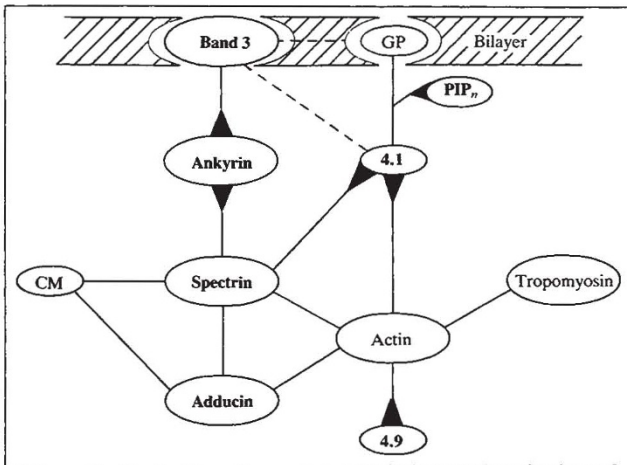

Schematic illustration of reported protein interactions in the red-cell membrane and in the skeleton. Proteins known to be phosphorylated are in bold text; arrowheads indicate interactions affected by phosphorylation. PIP$_n$, phosphatidylinositol phosphates; CM, calmodulin; GP, glycophorin.

Now, Branton and co-workers[1] have hit on something much more like an all-or-none effect of a phosphorylation. But this result too has elusive features. Branton and collaborators study protein 4.9, an actin-binding and bundling protein of inscrutable, even paradoxical function. In solution it exists as a trimer, containing two structurally similar subunits of relative molecular mass 48,000 (48K) and 52K. As the subunits are present in a ratio of 3:1, some of the trimers must contain only the 48K subunit. Both subunits are substrates for cyclic AMP-dependent and -independent kinases. The phosphorylation, it transpires, inhibits actin bundling and at a concentration of two phosphoryl groups per 48K subunit, annihilates it. The effect is reversible, for on dephosphorylation with alkaline phosphatase the activity returns. (Phosphorylation by protein kinase C does not affect bundling, but as only one mole of phosphorus is incorporated it is unclear whether this is because the kinase acts at different sites or causes too low a degree of phosphorylation.)

When bundling is maximal (dephosphorylated protein) a 4.9 trimer binds on average to 11–13 actin subunits (about one turn of the actin helix). The inactive, phosphorylated form also binds to actin filaments but at a concentration of about one trimer per 23 subunits (a strange number), and without effecting any bundling. Herein lies a mystery: here we have a protein with three effectively (or in some trimers, actually) identical actin-binding subunits, yet once it is phosphorylated, it ceases to crosslink actin but still binds. The multivalency that is implied by the bundling activity is not then after all a function of the subunit structure. Moreover, Branton and colleagues find that dephosphorylated monomeric protein 4.9, generated by addition of glycerol, retains its bundling activity in contrast to the phosphorylated subunit. Either, then, phosphorylation knocks out a binding site, or it distorts the geometry such that the sites cannot bind two filaments. But why in this case can a symmetrical trimer not make crosslinks when there is still one working site per subunit?

And what is the protein doing there anyway? By radioimmunoassay, Branton and colleagues[1] find 40,000 copies of 4.9 trimer per cell, which corresponds closely enough to one for each of the actin protofilaments that make up the network junctions. The average separation of these filaments in a regular lattice, such as the membrane skeleton appears to be[4], is far too large to make bundling a possibility. One might with an effort envisage a local, perhaps transient association of protofilaments if the lattice junctions were free to float in the membrane, but there is no evidence for such freedom. The complexity of the system is increased even further when adducin is considered. This calmodulin-binding protein, which, like protein 4.9, is phosphorylated by protein kinase C, also has actin-bundling activity[5].

Of the other phosphorylations shown in the figure, that of 4.1 may be important, for it affects its interaction with spectrin and actin to form a prototypic skeleton complex *in vitro*[6]. But a causal link between phosphorylation and cell functions (in particular shape control) has been established only for the phosphoinositides[7]. One possibility is that phosphorylation-regulated interactions are important at the time of synthesis and assembly of the membrane skeleton and/or cytoplasmic cytoskeleton in the erythroid precursors, and that some of the components simply remain in the mature cells as now useless relics of their early history. All the proteins in question, protein 4.9 included, have close analogues in other tissues. Because the cytoskeletons of other cells are in general much more dynamic than the red-cell membrane skeleton, it appears altogether more likely that phosphorylation of their constituents may play some indispensable role in non-erythroid — neuronal, for example — contexts. □

1. Husain-Chishti, A., Levin, A. & Branton, D. *Nature* **334**, 718–721 (1988).
2. Cianci, C.D., Giorgi, M. & Morrow, J.S. *J. cell. Biochem.* **37**, 301–315 (1988).
3. Lu, P.-W. *et al. J. biol. Chem.* **260**, 14958–14964 (1985).
4. Bennett, V. *A. Rev. Biochem.* **54**, 273–304 (1985).
5. Mische, S.M. *et al. J. Cell Biol.* **105**, 2837–2845 (1987).
6. Eder, P.S. *et al. Biochemistry* **25**, 1764–1770 (1986).
7. Ferrell, J.E., Jr & Huestis, W.H. *J. Cell Biol.* **98**, 1992–1998 (1984).

Lars Backman is in the Department of Biochemistry at the University of Umeå, Umeå, Sweden.

Daedalus

Seeing the strain

Despite great advances in the computer simulation of structures and finite-element analysis of components, engineering design is still a fairly black art. The actual distribution of stresses that a given load will induce in a structure, and the variety of loads that it will meet in service, can only be estimated. Every engineer offsets his ignorance with prudent but wasteful margins of safety.

Daedalus plans to change all this. DREADCO's chemists are synthesizing dyes whose chromophore groups, strung along a polymer backbone, are sterically very crowded. Even a very small dimensional change will cause a big alteration in the electronic overlap between neighbouring chromophore orbitals. As a result, the dye will change colour dramatically when squashed or stretched. The final product will be incorporated into DREADCO's strain-sensitive Loadlacquer®.

Painted with Loadlacquer and loaded, an engineering component will reveal its underlying stress pattern as a colourful map of contrasting hues, readily calibrated by DREADCO's shade card. It will blush coyly in regions of embarrassingly high stress but maintain a stern and unyielding colour where the metal is far too strong for the job. The designer will know immediately what modifications are needed.

Applied to a finished bridge or aircraft, say, Loadlacquer will discount the static load due to the structure itself, and reveal only the additional service loads as they come on. A heavy lorry crossing the bridge will be accompanied by a subtle discoloration of the girders beneath it; the wings of the cruising aircraft will flush in a rainbow gradation from the highly stressed wing roots to the lightly loaded tips, and shimmer subtly with the buffetings of air-turbulence. In either case, excessive loading will signal its own warning; impending disaster will be averted.

Loadlacquer may be even more popular in the domestic sector of the market. The rickety shelves and brackets put up by amateurs, and the precarious ladders they use to do so, could all benefit from its premonitory coloration. Loadlacquer string, giving due warning that it is about to snap, should be especially popular. And fabric woven from Loadlacquer thread could bring a whole new appealing dimension to the rag trade. Jogging outfits whose flashing and shimmering colours mirror their wearers' exertions; provocatively tight creations displaying a stress map of their tense equilibrium with the form inside; baby clothes whose gradually changing hue reveals when the baby has got too big for them — all will swell DREADCO's rosy bank account.

David Jones