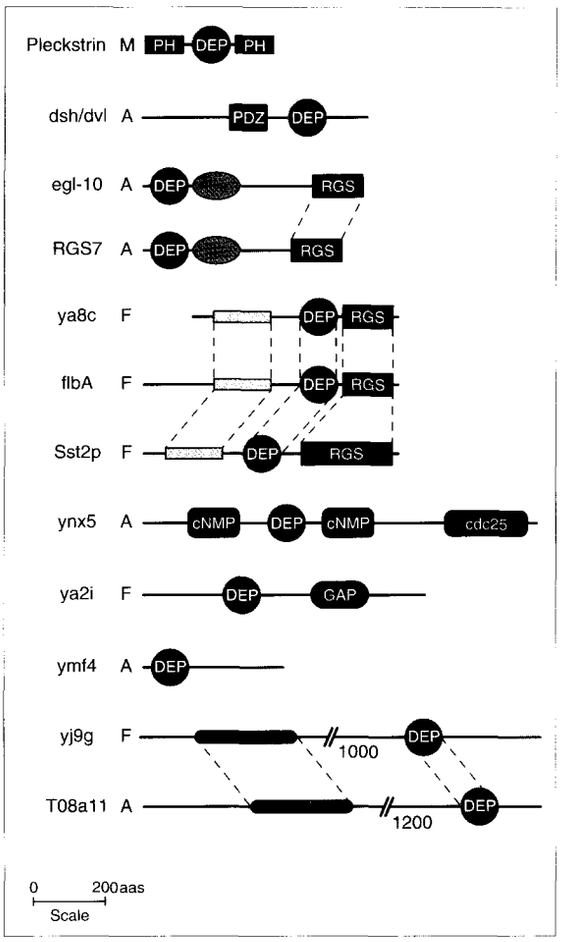

Figure 2

Domain organization of proteins that contain a DEP domain. Abbreviations used: A, animals; F, fungi; M, mammals. The DEP domains are 70% identical between RGS7 and egl-10 and only 20% between yj9g and T08a11. Within the families of proteins with similar modular architecture, DEP appears to be the most conserved domain; its presence in divergent eukaryotes including yeast also points to an important functional role.

although its molecular functions remain obscure. Analysis of the modular architecture of dsh reveals a significant sequence similarity to a region in Yj9g, a putative *Saccharomyces cerevisiae* open reading frame (BLASTP P-value for the probability of a chance hit $P < 10^{-7}$; Ref. 4). The remaining putative DEP-domain-containing proteins, including pleckstrin, were identified using a variety of iterative motif and profile searching procedures (Fig. 1a; for details of the search strategy used, see Ref. 5). The DEP domain is approximately 80 residues in length and structural predictions indicate it to be a globular domain with an $\alpha + \beta$ topology (Fig. 1a).

The domain organizations of the proteins identified (Fig. 1b) point to a role for DEP domains in regulating GTP–GDP exchange by Ras-like molecules: DEP domains occur in a guanine nucleotide dissociation stimulator (GDS) homologue (Ynx5), in two GTPase-activating protein (GAP) homologues (Ya2i and Yfe7), and in five regulator of G-protein signaling (RGS) proteins (flbA, Sst2p, Ya8c, egl-10 and RGS7). These latter molecules each contain a 'RGS' domain[6–9], which in human GAIP interacts directly with the G protein $G_{\alpha i3}$ (Ref. 10). Thus, unlike GDS-, GAP- and RGS-domain families, DEP domains probably do not interact directly with Ras-like GTPases. Rather they might regulate interactions between GDS, GAP or RGS domains and G proteins by co-localizing other, perhaps downstream, components of signaling pathways.

RGS proteins and pleckstrin are both known to be negative regulators of critical signaling pathways. RGS-domain-containing proteins negatively regulate both mammalian mitogen-activated protein (MAP) kinase activation by G-protein-linked receptors and the analogous pathway in yeast[7]. Similarly, pleckstrin has been shown to negatively regulate signaling pathways through its inhibition of phosphoinositide hydrolysis by a mechanism at or below the level of G proteins in the signaling pathway[2]. In both cases, inhibition is mediated by domains other than DEP (by RGS and PH domains, respectively), yet the presence of DEPs in these proteins indicates their participation in an additional level of regulation, perhaps by sequestration of additional molecules to sub-membranous complexes. A human dsh-like protein sequence fragment that contains a DEP domain (RACK8), and that is reported to bind PKC, has been deposited in databases (Kuroda *et al.*, unpublished; EMBL database accession number U48252). Thus, DEP, like the PH domain, is a candidate PKC-binding domain. DEP therefore can fulfil an important regulatory role complementary to the RGS domain.

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Corrigendum

In the May issue of *TiBS*, we published the Review article 'Iron–sulfur clusters as biosensors of oxidants and iron' by Tracey Rouault and Richard Klausner (*TiBS* 21, 174–177). A citation error in the legend of Fig. 2 has been brought to our attention. In the description of part **(b)**, the entrapped polysulfides should have Refs 30 and 35 associated with them and not 33 and 35 as printed.

We would like to apologize to our readers for this mistake.