Correspondence

# A lipid-binding domain in Wnt: a case of mistaken identity?

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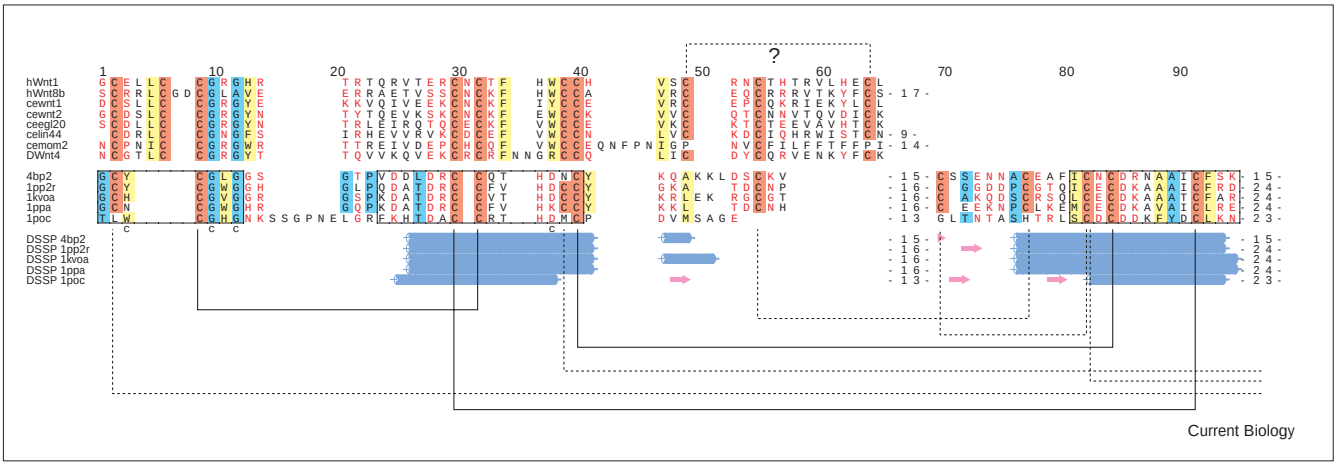
Several groups have recently presented convincing evidence for extensive membrane localisation during signalling between the secreted glycoprotein Wnt and its cell-surface receptor Frizzled ([1] and references therein). Aravind and Koonin [2] extended this by identifying a colipase-like domain in the Wnt antagonist Dickkopf. They suggested that this domain might be necessary for lipophilic interactions leading to membrane association during Wnt antagonism. They identified this colipase domain with PSI-BLAST [3], which found several highly conserved cysteine and other residues shared between the cysteine-rich Dickkopf protein and the colipase. This homology was subsequently backed up with a convincing alignment and a consideration of the known structure of the colipase. Recently, Reichsman *et al.* [4] have proposed a similar lipid-binding and membrane-localisation role in a region of Wnt proteins. Using BLAST, they identified a putative homology between the Wnt carboxyl terminus and the phospholipase A2 (PLA2) lipid-binding domain. Here, we analyse a diverse set of Wnt homologues and the known structures of PLA2s and argue against homology between Wnts and PLA2s.

PLA2s are a diverse superfamily of proteins. Three-dimensional structures are known for at least 15 species [5], and analysis of a structure-based sequence alignment [6] shows much variation across vertebrate and invertebrate PLA2s. Nevertheless, all known PLA2s contain a common core (boxes in Figure 1), comprising a calcium binding loop, two alpha helices and three conserved disulphide bonds (solid lines in Figure 1). The alignment of Reichsman *et al.* [4] corresponds to approximately half of the PLA2 core structure, and there is insufficient sequence in Wnt

**Figure 1**




Alscript [9] figure showing alignment of a selection of the carboxyl termini of Wnt homologues (top), PLA2s (middle) and DSSP [10] secondary structure assignments for the PLA2s (bottom; magenta arrows denote β strands; blue cylinders denote alpha helices). The alignment of Wnt homologues was performed using Clustal X [11]; alignment of PLA2 structures was performed with STAMP [6]. Boxed regions within the PLA2 homologues indicate regions of structural similarity [6]. Residue positions are coloured to denote conservation across Wnt or PLA2 sequences: red, polar; yellow background, hydrophobic; blue background, small; red background, cysteines. Disulphide pairings for PLA2s, taken from inspection of the three-dimensional structures, are shown below the alignment. Solid lines denote those that are common to all PLA2s shown; dashed lines denote those that are variable or not present in all structures. Note that some pairings involve cysteines that are not shown. A putative disulphide bond in Wnts, based on correlated Cys mutations, is shown as a dashed line above the alignment. Residues known to be involved in calcium binding in PLA2 are indicated by 'c' below the PLA2 sequences. Note that the Lys (K) at position 38 in sequence 1ppa is a site-directed mutation from Asp (D). Numbers within the sequences indicate sequence regions that have been deleted for clarity. Numbers given above the alignment refer to alignment position, and are meant only as a guide to the location of a particular set of aligned residues. Abbreviations and accession numbers: jWnt1, Human Wnt-1, P04628; hWnt8b, Human Wnt-8b, CAA71968; cewnt1, *Caenorhabditis elegans* Wnt-1, P34888; cewnt2, *C. elegans* Wnt-2, P34889; ceegl20, *C. elegans* egl20; AAD03603; celin44, *C. elegans* Lin44, A57234; cemom2, *C. elegans* mom2, AAC47749; DWnt4, *Drosophila melanogaster* DWnt4, P40589. PLA2 homologues are denoted by their four letter PDB [12] accession code, suffixed with a chain identifier where necessary.

homologues to form the carboxy-terminal portion of the PLA2 core. The alignment also shows that Wnts lack two pairings for the core disulphide bonds and an invariant calcium-coordinating aspartic acid residue (position 38 in Figure 1). Moreover, alignment of Wnt sequences to PLA2 places insertions in the first alpha helix, which would probably cause severe disruptions to the protein core. Few positions show conservation of residue properties (for example hydrophobic or small side-chain), and several cysteines found in Wnt homologues cannot easily be paired when put on to the PLA2 structure. We argue that these problems make homology and structural similarity between Wnt and PLA2 suspect. Instead, we suggest that the carboxy-terminal region of Wnt will adopt a different disulphide-rich structure. We cannot rule out the possibility that this region in Wnt is involved in lipid binding, but if this does occur, we suggest that it is likely to occur by a different mechanism.

The proposed homology between Wnt and PLA2 illustrates a common problem in the interpretation of results from sequence searches. The fact that the gapped BLAST [3] expect (E) value is ~$10^{-3}$ probably reflects a fortuitous match of cysteines. Exchange matrices used in programs like gapped BLAST (for example [7]) are largely derived from globular proteins, which usually contain few disulphide bonds. Extracellular, small, disulphide-rich proteins form an important subset of proteins for which alignments of cysteines should be treated differently. Mistaken homologies like that between PLA2 and Wnt and others (for example [8]) suggest that there is a pressing need to address this problem.

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## Phospholipases A2 and Wnts are unlikely to share a common ancestor

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Reichsman *et al.* [4] recently suggested that a carboxy-terminal region of Wingless/Wnt-1 and a lipid-binding domain found in class I and class II secreted phospholipases A2 (sPLA2s) are homologues. This proposal was based on 30–40% sequence identities among pairs of sequences approximately 50 amino acids in length drawn from both families and, additionally, the apparently significant expection (E) values from gapped BLASTP2 database searches. We were interested in evaluating further the significance of this intriguing prediction, given that pairs of sequences with 50 alignment positions in common can be structurally dissimilar, and therefore non-homologous, at up to 45% pairwise sequence identity [13].

Crystal structures show that the region of sPLA2 enzymes predicted to be homologous to Wingless/Wnt-1 corresponds to a non-compact amino-terminal fragment of the sPLA2 structure [14,15]. Moreover, the region covers only one of the two core alpha helices that are conserved between insect and vertebrate phospholipase A2 families [5,16]. As the region of proposed homology occurs at the carboxyl terminus of Wingless/Wnt-1, there is no possibility of extending the alignment to include these missing residues. The vertebrate sPLA2 structure contains seven disulphide bridges. Of these, only one is entirely contained within the suggested alignment of sPLA2 and Wnt-1. This means that either the remaining conserved cysteine residues in the proposed Wingless/Wnt-1 structure are unpaired or their disulphide-bridge pairings are not conserved among homologues. This scenario is unprecedented among conserved extracellular domain families. From this structural evidence, we conclude that the proposed region of homology with sPLA2 in Wingless/Wnt-1 represents an incomplete structural domain. As such, it is unlikely to fold in isolation.

Sequence similarity between sPLA2 enzymes and Wingless/Wnt-1 is mostly localised to cysteines that cannot participate in conserved disulphide bridges. Thus we conclude that the observed sequence similarities represent a random coincidence in the spacing of cysteine residues that are conserved separately by two distinct sequence families. This analysis indicates that the limited sequence similarities between sPLA2 enzymes and Wingless/Wnt-1 are not indicative of either a structural similarity or a common ancestry (homology). Consequently, we suggest that functional inferences based on the proposal of their homology should be treated with caution.

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## Susan Cumberledge, Frieda Reichsman and Helen M. Moore respond:

The alignments of sPLA2s and Wnts of Reichsman *et al.* [4] and Barnes and Russell [17] were generated using two different paradigms. Barnes and Russell compared the physico-chemical properties of the amino acid residues; we used the

Gonnet PAM 250 substitution matrix to evaluate amino acid similarities. PAM (percentage of acceptable point mutations) matrices have been widely recommended for detecting related proteins [18–20]. These substitution matrices are based on evolutionary relationships between proteins and are generated by analyzing point mutations in closely related proteins. Using the PAM matrices, we find that sPLA2 App-K49 residues 9–61 are more similar to the carboxy-terminal portion of Wingless than to the corresponding bovine sPLA2 sequences (44% versus 36% similarity). A total of 24 residues are conserved between sPLA2 and Wingless; 7 of these are cysteines. Note that App-K49 lacks the conserved Ala49, does not bind $Ca^{2+}$ and has no catalytic activity, yet retains the ability to bind membranes and disrupt phospholipid vesicles [21].

The first 60 residues of sPLA2 form the helix–loop–helix backbone that constitutes much of the lipid-binding pocket. The amino-terminal helix and loop are important for interfacial membrane binding, whereas residues in helix 2 contribute to the active site. As the rest of the App-K49 sequence has no similarity to Wingless, the two protein families probably have very different tertiary structures. Site-directed mutagenesis studies on sPLA2 [22] have shown that of the seven conserved disulphide bonds, only one, Cys29–Cys45, is required for lipase activity. Cys29 and Cys45 correspond to Cys428 and Cys445 in Wingless. These cysteines are conserved in the Wnts, and Cys445 is required for Wingless activity. Nonetheless, we agree that it is difficult to evaluate the significance of these sequence similarities without more information on Wnt function and structure.

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