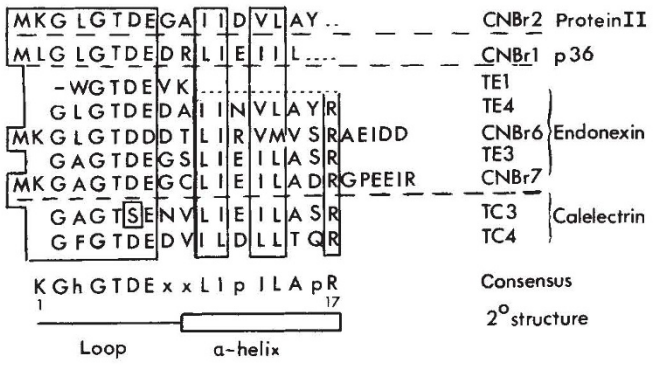

Fig. 3 Amino-acid sequences of endonexin CNBr peptides and tryptic peptides from endonexin (TE) and calelectrin (TC) were sequenced to completion using an Applied Biosystems model 470A gas-phase protein sequencer. Endonexin (p32.5) was purified from bovine liver[4], p36 from pig mesenteric lymph nodes[21] and protein II from pig intestinal epithelia[10]. Protein II cross-reacts immunologically with endonexin[22]. Methionines are shown at the amino termini of all CNBr peptides, because the protein amino termini appear to be blocked. Similarly, the tryptic peptides must each be preceded either by lysine or arginine. In the consensus sequence, h = hydrophobic residue, p = polar residue and x = variable residue. Secondary structure was predicted by a modification of the method of Chou and Fasman (Roberts and M.J.G., unpublished).

sequences TC3 and TC4 (Fig. 3). The amino-acid sequence of the most immunoreactive peptide, TC1, is unrelated to any sequence determined for mammalian proteins (not shown). The tryptic peptides and CNBr fragments sequenced so far account for approximately half of endonexin (150 residues) and more than 50% of these occur in the conserved repeat. It seems likely that more repeats will emerge after the complete sequence has been determined, since internal basic amino acids have led to the fragmentation of the putative repeat in TE1, the repeat contained within CNBr6 and in other sequences not shown. Substantially less calelectrin sequence has been determined (63 residues) and more repeats could be present.

The consensus repeat sequence reveals a pattern of conserved glycines, polar, nonpolar and charged residues within an exact length of 17 amino acids (Fig. 3). There is no statistically significant difference between the repeats in calelectrin and endonexin, which together have a homology of 68%. A search of the current protein sequence databases (3,500 sequences) failed to find significant homology between the sequences in Fig. 3 and any known protein.[16] No other significant homologies have been found and so the similarity of the primary structure of the proteins is currently restricted to the repeated sequences shown in Fig. 3.

When terbium was used as a spectroscopic probe for Ca^{2+}-binding sites in endonexin and calelectrin, multiple sites were present in both proteins[11,12]. Fluorescence emission spectra from protein-bound Tb^{3+} gave an excitation maximum at 285 nm, characteristic of tryptophan residues close to Tb^{3+} sites[11,12]. As there are at least five conserved repeats in endonexin, one of which (TE1) contains tryptophan, these repeats may form part of Ca^{2+}-binding sequences, despite lack of homology with the E-F hand of many Ca^{2+}-binding proteins[16]. Multiple binding sites are also indicated by the cooperative binding to liposomes.

It is striking that endonexin combines only with liposomes containing lipids possessing marked asymmetry in the bilayers of cell membranes. PE, PI and PA are found predominantly in the cytoplasmic leaflets of plasma or endoplasmic reticulum membranes[17]. PC, which is not bound by endonexin, has a less marked asymetrical distribution. PS, which is almost exclusively present in the cytoplasmic leaflet of the plasma membrane, is not bound by endonexin, but is bound by the analogous Ca^{2+}-binding protein synexin, which seems to be co-distributed with endonexin and other proteins described here[5,18].

The sarcoma virus tyrosine kinase substrate, p36, has also been shown to bind to liposomes containing PS or PI[19]. The presence of lipid shifts the Ca^{2+}-dependence of tyrosine phosphorylation from the millimolar to the micromolar level[19]. The demonstration of Ca^{2+}-dependent liposome binding by p36, endonexin and calelectrin[20], together with the conserved sequences described here, strengthens previous suggestions that these proteins are related in their structure and functions[3,4,8]. We speculate that the common sequence represents part of either a new type of Ca^{2+}-binding site or a lipid-binding site and that this feature will be common to other members of this new family of Ca^{2+}-binding proteins. The established lipid[19] and cytoskeleton[10] binding properties of p36 suggests that this protein and possibly the others described, could cross-link lipids in the bilayer to membrane cytoskeletal elements.

We acknowledge the support of EEC 'Stimulation Action' grant STI-U69-S-UK from the directorate general for Science Research and Development (for U.F.). We thank Drs V. Witzemann and V. P. Whittaker for critical comment on the manuscript, Drs P. Cohen (Dundee) and A. I. Magee (National Institute for Medical Research) for gifts of calcineurin B and pp60, respectively, N. Totty (Imperial Cancer Research Fund) for some sequence determinations, and A. Harris and J. Childs for technical assistance.

Note added in proof: After submission of this paper, the protein sequence deduced from human lipocortin cDNA was published[23]. This phospholipase A_2 inhibitor contains the concensus sequence described.

Received 16 December 1985; accepted 26 February 1986.

1. Walker, J. H. *J. Neurochem.* **39,** 815-823 (1982).
2. Walker, J. H., Obrocki, J. & Südhof, T. C. *J. Neurochem.* **41,** 139-145 (1983).
3. Südhof, T. C., Ebbecke, M., Walker, J. H., Fritsche, U. & Boustead, C. *Biochemistry* **23,** 1103-1109 (1984).
4. Geisow, M. J. *et al. EMBO J.* **3,** 2969-2974 (1984).
5. Creutz, C. E. *et al. J. biol. Chem.* **258,** 14664-14674 (1985).
6. Owens, R. J., Gallagher, C. J. & Crumpton, M. J. *EMBO J.* **1,** 1167-1170 (1984).
7. Moore, P. B. & Dedman, J. R. *J. biol. Chem.* **257,** 9663-9667 (1982).
8. Owens, R. J. & Crumpton, M. J. *Biochem. Essays* **1,** 61-63 (1984).
9. Gerke, V. & Weber, K. *EMBO J.* **3,** 227-233 (1984).
10. Gerke, V. & Weber, K. *J. biol. Chem.* **260,** 1688-1695 (1985).
11. Südhof, T. C., Walker, J. H. & Fritsche, U. *J. Neurochem.* **44,** 1302-1307 (1985).
12. Südhof, T. C. *Biochem. biophys. Res. Commun.* **123,** 100-107 (1984).
13. Soric, J. & Gordon, J. A. *Science* **230,** 563-566 (1985).
14. Sefton, B. M., Trowbridge, I. S., Cooper, J. A. & Scolnick, E. M. *Cell* **31,** 456-474 (1982).
15. Aitken, A. *et al. FEBS Lett.* **150,** 314-318 (1982).
16. Tufty, R. M. & Kretsinger, R. H. *Science* **187,** 167-169 (1975).
17. Op den Kamp, J. A. F. *A. Rev. Biochem.* **48,** 47-71 (1979).
18. Morris, S. J., Hughes, J. M. X. & Whittaker, V. P. *J. Neurochem.* **39,** 529-536 (1982).
19. Glenney, J. R. *FEBS Lett.* **192,** 79-82 (1985).
20. Südhof, T. C., Walker, J H. & Obrocki, J. *EMBO J.* **1,** 1167-1170 (1982).
21. Hexham, J. M., Totty, N. F., Waterfield, M. D. & Crumpton, M. J. *Biochem. biophys. Res. Commun.* **134,** 248-254 (1986).
22. Davies, A. A. & Crumpton, M. J. *Biochem. biophys. Res. Commun.* **128,** 571-577 (1985).
23. Wallner, B. P. *et al. Nature* **320,** 77-81 (1986).

Erratum

Danish Basin subsidence by Triassic rifting on a lithospheric cooling background

K. Sørensen

Nature **319,** 660-663 (1986)

IN this letter Figs 2 and 3 were transposed; the legends are correct as printed.

Corrigendum

Electron microscopy of frozen-hydrated biological material

M. Stewart & G. Vigers

Nature **319,** 631-636 (1986)

IN the numbered references 11, 16, 28, 38, 41 and 47, the author's name should read McDowall, A. W. The correct form is seen in ref. 39.