Hypothesis

The new MATH: homology suggests shared binding surfaces in meprin etramers and TRAF trimers

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Abstract Although apparently functionally unrelated, intracellular TRAFs and extracellular meprins share a region with conserved meprin and traf homology, MATH[1]. Both TRAFs and meprins require subunit assembly for function. By structural analysis of the sequences, we provide an explanation of how meprins, which form tetramers, and TRAF molecules, which form trimers, can share homology. Our analysis suggests it is highly likely that the same oligomerization surface is used. The analysis has implications for the widely distributed group of proteins containing MATH domains.

Key words: Membrane; Interdomain surface; Metalloendopeptidase; Tumor necrosis factor receptor

Meprins are tissue-specific metalloendopeptidases implicated in developmental, normal and pathological processes by hydrolyzing a variety of peptides and proteins [2–4]. Tumor necrosis factor (TNF) receptor-associated factors, or TRAFs, regulate cell growth signalling and apoptosis by interacting with membrane-bound receptors through their TRAF-C domains [5,6]. The MATH motif, defined by the homology between TRAF-C domains of TRAF proteins and a C-terminal region of meprins A and B [1], thus links proteins that appear functionally unrelated. Despite the different states of oligomerization of TRAFs and meprins, a homology noted here suggests unexpected commonalities of subunit interaction.

Both TRAFs and meprins require subunit assembly for function. Trimerization mainly by the TRAF-N trimeric coiled-coil motif and by TRAF-C domain interactions appears crucial for establishing appropriate connections to form signalling complexes with TNF receptor-1 and related death receptors [7–10]. Meprins A and B form membrane-bound tetrameric complexes, dimers of heterodimers, through interactions between MAM domains N-terminal to the MATH motif [11,12], and the tetrameric state was also observed within the secreted meprin A homo-oligomer [13]. A meprin mutant incapable of forming the tetrameric state lost activity toward proteins, but could still hydrolyze peptide substrates [12].

Residues in meprins A and B that are highly similar with TRAF-C domains are located throughout the β-barrel, including the eighth strand which was only recently included in the MATH motif [14] (Fig. 1). Buried residues in TRAF-C are well conserved in meprins, suggesting a conserved domain interior, and as meprin insertions align to surface-exposed regions of TRAF-C they are not likely to require an altered fold (Figs. 1, 2A).

Residues involved in trimerization are surprisingly well conserved between TRAFs and meprins (Fig. 1), suggesting that the oligomerization surfaces could be common to both the TRAF trimer and the meprin tetramer. The conserved interfacial residues cluster at the outer trimer interaction surface whereas inner residues, including the critical Y386 [7,8], are not conserved (Fig. 2A). A tetrameric arrangement of meprin domains (Fig. 2B) can be postulated that uses only the outer cluster of interfacial contacts. The coiled-coil region (TRAF-

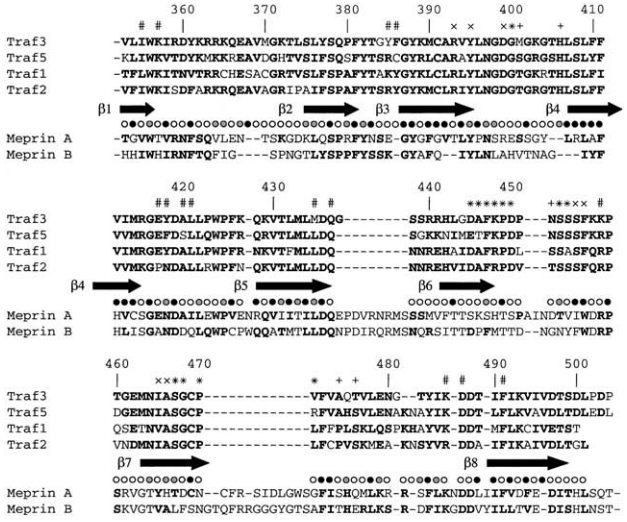

Fig. 1. Alignment of meprins A and B with TRAF-C domains of human traf1, 2, 3 and 5, which all bind to the same receptor and are thus likely to have the same fold and oligomerization state. Numbering is that of human TRAF-2. Arrows indicate β-strands in TRAF-2 [8]. Boldfaced residues show identities or conservations (I/L/V/F/Y, K/R/Q/N, D/E, and T/S) between TRAFs and meprins. The degree of amino acid surface exposure in a TRAF2 TRAF-C monomer is indicated by filled (< 5%), gray (5–15%) or open (> 15%) circles. Labellings indicate TRAF2 interactions in the trimer [8] (#), as well as receptor interactions with TNF-R2 [8] (+), TRADD [9] (×) or both (*).

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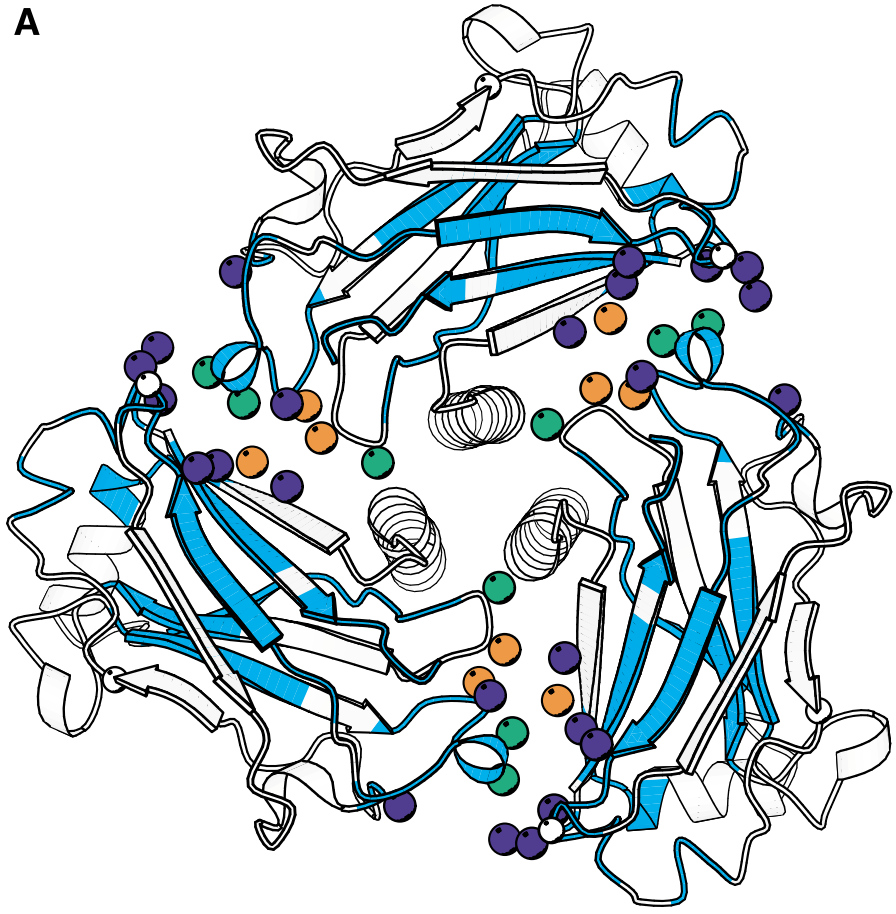

Fig. 2. A: The TRAF-N/TRAF-C trimer in TRAF2 [8]. Sky blue in the TRAF-C domain marks stretches of more than three residues that are 80% conserved between meprins A and B and the human sequences of TRAFs 1, 2, 3 and 5, which bind the same receptor. Residues in the TRAF-C domain of TRAF2 involved in trimerization [7,8] (# in Fig. 1) are highlighted with spheres, where residues conserved or identical with TRAF-Cs in (1) both meprins A and B are navy blue, (2) either meprin A or B is green, (3) neither meprin A nor B is yellow. Smaller white spheres indicate where insertions of more than three residues occur in meprins A and B vs the TRAF-C domain of TRAF2. B: Model for a tetrameric arrangement of MATH domains using coordinates from TRAF-C in TRAF2 [8]. The domains were manually adjusted to incorporate a fourth domain, while preserving the distances between conserved side chains in the outer interacting cluster (421, 491, 487, 458 and 435 [7,8]; ±2 Å) as well as the general orientation of MATH domains in the trimer arrangement. The annotation of spheres is as in a. Shading (pink) of the backbone indicates stretches of more than three residues that are non-identical between meprins A and B.

N) performs an integral structural function in TRAF proteins both by stabilizing trimeric interactions and by making intimate contacts with the TRAF-C domain [7,8]. The MAM domain would be able to perform a similar function in the meprin tetramer, where a flexible domain linkage is unlikely since secondary structure is consistently predicted in MAM directly adjacent to the meprin–TRAF homologous domain. Part of the MAM domain could be accommodated in the middle of the tetramer (Fig. 2B). Although meprins are extracellular and TRAFs intracellular, the C-terminal location of the membrane-spanning meprin B domain implies a similar orientation of the MATH oligomer relative to the membrane as that suggested for receptor-bound TRAF proteins [7]. This analogy between meprins and TRAFs supports the suggested involvement of meprins in signal transduction [4].

A poorly conserved region comprising residues of TRAF-C β-strands 6 and 7 houses the major ligand binding surface of TRAF proteins [7–10]. The corresponding meprin region is required for proteolysis of larger protein substrates by meprins [15], and could be involved in protein interactions as well for this purpose. Interestingly, meprins A and B differ significantly in this region (Figs. 1, 2B), suggesting distinct binding specificities for the two meprins if this region is involved in protein binding. Structure analysis of the meprin tetramer at a detailed molecular level is needed to evaluate the model and elucidate the role of sequence differences between meprins A and B in both the MAM and MATH homology regions.

In the diverse range of eukaryotic proteins containing MATH domains [14,16–18], surface residues buried by trimerization in TRAF proteins are variably conserved, suggesting that some of these proteins might also have distinct subunit assembly states. MATH domains in ubiquitin hydrolases, without any known multimerization domain, lack conserved interfacial residues. In *Caenorhabditis elegans*, MATH domains that are sequentially linked in arrays of two to six domains generally preserve both the inner and outer core of interfacial residues present in trimeric TRAFs. The human protein SPOP houses a MATH domain with only the outer interfacial core conserved, and is N-terminally linked to a POZ domain with homology to the tetramerization domain of the Aplysia Shaker protein [19]. The relative positioning of the oligomerization domain (N versus C) should be of little consequence, since both MATH domain termini can extend

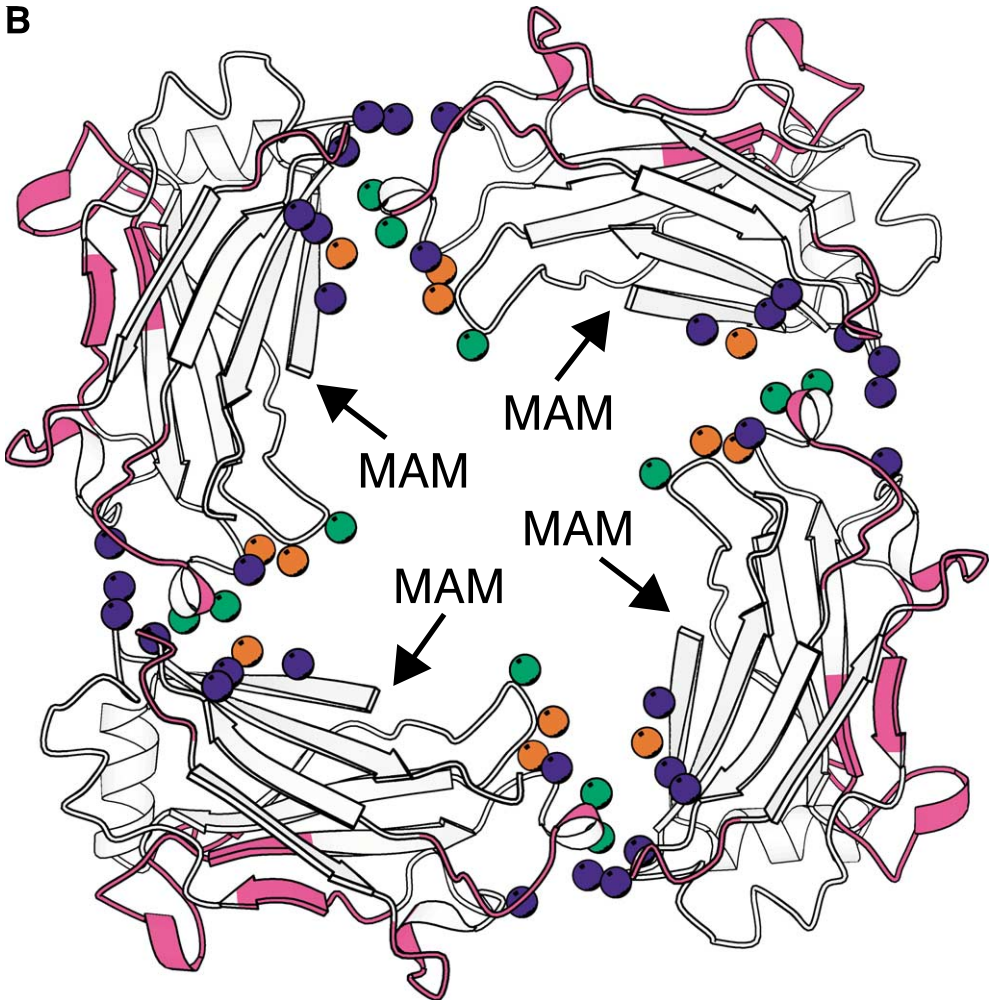

Fig. 2 (*Continued*).

towards the oligomerization center. Thus it appears that the MATH domain, as an independent folding unit, may take part in diverse modular arrangements defined by multimerization domains linked to it, as described previously for the winged HTH DNA binding motif [20]. A deeper understanding of the oligomerization of MATH-containing proteins awaits biochemical and structural evidence.

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