

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 $\sim 10^{-3}$ 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 expectation (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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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].

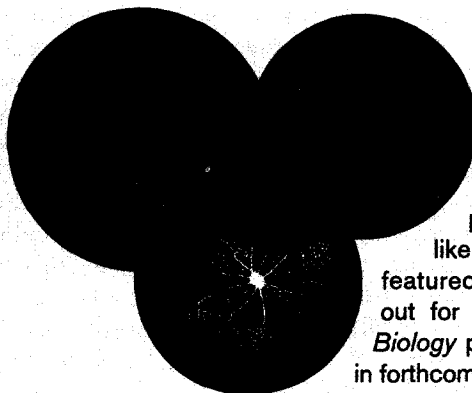
The first 60 residues of sPLA2 form the helix–loop–helix backbone that constitutes much of the lipid-binding pocket. The amino-terminal helix and loop are important for interfacial membrane binding, whereas residues in helix 2 contribute to the active site. As the rest of the App-K49 sequence has no 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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References

1. Reichsman F, Smith L, Cumberledge S: **Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction.** *J Cell Biol* 1996, **135**:819-827.
2. Aravind L, Koonin EV: **A colipase fold in the carboxy-terminal domain of the Wnt antagonists – the Dickkopfs.** *Curr Biol* 1998, **8**:R477-R478.
3. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res* 1997, **25**:3389-3402.
4. Reichsman F, Moore HM, Cumberledge S: **Sequence homology between Wingless/Wnt-1 and a lipid-binding domain in secreted phospholipase A2.** *Curr Biol* 1999, **9**:R353-R355.
5. Murzin AG, Brenner SE, Hubbard TJ, Chothia C: **SCOP: a structural classification of proteins.** *J Mol Biol* 1995, **247**:536-540.
6. Russell RB, Barton GJ: **Multiple protein sequence alignment from tertiary structure comparison: assignment of global and residue confidence levels.** *Proteins* 1992, **14**:309-323.
7. Henikoff S, Henikoff JG: **Amino acid substitution matrices from protein blocks.** *Proc Natl Acad Sci USA* 1992, **89**:10915-10919.
8. Johansson J, Curstedt T, Jornvall H: **Surfactant protein B: disulfide bridges, structural properties and kringle similarities.** *Biochemistry* 1991, **30**:6917-6921.
9. Barton GJ: **ALSCRIPT: a tool to format multiple sequence alignments.** *Protein Eng* 1993, **6**:37-40.
10. Kabsch W, Sander C: **Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features.** *Biopolymers* 1983, **22**:2577-2637.
11. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG: **The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.** *Nucleic Acids Res* 1997, **25**:4876-4882.
12. Bernstein FC, Koetzle TF, Williams GJ, Meyer Jr EE, Brice MD, Rodgers JR, Kennard O, Shimanouchi T, Tasumi M: **The Protein Data Bank: a computer-based archival file for macromolecular structures.** *J Mol Biol* 1977, **112**:535-542.
13. Rost B: **Twilight zone of protein sequence alignments.** *Protein Eng* 1999, **12**:85-94.
14. Dijkstra BW, Drenth J, Kalk KH: **Active site and catalytic mechanism of phospholipase A2.** *Nature* 1981, **289**:604-606.
15. Holland DR, Clancy LL, Muchmore SW, Ryde TJ, Einspahr HM, Finzel BC, Heinrichson RL, Watenpaugh KD: **The crystal structure of a lysine 49 phospholipase A2 from the venom of the cottonmouth snake at 2.0 Å resolution.** *J Biol Chem* 1990, **265**:17649-17656.
16. Scott DL, Otwinowski Z, Gelb MH, Sigler PB: **Complex structure of bee-venom phospholipase A2 in a complex with a transition-state analogue.** *Science* 1990, **250**:1563-1566.
17. Barnes MR, Russell RB: **A lipid-binding domain in Wnt: a case of mistaken identity?** *Curr Biol* 1999, **9**:R717-R718.
18. Schwartz RM, Dayhoff MO: In *Atlas of Protein Sequence and Structure*. Edited by Dayhoff MO. Washington, DC: National Biomedical Research Foundation; 1978, 5:353-362.
19. Collins JF, Coulson AFW, Lyall A: **The significance of protein sequence similarities.** *Comp App Biosci* 1988, **4**:67-71.
20. Altschul S: **Amino acid substitution matrices from an information theoretic perspective.** *J Mol Biol* 1991, **219**:555-565.
21. Dennis EA: **The growing phospholipase A2 superfamily of signal transduction enzymes.** *Trends Biochem Sci* 1997, **22**:1-2.
22. Zho H, Dupureur CM, Zhang X, Tsai MD: **Phospholipase A2 engineering. The roles of disulfide bonds in structure, conformational stability, and catalytic function.** *Biochemistry* 1995, **34**:15307-15314.

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