somatic alteration of the remaining allele in a tumour from a patient with a germline *hPMS2* mutation supports the idea that inactivation of both alleles of a mismatch repair gene is required for tumour formation. □

Received 21 June; accepted 22 July 1994.

1. Lynch, H. T. *et al. Gastroenterology* **104,** 1535–1549 (1993).
2. Peinado, M. A., Malkhosyan, S., Velazquez, A. & Perucho, M. *Proc. natn. Acad. Sci. U.S.A.* **89,** 10065–10069 (1992).
3. Ionov, Y., Peinado, A., Malkhosyan, S., Shibata, D. & Perucho, M. *Nature* **363,** 558–561 (1993).
4. Thibodeau, S. N., Bren, G. & Schaid, D. *Science* **260,** 816–819 (1993).
5. Aaltonen, L. A. *et al. Science* **260,** 812–816 (1993).
6. Strand, M., Prolla, T. A., Liskay, R. M. & Petes, T. D. *Nature* **365,** 274–276 (1993).
7. Parsons, R. P. *et al. Cell* **75,** 1227–1236 (1993).
8. Umar, A. *et al. J. biol. chem.* **269,** 14367–14370 (1994).
9. Peltomaki, P. *et al. Science* **260,** 810–812 (1993).
10. Lindblom, A., Tannergard, P., Werelius, B. & Nordenskjold, M. *Nature Genet.* **5,** 279–282 (1993).
11. Nystrom-Lahti, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **91,** 6054–6058 (1994).
12. Fishel, R. *et al. Cell* **75,** 1027–1038 (1993).
13. Leach, F. S. *et al. Cell* **75,** 1215–1225 (1993).
14. Palombo, F., Hughes, M. & Jiricny, J. *Nature* **367,** 417–418 (1994).
15. Bronner, C. E. *et al. Nature* **368,** 258–261 (1994).
16. Papadopoulos, N. *et al. Science* **263,** 1625–1629 (1994).
17. Adams, M. D. *et al. Science* **252,** 1651–1656 (1991).
18. Frohman, M. A., Dush, M. K. & Martin, G. R. *Proc. natn. Acad. Sci. U.S.A.* **85,** 8998–9002 (1988).
19. Kozak, M. *Cell* **44,** 283–292 (1986).
20. Prolla, T. A., Christie, D. M. & Liskay, R. M. *Molec cell. Biol.* **14,** 407–415 (1994).
21. Liu, B. *et al. Cancer Res.* (in the press).
22. Powell, S. M. *et al. New Engl. J. Med.* **329,** 1982–1987 (1993).
23. Van Der Luijt, R. *et al. Genomics* **20,** 1–4 (1994). (**Initials?**)
24. Dietz, H. C., *et al. Science* **259,** 680–683 (1993).
25. Prolla, T. A. *et al. Science* (in the press).
26. Kramer, W., Kramer, B., Williamson, M. S. & Fogel, S. J. *J. Bact.* **171,** 5339–5346 (1989).
27. Schuler, G. D., Altschul, S. F. & Lipman, D. J. *Proteins Struct. Funct. Genet.* **9,** 180–190 (1991).
28. Johnson, C. V. *et al. Meth. cell. Biol.* **35,** 73–99 (1991).
29. Sidransky, D. *et al. Science* **252,** 706–709 (1991).

ACKNOWLEDGEMENTS. We would like to thank R. Wang, C. Fischer, V. Madike, and the sequencing staff from HGS for technical support. This work was supported by grants from the Clayton Fund, the Academy of Finland, the Finnish Cancer Societies, the Sigrid Juselius Foundation, the Folkhalsan Institute of Genetics, the Cooperative Human Tissue Network (NCI) and the NIH. N.C.N. and N.P. contributed equally to this work.

Crystal structure of an isoleucine-zipper trimer

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SUBUNIT oligomerization in many proteins is mediated by short coiled-coil motifs[1,2]. These motifs share a characteristic seven-amino-acid repeat containing hydrophobic residues at the first (a) and fourth (d) positions. Despite this common pattern, different sequences form two-, three- and four-stranded helical ropes. We have investigated the basis for oligomer choice by characterizing variants[3] of the GCN4 leucine-zipper dimerization domain that adopt trimeric or tetrameric structures in response to mutations at the a and d positions. We now report the high-resolution X-ray crystal structure of an isoleucine-containing mutant that folds into a parallel three-stranded, α-helical coiled coil. In contrast to the dimer and tetramer structures[3,4], the interior packing of the trimer can accommodate β-branched residues in the most preferred rotamer at both hydrophobic positions. Compatibility of the shape of the core amino acids with the distinct packing spaces in the two-, three- and four-stranded conformations appears to determine the oligomerization state of the GCN4 leucine-zipper variants.

The GCN4-pII peptide, which differs from the wild-type GCN4 leucine zipper by isoleucine substitutions at four **a** and four **d** positions (Fig. 1*a*; Table 1), forms a parallel trimer in solution[3]. A similar isoleucine repeat occurs in the trimeric σ1 protein of reovirus serotype 1 (refs 5, 6) in haemagglutinins from several strains of human parainfluenza virus[7,8] and in trout axonemal dynein[9]. The trimeric coiled coils from influenza haemagglutinin[10,11] and the yeast heat-shock factor[12] (HSF) resemble the GCN4-pII sequence at the **d** positions of the heptad repeat, which contain predominantly β-branched residues (IIIIL in the five heptads of HSF).

The X-ray crystal structure of GCN4-pII at 1.8 Å resolution (Fig. 1) shows that three α-helical peptide monomers wrap in a gradual left-handed superhelix. The superhelix describes a cylinder that is ~24 Å wide and ~48 Å long. The isoleucine residues point into the core of the trimer, and cross-sectional layers containing isoleucine at the **a** positions alternate with layers containing isoleucine at the **d** positions (Fig. 1*d*). In seven of eight layers, the dihedral angles χ_1 and χ_2 of the isoleucine side chains are approximately −60,180, corresponding to the most abundant rotamer[13]. The isoleucine residues in the C-terminal **a** layer assume dihedral angles near +60,180. Residues at positions **e** and **g** pack against the isoleucines at **d** and **a**, respectively, to complete the hydrophobic core. Interhelical salt bridges form with high frequency between charged side chains at the **g** position of one heptad and the **e** position of a succeeding heptad (for

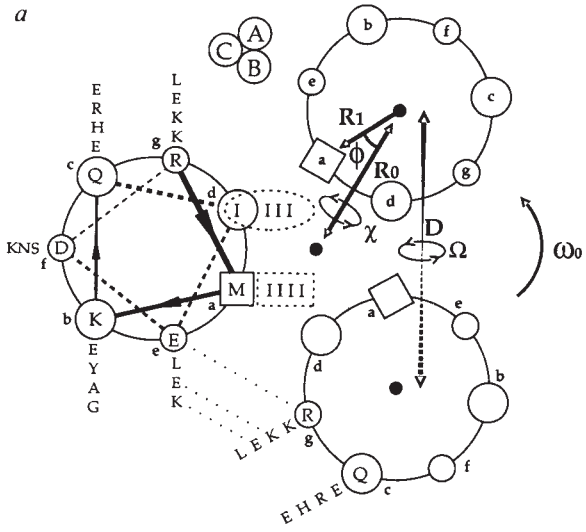
TABLE 1 Helix–helix interactions*

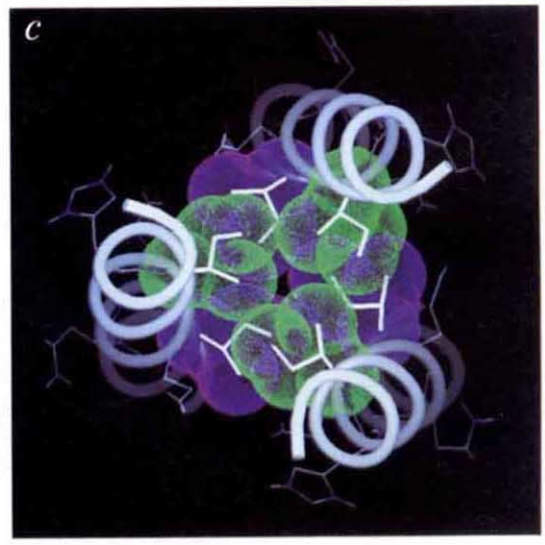
	Dimer		Trimer	Tetramer	Globular proteins[17]
GCN4 peptide variant†	pII‡ and pIL		pIII	pLII	
Residues at four **a** positions	V(N)	I	I	L	
Residues at four **d** positions	L	L	I	I	
Superhelix parameter					
Supercoil radius, R_0(Å)	4.9		6.7	7.6	
Residues per supercoil turn, ω_0	100		118	139	
Supercoil pitch (Å)	148		175	205	
Radius of curvature (Å)	118		124	149	
Superhelix crossing angle (χ)	23.4°		26.8°	26.0°	
Position **a** orientation angle, ϕ	21.6°		20.4°	19.8°	
α-Helix parameter[17]					
Residues per α-helix turn, n	3.62		3.60	3.59	3.64 ± 0.18
Rise/residue, d (Å)	1.51		1.53	1.52	1.51 ± 0.12
α-Helix radius (Cα), R_1(Å)	2.28		2.24	2.26	—
Pairwise helix-crossing angle, Ω	23.4°		23.2°	18.3°	19° ± 24°
Pairwise interhelix distance, D (Å)	9.8		11.5	10.6	10.2 ± 2.0
Interhelical salt bridges					
g to **e**	3 of 6		8 of 9	5 of 12	
g to **b** and **c** to **e**	0 of 8		1 of 12	9 of 16	

* Superhelical characteristics were obtained by fitting the Cα backbones to a supercoil parametrization suggested by Crick[22] (Fig. 1a). The radius (R_0), frequency (ω_0) and pitch of the superhelix, and the radius (R_1) and phase (ϕ) of the α-helix were treated as variables. The frequency of the α-helix was fixed at 4π radians per 7 amino acids to preserve the periodicity of the heptad structure. α-helix parameters were calculated as defined in ref. 17. The properties of α-helices in globular proteins were taken from that reference and are expressed as the mean value ±2 standard deviations. The values of D and Ω for globular proteins are averages for the 3–4 ridges-into-grooves packing class[17]. Consistent with earlier analyses[3,22,23], the superhelical radius and pitch, and the radius of helix curvature increase with the number of strands in the coiled coil.

† The leucine zipper variants were derived from the wild-type GCN4 coiled-coil peptide, GCN4-p1 (ref. 24), by collective mutation to the indicated residues at four **a** positions (dashed box in Fig. 1*a*) and four **d** positions (dashed oval in Fig. 1*a*)[3].

‡ Geometric properties of the dimer conformation were obtained from the structure of GCN4-p1 (ref. 4) because the crystallographic analysis of GCN4-pIL has not been completed. The GCN4-p1 sequence contains four leucines at the varied **d** positions, and three valines and one asparagine at the varied **a** positions. Interestingly, replacement of the asparagine residue (Asn 16) by valine generates a peptide, GCN4-pVL, that forms a mixture of dimers and trimers[3]. This result indicates that the buried polar side chain of Asn 16 directs dimer formation.





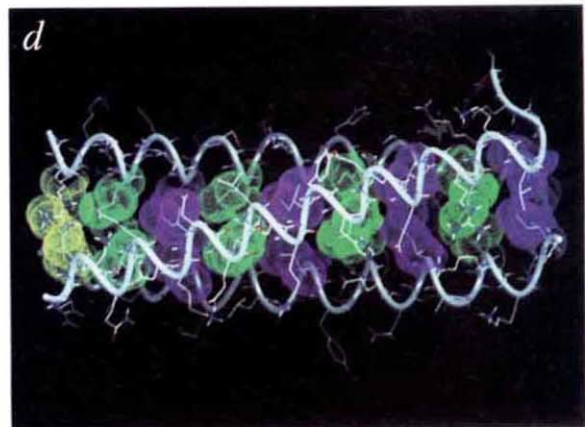


FIG. 1 Crystal structure of the GCN4-pII trimer. *a*, Helical wheel projection of residues 1 (Arg) to 32 (Glu) of the GCN4-pII sequence. View is from the N terminus, and residues in the first two helical turns are boxed or circled. Heptad positions are labelled **a**–**g**. The R_0, ω_0, α, ϕ and R_1 variables from a coiled-coil parameterization suggested by Crick[3,22] and the D and Ω variables defined in ref. 17 are illustrated. *b*, A portion of the 6.0–1.8 Å resolution $2F_o - F_c$ electron-density map superimposed on the final model. A side view of residues Ser C14 to Asn C21 is shown. *c*, Axial view of the GCN4-pII trimer. The van der Waals surfaces are coloured purple for residues at **a** positions and green for residues at **d** positions. *d*, Side view of the trimer. The van der Waals surfaces of the N-terminal methionine layer are yellow.

METHODS. The GCN4-pII peptide, Ac-R MKQIEDK IEEILSK IYHIENE IAR IKKLIGER (where mutant **a** residues are underlined and mutant **d** residues double-underlined; Ac, acetyl), was crystallized by vapour diffusion from 100 mM sodium citrate, pH 4.8, 600 mM NaBr and 5% PEG 400. The crystals had $P2_1$ symmetry ($a = 35.32$ Å, $b = 39.67$ Å, $c = 36.97$ Å, $\beta = 98.38°$) with a trimer in the asymmetric unit. Data to 1.8 Å resolution were collected using an *R*-Axis image plate detector (7,052 unique reflections, $R_{merge} = 0.045$). An idealized model was built[25] using an empirical method for calculation of coiled-coil structures based on an algebraic parameterization[22]. The model was used in a combined rotation–translation search (with 6–2.9 Å resolution data $> 2\sigma$) along a non-crystallographic 3-fold symmetry axis (located with GLRF[26]) using the program XPLOR[27]. The coordinates corresponding to the peak solution (initial $R = 52\%$) were annealed and rebuilt into simulated-annealing omit maps[28]. Water molecules were added and the coordinates and individual *B* values were refined using TNT[29] and FRODO[30]. The final model, which includes 96 amino acids of the trimer and 48 water molecules, has a crystallographic *R*-factor of 0.175 (6–1.8 Å data) with root-mean-square deviations from ideal bond lengths and bond angles of 0.013 Å and 2.4° respectively. All main-chain dihedral angles fall within allowed regions of the Ramachandran plot, and most side chains assume well populated rotamer conformations. The coordinates of the search model and the refined structure differ by a root-mean-square deviation of 0.46 Å for main-chain and core side-chain atoms. These results demonstrate the feasibility of using a predicted structure to initiate crystallographic analysis. The coordinates have been deposited in the Brookhaven Protein Data Bank.

example, Glu B22–Lys C27; Table 1 and Fig. 1*a*). This observation supports proposals that heterotrimer formation could be directed by electrostatic attraction[14] or repulsion[15,16] between these residues.

Although the pitch, radius and residues per turn of the superhelices in the trimer (GCN4-pII), dimer (GCN4-pI) and tetramer (GCN4-pLI) differ greatly, the individual α-helices are virtually identical on a local scale (Table 1). Moreover, pairs of adjacent helices in the three structures have similar separations (D) and crossing angles (Ω). This conserved pairwise arrangement of helices corresponds to the optimal geometry for one class of helix association in globular proteins[17] (Table 1).

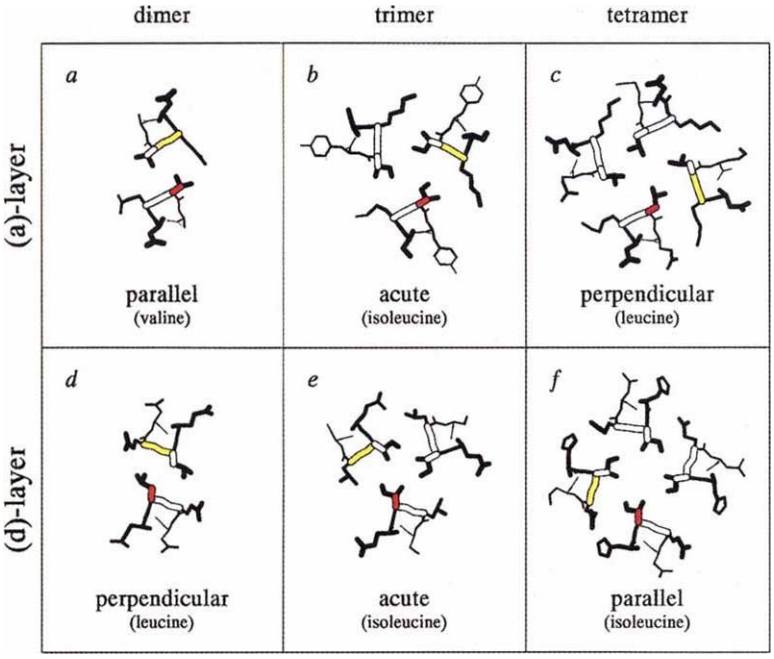


FIG. 2 Packing differences in parallel coiled coils[3,18]. Helix cross-sectional layers centred on the **a** positions (containing the residues at positions **f**, **g**, **a** and **b**) and on the **d** positions (containing the residues at positions **c**, **d**, **e** and **f**) are depicted[31]. Knobs formed by the side chains of one helix fit into holes formed by the spaces between side chains on a neighbouring helix. Looking from the N terminus down the superhelix axis, each knob at an **a** position (red) packs into a hole formed between the **g** and **a** residues (yellow) and two **d** residues in adjacent layers (not shown) of the anti-clockwise-related monomer. Similarly, each knob at a **d** position (red) packs into a hole formed between the **d** and **e** residues (yellow) and two **a** residues in adjacent layers (not shown) of the clockwise related monomer. *a*, Position **a** of the dimer: the Cα–Cβ bond of each valine knob (red) is oriented parallel to the Cα–Cα vector at the base of the hole into which it projects (yellow). *b*, Position **a** of the trimer; the Cα–Cβ bond of each isoleucine knob (red) forms an acute angle (~50°) with the Cα–Cα vector at the base of the recipient hole (yellow). *c*, Position **a** of the tetramer; the Cα–Cβ bond of each leucine knob (red) is perpendicular to the Cα–Cα vector at the base of the apposed hole (yellow). *d*, Perpendicular packing at position **d** of the dimer. *e*, Acute packing at position **d** of the trimer. *f*, Parallel packing at position **d** of the tetramer.

The GCN4-pII trimer shows 'knobs-into-holes' packing, in which the side chains (knobs) at positions **a** and **d** fit into the spaces (holes) between four residues on a neighbouring helix[18]. In both the **a** and **d** layers, the Cα–Cβ bond of the knob side chain makes an acute angle with the Cα–Cα vector defining the base of the apposed hole (Fig. 2*b*, *e*). This shared geometry results in a similar placement of atoms around the side chains at positions **a** and **d** (Fig. 3*b*). However, the acute angles in the two types of layers differ by ~25° (Fig. 3*b*). In addition, the third helix of the trimer sits to the C-terminal side of the hole in the **a** layer (anti-clockwise from hole in Fig. 2*b*) but to the N-terminal side of the hole in the **d** layer (clockwise from hole in Fig. 2*e*). Thus, for example, a threonine knob residue with a (−) χ_1 dihedral angle would direct its hydroxyl group into the hydrophobic interface at an **a** position, but towards solvent at a **d** position.

The interior packing of the trimer differs from that seen in the dimeric and tetrameric leucine-zipper variants. In the GCN4-pI dimer, the Cα–Cβ bond of the knob side chain at position **a** points out of the interface (parallel packing; Fig. 2*a*), and the Cα–Cβ bond of the knob side chain at position **d** points directly at the adjacent helix (perpendicular packing; Fig. 2*d*). This pattern is reversed in the GCN4-pLI tetramer (Figs 2*c*, and 3*a*, *c*), which shows perpendicular packing in the **a** layers and parallel packing in the **d** layers. Thus the packing geometries in the dimer, trimer and tetramer are distinct from one another at both hydrophobic positions (Fig. 2).

The three modes of knobs-into-holes packing exhibit different preferences for specific amino acids at the knob position. In dimeric coiled-coil sequences, the **a** positions (parallel packing) are enriched for the β-branched residues valine and isoleucine, whereas the **d** positions (perpendicular packing) are sharply depleted of β-branched residues and enriched for leucine[19,20]. In trimeric coiled-coil sequences, however, leucine and the β-branched amino acids are approximately equally represented and distributed evenly between the **a** and **d** positions[21] (consistent with the geometric similarity of the packing at **a** and **d** in the trimer; Fig. 3*b*). Thus parallel geometry favours β-branched residues, perpendicular geometry disfavours β-branched residues and favours leucine, and acute geometry shows little preference.

The oligomerization states of the GCN4 leucine-zipper mutants are apparently dictated by matching the core residues, determined by sequence, with appropriate packing geometry, determined by structure. The dimer of GCN4-pIL and the tetramer of GCN4-pLI, for example, place the leucine residues in perpendicular geometry and the isoleucine residues in parallel geometry. By contrast, a dimer or tetramer of GCN4-pII would mismatch four of the eight buried isoleucines with perpendicular geometry. The trimer conformation, however, places all the isoleucines in acute geometry, thereby accommodating β-branched amino acids at both the **a** and **d** positions.

The packing properties of the β-branched residues isoleucine and valine are not identical. For example, the peptide

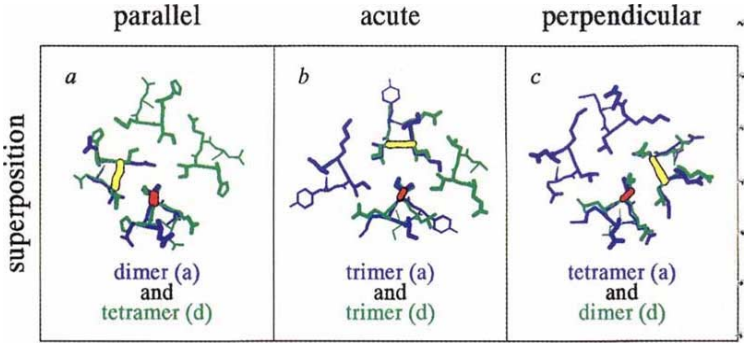


FIG. 3 Superposition[31] of parallel, perpendicular and acute knobs-into-holes packing. *a*, Parallel packing in the dimer **a** layer (green) and the tetramer **d** layer (blue). *b*, Acute packing in the trimer **a** layer (green) and the trimer **d** layer (blue). *c*, Perpendicular packing in the tetramer **a** layer (green) and the dimer **d** layer (blue).

GCN4-pVL forms both dimers and trimers and the peptide GCN4-pLV is trimeric[3]. Comparison of the oligomerization properties of GCN4-pVL and GCN4-pIL (dimer), or GCN4-pLV and GCN4-pLI (tetramer), suggests that isoleucine exhibits a stronger perference than valine for parallel over acute geometry. Thus, the GCN4 variant coiled coils provide a striking example of how side-chain shape and packing can dramatically affect the global fold of a protein. □

Received 27 April; accepted 8 July 1994.

1. Cohen, C. & Parry, D. A. D. *Proteins* **7,** 1–15 (1990).
2. Cohen, C. & Parry, D. A. D. *Science* **263,** 488–489 (1994).
3. Harbury, P. B., Zhang, T., Kim, P. S. & Alber, T. *Science* **262,** 1401–1407 (1993).
4. O'Shea, E. K., Klemm, J. D., Kim, P. S. & Alber, T. *Science* **254,** 539–544 (1991).
5. Duncan, R., Horne, D., Cashdollar, L. W., Joklik, W. K. & Lee, P. W. K. *Virology* **174,** 399–409 (1990).
6. Strong, J. E., Leone, G., Duncan, R., Sharma, R. K. & Lee, P. W. K. *Virology* **184,** 23–32 (1991).
7. Elango, N., Coligan, J. E., Jambou, R. C. & Venkatesan, S. *J. Virol.* **57,** 481–489 (1986).
8. Coelingh, K. L. V. W., Winter, C. C. & Murphy, B. R. *Virology* **162,** 137–143 (1988).
9. Garber, A. T., Retief, J. D. & Dixon, G. H. *EMBO J.* **8,** 1727–1734 (1989).
10. Wilson, I. A., Skehel, J. J. & Wiley, D. C. *Nature* **289,** 366–373 (1981).
11. Carr, C. M. & Kim, P. S. *Cell* **73,** 823–832 (1993).
12. Sorger, P. K. & Nelson, H. C. *Cell* **59,** 807–813 (1989).
13. Ponder, J. W. & Richards, F. M. *J. molec. Biol.* **193,** 775–791 (1987).
14. Beck, K., Dixon, T. W., Engel, J. & Parry, D. A. *J. molec. Biol.* **231,** 311–323 (1993).
15. O'Shea, E. K., Rutkowski, R. & Kim, P. S. *Cell* **68,** 699–708 (1992).
16. O'Shea, E. K., Lumb, K. J. & Kim, P. S. *Curr. Biol.* **3,** 658–667 (1993).
17. Chothia, C., Levitt, M. & Richardson, D. *J. molec. Biol.* **145,** 215–250 (1981).
18. Crick, F. H. C. *Acta crystallogr.* **6,** 689–697 (1953).
19. Hu, J. C. & Sauer, R. T. in *Progress in Nucleic Acids and Molecular Biology* (eds Eckstein, F. & Lilley, D. M. J.) 82–101 (Springer, Berlin, 1992).
20. Conway, J. F. & Parry, D. A. D. *Int. J. biol. Macromol.* **12,** 328–334 (1990).
21. Conway, J. F. & Parry, D. A. D. *Int. J. biol. Macromol.* **13,** 14–16 (1991).
22. Crick, F. H. C. *Acta crystallogr.* **6,** 685–689 (1953).
23. Seo, J. & Cohen, C. *Proteins* **15,** 223–234 (1993).
24. O'Shea, E. K., Rutkowski, R. & Kim, P. *Science* **243,** 538–542 (1989).
25. Harbury, P. H. thesis, Harvard Univ. (1994).
26. Tong, L. & Rossmann, M. G. *Acta. crystallogr.* A**46,** 783–792 (1990).
27. Brunger, A. T., Kuriyan, J. & Karplus, M. *Science* **235,** 458–460 (1987).
28. Brunger, A. T. *J. molec. Biol.* **203,** 803–816 (1988).
29. Tronrud, D. E., TenEyck, L. F. & Matthews, B. W. *Acta crystallogr.* A**43,** 489–501 (1987).
30. Jones, T. A. *J. appl. Crystallogr.* **11,** 268–272 (1978).
31. Kraulis, P. J. *J. appl. Cryst.* **24,** 946–950 (1991).

ACKNOWLEDGEMENTS. We thank M. Milhollen for peptide synthesis. This work was supported by grants from the National Institutes of Health (to P.S.K. and T.A.) and the Lucille P. Markey Charitable Trust (to T.A.).

CORRECTION

Flattening of the sea-floor depth–age curve as a response to asthenospheric flow

Jason Phipps Morgan & Walter H. F. Smith

Nature **359,** 524–527 (1992)

WE are grateful to Carol and Seth Stein[1] for alerting us to errors in our discussion of the results shown in Fig. 2*b* of our Letter. In the calculations shown in this Figure, the asthenospheric viscosity beneath the South Atlantic lithosphere was 4.2×10^{19} Pa s and the African plate was assumed to be stationary, instead of migrating eastward at 10 mm yr^{-1} as stated in the Letter. In addition, the thermal subsidence rate and ridge-crest depth, which were not mentioned, were 280 m $Myr^{-1/2}$ and 2,200 m respectively. In contrast, the values used for the Pacific plate were, as stated, 300 m $Myr^{-1/2}$ and 2,644 m. Hence the fit to the Pacific and South Atlantic plates results from assuming subsidence rates differing by 7% and viscosities differing by a factor of 21. □

1. Stein, C. & Stein, S. *Geophys. Res. Lett.* **21,** 709–712 (1994).

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Supplementary information is material relevant to Articles or Letters which cannot, for lack of space, be published in full, but which is available from *Nature* on request.

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