N-terminal bundle of Vh establish that the structure of this domain is regulated by contacts with cytoskeletal proteins. For example, the binding of talin between helices α1 and α2 displaces Vt, and α-actinin binding to Vh also displaces Vt, yet talin and α-actinin bind to Vh in a mutually restricted manner. The exclusive interactions of these α-helices with Vh would thus serve to properly direct cytoskeletal and signalling decisions induced by focal adhesions versus adherens junctions (Fig. 5).

The remarkable structural changes in the α-helices of the N-terminal bundle of Vh establish helical bundle conversion as a mechanism of control in signal transduction pathways. Helical bundle conversion is fundamentally different from helical exchange, where the binding of one α-helix simply displaces the binding of another without altering the structure of the displaced helix (Supplementary Fig. S4). Rather, helical bundle conversion provokes dramatic changes in the conformation of α-helices, allowing proteins to expand their repertoire of structures, substrates and functions. □

Methods

Detailed information on methodological aspects of X-ray data collection, structure determination and refinement is provided as Supplementary Information.

Protein preparation

Vinculin complementary DNA (GenBank accession number NM 003373) was generated by polymerase chain reaction with reverse transcription (RT–PCR) of human fibroblast messenger RNA, and was cloned into the pET3 expression vector (Novagen). The final constructs were N-terminal hexahistidine fusion tags proceeded by Met-Glu and included amino acids 1–258 for human Vh and 879–1066 for human Vt. Proteins were expressed in *Escherichia coli* BL21(DE3) or in B834(DE3) for selenomethionine (SeMet) incorporation. Cells were lysed in Tris-HCl (pH 8), 0.5 M NaCl and PMSF, and expressed proteins were purified using a chelating nickel affinity column (Amersham). The protein was eluted over a gradient to 0.5 M imidazole. Vh was purified using an anion-exchange column (Q Sepharose, Amersham), whereas Vt was purified using a cation-exchange column (SP Sepharose, Amersham) and a Superdex 75 gel-filtration column in 200 mM NaCl and 10 mM Tris-acetate (pH 7.6). Proteins were dialysed into Tris-acetate (pH 7.6), 10 mM dithiothreitol (DTT) and 1 mM EDTA. For Vt dialysis buffer also included 150 mM NaCl. Proteins were concentrated to 10 mg ml^{-1}, aliquoted and stored at −20 °C.

Vh–Vt crystallization

Native Vh–Vt crystals and native Vh in complex with SeMet-substituted Vt crystals were obtained by vapour diffusion by equilibrating 1.5 mol Vt per mol of Vh against a reservoir containing 15% polyethylene glycol of molecular mass 3350 Da (PEG-3350), 50 mM NaCl, 100 mM Tris (pH 8), and 10 mM DTT. Crystals of approximate dimensions $0.2 \times 0.15 \times 0.05$ mm^3 appeared overnight at room temperature. These crystals belong to space group $P2_12_12$ with one heterodimer in the asymmetric unit, a solvent content of 46%, and a volume to mass ratio of 2.3 Å^3 Da^{-1}. Crystals were cryoprotected in 30% PEG-3350.

Vh–VBS3 crystallization

Talin VBS3 (residues 1944–1969) was synthesized and purified by high-performance liquid chromatography in our in-house facility. Initial crystallization conditions were identified at the Hauptman–Woodward Institute. Native and SeMet Vh–VBS3 crystals were obtained by equilibrating 2.4 mol VBS3 per mol Vh against a reservoir of 2% MPD, 100 mM citric acid (pH 4) and 100 mM CdCl. Crystals of approximate dimensions of $(0.15 \text{ mm})^3$ appeared within one week at room temperature. These crystals belong to space group $I4_132$ with one heterodimer in the asymmetric unit, a solvent content of 44% and a volume to mass ratio of 2.2 Å^3 Da^{-1}. The Vh–VBS3 crystals were cryoprotected in paratone oil.

Received 28 August; accepted 9 December 2003; doi:10.1038/nature02281.
Published online 31 December 2003.

1. Zamir, E. & Geiger, B. Molecular complexity and dynamics of cell-matrix adhesions. *J. Cell Sci.* **114,** 3583–3590 (2001).
2. Pokutta, S. & Weis, W. I. The cytoplasmic face of cell contact sites. *Curr. Opin. Struct. Biol.* **12,** 255–262 (2002).
3. Xu, W., Baribault, H. & Adamson, E. D. *Vinculin* knockout results in heart and brain defects during embryonic development. *Development* **125,** 327–337 (1998).
4. Winkler, J., Lunsdorf, H. & Jockusch, B. M. The ultrastructure of chicken gizzard vinculin as visualized by high-resolution electron microscopy. *J. Struct. Biol.* **116,** 270–277 (1996).
5. Gilmore, A. P. & Burridge, K. Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4–5-bisphosphate. *Nature* **381,** 531–535 (1996).
6. Miller, G. J., Dunn, S. D. & Ball, E. H. Interaction of the N- and C-terminal domains of vinculin. Characterization and mapping studies. *J. Biol. Chem.* **276,** 11729–11734 (2001).
7. McGregor, A., Blanchard, A. D., Rowe, A. J. & Critchley, D. R. Identification of the vinculin-binding site in the cytoskeletal protein α-actinin. *Biochem. J.* **301,** 225–233 (1994).
8. Johnson, R. P. & Craig, S. W. F-actin binding site masked by the intramolecular association of vinculin head and tail domains. *Nature* **373,** 261–264 (1995).
9. Wood, C. K., Turner, C. E., Jackson, P. & Critchley, D. R. Characterization of the paxillin-binding site and the C-terminal focal adhesion targeting sequence in vinculin. *J. Cell Sci.* **107,** 709–717 (1994).
10. Bakolitsa, C., de Pereda, J. M., Bagshaw, C. R., Critchley, D. R. & Liddington, R. C. Crystal structure of the vinculin tail suggests a pathway for activation. *Cell* **99,** 603–613 (1999).
11. Bass, M. D., Smith, B. J., Prigent, S. A. & Critchley, D. R. Talin contains three similar vinculin-binding sites predicted to form an amphipathic helix. *Biochem. J.* **341,** 257–263 (1999).
12. Ling, K., Doughman, R. L., Firestone, A. J., Bunce, M. W. & Anderson, R. A. Type I gamma phosphatidylinositol phosphate kinase targets and regulates focal adhesions. *Nature* **420,** 89–93 (2002).
13. Di Paolo, G. *et al.* Recruitment and regulation of phosphatidylinositol phosphate kinase type 1 gamma by the FERM domain of talin. *Nature* **420,** 85–89 (2002).
14. Pokutta, S. & Weiss, W. I. Structure of the dimerization and β-catenin-binding region of α-catenin. *Mol. Cell* **5,** 533–543 (2000).
15. Yang, J., Dokurno, P., Tonks, N. K. & Barford, D. Crystal structure of the M-fragment of α-catenin: implications for modulation of cell adhesion. *EMBO J.* **20,** 3645–3656 (2001).
16. Pokutta, S., Drees, F., Takai, Y., Nelson, W. J. & Weis, W. I. Biochemical and structural definition of the l-afadin- and actin-binding sites of α-catenin. *J. Biol. Chem.* **277,** 18868–18874 (2002).
17. Weiss, E. E., Kroemker, M., Rudiger, A.-H., Jockusch, B. M. & Rudiger, M. Vinculin is part of the cadherin-catenin junctional complex: complex formation between α-catenin and vinculin. *J. Cell Biol.* **131,** 755–764 (1997).
18. Watabe-Uchida, M. *et al.* α-catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *J. Cell Biol.* **142,** 847–857 (1998).
19. Johnson, R. P. & Craig, S. W. An intramolecular association between the head and tail domains of vinculin modulates talin binding. *J. Biol. Chem.* **269,** 12611–12619 (1994).
20. Bass, M. D. *et al.* Further characterization of the interaction between the cytoskeletal proteins talin and vinculin. *Biochem. J.* **362,** 761–768 (2002).
21. Lawrence, M. C. & Colman, P. M. Shape complementarity at protein/protein interfaces. *J. Mol. Biol.* **234,** 946–950 (1993).
22. Johnson, R. P. & Craig, S. W. An intramolecular association between the head and tail domains of vinculin modulates talin binding. *J. Biol. Chem.* **269,** 12611–12619 (1994).
23. Hayashi, I., Vuori, K. & Liddington, R. C. The focal adhesion targeting (FAT) region of focal adhesion kinase is a four-helical bundle that binds paxillin. *Nature Struct. Biol.* **9,** 101–106 (2002).
24. Hoellerer, M. K. *et al.* Molecular recognition of paxillin LD motifs by focal adhesion targeting domain. *Structure* **11,** 1207–1217 (2003).

Supplementary Information accompanies the paper on **www.nature.com/nature.**

Acknowledgements We thank J. Cleveland for many helpful discussions. We also thank V. Morris, K. Brown and C. Kirby for technical assistance; C. Vonrhein for expert advice and help with autoSHARP; C. Ross for maintaining the X-ray and computing facilities; L. Messerle for the tantalum compound; and M. Kastan for critical review of the manuscript. We are grateful to the staff at the Advanced Photon Source, COM-CAT, SBC-CAT and SER-CAT, and at the Advanced Light Source, Lawrence Berkeley Laboratory, 5.0.2, for synchrotron support. This work was supported in part by the Cancer Center Support (CORE) Grant and by the American Lebanese Syrian Associated Charities (ALSAC). P.B. is a Van Vleet Fellow.

Competing interests statement The authors declare that they have no competing financial interests.

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The atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession codes 1RKE and 1RKC for the Vh–Vt and Vh–VBS3 structures, respectively.

erratum

Hydrocarbons and the evolution of human culture

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Nature **426,** 318–322 (2003).

In Table 1 of this Insight, the USGS estimate of world oil ultimate recovery should have cited ref. 10, not ref. 11. References 10 and 11 should read as below[10,11]. In addition, in ref. 17, P. Tharakan's surname was misspelt as Tharkan. □

10. United States Geological Survey (USGS) *U.S. Geological Survey World Petroleum Assessment 2000 - Description and Results.* Version 1.1 (USGS Digital Data Series DDS-60, United States Geological Survey, Washington DC, 2000).
11. Energy Information Administration, United States Department of Energy (EIA/DOE) *Long-term World Oil Supply: A Resource Base/Production Path Analysis* (US DOE, Washington DC, 2000); at ⟨http://tonto.eia.doe.gov/FTPROOT/presentations/long_term_supply/index.htm⟩.