rise[3] in the late glacial and early Holocene. However, from the bulk reservoir change of 1.35×10^{12} tonnes of carbon for terrestrial organic carbon inferred in ref. 2 and from the present-day global caliche carbonate estimate of 0.93×10^{12} tonnes inferred by Schlesinger[4], it seems that CO_2 uptake may well have been of the same order as the rate at which CO_2 was leaving the oceans. It also seems advisable that those who wish to explain the time course of the atmospheric CO_2 rise during deglaciation seriously consider the role of the terrestrial system as a damping agent on any oceanically induced changes.

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1. Kern, R. A. & Schlesinger, W. H. *Nature* **357**, 447–449 (1992).
2. Adams, J. M. *et al. Nature* **348**, 711–714 (1990).
3. Neftel, A., Oeschger, H., Schwander, B. & Zumbrunn, R. *Nature* **295**, 220–223 (1982).
4. Schlesinger, W. H. *Geochim. cosmochim. Acta* **49**, 57–66 (1985).

Whither Pentastomida?

SIR — In his otherwise fine summary in News and Views[1] of the controversy surrounding limb homology and arthropod origins, Shear may have left readers with the impression that the enigmatic phylum Pentastomida still lies in limbo, awaiting a databased call to join the soon-to-be-erected umbrella phylum Lobopodia. Given their truly peculiar morphology, uncertainty over pentastomid affinities is understandable. However, Abele *et al.*[2] have recently provided molecular evidence that, among the taxa they examined (a crustacean, a chelicerate, a myriapod, an insect and an annelid worm), the pentastomids are most closely related to the subphylum Crustacea. Hence, rather than being an early proto-arthropod line of a rank comparable to onychophorans or tardigrades, as Shear implies, pentastomids fall, on molecular evidence, securely within the existing phylum Arthropoda, as some have argued all along[3,4].

Shear is to be commended for striving to keep a broad audience aware that the world of living invertebrates is still populated by weird and wonderful creatures whose affinities to other phyla remain obscure. One can only wonder in this age of increased concerns about biodiversity how many people have even heard of the Pentastomida, much less have even a vague impression of what these animals look like. I'd be willing to bet a litre of fine Canadian rum that fewer than 1% of the readers of *Molecular Biology and Evolution*, where the original molecular data were published, and perhaps even of *Nature* itself, could say without running to an invertebrate zoology textbook whether members of this phylum swim or fly, jump or crawl, chirp or sing, are brightly coloured or dull, or have legs, eyes, jaws or a tail, let alone have any thoughts on whether these animals should be placed within the Arthropoda or not. Any takers?

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1. Shear, W. A. *Nature* **359**, 477 (1992).
2. Abele, L. G., Kim, W. & Felgenhauer, B. E. *Molec. biol. Evol.* **6**, 685–691 (1989).
3. Wingstrand, K. G. *Biol. Skr.* **19**, 1–72 (1972).
4. Riley, J., Banaja, A. A. & James, J. L. *Int. J. Parasit.* **8**, 245–254 (1978).

Lipid–cytoskeleton interactions

SIR — Fukami *et al.* have shown the requirement of the acidic phospholipid phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) for α-actinin function[1]. Among other lines of evidence for this notion, the authors find that endogenous and also added $PtdInsP_2$ remains bound to α-actinin even after SDS–PAGE and transfer to nitrocellulose. They conclude from this and by other methods that there is a very tight complex of $PtdInsP_2$ to α-actinin.

I have been working for some time on the interaction of lipids with the cytoskeletal protein vinculin, and it has been clearly established that this protein interacts with acidic phospholipids such as phosphatidylserine or phosphatidylinositol, as shown by hydrophobic photolabelling and gel-filtration chromatography[2–4]. However, we could not find significant binding of ^{14}C-labelled phosphatidylserine to vinculin after SDS–PAGE of a vinculin–lipid mixture. This is to be expected, as a strong detergent such as SDS is expected to dissociate non-covalent lipid–protein complexes.

Surprisingly, we found that substantial amounts of ^{14}C-labelled phosphatidylinositol remained bound to vinculin after separation of a phosphatidylinositol–vinculin mixture by SDS–PAGE. This binding could, however, be prevented by inclusion of antioxidants such as mercaptoethanol or butylated hydroxytoluene in the medium during lipid sonication and subsequent incubation with vinculin, and by keeping the samples carefully under N_2. Moreover, 'binding' after SDS–PAGE was found only with certain batches of phosphatidylinositol (my unpublished observations).

These results point to covalent crosslinking of reactive lipid breakdown products to protein, due to oxidative deterioration of polyunsaturated fatty acid side chains. Specific crosslinking may occur as a result of a close protein–lipid interaction, and may depend on the degree of unsaturation of the fatty acids. Free radical trapping agents such as butylated hydroxytoluene prevent this crosslinking. Generally, care should therefore be taken to exclude oxidative processes when examining protein–lipid interactions, and techniques other than SDS–PAGE should also be used, to substantiate the conclusions.

Fukami *et al.* present other lines of evidence for their conclusions, and I do not challenge them. But our experience shows that care is needed in evaluating lipid–protein interactions of this type.

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FUKAMI REPLIES — As Niggli mentions, phosphatidylinositol can bind to vinculin through its oxidized polyunsaturated fatty acid. Such binding is probably diminished in the presence of antioxidants such as mercaptoethanol or butylated hydroxytoluene. In our experiments[1], we carefully checked the binding of phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) to α-actinin. The binding of $PtdInsP_2$ to α-actinin could be observed in the presence of 50 mM mercaptoethanol. Moreover, the binding was very specific to $PtdInsP_2$. We could not detect the binding of phosphatidylinositol or phosphatidylinositol 4-phosphate to α-actinin in western blot analysis. We also demonstrated the presence of $PtdInsP_2$-bound α-actinin even after striated muscles were solubilized directly by SDS sample buffer containing fresh mercaptoethanol. This binding seemed to be specific to α-actinin because we did not detect other $PtdInsP_2$-bound proteins by western blot analysis. Finally, we showed that $PtdInsP_2$ is specially localized in the Z-bands of striated muscle, where α-actinin is present. All these data suggest that $PtdInsP_2$ binds to α-actinin physiologically, though SDS–PAGE and western blot analysis alone may have some controversial aspects for the detection of lipids bound to proteins.

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1. Fukami, K. *et al. Nature* **359**, 150–152 (1992).
2. Ito, S., Werth, D., Richert, N. D. & Pastan, I. *J. biol. Chem.* **258**, 14,626–14,631 (1983).
3. Niggli, V., Dimitrov, D., Brunner, J. & Burger, M. M. *J. biol. Chem.* **261**, 6912–6918 (1986).
4. Niggli, V., Sommer, L., Brunner, J. & Burger, M. M. *Eur. J. Biochem.* **187**, 111–117 (1990).