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" ! ... Comment ... ? "

Microbiology* Comment** provides a platform for readers of ***Microbiology to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders
Editor-in-Chief

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) (3). 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 (5) and a recent review discusses the importance of bacterial lipocalins for understanding lipocalin evolution (4).

Perhaps the most interesting feature of the bacterial lipocalins is that they are membrane 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

Table 1. Identity and similarity of proteins

Primary structures (excluding signal peptides) were compared between *E. coli* Blc (EcBlc; GenBank U21726), *Citrobacter freundii* Blc (CfBlc; GenBank U21727), *Vibrio cholerae* VlpA (VcVlp; GenBank X64097), *Homo sapiens* ApoD (HsApd; GenBank J02611), *Mus musculus* ApoD (MmApd; GenBank L39123), *Rattus norvegicus* ApoD (RnApd; GenBank X55572), *Oryctolagus cuniculus* ApoD (OcApd; GenBank L42979) and *Schistocerca americana* Lazarillo (SaLaz; GenBank Z38071). Percentage identities and similarities (in parentheses) were derived from pairwise alignments using the GAP algorithm (Genetics Computer Group, University of Wisconsin).

	EcBlc	CfBlc	VcVlp	SaLaz	HsApd	MmApd	RnApd	OcApd
EcBlc	100	(95)	(71)	(44)	(57)	(59)	(60)	(55)
CfBlc	89	100	(70)	(46)	(54)	(62)	(58)	(55)
VcVlp	50	49	100	(51)	(52)	(54)	(54)	(51)
SaLaz	21	24	31	100	(49)	(53)	(50)	(56)
HsApd	34	34	24	30	100	(81)	(82)	(87)
MmApd	37	39	28	33	74	100	(94)	(89)
RnApd	38	38	28	30	73	91	100	(89)
OcApd	36	35	25	34	78	82	80	100

▶ *GUIDELINES*

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of ***Microbiology*** do not necessarily agree with the views expressed in ***Microbiology* Comment**.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.

that two distinct lines of vertical descent have occurred. Perhaps a subtle difference in ligand specificity may eventually distinguish these two groups of putative porphyrin-binding proteins. The possibility of defining the precise biochemical nature of the ligands is greatly increased now that bacterial lipocalin homologues are available.

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1. Barker, A. & Manning, P.A. (1997). VlpA of *Vibrio cholerae* O1: the first bacterial member of the α_2-microglobulin lipocalin superfamily. *Microbiology* **143**, 1805–1813.
2. Bishop, R.E. & Weiner, J.H. (1996). 'Outlier' lipocalins more than peripheral. *Trends Biochem Sci* **21**, 127.
3. Bishop, R.E., Penfold, S.S., Frost, L.S., Höltje, J.-V. & Weiner, J.H. (1995). Stationary phase expression of a novel *Escherichia coli* outer membrane lipoprotein and its relationship with mammalian apolipoprotein D: implications for the origin of lipocalins. *J Biol Chem* **270**, 23097–23103.
4. Flower, D.R. (1996). The lipocalin protein family: structure and function. *Biochem J* **318**, 1–14.
5. Flower, D.R., Sansom, C.E., Beck, M.E. & Attwood, T.K. (1995). The first prokaryotic lipocalins. *Trends Biochem Sci* **20**, 498–499.

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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1. Barker, A. & Manning, P.A. (1997). VlpA of *Vibrio cholerae* O1: the first bacterial member of the α_2-microglobulin lipocalin superfamily. *Microbiology* **143**, 1805–1813.
2. Barker, A., Clark, C.A. & Manning, P.A. (1994). Identification of VCR, a repeated sequence associated with a locus encoding a hemagglutinin in *Vibrio cholerae* O1. *J Bacteriol* **176**, 5450–5458.
3. Clark, C.A., Purins, L., Kaewrakon, P. & Manning, P.A. (1997). VCR repetitive sequence elements in *Vibrio cholerae* chromosome constitute a mega-integron. *Mol Microbiol* **26**, 1137–1143.
4. Manning, P.A. (1991). Molecular analysis of potential adhesins of *Vibrio cholerae* O1. In *Molecular Pathogenesis of Gastrointestinal Infections*, pp. 139–146. Edited by T. Wadstrom and others. New York: Plenum.
5. Manning, P.A. (1995). The molecular biology of bacterial enteric pathogens. *Hong Kong J Pediatr* **1** (Suppl.), 17–27.