Enterobacterial lipocalins precede Vibrio homologue

The lipocalin protein superfamily was once restricted to eukaryotes, but the recent discovery of bacterial lipocalins has sparked interest in their evolutionary origins. A recent report by Barker & Manning, published in Microbiology, claims to have identified the first bacterial lipocalin in the form of a membrane lipoprotein of Vibrio cholerae known as VlpA (1). However, the first report of a bacterial lipocalin was published 2 years previously with the discovery of an outer-membrane lipoprotein of Escherichia coli known as Blc (for bacterial lipocalin) (2). The report of Blc from E. coli also described homologues from the closely related Citrobacter freundii as well as the V. cholerae VlpA, which was then present in GenBank. Independently, Flower and co-workers noted that VlpA belonged to the lipocalin superfamily (3) and a recent review discusses the importance of bacterial lipocalins for understanding lipocalin evolution (4).

Table 1. Identity and similarity of proteins

<table>
<thead>
<tr>
<th></th>
<th>EcBlc</th>
<th>CfBlc</th>
<th>VcVlp</th>
<th>SaLaz</th>
<th>HsApoD</th>
<th>MmApoD</th>
<th>RnApoD</th>
<th>OcApoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcBlc</td>
<td>100</td>
<td>(95)</td>
<td>(71)</td>
<td>(44)</td>
<td>(57)</td>
<td>(59)</td>
<td>(60)</td>
<td>(55)</td>
</tr>
<tr>
<td>CfBlc</td>
<td>89</td>
<td>100</td>
<td>(70)</td>
<td>(46)</td>
<td>(54)</td>
<td>(62)</td>
<td>(58)</td>
<td>(55)</td>
</tr>
<tr>
<td>VcVlp</td>
<td>50</td>
<td>49</td>
<td>100</td>
<td>(51)</td>
<td>(52)</td>
<td>(54)</td>
<td>(54)</td>
<td>(51)</td>
</tr>
<tr>
<td>SaLaz</td>
<td>21</td>
<td>24</td>
<td>31</td>
<td>100</td>
<td>(49)</td>
<td>(53)</td>
<td>(30)</td>
<td>(56)</td>
</tr>
<tr>
<td>HsApoD</td>
<td>34</td>
<td>34</td>
<td>24</td>
<td>30</td>
<td>100</td>
<td>(81)</td>
<td>(82)</td>
<td>(87)</td>
</tr>
<tr>
<td>MmApoD</td>
<td>37</td>
<td>39</td>
<td>28</td>
<td>30</td>
<td>74</td>
<td>100</td>
<td>(94)</td>
<td>(89)</td>
</tr>
<tr>
<td>RnApoD</td>
<td>38</td>
<td>38</td>
<td>28</td>
<td>30</td>
<td>73</td>
<td>91</td>
<td>100</td>
<td>(89)</td>
</tr>
<tr>
<td>OcApoD</td>
<td>36</td>
<td>35</td>
<td>25</td>
<td>34</td>
<td>78</td>
<td>82</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

lipoproteins and are most closely related to those eukaryotic lipocalins that also articulate with membranes by hydrophobic anchors. Since eukaryotes do not possess lipoprotein modification machinery, the eukaryotic homologues have devised alternative membrane anchor mechanisms. As discussed previously (2, 3), the bacterial lipocalins are homologous to apolipoprotein D (ApoD), which is associated with the nervous system and with the high-density lipoprotein fraction of mammalian plasma, and to Lazarillo, which is associated with the nervous system of the American grasshopper Schistocerca americana. However, ApoD interacts with membranes by a solvent-exposed hydrophobic surface loop, and Lazarillo possesses a glycosylphosphatidylinositol anchor. Therefore, these homologous proteins have acquired three evolutionarily distinct membrane anchor mechanisms.

In making their claim to have discovered the first bacterial lipocalin, Barker & Manning have not only overlooked Blc, but failed to place VlpA in its appropriate evolutionary context. As shown in Table 1, the enterobacterial Blc proteins are more closely related to the ApoD proteins than they are to Lazarillo, whereas VlpA is more closely related to Lazarillo than to ApoD. Since no lipocalin has yet been identified from an archaeon, the possibility remains that the bacterial lipocalins were acquired by horizontal transmission from a eukaryote, but the presence of two distinct subgroups (VlpA–Lazarillo and Blc–ApoD) suggests...
that two distinct lines of vertical descent have occurred. Perhaps a subtle difference in ligand specificity may eventually distinguish these two groups of purative porphyrin-binding proteins. The possibility of defining the precise biochemical nature of the ligands binding proteins. The possibility of defining these two groups of putative porphyrin-occurred. Perhaps a subtle difference in that two distinct lines of vertical descent have increased now that bacterial lipocalin homologues are available.

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Authors’ reply

Origin of Vibrio cholerae lipocalin: which gene came first?

We wish to acknowledge an oversight in not referring to the work of Bishop and colleagues. We were aware of the homologies of VlpA to the family of lipocalins at the time of our database submission (9th January 1992 compared with 24th February 1995 for the blc sequence of Dr Weiner) and this had been reported (4, 5), although the first detailed discussion of vlpA from this laboratory (1) post-dated the publications of Drs Bishop and Weiner.

Interestingly, the lipocalin gene (vlpA) in Vibrio cholerae, as reported previously, varies significantly in copy number and chromosomal location (1). It is now clear that vlpA is associated with a mega-integron (3), which is essentially a gene-capture system involving site-specific recombination of gene cassettes containing the VCR repeat element and constitutes about 5% of the V. cholerae chromosome (2). The multiple copies observed in different V. cholerae strains are a consequence of insertion at different sites (P. Kaewrakon & P.A. Manning, unpublished).

Thus, although the V. cholerae lipocalin was the first reported in the database, the gene itself has probably been pilfered from another organism, possibly a member of the Enterobacteriaceae.

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