substitutions of isoleucine for leucine.

We wish to caution against the too ready acceptance of the significance of leucine heptad repeats found in databank searches. In particular, we address the following questions concerned with the statistical background of such searches. How many leucine heptad repeats might one expect to find merely by virtue of chance? Are leucine heptad repeats relatively more frequent in proteins than heptad repeats of other amino acids (taking into account the biases in residue composition)? Are heptad repeats of leucine more frequent than repeats of other period length?

In the table we list the probability of occurrence of 4 or 5 leucine repeats in random protein sequences of given size and leucine content. Natural protein sequences are not random, but the tabulated probability values may serve as a reference in estimating the statistical significance of the observed repeats. It is seen, for example, that 4 repeats in a 300–500 residue sequence of average leucine content (10%) occur with only 3–4% probability, but that this probability is considerably increased for sequences of higher leucine content or greater length.

We have screened in excess of 450 distinct mammalian proteins of mean length 450 residues and average leucine content 9.7% for occurrences of the motif L-X_6-L-X_6-L-X_6-L, where L is leucine and X is any other amino acid. The motif is found in more than 30 of the sequences, but in only about half of them, including ribophorin I, spectrin and interleukin-3, is X never a proline. Heptad repeats of other amino acids occur much less than half as frequently as the leucine heptads, in agreement with the fact that leucine is by far the most common amino acid over all the proteins analysed. The second most frequent heptad repeat (occurring more than five times as often as expected) contains glutamic acid residues instead of leucine (as, for example, in the $i+3$th positions of the Fos leucine zipper).

Probability of observing a success run of r periodically repeated leucines in a sequence of length n for a given frequency f of leucine in the sequence.

	$r = 4$			$r = 5$		
n/f	6.5%	10.0%	13.5%	6.5%	10.0%	13.5%
200	0.003	0.02	0.06	0.001	0.002	0.008
300	0.005	0.03	0.08	0.001	0.003	0.01
500	0.008	0.04	0.13	0.001	0.004	0.02
1000	0.02	0.09	0.25	0.001	0.009	0.04

The probability is closely approximated by $(1 - fx)/[(r + 1 - rx)(1-f)x^{n+1}]$, where x solves $(1 - f)x(1 + fx + .. + f^{r-1}x^{r-1}) = 1$ (ref. 7). A lower bound for the probability of observing an r-repeat of any (not predetermined) amino acid is given by the same formula with values $r - 1$ and $f = 5\%$ (0.03–0.06 for $r = 4$ and $n =$ 300–500).

For the same set of proteins, leucine repeats of length 4 with spacing 5 or 7 (rather than 6) are found in about 20 proteins each. Also, spacings 3 and 6 are preferred over spacings 4,5,7 and 8 between (not necessarily nearest) neighbouring leucines. This relative excess of leucine heptad repeats is consistent with the role of leucines in establishing hydrophobic faces of α-helices, including those involved in coiled coil interactions. Interestingly, the same preference for spacing 6 holds for glutamic acid residues, possibly reflecting their role in establishing hydrophilic faces of α-helices.

Thus the leucine repeat by itself occurs widely on both probabilistic and empirical grounds. This abundance undoubtedly reflects the particular structural role of the motif but it also warrants a cautioning note with respect to the prolific citation of leucine zippers, particularly when pattern searches allow for substitutions in the leucine positions. While one might rightly want to accommodate true variations in primary sequence yielding equivalent three-dimensional structures, attention ought to be given to the concomitant increase in the level of false positive occurrences. Based on our probability estimates and data work, we think that perhaps as many as two of every three leucine zipper motifs are simply chance occurrences.

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1. Landschulz, W.H., Johnson, P.F. & McKnight, S.L. *Science* **240**, 1759–1764 (1988).
2. O'Shea, E.K., Rutkowski, R. & Kim, P.S. *Science* **243**, 538–542 (1989).
3. Landschulz, W.H., Johnson, P.F. & McKnight, S.L. *Science* **243**, 1681–1688 (1989).
4. Buckland, R. & Wild, F. *Nature* **338**, 547 (1989).
5. McCormack, K *et al. Nature* **340**, 103 (1989).
6. White, M.K. & Weber, M.J. *Nature* **340**, 103–104 (1989).
7. Feller, W. *An Introduction to Probability Theory and its Applications* 3rd edn Vol. 1 325 (Wiley, New York, 1968).

Moving proteins

SIR—The use of Con A labelled with 40-nm gold particles to show forward movement of membrane glycoproteins in locomotor cells[1] is exciting, but it should not be forgotten that during the past decade, several groups in the leukocyte chemotaxis field have reported forward distribution of cell-surface receptors in polarized and actively moving leukocytes, albeit using less refined techniques. Many workers have considered that this distribution is the result of an anterior movement of membrane proteins rather than their reinsertion at the leading edge.

Anterior distribution has been reported for Fc receptors[2-4] and for receptors for a chemoattractant peptide[5]. A variety of functionally unrelated proteins, including Thy-1 (ref. 4) and CD45 (ref. 6) also show forward distribution on locomotor lymphocytes. This anterior distribution is independent of the ligand used to stimulate polarization and locomotion, and occurs rapidly[7]. We have suggested[8] that forward movement of chemotaxis receptors is an intrinsic component of chemoattractant-induced polarization, that it is intrinsic to the directional locomotor response, reinforcing the persistence of that response in cells moving up a gradient, and that it also accounts for the long stretches of persistent locomotion in a given direction seen in cells moving randomly when the chemoattractant is uniformly distributed.

A question remains about mechanism. Kucik *et al.* discuss myosin I which is linked to both actin and to membrane sites, as a possible motor. However, Thy-1, a protein which is inserted by a phosphatidyl-linkage into the outer leaflet of the lipid bilayer, nevertheless moves forward and maintains an anterior distribution in motile lymphocytes, indicating that linkage of cytoskeleton to forward-moving proteins may be indirect.

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1. Kucik, D., Elsen, E. & Sheetz, M. *Nature* **340**, 315 (1989).
2. Wilkinson, P.C., Michl, J. & Silverstein, S.C. *Cell Biol. Int. Rep.* **4**, 736 (1980).
3. Walter, R., Berlin, R. & Oliver, J. *Nature* **286**, 724 (1980).
4. Shields, J.M. & Haston, W.S. *J. Cell Sci.* **74**, 75–93 (1985).
5. Sullivan, S.J., Daukas, G. & Zigmond, S.H. *J. Cell Biol.* **99**, 1461–1467 (1984).
6. Haston, W.S. & Wilkinson, P.C. *Curr. Opinion Immunol.* **1**, 5–9 (1988).
7. Haston, W.S. & Shields, J.M. *J. Cell Sci.* **82**, 249 (1986).
8. Haston, W.S. & Wilkinson, P.C. *J. Cell Sci.* **87**, 373 (1987).

SIR—Kucik *et al.*[1] state that "no forward transport of particles has previously been reported" on locomoting cells and, again, that "only rearward transport of surface particles had been observed". This is not the case. As detailed in my recent review[2] of the entire literature on the use of inert particles as markers for cell surface membrane dynamics during cell motility, workers have observed the forward movement of inert marker particles (carmine, India ink, carbon particles) along the surface of locomoting freshwater amoebae.

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1. Kucik, D., Elsen, E. & Sheetz, M. *Nature* **340**, 315 (1989).
2. Bloodgood, R. A. in *Microspheres: Medical and Biological Applications* (eds Rembaum, A. & Tokes, Z.) 165–192 (CRC, Boca Raton, 1988).